## INVITED SPEAKERS (INV)

**INV01**
**Redefining Virulence: Bacterial Gene Expression during Human Infection**
H. L. T. Mobley
*University of Michigan Medical School, Department of Microbiology and Immunology, Ann Arbor, United States*

Investigators identifying virulence genes at first did so by examining transposon mutants or individual gene mutations. Mutants of bacterial pathogens were then assessed in animals, whose symptoms mimicked human disease. Later, genome-wide screens (STM, IVET, IVIAT) were developed whereby genes and proteins that influence virulence could be identified. These efforts led to our conventional view of microbial virulence, with its focus on adhesins, iron acquisition, toxins, secretion, and motility, as well as on those bacteria with genes such as on horizontally transferred pathogenicity-associated islands that are not found in commensal strains. Now, however, we also must consider what metabolic pathways are in play when microbial pathogens infect their hosts. How are these bacteria metabolizing available molecules to colonize a particular body site? Which import and export systems are active during infection? Using Extraintestinal *E. coli* as an example, we demonstrate the importance of measuring gene expression during actual infections either in animal models of infection or in humans themselves, using microarray, RNA-seq, or Tn-seq. Using data from each of these approaches, virulence can be redefined as the sum of classical virulence factors, requisite metabolic pathways, and key import and export pumps. Indeed, measuring gene expression *in vivo* is critical to defining virulence of bacterial pathogens.

**Presentation:** Sunday, September 27, 2015 from 16:40 – 17:25 in room Congress Saal.

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**INV02**
**Multi-resistant Gram-negative zoonotic pathogens - a global threat**
L. H. Wieler
*Robert Koch Institute, Berlin, Germany*

Surveillance of resistant pathogens proofs a constant increase of Multi-resistant Gram-negative bacteria (MRGN) in certain infectious diseases. In veterinary medicine surveillance is mostly restricted to MRGN occurrence or contamination of livestock or food originating from animals. While increasing numbers of nosocomial and wound infections by MRGN are reported in companion animals, lack of surveillance in this area hinders a sound risk assessment and implementation of intervention strategies. In contrast, the medical area concentrates surveillance on isolates from diagnostic laboratories and particular clinical settings as well as specific infectious diseases. These and further differences in surveillance methodology hinder sound analyses of transmission pathways, zoonotic adaptation mechanisms etc. - facts that are further complicated by the increasing evidence of MRGN in wildlife and the environment. This global threat increases the importance of community-associated MRGN.
To gain more insights into phylogeny, relationships and possible transmission routes, bacteria are routinely analyzed by Multi-locus sequence typing (MLST), defining Sequence Types (STs) and also increasingly by comparative whole-genome sequence analyses (WGSA). This paper presents current data on the microevolution of MGRN concentrating on particular pandemic lineages of *E. coli* MRGN from various habitats. The analyses of strains of pandemic ST 131 and the recently recognized ST 648 (Ewers et al. 2014; JAC 2014, 69:1224-30), isolated from humans, livestock, companion animals and wildlife as well as of ST 410 reveals novel insights into their microevolution and possible adaptation mechanisms. Our data once again corroborate the need of future integrated surveillance, linking human, animal and environmental health.

**Presentation:** Monday, September 28, 2015 from 10:30 – 11:00 in room Congress Saal.

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**INV03**
**Breath-taking viral zoonosis: Lessons from influenza viruses**
T. Wolff
*Robert Koch-Institut, Division 17, Influenza viruses and other Respiratory Viruses, Berlin, Germany*

The World Health Organization recently expressed concerns about an unprecedented diversity and geographical distribution of influenza viruses currently circulating in animal reservoirs. This includes an increase in the detection of animal influenza viruses that co-circulate and exchange viral genes giving rise to novel virus strains. As the avian and porcine host reservoirs have in the past contributed essentially to the genesis of human pandemic influenza viruses causing waves of severe respiratory disease on a global scale, this is a notable situation.
Zoonotic transmissions of avian influenza viruses belonging to the H5N1 or H7N9 subtypes have been well documented in recent years. More than 800 human infections with highly pathogenic avian H5N1 viruses have been described with a stunning case fatality of > 50% since 2003, and an upsurge of cases in Egypt in early 2015. Moreover, three waves of human infections with a novel avian reassortant influenza virus of the H7N9 subtype causing severe or lethal lower respiratory tract disease in many patients have been recorded since spring 2013 in China. Significantly, those H7N9 viruses appear to be benign in poultry, the suspected vector species, creating additional challenges for the detection and control of such zoonotic transmissions. Fortunately, both H5N1 and H7N9 influenza viruses have so far shown only a very limited capability to transmit among humans. Finally, spill-over infections of porcine influenza A viruses of different subtypes (H1N1v, H3N2v, H1N2v) to humans have been detected at an increased rate in recent years.
Influenza viruses are characterized by unusually high diversity and changeability of their segmented negative strand RNA genomes enabling them to adapt to new host species or to evade from selective pressures such as antiviral therapy. This presentation will summarize current situations on zoonotic influenza viruses, address recent molecular analyses of genetic polymorphisms accompanying interspecies transmission and discuss possible reasons for the pathogenicity of some animal influenza viruses in humans.

**Presentation:** Monday, September 28, 2015 from 11:00 – 11:30 in room Congress Saal.

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**INV04**
**Novel antimicrobial resistance genes in staphylococci of animal, human, and environmental origin**
S. Schwarz
*Friedrich-Loeffler-Institut, Bundesinstitut fuer Tiergesundheit, Neustadt, Germany*

Abstract has not been submitted.

**Presentation:** Monday, September 28, 2015 from 11:30 – 12:00 in room Congress Saal.

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**INV05**
**Foodborne infections: impact of subtyping isolates**
F. Allerberger
*AGES, Public Health, Vienna, Austria*

Subtyping of isolates is essential for active surveillance. However, typing results can miss epidemiological relations due to under-discrimination and due to over-discrimination. The example of salmonellosis, where incidence has dropped by approx. 80% during the last decade, is impressively underling the potential of public health interventions targeting microbiologically proven transmission chains. For *Salmonella*, serotyping, phage-typing, and antibiotic resistance profiling are old but still efficient work-horses, with pulsed-field gel electrophoresis (PFGE), multiple-locus variable number tandem repeat analysis (MLVA), CRISPR

(clustered regularly interspaced short palindromic repeats) strain characterization, and next-generation sequencing (NGS) often used for confirmation only. The example of campylobacteriosis displays the arduous situation without availability of adequate subtyping methods. The development and widespread application of multilocus sequence typing (MLST) of *Campylobacter* spp. has recently informed source attribution studies, but so far routine subtyping of all isolates is an exception. Enterohaemorrhagic *Escherichia coli* (EHEC) and *Listeria monocytogenes* are rare but, due to their high case-fatality, extremely important food-borne pathogens. By law, in Austria every human isolate and every food isolate has to be submitted to the respective national reference laboratory, where PFGE is performed. While in Austria every human isolate of *Salmonella*, EHEC and *L. monocytogenes* is subtyped (no routine subtyping for *Campylobacter* spp.), other foodborne pathogens are subtyped as needed only. From January 2013 to August 2014, 1589 hepatitis A (HA) cases were reported associated with an outbreak affecting 11 EU member states, with mixed frozen berries as the vehicle of infection. Subtyping of HA virus (HAV) by performing RT- PCR targeting the HAV polymerase gene was a prerequisite to recognize this outbreak. Increasingly whole-genome single nucleotide polymorphism-based approaches are used to identify the source of foodborne infections and to clarify the epidemiology of outbreaks. The continuing introduction of new bioinformatics tools for rapid comparison of SNPs and open-access NGS databases will simplify surveillance and speed up outbreak investigation.

**Presentation:** Monday, September 28, 2015 from 13:30 – 14:00 in room Congress Saal.

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**INV06**

**MRSA and ESBL in Denmark - two prevention strategies - two outcomes**

R. Skov
*Statens Serum Institut, Microbiology and Infection, Copenhagen, Denmark*

The worldwide increase in resistance among bacteria is very worrisome. For MRSA and E.coli ESBL and CPE the problems has been aggravated the last 1-2 decades by transmission and persistence of resistant strains not only in hospitals but also in the community which challenges infection control practices.
In Denmark MRSA has been controlled vigorously since the mid 1970s. For a long time this was driven by the profession lead by clinical microbiologists and infection control nurses (with a very high degree of consensus and compliance). Around year 2000 major changes was seen in the MRSA epidemiology: 1) we began to see community acquired MRSA and experienced a large 4-year lasting hospital associated outbreak in one of the Danish counties. These changes gave rise to a national guideline for prevention of MRSA issued by the National Board of health (2006) – The guideline addressed both hospitals, nursing homes and community including screening procedures, isolation and barrier precautions and treatment of carriage.
For ESBL the situation has been somewhat different. ESBL was only sporadic until the mid-2000s but from 2006 the situation changed into an endemic state with an prevalence of 5-15% of bloodstream isolates of K. pneumoniae and E. coli. In contrast to MRSA, neither professional consensus nor agreement of developing a national guideline for ESBL producing enterobacteriaceae has been achieved. This is also the case for vancomycin resistant enterococci and for carbapenemase producing enterobacteriaeceae (CPE).
For MRSA, Denmark has been challenged by the emergence of LA-MRSA and the incidence is rapidly rising( ~3000 new cases in 2014). Despite this, the prevalence of hospital acquired MRSA infections continues to be very low (N = 49 in 2014), with only 50 cases of MRSA bacteremia (3% of S. aureus bacteremia. For ESBL the incidence has leveled off at a quite high level of 7% (N = 300) cases of E. coli ESBL bacteremia and 9% (N = 70) cases of ESBL K. pneumonia. VRE clinical cases has increased rapidly the last couple of years from 54 in 2012 to increase from 54 clinical cases in 2012 for >300 in 2014. For CPE the actual numbers are still low but has gone from "0" to >50 in over the last 5 years.
In the talk the development in Denmark for MRSA, ESBL, CPE and VRE will be presented and discussed in relation to differences in infection control practices and presence/absence of national guidelines.

**Presentation:** Monday, September 28, 2015 from 14:00 – 14:30 in room Congress Saal.

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**INV07**

**Controlling HAI in Germany and France: similarities and differences**

J.-C. Lucet
*Hôpital Bichat - Claude-Bernard, Infection Contact Unit, Paris, France*

Abstract has not been submitted.

**Presentation:** Monday, September 28, 2015 from 14:30 – 15:00 in room Congress Saal.

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**INV08**

**Real-time Monitoring of Multi-resistant Bacteria in a University-Hospital by Whole Genome Sequencing**

A. Mellmann
*University Hospital Muenster, Institute of Hygiene, Muenster, Germany*

Driven by the rapid development of next generation sequencing (NGS) technologies, in the near future shotgun whole genome sequencing (WGS) of bacterial pathogens will be applied in clinical microbiology and infection control to unravel both the molecular epidemiology and further information such as the pathogenicity make-up and antibiotic resistance traits. Whereas the laboratory workflow to generate WGS data is nowadays already quite convenient and suitable for integration into a routine laboratory environment, data analysis and interpretation is still the major obstacle for broad usage of WGS. This presentation will demonstrate the applicability of WGS in a routine clinical laboratory. Over a 6-month period, all multidrug-resistant bacteria that were isolated from patients at the University Hospital Muenster, Germany, were subjected to WGS in a prospective manner to monitor their molecular epidemiology and to determine potential transmission events. Here, not only the molecular data will be shown but also the technical feasibility, the potential impact of patient care, and the cost-effectiveness of prospective WGS in a routine environment will be discussed.

**Presentation:** Tuesday, September 29, 2015 from 10:30 – 11:00 in room Congress Saal.

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**INV09**

**Genomic epidemiology of bacterial pathogens: harnessing high-throughput sequencing to monitor the global emergence of virulent and multiresistant clones**

S. Brisse
*Institut Pasteur, Microbial Evolutionary Genomics, Paris, France*

High-throughput sequencing has revolutionized our abilities to track and characterize novel bacterial strains as they emerge and disseminate globally. Genome-based typing systems are being developed to replace or complement classical epidemiological markers and have the potential to provide ultimate resolution for strain discrimination. Genome sequences may also be used to extract medically important features such as virulence and resistance genes in real time. Examples of how high-throughput genomic sequencing is changing population biology, molecular surveillance and outbreak investigation of bacterial pathogens will be presented, with a focus on hospital-acquired and food-borne infections. Bioinformatics tools that enable international collaboration on epidemiological surveillance will be presented.

**Presentation:** Tuesday, September 29, 2015 from 11:00 – 11:30 in room Congress Saal.

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**INV10**
**Rapid Pathogen Identification by Metagenomic NGS of Clinical Specimens**
S. Miller
*University of California, Laboratory Medicine, San Francisco, United States*

Conventional diagnosis of acute infectious disease relies on the ability to cultivate growth in a variety of media, amplify specific nucleic acid targets, or identify a specific antigen or immune response, leading to a continually expanding number of diagnostic tests. The entire panel of microbiologic testing can in principle be performed using metagenomic next-generation sequencing (NGS), which can detect nucleic acid from all types of pathogens, except prions. This can be particularly useful for immunocompromised and transplant patients, where the range of potential pathogens is extremely broad. We have developed a protocol for unbiased metagenomic NGS of clinical samples to enable pan-pathogen detection, and determined performance characteristics for a variety of specimen types. Randomly amplified nucleic acid is sequenced and subjected to bioinformatics analysis where human sequences are removed and microbial sequences identified and classified according to taxonomic status using the SURPI pipeline (Sequence-based Ultra-Rapid Pathogen Identification). Data visualization tools allow the clinical microbiologist or laboratorian to interpret complex metagenomic data and identify sequences of potential infecting organisms, and to perform genomic and epidemiologic evaluations. This presentation will discuss the ongoing development of clinical metagenomic NGS, and challenges and strategies for implementation within the clinical laboratory.

**Presentation:** Tuesday, September 29, 2015 from 11:30 – 12:00 in room Congress Saal.

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**INV11**
***Staphylococcus aureus* alpha-toxin in the host-pathogen interaction**
J. Bubeck Wardenburg
*University of Chicago, Critical Care Departments of Pediatrics and Microbiology, Chicago, United States*

*Staphylococcus aureus* alpha-toxin is a pore-forming cytotoxin known to exert complex effects on an array of targeted host cells. While many of these effects have been studied in isolation or in the context of single organ-system disease, the pleiotropic actions of the toxin suggested that it may coordinate molecular and cellular events in systemic disease. Multi-organ failure is associated with mortality in bacterial sepsis. Platelet and immune cell activation contribute to organ injury during sepsis, but the mechanisms by which bacterial virulence factors initiate these responses remains poorly defined. We demonstrate that during lethal sepsis, *Staphylococcus aureus* αlpha-toxin simultaneously alters platelet activation and promotes neutrophil inflammatory signaling through interactions with its cellular receptor ADAM10. Platelet intoxication prevents endothelial barrier repair and facilitates formation of injurious platelet-neutrophil aggregates, contributing to lung and liver injury that is mitigated by ADAM10 deletion on platelets and myeloid lineage cells. While platelet or myeloid-specific ADAM10 knockout does not alter sepsis mortality, double knockout animals are highly protected. These results define a pathway by which a single bacterial toxin utilizes a widely-expressed receptor to coordinate progressive, multi-organ disease in lethal sepsis. As an expression-enhancing ADAM10 polymorphism confers susceptibility to severe human sepsis, these studies highlight the importance of understanding the molecular basis of the host-pathogen interaction.

**Presentation:** Tuesday, September 29, 2015 from 13:30 – 14:00 in room Congress Saal.

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**INV12**
**Interaction of Salmonella enterica with the intestinal mucosa: Role of the giant non-fimbrial adhesin SiiE**
M. Hensel
*Universitaet Osnabrueck, Fachbereich 5: Biologie/Chemie, Osnabrueck, Germany*

Please see page 173.

**Presentation:** Tuesday, September 29, 2015 from 14:00 – 14:30 in room Congress Saal.

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**INV13**
**Transfer of Shiga toxin within host extracellular microvesicles**
D. Karpman*[1]
[1]*Lund University, Department of Pediatrics, Clinical Sciences, Lund, Sweden*

This study describes a novel mechanism of transfer of bacterial toxin to target organ cells within host blood cell-derived microvesicles in the circulation. This was achieved by studying pathogenetic mechanisms of Enterohemorrhagic *E. coli* (EHEC) infection. EHEC are non-invasive bacteria. The transfer of bacterial virulence factors via the circulation to target organs is essential for disease development. Previous studies from our group and others have shown that the toxin does not circulate in free form but rather bound to blood cells. We have shown that toxin-stimulated blood cells release microvesicles and that patients exhibit high levels of blood cell-derived microvesicles carrying complement and tissue factor. In the present study we demonstrated that Shiga toxin binds to blood cells, undergoes endocytosis and is expelled within blood cell-derived microvesicles. These microvesicles then circulate and bind to target organ cells in the kidney from where they migrate with their cargo within cells and release toxin along the way. Shiga toxin circulating within host blood cell-derived microvesicles evades the host immune system but retains its toxicity. Blood cell-derived microvesicles containing bacterial toxin may pass from cell to cell within the kidney and even through basement membranes. Shiga toxin may thus affect cells in a toxin receptor-independent manner.

**Presentation:** Tuesday, September 29, 2015 from 14:30 – 15:00 in room Congress Saal.

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**INV14**
**Multiple identities and roles of HMGBI in sterile inflammation**
M. Bianchi
*San Raffaele University, Genetic and Cell biology, Milano, Italy*

Abstract has not been submitted.

**Presentation:** Wednesday, September 30, 2015 from 10:30 – 11:00 in room Congress Saal.

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**INV15**
**Innate immunity and susceptibility to tuberculosis**
S. Nejentsev*[1]
[1]*University of Cambridge, Department of Medicine, Cambridge, Great Britain*

Tuberculosis (TB) is a major public health problem in the developing world and an increasing threat in developed countries. Human genetic factors determine susceptibility to *Mycobacterium tuberculosis* infection and can predispose to clinical TB. Discovery of human genes involved in responses to mycobacterial infection and susceptibility to TB can highlight novel mechanisms in the disease pathogenesis. I will discuss results of our large genome-wide association study of TB susceptibility and a recent study investigating genetic control of transcriptional responses to mycobacterial infection in human macrophages.

**Presentation:** Wednesday, September 30, 2015 from 11:00 – 11:30 in room Congress Saal.

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**INV16**

**S100 alarmins: linking sterile inflammation and infections**

J. Roth

*University of Muenster, Institute of Immunology, Muenster, Germany*

Innate immune cells respond to invading pathogens via recognition of conserved microbial patterns by so-called pattern recognition receptors (PRR). In contrast, the term "sterile inflammation" describes inflammatory processes not triggered by microbial agents but rather induced by endogenous molecules released during tissue damage or cell stress. These endogenous triggers, called alarmins, act as extracellular danger signals triggering inflammation when released by damaged or activated cells. S100A8 (synonym myeloid related protein, MRP8) and S100A9 (MRP14) are the most abundant alarmins released by activated phagocytes during many diseases, like infections, arthritis, allergies, autoimmune diseases, inflammatory bowel, pulmonary or cardiovascular diseases. Interestingly, S100A8/S100A9 promote inflammation as endogenous ligands of Toll-like receptor 4 (TLR4) a well-known receptor for the microbial product endotoxin. In my presentation I will describe a new mechanism of an alarmin-TLR4 interaction and unravel a novel molecular pathway by which auto-inhibitory regulation of S100A8/S100A9 activity restricts TLR4-driven inflammation to local sites of infection or injury.

**Presentation:** Wednesday, September 30, 2015 from 11:30 – 12:00 in room Congress Saal.

# MICROBIOLOGICAL DIAGNOSTICS (DVP)

**001/DVP**

**Novel growth monitor for antibiotic susceptibility testing of bacteria**

M. Droege
*Fraunhofer FIT, BioMOS, Sankt Augustin, Germany*

Sepsis is the third most common cause of death in Germany. Each year, 154.000 people in Germany are suffering from sepsis, from which 56.000 die (SepNet study 2003/04). The cause of the high mortality rate of over 36% is mainly the long period of the presumptive diagnosis and introduction of an appropriate therapy. In addition, the increasing spread of multi-resistant bacteria causes some cases of the disease which are no longer treatable with the limited range of antibiotics. A targeted and effective antibiotic therapy, can only be done if the sensitivities, or the resistance of the sepsis pathogens are known (antibiotic susceptibility testing). Since this information is available in the current clinical standard workflow application at the earliest after 24-72h hours, Fraunhofer FIT has developed a novel miniaturized growth monitor, which significantly reduces the time to determine the susceptibility of the pathogens.
The growth monitor consists of a microculture chip and microscopic optics that pictures the image onto a camera chip. The images are analyzed with a specially developed, trainable software that calculates the growth curves as well as the resulting sensitivity of pathogens to the antibiotics tested. The microculture chip represents the miniaturization of agar plates, which are established in microbiology for decades. Caused by the analogy to the standard method, the growth monitor is easy to integrate into existing clinical workflows and provides a low acceptance threshold for physicians and clinical staff. Due to microscopic observation of the growth behavior of individual cells and the parallel-running analysis, the results regarding the sensitivity / resistance of pathogens to the antibiotics, may already be present after 2-3 hours. The microscopic images are available to the physician at any time, so that an additional control, for example on the morphology of individual pathogens can be performed.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**002/DVP**

**DiAL-FISH for the rapid detection and identification of bacterial agents**

K. Aistleitner*[1], K. Stoecker[1], T. Sieper[1], I. Stuerz[1], R. Woelfel[1]
[1]*Bundeswehr Institute of Microbiology, Dept. of Bacteriology and Toxinology, Munich, Germany*

Fluorescence in situ hybridization (FISH) is a powerful method for the cultivation-independent in situ detection and identification of microorganisms. Since its establishment over two decades ago it has become an essential tool in microbial ecology. As it is an easy, robust, cheap and rapid method, FISH is also occasionally used in clinical settings. However, until recently only three bacterial species could be identified simultaneously in one hybrization step, making the identification of larger sets of bacterial species laborious and time-consuming, thereby impairing the use of FISH in many diagnostic approaches.
Here we report on the development of an rRNA-targeted FISH based diagnostic algorithm (DiAL-FISH) allowing for the cultivation-independent rapid detection, identification and quantification of up to thirteen bacterial pathogens in clinical samples. To overcome the above mentioned limitation of FISH based diagnostics we combined group- and species-specific multicolored double-labeled oligonucleotide probes, facilitating the specific identification of a species by only two hybridizations in less than four hours.
Currently, the algorithm relies on a mixture of newly designed and previously published probes and targets thirteen bacterial pathogens, but may be extended to a larger number of organisms. Target organisms include for example *Yersinia pestis*, *Brucella spp.*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Vibrio cholerae* and *Francisella tularensis*. In contrast to conventional FISH, which uses formamide to adjust the stringency of probe binding, we aim to substitute for this reagent by non-toxic urea, as the toxicity of formamide renders a fume hood mandatory and thus sometimes limits the application of FISH. Interestingly, usage of urea so far not only results in the specific detection of bacteria, but also in brighter fluorescence signals for some probes.
In summary, DiAL-FISH allows the fast and direct visualization of rarely occurring, but important bacterial pathogens in a straightforward and robust manner. In addition, it provides a different and independent laboratory method that supplements PCR-based detection methods for these bacteria.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**003/DVP**

**Fast and direct antibody microarray-based detection and discrimination of *Legionella pneumophila* serogroups**

E. Mueller*[1], P. Miethe[2], A. Helming[2], R. Ehricht[1]
[1]*Alere Technologies GmbH, Research&Development, Jena, Germany*
[2]*FZMB GmbH, Bad Langensalza, Germany*

*Legionella pneumophila* is the predominating causative agent of Legionnaires' disease. It is responsible for about 90% of cases of legionellosis. *L. pneumophila* comprises approximately 15 known serogroups out of which serogroup 1 is the most significant one. SG-1 causes more than 95% of travel-associated cases of Legionnaires' disease and more than 85% of community-acquired cases, but only about 50% of nosocomial infections. In Germany, about 600 cases of Legionnaires' disease per year are reported and documented. However, the true case number is probably as high as 15,000-20,000 per year according to estimates by the CAPNET pneumonia study group, i.e., approximately 4% of all cases of community-acquired pneumonia. For epidemiological purposes, such as outbreak investigations, there is a big demand for assays that facilitate a rapid discrimination of serogroups (SG) and strains of legionella.
A specific protein array was developed using antibodies in order to discriminate *Legionella pneumophila* serogroups 1 to 14. Eleven antibodies were spotted in various dilutions and with different additives. Tests were performed with harvested culture material of 26 known *Legionella pneumophila* strains. These included 12 different variants of SG-1 as well as representatives for SGs 2 to 14. Antigens were detected using biotinylated detection antibodies; and for that purpose the same 11 monoclonal antibodies as well as one additional polyclonal antibody were used. After staining with streptavidin-horseradish peroxidase conjugate and a precipitating dye, arrays were scanned and resulting images were analysed. Detection antibodies were used alone and in various combinations in order to generate strain-/SG-specific patterns. This was achieved by using two different mixtures of detection antibodies with two antibody microarrays per sample.
The next step will be to construct and to expand a database of specific patterns by testing more known strains and eventually by screening unknown isolates that are to be assigned by pattern comparison to the database. Compared to ELISA, this method can operate faster and with much smaller quantities of antibodies thus saving major expenses. Compared to sequence-based typing (SBT/MLST), this approach is much faster and this might be useful especially in outbreak situations.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**004/DVP**

**Analysis of Epstein-Barr virus (EBV) serology using the "Liaison® EBV Guide to the Interpretation of the Results" by DiaSorin**

A. Frister[1], C. J. Téllez-Castillo*[1], C. Guenther[1], B. Poetschke[1]
S. Neumann[1], R. Findeisen[1]
[1]*Oberlausitz-Kliniken gGmbH, Institut fuer Labordiagnostik, Mikrobiologie und Krankenhaushygiene, Bautzen, Germany*

**Introduction:** Epstein-Barr virus (EBV) is one of the most common human viruses. The seroprevalence for people older than 30 years is more than 90%.
**Objectives:** Definite confirmation or exclusion of an acute primary infection with EBV is of great importance in differential diagnosis.
**Material & Methods:** The analyses were performed at the hospital Bautzen of Oberlausitz-Kliniken gGmbH. Quantitative automated luminometric immunoassays for the determination of EA-IgG, EBV-IgM, VCA-IgG and EBNA-IgG were used at LIAISON XL (DiaSorin Deutschland GmbH). The interpretation according to the "Liaison® EBV Guide to the interpretation of the results" (Table 1) was done and additional EA-IgG testing was evaluated.
**Results:** We analyzed 406 sera from 208 male and 198 female patients. The status of EBV-infection is shown in Table 2.
**Conclusions:** Testing of the three parameters EBV-IgM, VCA-IgG and EBNA-IgG without EA-IgG is sufficient for efficient EBV serology.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

**Table 1:** Interpretation of the results

| Suggested interpretation | EBV IgM [U/ml] | VCA IgG [U/ml] | EBNA IgG [U/ml] |
|---|---|---|---|
| Negative | <20 | <20 | <20 |
| Suspected primary infection (onset) | ≥20 | <20 | <20 |
| Acute phase of primary infection | ≥20 | ≥20 | <20 |
| Transient phase | ≥40 | ≥20 | ≥20 |
| Past infection | <40 | ≥20 | ≥20 |
| Past infection | <20 | ≥20 | ≥5 |
| Unresolved (VCA IgG only) | <20 | ≥20 | <5 |
| Unresolved (repetition of all tests necessary) | Other combinations | | |

**Figure 2**

**Table 2:** Results

| Status of EBV-Infection | N | Gender | | Age (Years) | |
|---|---|---|---|---|---|
| | | Male | Female | Mean | SD |
| Negative | 73 | 43 | 30 | 6,4 | 7,3 |
| Suspected primary infection (onset) | 12 | 4 | 8 | 6,3 | 5,6 |
| Acute phase of primary infection | 40 | 19 | 21 | 18,1 | 16,1 |
| Transient phase | 9 | 4 | 5 | 24,5 | 19,7 |
| Past infection | 236 | 117 | 119 | 45,8 | 25,1 |
| Unresolved (VCA IgG only) | 23 | 13 | 10 | 39,6 | 26,6 |
| Unresolved (repeat all the tests) | 6 | 5 | 1 | 31,3 | 18,5 |

**005/DVP**

**Dual-temperature microbiological control of cellular products: sense or nonsense for bacterial screening of platelet concentrates**

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[1]*Institut fuer Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum NRW, Bad Oeynhausen, Germany*

**Background:** An experimental study by the Paul-Ehrlich institute (PEI) demonstrated that temperatures between 35-37°C are too high for the growth of some bacterial strains (e.g. *Pseudomonas fluorescens*). Therefore, the PEI passed a statement including the requirement of a dual-temperature microbiological control of haematopeotic stem cell preparations which was further assumed in a draft manuscript regarding the amendment of chapter 2.6.27 of the European pharmacopoeia in 2013. We analyzed the growth kinetics of several bacteria in platelet concentrates (PCs) under standard storage conditions and compared the results of the microbiological control at incubation temperatures of 25 °C and 35 °C.
**Methods:** In total, PCs were inoculated with 37 strains (3-6 donors per strain) from different origins (PC isolates, reference strains) and stored for 3 days at 20-22°C under constant agitation. Subsequently, inoculated PCs were split to inoculate aerobic and anaerobic culture bottles (BacT/Alert AST/NST), each with 5 mL of sample and culture bottles were incubated at 25 °C and 35 °C using the automated BacT/Alert Dual temperature system.
**Results:** All tested strains of *Staphylococcus* spp. (n=10), *Streptococcus* spp. (n=4), *Bacillus* spp. (n=4) and *Pseudomonas aeruginosa* (n=5) showed bacterial proliferation within three days in most inoculated PCs, revealing a faster growth kinetic at an incubation temperature of 35 °C. Tested *P. putida* (n=3) strains showed a noticeable reduced capability to grow in PCs. Nonetheless, those having a growth capability revealed faster growth kinetics at an incubation temperature of 35 °C. Exclusively one of four tested *P. fluorescens* strains (strain ATCC 13525) was able to grow in PCs showing a faster growth kinetic at an incubation temperature of 25 °C but also detection at 35 °C.
**Conclusion:** The commonly detected bacteria involved in bacterial contamination of PCs showed no reduced detectability at 35°C incubation for microbiological control. Only one *P. fluorescens* strain showed an enhanced growth at 25°C, but the microbiological control at 35°C did not fail to identify this contamination. In conclusion, screening of PCs using a dual-temperature setting for the microbiological control impresses at the moment as "using a sledgehammer to crack a nut".

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

**006/DVP**

**Rapid Broad-Range Detection and Identification of Pathogenic Bacteria and Candida from Patients with Suspected Pneumonia using PCR/ESI-MS Technology**

M. Picard-Maureau*[1], T. Brodegger[2]
[1]*Abbott GmbH & Co. KG, Wiesbaden, Germany*
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**Question**: Currently available molecular tests to detect pathogenic bacteria and Candida from patients with suspected lower respiratory tract infections have limitations regarding the broadness of the pathogen spectrum. Multiplex PCR approaches detect a limited number of major pathogens, often with a decreased sensitivity. We assessed the performance and usability of PCR/ESI-MS technology, a combination of broad-range PCR and Electrospray Mass Spectrometry, in comparison to culture-based standard of care technologies, using the Abbott IRIDICA system with the BAC LRT Assay, allowing to detect and identify a broad range of more than 750 bacteria and Candida from lower respiratory tract specimen.
**Methods**: 109 endotracheal aspirate samples have been collected from patients with suspected pneumonia. Each sample was analyzed in parallel with two different methods: Culture-based analysis including identification with Bruker MALDI-TOF/Biomerieux Vitek-2, and PCR/ESI-MS analysis with the Abbott IRIDICA system and the BAC LRT Assay. The results were compared in conjunction with the clinical picture.
**Results**: The results were concordant between culture-based and PCR/ESI-MS in 38 cases, (concordant negative in 3 cases, concordant positive in 35 cases). Discrepancies have been observed in 71 cases (65.1%), in 62 (56.9%) cases discrepancies were observed with respect to potentially pathogenic bacteria & Candida. In these samples 17 additional potentially pathogenic bacteria & Candida species were detected by culture-based methods in 16 samples while 79 additional potentially pathogenic bacteria & Candida species were detected in 59 samples by PCR/ESI-MS.
**Discussion**: PCR/ESI-MS technology (Abbott IRIDICA system) allows the detection of a significantly broader spectrum of bacteria & Candida compared to currently available PCR-based methods. The turnaround-time of the IRIDICA system was 6-8 h, while culture-based results are normally available after 1-2 days.
Both methods allowed the identification of 115 pathogens. 18 were identified by PCR/ESI-MS and culture concordantly (15.7%) while another 18 species (15.7%) were only identified by culture-based methods and another 79 species by PCR-ESI-MS only (68.7%).

Especially S. pneumoniae (26), S. pseudopneumoniae (9) and H. influenzae (5) have not been detected by culture-based methods.
The IRIDICA system showes the potential for improving current culture-based diagnostic methods by identifying additional pathogenic bacteria & Candida species, and by getting results within 6-8 h, allowing earlier potential optimization of therapy.
**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**007/DVP**
**Metabolic activity of selected acidogenic bacteria reflects caries activity**
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**Background:** Dental caries is a polymicrobial disease with many associated taxa, such as *Streptococcus mutans, lactobacilli, bifidobacteria,* and *Scardovia wiggsiae.* The extended ecological plaque hypothesis proposes changes in the environment disrupting the homeostasis between the microflora and the host as the main reason for dental decay, which results from enamel and dentin demineralization by acids fermented from nutritional sugars. Until now no reliable microbiological method exists to decide whether a caries lesion is active or inactive, required as key information for further treatment. It is our hypothesis that an increase in the metabolic activity of the aciduric and acidogenic bacteria reflected by a rise in ribosomes per cell could serve as a measure for caries activity.
**Objective:** The objective was to elucidate the metabolically active portion of caries-associated bacteria in three categories of clinical samples.
**Material and methods:** Plaque samples from 13 caries-active individuals with cavitated dentin caries (CAC) and plaque samples from a sound dental control surface (CAS) from the same individuals were taken. Further 10 samples were taken from sound surfaces of caries-free individuals (CFS). DNA and RNA were purified and the ribosomal 16S-rRNA and 16S-rRNA-gene were amplified by q-PCR or rt-q-PCR with species- (*S. mutans, S. wiggsiae*) or genus- (*lactobacilli, bifidobacteria*) specific primers as well as with universal primers. Bacterial counts per mg and relative metabolic activity were calculated. A *Fusobacterium* genus-specific PCR served as control as members of this genus are not caries-associated but almost always present in oral biofilm.
**Results:** Biofilm samples from caries-free individuals (CFS) showed low total cell numbers but highest values for total ribosomes and thus high metabolic activity. This might reflect the re-growth of healthy plaque after regular tooth-brushing events. An increasing trend for caries-associated taxa tested and - interestingly - a concomitant inverse trend for *fusobacteria* in the relative metabolic activity was observed in the order CFS > CAS > CAC.
**Conclusions:** The present study suggests different metabolic activity patterns for biofilms from caries-free and caries-active individuals. As especially *lactobacilli* showed a tendency for over-average relative metabolic activity in CAC, a major contribution in cariogenic processes can be expected. Biofilm from caries-free individuals showed highest overall metabolic activity but lowest presence and metabolic activity for caries-associated bacteria. The relative metabolic activity in marker species, both for health and disease, could function as caries activity measure and indicator for treatment need, but further studies are needed to confirm these findings.

This presentation is a part of a study that is also presented at the 62nd ORCA Congress in Brussels, Belgium.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**008/DVP**
**Application of the Alere™ PBP2a Culture Colony Test for the detection of MRSA from a chromogenic selective medium**
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The use of chromogenic selective media for MRSA screening diagnostics has greatly reduced the time between retrieval of swabs and reporting a preliminary result to the ward. If colonies indicative for MRSA grow, it has become common practice for many laboratories to perform tests with chromogenic agar-derived colonies for rapid confirmation of species affiliation and methicillin-resistance (e.g. PBP2a assay). This is limited by the fact that some PBP2a detection assays are only validated by the manufacturer for cultures from non-chromogenic agars. Hence, in this study, we evaluated whether using colonies from a chromogenic agar produced reliable results when subjected to the Alere™ PBP2a test.
Methods: Between April 2014 and January 2015, colonies growing on chromID MRSA agar (bioMérieux) were directly tested by Alere™ PBP2a Culture Colony Test without subculture on non-selective media. Subsequently, PBP2a test results were confirmed by susceptibility testing using VITEK™ 2 automated system (applying EUCAST clinical breakpoints) and PCR targeting an *S. aureus*-specific marker gene and *mecA* (GenoType MRSA, Hain Lifescience) and, if *mecA*-negative, *mecC* by an in house-procedure*. Only the first isolate per patient was included.
Results: During the study period, 490 pheno- and genotypically confirmed MRSA were included. The PBP2a test yielded a positive result when performed on colonies from chromogenic agar in 488/490 cases (99.6%). The two (0.4%) PBP2a negative, but genotypically confirmed MRSA isolates were *mecC*-positive and belonged to *spa* type t843. Three isolates that grew on chromogenic agar and were PBP2a/*mecA*/*mecC* negative were phenotypically resistant to cefoxitin representing potential borderline *S. aureus* (BORSA) strains.
Discussion: We found that the Alere™ PBP2a immunochromatographic assay tested yielded reliable results when performed directly on colonies from the chromogenic agar tested, which greatly facilitates routine diagnostics. Only *mecC*-positive strains, which represented 0.4% of the isolates, were not correctly identified as being MRSA by the PBP2a test.
* Kriegeskorte et al., Human MRSA isolates with novel genetic homolog, Germany. Emerg Infect Dis. 2012;18:1016-8.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**009/DVP**
**Evaluation of the eazyplex®MRSA system for the detection of MRSA and MSSA strains**
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**Introduction**: The rapid detection and meticulous identification of methicillin-resistant *Staphylococcus aureus* (MRSA) strains is essential for disease management and outcome as well as surveillance purposes. The heterogeneity of the SCC*mec* element including the methicillin resistance-encoding gene presents a challenge for the molecular diagnostics of MRSA.
**Objectives**: To study a novel multiple loci-targeting, freeze-dried, ready-to-use LAMP-based amplification system (eazyplex®MRSA) for ultra-rapid MRSA detection combining species discrimination between *S. aureus* and *S. epidermidis* with the detection of *mecA* and *mecC* genes, a well-characterized collection of staphylococcal challenge strains was tested.
**Methods**: Overall, 53 clinical MRSA strains were tested comprising the most prevalent *spa* types and including four *mecC*-positive isolates of four different *spa* types. In addition, 40 MSSA isolates from various German studies and 54 non-*S. aureus*

staphylococcal type and reference strains were included, comprising different staphylococcal species and subspecies.
**Results**: All MRSA and MSSA challenge isolates were identified correctly as belonging to the species *S. aureus* and the detection of *mecA*/*mecC* genes was accurate in all cases. Testing of non-*S. aureus* strains demonstrated reliable results, two type strains (*S. fleurettii* DSM 13212 and *S. hominis* subsp. *novobiosepticus* ATCC 700236) showed unexpected findings of a positive *mecA* signal. However, re-testing by other approaches confirmed the presence of the *mecA* gene.
**Conclusions**: Applying a collection of challenge strains, eazyplex®MRSA system has proved to be suitable for identification of MRSA and distinction between MSSA and non-*S. aureus* strains. The evaluation of this assay for the direct detection of MRSA from screening samples warrants further investigation.

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**010/DVP**
**Rapid phenotypic MRSA detection by a real-time laser-scattering method**
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**Introduction:** Rapid differentiation between methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* isolates is crucial for the initiation of an appropriate and targeted antimicrobial therapy. BacterioScan$^{TM}$216R (BacterioScan Inc., St Louis, US) is a novel device, which uses laser-scattering technology to rapidly quantify bacteria in fluid samples.
**Objectives:** This study aimed (i) to determine the potential of this method to differentiate between MRSA and MSSA strains and (ii) to determine the incubation time required for reliable results.
**Materials and methods:** Suspensions of $5x10^5$ cfu/ml of *S. aureus* ATCC 29213 (MSSA) and *S. aureus* ATCC BAA-44 (MRSA) reference strains were prepared in Brain-Heart-Infusion (BHI) broth and incubated in the BacterioScan$^{TM}$216R device with and without addition of cefoxitin applying the breakpoint concentration of 4 µg/ml. Incubation was accomplished at 36°C for up to 24 hours, measurements were taken automatically approx. every 3 min for each sample. The tests were performed in triplicate, sterile BHI control was included.
**Results:** Detection of MRSA or MSSA phenotype was possible within 2.5 hours after start of incubation. Reproducible categorization as methicillin-susceptible was obtained based on a distinguishing criterion of at least 1 $\log_{10}$ cfu/ml difference between the growth control curve and the growth curve of the same strain with addition of cefoxitin. In MRSA, growth curves under addition of cefoxitin were comparable with the growth control without antibiotic and the above-mentioned criterion was not achieved. Sterility control curve remained under the device's detection level.
**Conclusion:** Real-time laser-scattering method using BacterioScan$^{TM}$216R possesses potency for rapid phenotypic differentiation between MSSA and MRSA. Future optimization of inoculum size, broth, cut-off criteria and other conditions may allow even shorter time to result. Further investigations are warranted for the validation of the method on a large collection of clinical MRSA and MSSA strains.
**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**011/DVP**
**Validation of the Xpert® Carba-R Assay for detection of carbapenemase genes in cultured *Enterobacteriaceae* strains**
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**Question**: Only limited treatment options are available for carbapenemase-producing *Enterobacteriaceae* (CPE). Reliable recognition of CPE is important for implementation of appropriate infection control measures and therapy and can be achieved by phenotypic as well as molecular methods. One advantage of the latter is detection of different carbapenemase genes which can be helpful in outbreak detection. The most important carbapenemases in Germany are KPC, VIM, NDM and OXA-48 like enzymes. The aim of this study was to validate a fully automated assay on cultured strains from a collection of *Enterobacteriaceae* with reduced susceptibility to carbapenems.
**Methods**: Previously characterized *Enterobacteriaceae* from Germany with reduced susceptibility to carbapenems (n = 197) and different resistance mechanisms were grown on Columbia blood agar. Bacterial colonies were suspended and adjusted to an optical density equivalent to McFarland 0.5 and diluted 1:10 in 0.9% saline, of which 10 µl were added into Sample Reagent (Cepheid). Approximately 1.7 ml of this suspension was transferred into the Xpert® Carba-R cartridge (Cepheid), which was further handled according to manufacturer's instructions.
**Results**: Testing of eight strains (4.1%) produced an error making retesting necessary. Repeated tests gave true positive results. Sensitivities were 100% (95% CI: 89.7% to 100%) for $bla_{KPC}$ , 100% (95% CI: 88.1% to 100%) for $bla_{VIM}$, 100% (95% CI: 15.8% to 100%) for $bla_{IMP\text{-}1\ like}$, 100% (95% CI: 86.3% to 100%) for $bla_{NDM}$, and 85% (95% CI: 73.4% to 92.9%) for $bla_{OXA\text{-}48\ like}$. All isolates with $bla_{OXA\text{-}181}$ and $bla_{OXA\text{-}232}$ gave false-negative results, whereas all strains with $bla_{OXA\text{-}48}$, $bla_{OXA\text{-}162}$, $bla_{OXA\text{-}204}$ and $bla_{OXA\text{-}244}$ were identified. Of note, with the exception of $bla_{OXA\text{-}181}$ and $bla_{OXA\text{-}232}$ also strains harbouring two carbapenemases were correctly detected. No false positive results were recorded.
**Conclusions**: The Xpert® Carba-R Assay reliably detects $bla_{KPC}$, $bla_{VIM}$, $bla_{NDM}$ and $bla_{OXA\text{-}48}$. However, detection fails for genes coding for certain OXA-48 like enzymes such as OXA-181 and OXA-232 and the user should be aware of this limitation.

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**012/DVP**
**HIV- and syphilis-counselling and -testing in the public health service in North Rhine-Westphalia (NRW)**
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**Background:** Since 1987 the NRW Centre for Health (LZG.NRW) offers free, anonymous HIV testing and counselling under the „state programme to combat the AIDS epidemic", which is implemented by the local health authorities in NRW. This programme was commissioned by the Ministry of Health NRW and expanded also offering syphilis testing and HIV point-of-care-testing.
**Methods:** Since 2011 laboratory Krone (Bad Salzuflen) conducted and analysed the HIV and syphilis testing on behalf of the LZG.NRW. As part of the analysis for diagnosis or exclusion of antibody-negative acute HIV infection, RT-PCR is carried out following a reactive screening test and a negative or inconclusive immunoblot.
Point-of-care-testing for HIV was carried out in 23 health centres in NRW. A positive rapid test result was followed by further serum analysis at laboratory Krone. People at high risk for HIV infection or suspected early infection were directly laboratory tested without previous rapid test.
**Results:** Overall, HIV testing rates increased by 13% between 2011 and 2014. The proportion of first-time laboratory confirmed

HIV-positive tests increased from 1.0% to 1.2%. The proportion of antibody negative, p24 antigen positive acute HIV tests of all initial HIV-positive findings was 4.2% in 2013 and 4.7% in 2014.
In 46 local health centres syphilis diagnostics increased by 81% between 2011 and 2014; at the same time first-time antibody positive syphilis tests increased by 43% and positive tests showing active or latent syphilis requiring treatment increased by 58%.
**Conclusions:** The differentiated HIV counselling and testing at the public health services in NRW has been increasingly adopted between 2011 and 2014. Reasons for the increased proportion of confirmed positive laboratory findings: a targeted selection of laboratory testing for people at high risk; an amended investigation algorithm with additional detection of acute infections of early Fiebig stage II and III (about 3 weeks p.i.); the "screening effect" of HIV rapid tests. With this testing strategy it is possible to exclude HIV infection 6 weeks after high-risk exposure according to the European guidelines 2014.
Due to increasing syphilis diagnostics at the public health departments in NRW more active and latent infections were detected in 2014 compared to previous years.

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**013/DVP**

**Improvement of a DNA microarray for molecular typing of non-cholera *Vibrio* spp.**

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**Introduction:** Our work is focused on gram-negative aquatic non-cholera *Vibrio* spp. which can cause gastroenteritis or wound infections after consumption of contaminated seafood or after direct seawater contact.
Our aim is to establish a typing tool that allows for easy, fast and economic detection of pathogenic non-cholera *Vibrio* species in routine diagnostics. Therefore, we improved a previously developed DNA microarray (version 1.0, Eichhorn et al., 2012) to a refined version which covers over 90 genes represented by 222 DNA probes, enabling us to determine *Vibrio* species, virulence determinants and antibiotic resistance genes of different *Vibrio* spp. Furthermore, the presence of characteristic species specific genes will help us to distinguish and cluster within the *Vibrio* spp. *V. vulnificus*, *V. parahaemolyticus* and *V. alginolyticus*.
**Methods:** We evaluated optimal hybridization and labeling conditions for the array by comparing experiments with reference strains to computed *in silico* hybridizations. In detail, we compared different temperatures, DNA and primer concentrations by pattern matching and we evaluated every experimental step to identify conflicting conditions. Sensitivity and specificity of the probes was determined with a large set of control strains.
For easy and rapid analysis of larger sets of *Vibrio* isolates we created a semi-automated analysis tool that generates a short experiment report including species affiliation, genomic profile and pattern match, from the hybridization readout of the array scanner. Based on such reports, we created a dendrogram of more than 40 *V. vulnificus* strains originating from the Baltic Sea.
**Results:** Optimum hybridization conditions are achieved at a hybridization temperature of 55 °C and a washing temperature of 50 °C with template DNA concentrations between 1 and 2 µg. The customized analysis report tool helps to identify the *Vibrio* spp. easily. Genetic relationships within the evaluated *V. vulnificus* strains will be compared to published data regarding their pathogenic potential, biotype association and cluster formation.
**Conclusions:** Thus, we have substantially expanded a previously developed DNA microarray and extensively evaluated array version 2.0 for detection of *Vibrio* spp. and identification of potential virulence markers. Additionally we have customized a user interface allowing for easy analysis of experimental data. Potential fields of application are identification of genetic relationships among non-cholera *Vibrio* spp., as well as detailed molecular typing of individual *Vibrio* isolates including detection of virulence markers and resistance genes.

**Reference**

*Eichhorn et al. (2012). Design and evaluation of a DNA oligonucleotide array specific for Vibrio spp.. 64th Annual Meeting of the German Society for Hygiene and Microbiology (DGHM) 2012, Hamburg.*

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**014/DVP**

**Mycobacteria Identification by MALDI Biotyper System: Evolution of Database Content and Evaluation Criteria**

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**Question:** Identification of mycobacteria by Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry demands for an optimized preparation method and a corresponding database. Mycobacteria Library versions were released in 2012, 2014 and this year with 173, 313 and 853 references, respectively. Here we present results obtained for mass spectra compared to different database versions with focus on sensitivity and specificity. In addition, an adaptation of threshold values for low and high confidence level intervals for mycobacteria is proposed.
**Methods:** Mycobacterial isolates (n = 1176) were inoculated on solid Loewenstein-Jensen medium or in liquid BD BACTEC™ MGIT™ tubes (BD, Heidelberg). In addition, patient material was inoculated in MGIT™ tubes (n = 93). Mass spectra were compared to Mycobacteria Libraries using MALDI Biotyper 4.0 software (Bruker Daltonik, Bremen, Germany). Reference method for study isolates was GenoType® Mycobacterium CM (Hain Lifescience, Nehren, Germany). Sequencing of 16S rRNA gene or ITS sequence was performed for a few isolates.
**Results:** Out of 1176 analyses of pure cultures log(score) values were ≥ 2.0 for 78.4 % and < 2.0 and ≥ 1.7 for 14.9 % representing the current high and low confidence identification results with database version 2.0. Very few discrepancies were observed (n = 8) for very closely related species with log(score) values below 2.0. These known restrictions of MALDI-TOF MS are marked with alerts by the system.
Using the adapted values and database version 3.0, 96.6 % and 2.6 % of high and low confidence level identifications were obtained, respectively. The update of the database to version 3.0 eliminated seven of eight discrepancies.
Out of 93 enrichment cultures of directly inoculated clinical specimens 78 % resulted in log(score) values ≥ 2.0. With adapted values, 95 % were considered as high confidence and 5 % as low confidence identifications. All these species identifications were correct.
**Conclusion:** Similarity between sample and database mass spectra is expressed by log(score) values. A higher number of references per species covers its potential natural variability and increases log(score) values. Furthermore, there can be other influences like human, instrument or medium based variability due to practical knowledge, instrument settings or liquid / solid medium, respectively. Such potential minor variations are balanced by several references per species.
Results of more than 1150 analyses demonstrated, that it is possible to lower threshold values without risking false positive species identification results.
In conclusion, these two improvements have led to an increased sensitivity without a decrease of specificity. As a benefit, time consuming repetitions and additional tests can be avoided. This advantage was demonstrated in a routine laboratory with patient inoculated cultures.

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**015/DVP**

**Culture-Independent Detection of Infective Endocarditis**

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**Introduction:** Accurate and fast diagnosis of infective endocarditis (IE) is of vital importance for patient outcome.
**Material & Methods:** Specimens from 40 fresh frozen heart valves were cultured on Columbia/chocolate agar plates and incubated in brain-heart broth for 7 days. In cases of no bacterial growth after 24 h, tissue specimens were evaluated with a new POCT multiplex-PCR device (Unyvero™, Curetis, Holzgerlingen, Germany) for the detection of heart valve infections and compared to 16S rDNA PCR results. The Unyvero™ i60 ITI (implant and tissue infection) cartridge is advertised to detect several grampositive/-negative bacteria and fungi, together with some of the most important antibiotic resistance genes.
**Results:** Fresh frozen heart valves were examined in routine bacteriology laboratory and with the Unyvero™ i60 ITI Cartridge. 16S rDNA PCR was negative in 20 cases, and positive in 20 cases. All 20 amplificates were sequenced and identified as *Staphylococcus spp (n*=6), *Enterococcus faecalis (n*=4), *Streptococcus spp. (n*=4), *Leifsonia shinshuensis (n=1*), *Granulicatella elegans / G. adjacens (n=2*), *Abiotrophia adjacens (n=1).* One case was positive in 16S PCR without signal in sequencing. The ITI cartridge signal was positive in 13 cases [*Staphylococcus aureus* (n=5), *Enterococcus spp. / E. faecalis* (n=5), ConS (n=1), *Granulicatella adjacens* (n=1), *Abiotrophia adjacens* (n=1)] and negative in 27 cases. Problems with invalid targets measuring the full resistance panel occurred in 11 out of 40 cartridges. Antibiotic resistances were found in 4 specimens [1: vanB, rpoB, oxa-58, ndm; 2: ermC, oxa-48, aacvA4; 3: rpoB; 4: aac(6)/aph(2)). From these, two specimens (1; 2) were without any pathogen identification. The detected pathogen related to the detected rpoB resistance was *S. aureus* and the detected species related to the detected aac(6)/aph(2) resistance was *E. faecalis*.
**Discussion:** Problems with invalid targets measuring the full resistance panel occurred in 11 out of 40 cartridges. When comparing both methods, identification was consistent in 9 cases (4 x for *E. spp./E.faecalis*, 3 x for *S. aureus*, *1 x for Granulicatella adjacens, 1 x for Abiotrophia adjacens*) and divergent in 9 cases (2 x *Streptococcus spp.* (Seq.) versus 2 x *S. aureus* (ITI), 2 x *Streptococcus spp.* (Seq.) versus negative result (ITI), 1 x *Leifsonia* (seq.) vs. negative result (ITI); 3 x S. aureus (seq). vs. negative (ITI); 1 x *Granulicatella adjacens* (seq) vs. negative result (ITI). Bacteria from the HACEK group (*Aggregatibacter aphrophilus, Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae*) and Streptocci (except. A and B) are not covered in the ITI panel.
**Conclusion:** The Unyvero ITI cartridge is a useful tool for IE diagnosis. It can be easily integrated into the lab work flow and is less laborious. However, for its application in routine IE diagnosis the multiplex system needs to be optimized and extended to include targets for viridans streptococci and the HACEK group. In addition, problems with invalid resistance and pathogen target detection need to be fixed.

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**016/DVP**

**Evaluation of the artus® *M. tuberculosis* RG PCR Kit (Qiagen, Hilden, Germany) performance in respiratory and non-respiratory specimen in a low incidence country**

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**Introduction**: Rapid and reliable detection of tuberculous mycobacteria is still challenging but of high clinical importance. In the last decade various commercial nucleic acid amplification tests (NAAT) have been established, aiming for specific detection of MTC (*Mycobacterium tuberculosis* complex) directly from clinical samples.
**Objective:** Our aim was to determine the sensitivity and specificity of the artus® *M. tuberculosis* RG PCR Kit (Qiagen, Hilden, Germany) in respiratory and non- respiratory specimens.
**Design:** Samples were gathered from January 2011 until December 2014 at the Institute of Microbiology in Wuerzburg, Germany. We included 436 non- respiratory and 1833 respiratory specimens (incl. gastric juice) for a retrospective, monocentric evaluation of the artus® *M. tuberculosis* RG PCR Kit. Samples from patients with clinically suspected TB or from Ziehl-Neelsen (ZN) positive specimens have been tested by artus® PCR as well as by conventional culture techniques on fluid and solid culture media following our standard operating procedures.
To determine sensitivity, specificity, negative and positive predictive value (NPV and PPV) we compared our NAAT results with the results of conventional culture techniques, taking previous MTC findings and clinical data into account.
**Results:** For respiratory specimens we obtained an overall specificity of 98,75%, a sensitivity of 62,33% a PPV of 94,12% and a NPV of 98,52%.
For non-respiratory specimen we obtained an overall specificity of 97,07% a sensitivity of 37,04% a PPV of 71,43% and a NPV of 95,89%.
The test performed better in ZN positive materials reaching a specificity and sensitivity of up to 100% each.
**Conclusions:** Our results show that the artus® *M. tuberculosis* Kit performs well in ZN positive materials for respiratory and non-respiratory samples alike, although the overall sensitivities are unsatisfactory, particularly in ZN negative specimens.

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**017/DVP**

**Change of bacterial nature and growth in iron-containing drinking water samples**

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The detection of bacterial contamination is an important topic for the assessment of drinking water quality, and the groups of Coliforms inclusive *E. coli* as well as *Pseudomonas aeruginosa* require regulatory safeguards for different water systems. The basis of the analytical methods is the bacterial cultivability on solid media. To strengthen sensitivity the membrane filtration technique for 100 ml water sample volumes with background flora is used followed by incubation on selective media. The Chromogenic Coliform Agar (CCA) contains chromogenic substrates for determination of enzymatic galactosidase and glucuronidase activities resulting in dark blue coloring *E. coli*, red colored Coliforms and colorless *Pseudomonas aeruginosa*. However, the cultivability has limitations such as lack of specifity and sensitivity, antagonistic organism interference and the chemical and physical composition of water samples.
Our study focused on recovery and phenotypical changes of bacteria in iron-containing supply water samples which are prepared by spiking with *E. coli, Klebsiella pneumoniae*, *Citrobacter freundii* and *Pseudomonas aeruginosa*.

We showed that high iron concentrations repressed growth of *Enterobacteriaceae* and fluorescence characteristics of *Pseudomonas* were changed. *Klebsiella* produced specific color only after > 24 h incubation. Our results revealed that confirmation of Coliforms is necessary by reason of red colored but oxidase positive colony growth as *Aeromonas* strains.
In conclusion, the quantification of bacteria on CCA plates is a reliable routine technique for determination of Coliforms and *E. coli* with limitation of analyzing un-buffered water which may result in false negative outcomes.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**018/DVP**

**Screening for biofilm formation of *E. coli* pathotypes and determination of the effect of antimicrobial peptides on biofilm formation by automated fluorescence microscopy**

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**Introduction**: Biofilms are communities of microorganisms, which live in a self-produced matrix of extracellular polymeric substances. Biofilm mediated infections are hard to treat due to their resistance to immune defense and antibiotics. Therefore it is important to develop tools to investigate biofilms for therapy improvement or to find new antimicrobial substances.
**Objectives**: The aim of this work is to build a fully automated large-scale screening platform, which can be used to examine the ability of biofilm formation under different culture conditions. Most importantly we aim to determine the effects of antimicrobial substances. As a first application biofilm formation of different *E. coli* pathotypes was examined. In addition we determined the effect of several antimicrobial peptides (AMP) on biofilm formation.
**Materials and Methods**: We enhanced our previous published VideoScan technology, which is based on fully automated fluorescence microscopy to perform high-throughput screening of biofilms. VideoScan enables the analysis of multiplex assays such as microbead or cell-based assays [Roediger et al. 2013; Froemmel et al. 2013]. We used a 96 well plate format for the formation of biofilms, which are visualized with Live/Dead staining followed by a VideoScan analysis. This analysis represents a two-step evaluation realized by our software and image processing. In the first step the fluorescence intensity of the biofilm is measured and in a second step overview pictures of biofilms are taken. Fluorescence conjugated beads are used as a reference signal for the fluorescence intensity measurement. For the screening we analysed 186 *E. coli* strains representing 8 different pathotypes (e.g. UPEC, ETEC, EAEC, CAEC, AFEC) for biofilm formation using 4 different media. Furthermore we examined the ability of different AMP to prevent biofilm formation for selected strains by coincubation.
**Results**: With VideoScan we discovered 110 biofilm formers out of 186 *E. coli* strains. The majority of biofilm formers could be detected in the groups of EAEC (96%) and UPEC (83%), followed by SAEC (61%), CAEC (58%), AFEC (54%), EPEC (46%) and HFEC (46%). Only very few and weak biofilm formers were found in the pathotype of ETEC (29%). Coincubation of bactericidal AMP (modified from K. Rapsch et al. 2014; sequence confidential) with a strong biofilm former revealed an inhibition of biofilm formation in already low concentrations (4µM).
**Conclusion**: With our VideoScan technology it is possible to study biofilms in a fully automated large-scale screening. With this technique we could detect a considerable number of biofilm formers out of 186 *E. coli* strains. In addition we demonstrated the bactericidal effect of different AMP resulting in inhibition of biofilm formation. In a next step classification of biofilm formation e.g. weak or strong biofilm former will be developed based on fluorescence intensity measurements.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**019/DVP**

**Identification of Highly Pathogenic Microorganisms using MALDI-TOF Mass Spectrometry - Results of an Inter-Laboratory Ring Trial**

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In the case of a release of highly pathogenic bacteria (HPB) there is an urgent need for rapid, accurate and reliable diagnostics. MALDI-TOF mass spectrometry is a rapid, accurate and relatively inexpensive technique which is becoming increasingly important in microbiological diagnostics to complement classical microbiology, PCR and genotyping of HPB. In the present study, the results of a joint exercise with eleven partner institutions from nine European countries are presented. In this exercise ten distinct microbial samples, among them five HPB, *Bacillus anthracis*, *Brucella canis*, *Burkholderia mallei*, *Burkholderia pseudomallei* and *Yersinia pestis* were characterized under blinded conditions. Microbial strains were inactivated by high-dose γ-irradiation before shipment. Preparatory investigations ensured that this type of inactivation induced only subtle spectral changes with negligible influence on the quality of the diagnosis. Furthermore, pilot tests on non-pathogenic strains were systematically conducted to ensure the suitability of sample preparation and to optimize and standardize the workflow for microbial identification.
The analysis of the microbial mass spectra was carried out by the individual laboratories on the basis of spectral libraries available on site. All mass spectra were also tested against an in-house HPB library at the Robert Koch Institute (RKI). The average identification accuracy equaled 77% in the first case and improved to > 93% when the spectral diagnoses were obtained on the basis of the RKI library. The compilation of complete and comprehensive databases with spectra from a broad strain collection is therefore considered of paramount importance for accurate microbial identification.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**020/DVP**

**The fully automated DiaSorin LIAISON® in comparison to established ELISA and gold standard for stool diagnosis of *Campylobacter***

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**Introduction**: *Campylobacter* are commensal organisms that populate primarily humans and domestic animals. *Campylobacter* are widely recognized as the most common cause of bacterial food-borne diarrheal disease worldwide and are the most commonly reported zoonotic-cause for human gastroenteritis.
Currently, culture is the gold standard for detection of *Campylobacter* infection, with a detection rate of approximately 60% for positive samples.
To improve the laboratory efficiency the fully-automated random-access DiaSorin LIAISON® based on chemiluminescence technology (CLIA) was evaluated against several ELISA systems and the culture method.
**Materials/Methods:** Samples from the routine or archive were used on several systems. Campylobacter assays were used from r-Biopharm RIDASCREEN® and Seramun Serazym® as ELISA and LIAISON® as chemiluminescent (CLIA) *in vitro* diagnostic as well as the standard culture. Over 330 samples were used in parallel and performed according to manufacturer's manual.
**Results:** The LIAISON® assays recognizes achieves excellent concordance with the compared ELISA assays with over 95% and a concordance with the culture with over 90%. The performance demonstrates a high specificity (>95%) and sensitivity (>95%). The sensitivity for EIA and CLIA antigen assays is than with the

culture which shows a sensitivity of 60-95%. The LIAISON® needed ca. 40% of the time compared to EIA systems.
**Discussion:** The DiaSorin LIAISON® *Campylobacter* test is a sensitive screening method to identify stool specimens. The LIAISON® *Campylobacter* assay has a comparable performance to currently used routine methods and shows due the used CLIA technology the same or better performance in terms of sensitivity and specificity making a switch of the routine to the new technology more easy. Reduced hands-on time and short incubation times leads to a faster availability of the results in ca. 40% of ELISA time. The LIAISON® *Campylobacter* assays are suitable for the routine usage in diagnosis of *Campylobacter* infection.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**021/DVP**
**Evaluation of Unyvero™ ITI - a rapid PCR-based detection system for microorganisms and antibiotic resistances in Implant and Tissue Infections**
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Implant and Tissue Infections are often difficult to diagnose due to time consuming laboratory testing, the emergence of biofilms or underrepresentation of anaerobic microorganisms. In many cases, patients can only be treated properly if the pathogens and their resistances to antibiotics are known. Molecular diagnostics could be a powerful tool to solve this problem due to its high sensitivity, quickness and ability to multiplex. The Unyvero™ Cartridge i60 is able to detect 114 analytes simultanously within approximately 5 hours allowing to distinguish between 91 microorganisms and 23 resistance markers.
Implant and tissue infections are grouped into different sub-classes such as implants, surgical sites, burn wounds, deep skin and tissue etc. and the Unyvero System is open to various sample materials. Basically we constrained this study to swabs of wounds in different locations. However, the Brain Heart Infusion Broth inoculations from four of the swabs were also subjected to multiplex-PCR testing in order to check for anaerobic bacteria under the identification threshold in the primary samples.
In total 52 clinical specimens were tested by Unyvero i60 in comparison to cultural analyses. Of these, multiple bacteria were detected in 13 (25%) and 14 (26.9%) by PCR assay and conventional culture, respectively. A single pathogen has been shown in 13 (25%) and 17 (32.7%) patients. Six different genes conferring drug resistance were detected in a total of 13 (25%) cases.
Furthermore the results obtained from PCR analyses differed from those obtained from the microbiological approach for 24 (46.1%) specimens, therefore indicating that PCR-based diagnostics could improve ITI treatment not only in terms of time saving but also in terms of sensitivity. However, clinical relevance has to be discussed as well as linkage between pathogens and resistance genes should be verified by microbiological approaches.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**022/DVP**
**Highly sensitive detection of *B. pseudomallei* in blood samples using selective enrichment of bacterial DNA**
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*Burkholderia pseudomallei* is a natural inhabitant of soil and water in many parts of the world and causative agent of the potentially fatal disease melioidosis. Severe sepsis is among the most common clinical presentations of melioidosis. Rapid direct detection of *B. pseudomallei*, from blood is crucial for timely therapeutic interventions, but sensitive molecular detection from blood remains a challenge. In this study we evaluated the Looxster® protocol, based on enrichment of unmethylated bacterial DNA, for the extraction of *B. pseudomallei* DNA from blood and subsequent detection by a published quantitative type three secretion system 1-*orf2* PCR assay. In spiked blood samples the analytical sensitivity was < 10 *B. pseudomallei* CFU/ml, which is higher than the reported sensitivity of other blood DNA extraction kits. We then tested EDTA blood samples from blood culture positive melioidosis patients admitted toMahosotHospital,Vientiane,Laos and found 19 out of 20 tested patients to be positive, whereas all controls were negative. Future prospective studies will be needed to determine the sensitivity and specificity of this molecular detection method as a diagnostic tool in a clinical setting.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**023/DVP**
**Development of a molecular diagnostic method for rapid detection of *Leptospira spp.* in a mobile laboratory environment**
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Leptospirosis is a bacterial disease caused by spirochete bacteria from the genus *Leptospira* and can be grouped into currently over 200 serovars. It is transmitted by urine of infected rodents. Although rats, mice and moles are considered as primary reservoirs also a wide range of other mammals may serve as secondary hosts. In humans, the infection with *Leptospira* causes a wide range of symptoms, typically including biphasic fever. However, without timely microbiological confirmation Leptospirosis might be misdiagnosed as another infectious disease, especially in an outbreak-situation like the current Ebola outbreak in West Africa. Hence, a proper discrimination between different fever-causing pathogens is especially important at an early phase of the diseases.
Here we describe the development of a novel qPCR diagnostic assay, which allows sensitive and rapid diagnosis of Leptospirosis in a mobile diagnostic laboratory environment even in limited resource settings. On basis of a computational comparison of the sequence of the highly conserved *Leptospira Major Outer Membrane Protein* (LipL32) gene available in GenBank, we designed a pair of primers and a FAM-BBQ labeled probe. For use within mobile laboratory in a rural African setting primers and probe were adapted and validated on lyophilized qPCR chemistry (OmniMix HS, Cepheid). Analytical sensitivity was evaluated using cloned and quantified target DNA. The assay showed a dynamic range of at least 7 $\log_{10}$ and demonstrated good intra- and interassay precision (coefficient of variation: CV % 1,14 and CV % 1,31 respectively). Analytical specificity was tested by negative amplification of a set of 15 different pathogens, which might cause similar clinical diseases or showed a relevant degree of genetic relationship to Leptospira. A probit regression model was used to quantify the analytical sensitivity of the qPCR assay. The 95% detection level was found to be 7,71 copies per reaction (CI 6-9 cps/rx).

The high sensitivity, the wide linear range, the good reproducibility of this assay and its usage of lyophilized qPCR chemistry render it especially useful for the diagnosis of Leptospirosis in the challenging environment of a mobile laboratory in an outbreak setting.

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**024/DVP**

***Prevotella quinquagintesima* sp. nov., isolated from a human clinical wound swab**

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*Prevotella* sp. are obligate anaerobes which have been classified as new genus to include species formerly contained in the genus *Bacteroides* (Shah and Collins, 1990). They are part of the human oral and gastrointestinal microbiota and can be involved in infections if translocated to other body sites. One strain (A1336) of obligately anaerobic, Gram stain-negative bacillus was isolated from a human wound swab and characterized phenotypically as well as genotypically. Electron microscopy revealed rod-shaped bacteria with intercellular matrix adhesion. The organism was moderately proteolytic (API rapid ID 32A profile 0501 4502 0 0) and sensitive to bile. The G+C content of the DNA was 43.2 mol%, as determined by HPLC (DSMZ). The cellular fatty acid profile was determined by GC (DSMZ) and differed from profiles of other *Prevotella* sp. available in the MIDI database (MIDI Inc., Newark). Phylogenetic analysis was based on full-length 16S rRNA gene sequence and showed the strain to belong to the genus *Prevotella*, but to be different from all the 49 recognized species. In the closest sequence similarity phylogenetic tree constructed by the maximum likelihood model, *Prevotella bergensis*, *Prevotella dentalis* and *Prevotella multisaccharovorax* were of closest relationship with bootstrap values of only 73, 79 and 64 respectively for these branches as based on 2000 replications. In the view of phenotypic and biochemical properties as well as gene sequencing, strain A1336 is considered to represent a novel species within the genus *Prevotella*, for which the name *Prevotella quinquagintesima* sp. nov. is proposed (L. indecl. *quinquaginta* fifty, designating this isolate as the fiftieth species of *Prevotella* to be named).

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**025/DVP**

**Performance of CHROMagar MRSA II in identification and recovery of newly emerging methicillin-resistant *Staphylococcus aureus* carrying *mecC* gene**

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**Introduction**: A divergent *mec*A homologue called *mec*C has been recently described in *Staphylococcus aureus*. Methicillin-resistant *S. aureus* (MRSA) isolates carrying *mec*C have been recovered from humans and animal infections, and a potential zoonotic transmission has been demonstrated. Due to the frequently low MICs for cefoxitin and oxacillin of these strains, it has been suggested that *mec*C-MRSA isolates might not be detected by using MRSA selective media.
**Objectives**: The aim of our study was to evaluate the performance of the chromogenic medium BBL™ CHROMagar™ MRSA II to detect *mecC*-MRSA
Strains and Methods: Swabs dipped in 0.5 McFarland suspensions originating from a collection of clinical and veterinary *mecC*-MRSA were spread onto the surface of chromogenic agar plate and incubated at 37°C for 24 h. Any growth was considered positive.
**Results**: All tested *mecC*-MRSA strains, most of them belonging to CC130, could be recovered on CHROMagar MRSA II and most of the isolates showed as mauve colonies on this medium. Unexpectedly, all tested CC130 strains originating from bovine mastitis from Bavaria appeared violet.
**Conclusions**: *mecC*-MRSA strains are capable to grow on CHROMAgar MRSA II and therefore it could be a adequate selective medium when used as screening when infections or colonisation with these strains are suspected. However we observed unexpectedly violet growth in CC130 isolates causing bovine mastitis obtained from cattle in Bavaria that could be dismissed as contaminants when no other further tests were performed. It still needs to be clarified whether the different colony appearance was related to a host-specific adaption or to an accidental mutation in a few epidemiologically linked isolates.

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**026/DVP**

**Evaluation of GenoType Bacident for the Detection of Pathogens Directly from Clinical Specimens**

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**Background:** 16s/18s-based PCR methods are often less sensitive than targeted PCR methods detecting pathogens. We set out o compare the usefulness of a target-based method, GenoType BacIdent to broad-range PCR from primarily sterile sites.
**Methods:** The GenoType Bac Ident system uses a DNA-strip technique to detect 14 bacteria with simultaneous detection of mecA, vanA and vanB genes designed primarily to detect blood culture pathogens. All suitable bacteriology specimens were subjected to routine culture, broad- range PCR (targeting 16s/18s sequences) and GenoType. The specimens included were: aspirates 133 (65 %), tissue 52 (25 %) and CSF 21 (10 %).
**Results:** A total of 206 specimens were investigated between November 2013 and June 2014. GenoType detected a pathogen in 72 (35 %) specimens; culture was positive in 64 (31 %); and the PCR was positive in 39 (19 %) specimens. A total of 33 (16 %) specimens were positive in both GenoType and PCR and 122 (59 %) negative in both. Five specimens (2 %) were positive in the PCR and negative in the GenoType and 38 (18 %) positive in GenoType and negative in the PCR. The sensitivity and specificity for GenoType was 86 % and 76 % respectively. The GenoType results corresponded to culture in 48 positive specimens and 115 negative specimens. Culture was positive in 11 specimens negative in Genotype and in 24 specimens the GenoType was positive with a negative culture result. The sensitivity and specificity of GenoType compared to culture was 81 % and 83 % respectively. Of the discrepant results; in 33 specimens GenoType detected a single pathogen not detected by PCR and in a further 12 specimens more than one pathogen, of which only one (7) or none (5) was detected by PCR. In 3 specimens PCR detected a pathogen not detected by GenoType and in 8 specimens the amplification in

GenoType was inhibited. In 6 specimens a pathogen was detected by PCR, which was not included in the GenoType panel. Of 7 culture negative and GenoType positive specimens, 4 were also negative in the PCR.
**Conclusions:** The GenoType method is both more sensitive than broad-range PCR in detecting the 14 bacteria targeted and more sensitive than culture. The study also demonstrates the relative lack of sensitivity of broad-range PCR using universal 16s and 18s sequences compared to targeted PCR.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**027/DVP**

**Improving the Differentiation of *Bacillus anthracis* from Closely Related Members in the *Bacillus cereus sensu lato* Group via MALDI-TOF MS**

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**Introduction**: Rapid and reliable differentiation of *Bacillus* (*B.*) *anthracis* from closely related species of the *B. cereus sensu lato* group is important. Especially, differentiation of *B. anthracis* from *B. cereus* is crucial as both pathogens cause infections in humans, however, severities of the diseases associated with the two species and corresponding treatment and disease management differ remarkably. While *B. anthracis* causes often-fatal anthrax disease (especially inhalational-, injectional-, and gastrointestinal anthrax), *B. cereus* is mainly associated with non-lethal foodborne diarrheal or emetic illnesses. Identification of these species in diagnostic laboratories based on biochemical or molecular biological methods is often not sufficiently unambiguous. Nowadays, identification of many bacteria is facilitated by matching specific patterns in the corresponding protein or peptide mass spectra to a variety of mass spectra deposited in databases usingMatrixAssistedLaserDesorption/Ionization-TimeofFlightmassspectrometry (MALDI-TOF MS). However, current commercial databases often have a low-resolution power for unambiguous differentiation of highly pathogenic bacteria from closely related species.
**Aim**: Therefore, it was the aim herein to generate a dedicated database that facilitates the differentiation of the species within the *B. cereus s.l.* group with focus on *B. anthracis*
**Material and Methods**: Protein mass spectra of 189 verified and diverse *Bacillus* strains were generated using MALDI-TOF MS and subsequently analyzed with unsupervised and supervised statistical methods, such as principle component analysis (PCA) and shrinkage discriminant analysis (SDA), to identify specific putative biomarkers in the protein spectra of *B. anthracis* in comparison to closely related *Bacillus* spp..
**Results**: Thereby, 7, 10, 18, and 14 *B. anthracis* specific biomarker candidates were identified not present in *B. cereus*, *B. mycoides*, *B. thuringiensis*, and *B. weihenstephanensis*, respectively. Main spectra (MSP) of a defined collection of *Bacillus* strains were generated and added to a dedicated in-house database. By validating the database with 15 *B. anthracis* and 14 *B. cereus* strains results with improved score values and no false identifications were achieved as compared to the commercial database.
**Summary**: A more detailed database for matching and identifying *Bacillus* spp. with MALDI-TOF MS was generated by means of current state statistical methods applied on MSPs from a diverse collection of *Bacillus* spp.. The identification of *B. anthracis* and differentiation from other strains of the *B. cereus s.l.* group via MALDI-TOF MS was herein remarkably improved.
**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**028/DVP**

**Performance of the cobas® MRSA/SA Test for the Detection of *Staphylococcus aureus* and MRSA**

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**Question**: Nucleic acid amplification tests have proven to be reliable, rapid and sensitive tools for the detection of *Staphylococcus aureus* (SA) and methicillin-resistant *S. aureus* (MRSA) DNA from nasal specimens. The objective of this study was to evaluate the newly developed **cobas**® MRSA/SA Test performed on the **cobas**® 4800 system. Geographical inclusivity using characterized isolates from around the world and clinical performance using nasal swabs from patients representative of the United States as part of a large, multicenter clinical trial were assessed.
**Methods**: MRSA isolates (n=281) and SA isolates (n=85) collected from diverse
geographic locations were evaluated with the **cobas**® MRSA/SA Test. Clinical utility evaluation of nasal specimens (MSwab, Copan, Brescia, Italy), with the **cobas**® MRSA/SA Test and direct chromogenic and enrichment culture was performed. Sensitivity, specificity, PPV and NPV values were calculated by comparing **cobas**® MRSA/SA Test results with direct chromogenic culture combined with enrichment culture. Discrepant analysis was performed on all discordant samples using the Cepheid Xpert™ SA Nasal Complete test, and a non-selective direct and non-selective enrichment culture.
**Results**: Geographical inclusivity studies showed the **cobas**® MRSA/SA Test detected 98.6% (277/281) of MRSA strains and 100% (85/85) SA strains. Clinical utility studies (n=2504) identified 160 MRSA-positive and 660 SA-positive specimens. The sensitivity, specificity, prevalence, PPV and NPV for the **cobas**® MRSA/SA Test compared to direct chromogenic culture combined with enrichment culture was 93.1% (149/160) and 97.5% (2281/2340), respectively, with a prevalence, PPV and NPV of 6.4%, 71.6% and 99.5%, respectively. The sensitivity and specificity for SA compared to combined direct and enrichment culture was 93.9% (620/660) and 94.2% (1734/1841), respectively, and the prevalence, PPV and NPV for SA compared to combined direct and enrichment culture was 26.4%, 85.3% and 97.7%, respectively. Discrepant analysis corroborated the **cobas**® MRSA/SA Test results for 5 of 11 MRSA false negative samples, 20 of 59 MRSA false positive samples, 31 of 40 SA false negative samples and 24 of 107 SA false positive samples.
Conclusion: The **cobas**® MRSA/SA Test, performed on the automated **cobas**® 4800 system, detects a broad range of MRSA and SA strains and displayed excellent performance compared to direct chromogenic and enrichment culture for the detection of *Staphylococcus aureus* and MRSA from clinical samples.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**029/DVP**

**Evaluation of Stool Specimens with the cobas® Cdiff Test Performed on the cobas® 4800 System for the Detection of *Clostridium difficile* Toxin B Compared with Toxigenic Culture**

O. Liesenfeld*[1,2], S. Young[3], T. Davis, Jr.[4], Z.- X. Wang[5]
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**Question**: Nucleic acid amplification tests have proven to be reliable, sensitive tools for the detection of *Clostridium difficile* from stool samples. The objective of this study was to evaluate performance characteristics of the newly developed **cobas**® Cdiff Test using prospectively collected stool specimens from patients representative of the United States as part of a large, multicenter clinical trial.

**Methods**: Stool specimens from patients suspected of *C. difficile* infection (CDI) were collected at 5 geographically diverse sites across the US. An aliquot of stool from each patient was sent to a central lab for toxigenic culture and one aliquot was evaluated with the **cobas**® Cdiff Test at 1 of 3 designated sites. The sensitivity, specificity and accuracy were calculated by comparing **cobas**® Cdiff Test results with the combined results from direct and enriched culture followed by cytotoxigenic culture (toxigenic culture). Discrepant analysis was performed on all samples with discordant results, using the Xpert® *C difficile* Epi Test.

**Results**: Specimens were collected from 683 subjects, 306 males (44.8%) and 377 females (55.2%) with a mean age of 56 years (range 3 to 99). Testing is complete on 555 subjects with 139 positive by toxigenic culture. The sensitivity, specificity and accuracy of the **cobas**® Cdiff Test compared to toxigenic culture was 94.2% (131/139), 98.3% (409/416) and 97.3% (540/555), respectively. Of the 8 specimens with negative **cobas**® Cdiff Test results compared topositive results by toxigenic culture, 3 have been tested by the Xpert® *C difficile* Epi Test and shown to be negative for *C. difficile*. Of the 7 specimens with positive **cobas**® Cdiff Test results vs. negative results by toxigenic culture, 3 were *C. difficile* positive by Xpert® *C difficile* Epi Test.

**Conclusion**: The results show the **cobas**® Cdiff Test, performed on the automated **cobas**® 4800 system, displayed excellent performance compared to toxigenic culture when evaluating clinical specimens for the presence of toxigenic *C. difficile.* These results support the use as an aid in the diagnosis of CDI in humans to allow appropriate patient management including treatment and infection control measures.

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**030/DVP**

**Rapid susceptibility testing directly from positive blood cultures using the novel MALDI biotyper based MS-ASTRA method**

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**Introduction**: While current methods for susceptibility testing usually require an overnight incubation, novel MALDI-TOF MS assisted techniques are able to detect antibiotic resistances within a few hours.

**Objectives**: Recently a new semi-quantitative method for MALDI-TOF MS assisted susceptibility testing (MS-ASTRA) was introduced. We adapted this technique for the use with bacteria directly isolated from blood cultures flasks at the time they are flagged as positive.

**Material & Methods**: The study evaluated 30 patient derived BCs containing Gram-negative rods. Bacterial cells were incubated in two different media: Mueller-Hinton medium and Mueller-Hinton medium supplemented with antibiotic. Two antibiotic substances were tested (cefotaxime and ciprofloxacin). After an incubation time of 2.5 hours bacteria were lysed. Extracts were spiked with an internal standard before MALDI-TOF MS analysis. An automated algorithm was used to compare the normalized peak-intensities obtained from both setups. A fix cut-off value was defined to distinguish between resistant and sensitive isolates. Results were validated by comparison with classical E-test method.

**Results:** In total 7 different Gram-negative species were tested. 14 BCs containing *E. coli*, 7 *K. pneumoniae*, 4 *E. cloacae*, 2 *P. aeruginosa*, 1 *K. oxytoca*, 1 *E. aerogenes*, and 1 *S.enterica* S. Typhi. Unambiguous results could be obtained for all isolates. Classification into susceptible and resistant strains was in complete accordance with the conventional method (E-test).

**Conclusion:** With the method described here we were able to detect resistance against 3rd generation cephalosporines and ciprofloxacin within 2.5 hours directly from blood cultures. Clear advantages of this approach are the simple setup, the short incubation and hands-on time and the necessity of very little biomaterial. In addition, based on the way this test operates, presumably it can be applied to all bacterial species, regardless of the mechanism causing the resistance, and to all types of antibiotic substances with their different modes of action. The combination of rapid species identification by MALDI-TOF MS and this MS-based resistance essay could be a promising perspective for diagnostic routine and enable an earlier re-evaluation of the antibiotic therapy.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**447/DVP**

**The significance of quantitative determination of IgG antibodies against the variable major protein VlsE for diagnosis and clinical activity of Lyme borreliosis. A retrospective study 2001-2014**

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**Introduction:** The diagnosis of Lyme borreliosis is determined under consideration of the anamnesis (i.e. the tick exposition) and the clinical symptoms. It can only be determined for certain in the early phase of the disease and only if an *Erythema migrans* appears. The extremely high number of unspecific symptoms makes a clinical diagnosis exceptionally difficult without the further help of a laboratory for instance.

In the laboratory the microbial detection via PCR/NAT is proof, but is only useful for a small percentage of patients, so that appropriate serological diagnostic tests have to be performed.

It is also difficult to interpret: Estimates as to the activity of the infection, as well as a categoricaldoubtless diagnosis isnot possible.

**Objective:** In order to make the diagnostics easier to interpret, more precise and thereby more secure, a dynamically reacting, activity- and pathogen-associated, quantifiable parameter with a high specificity for Borelia burgdorferi s.l., needed to be found.

As a solution the variable but highly specific major protein (VlsE) of Borrelia burgdorferi (B.b. s.l.)

was used as the target antigen. It was first described in 1997 by *Zhang et al*. and it possesses the desired properties of a key parameter.

**Materials and Methods:** For the study we used patient sera from our Lyme clinic, which had been stored since 2001 at -80 degrees Celcius. As testing procedure we used a classic IgG or IgM IFT, as well as an IgG or IgM Elisa without VlsE antigen. In addition we used 2 indirect IgG Elisas with recombinant VlsE antigen of B.b. s.s (standard) or B. b. s.s. **and** B. afzelii (*EUROIMMUN®)*. Both VlsE tests are validated for quantitative determination. Units are

(RU/ml). Confirmation in each case was performed using a full-antigen **and** a line immunoblot.
**Results:** 1. The lack of anti-VlsE antibodies excludes an active chronic Lyme infection almost certainly (above 98%).
2. The quantitative determination of anti-VlsE antibodies is highly suitable for the confirmation of a diagnosis and as an activity marker in follow-up checks of patients with active chronic Lyme borreliosis before and after therapy.
3. The blackening degree of VlsE-type chips in the line blot documents the infection with the individual VlsE type very well. A negative VlsE-type blot excludes an active chronic Lyme infection almost certainly. Exception: rare VlsE types such as. *B. spielmanii*.
4. The quantitative determination of anti-VlsE IgG with 2 different tests is sufficient for general screening of Lyme borreliosis. Furthermore, IgM antibodies should be investigated.
**Summary:** The quantitative determination of IgG antibodies against VlsE is the method of choice in order to prove a Lyme infection.

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## EUKARYOTIC PATHOGENS (EKP)

**031/EKP**
**An invertebrate infection model predicts *Candida glabrata* organ burden in mice**
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The study of microbial pathogens requires suitable infection models. While generally mice models are considered the gold standard for mimicking human infections, alternative infection hosts are used ever more frequently in recent years. However, non-mammalian models are often seen critically due to their physiological differences to humans and other mammals.
Our aim was to establish the suitability of an invertebrate infection model - Toll pathway-deficient *Drosophila melanogaster* - for fungal virulence factor screenings, and compare it to the established murine model. To this end, we used a recently created library of deletion mutants of *Candida glabrata* [1], an important fungal pathogen of humans and the second most frequent cause of disseminated candidiasis. Using two virulence indices, one based on the mean survival time of flies after infection, and the other on relative fungal burdens in different mice organs, we tested several hundred *C. glabrata* mutants for their virulence potential in both models.
Our results indicate that overall, reduced mean survival times of flies can largely predict a significant decrease in organ burden in mice [2]. Importantly, the predictive power of fruit fly survival is much better than *in vitro* growth rates alone. Hypo- and hypervirulence of individual mutants are largely in agreement in both models. For example, early steps in N-glycosylation are required in both, flies and mice. Specific differences were found, among others, for mutants defective in the oxidative stress response or polarized growth.
Overall, the *Drosophila* model has proven to be a suitable host to detect - to a large extent - mutants with likely defects in murine organ burden. With this system, large-scale pre-screens for virulence factors are hence possible, which would be ethically, financially, and practically challenging with a vertebrate host. Mutants leading to reduced or increased fly survival times can then be characterized in more detail by individual testing in suitable murine models, reducing the total number of mice required in these experiments.

**Reference**
[1] Schwarzmueller et al. PLoS Pathog (2014)
[2] Brunke, Quintin et al. Dis Model Mech (2015)

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

**032/EKP**
**Growth of the humanpathogenic mold *Aspergillus fumigatus* is negatively affected by factors secreted from the non-humanpathogenic species *Aspergillus niger* and *Aspergillus nidulans***
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*Aspergillus fumigatus* is currently the major airborne fungal pathogen causing life-threatening, systemic infections in severely immuno-compromised patients. Even under therapy the mortality of invasive aspergillosis remains in the range of 40-60%, which underlines the need for new therapeutic options.
As a saprophyte, *A. fumigatus* feeds on decaying organic material and in its natural habitat it has to compete with a wide range of fungi and other microorganisms. The antagonistic interactions between fungi and bacteria are well known and led to the identification of many antibiotic compounds. In contrast, we know little about antagonistic interactions between different fungi. We found that *A. nidulans* and *A. niger*, but not the pathogenic species *A. terreus*, inhibit germination and growth of *A. fumigatus*. This effect is also observed when the two fungal species are separated in a two-chamber system indicating that a soluble factor is responsible for this effect.
Supernatants of co-cultures of *A. nidulans* or *A. niger* with *A. fumigatus* showed a stronger inhibitory activity on *A. fumigatus* than supernatants of *A. nidulans* or *A. niger* grown individually. This indicates an enhanced production of the inhibiting factor(s), if the two fungal species are in competition with each other. Filtration experiments with different pore sizes furthermore suggest the existence of at least two different and novel factors that limit the growth of *A. fumigatus*.
In *A. niger* the 51 amino acids long antifungal protein Anafp is already known (Lee et. al. 1999). But as we still found a high negative effect on growth in *A. niger* supernatant that after filtration only could contain molecules that are smaller than 3 kDa we believe in the existence of another still unknown, small but strong suppressor of *A. fumigatus*.
*A. nidulans*, on the other hand, does not conserve the Anafp gene. Moreover the size of its secreted inhibtor is above 10 kDA.
This study deals with the identifiction and characterization of the two independent inhibitors of *A. fumigatus*, which could in future play an important role in the therapy of invasive aspergillosis.

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**033/EKP**
**Impairing fluoride export in the human-pathogenic fungus *Aspergillus fumigatus* mitigates voriconazole resistance**
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The halide fluoride is ubiquitous in the environment and also harmful at high concentrations to prokaryotic as well as eukaryotic cells. In order to keep intracellular fluoride concentrations low, $F^-$ export activities have evolved that constitute the crcB or FEX protein family. Based on conservation of functional domains in these transporters, we were able to identify the only FEX orthologue in the filamentous fungus *Aspergillus fumigatus*, the *fexA* (AFUA_2G16210) gene product. Expression studies in the presence of varying levels of fluoride indicate that *fexA* expression is low and might be regulated at the post-transcriptional level. Targeting and deleting the fluoride efflux pump-encoding *fexA* gene resulted in a strain impaired in fluoride export as demonstrated by an increased sensitivity towards this halide but,

for instance, not chloride. Furthermore, sensitivity of *A. fumigatus* towards fluoride was characterized to be pH-dependent. Based on the fact that the first-line antimycotic azole to treat invasive aspergillosis is a fluorine-containing molecule, we became interested in the effect of increased fluoride sensitivity on voriconazole resistance. Therefore, the *fexA* gene was deleted in a voriconazole resistant isolate and minimal inhibiting concentrations were determined to reveal that MICs of voriconazole were decreased when fluoride export became impaired. Our data suggest the FexA efflux pump as antifungal target and demonstrate its relevance for resistance against fluorinated compounds.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**034/EKP**

**Identification and characterization of *Candida albicans* factors that modulate cytokine production in distinct epithelial cell types**

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Disseminated candidiasis is a life-threatening infection in intensive care patients. An intravenous infection model of mice is commonly used to investigate this disease. In mice, *Candida albicans* is cleared from liver and spleen, whereas the pathogen proliferates in the kidney. As infection progresses, a distinct production of proinflammatory cytokines and progressive accumulation of immune cells are contributing to renal pathology. In contrast, the proinflammatory response in liver and spleen regresses over time. The molecular mechanisms of these different organ responses are not yet understood.
We hypothesize that fungal factors elicitating distinct epithelial responses contribute to organ-specific outcomes. Therefore, a large-scale screening approach was used: We analyzed a collection of 1100 *C. albicans* knock-out mutants for their ability to damage human oral, intestinal and renal epithelial cells and examined the host cell response by quantification of the proinflammatory cytokines IL-6 and IL-8. Not surprisingly, host cells produced less cytokines upon infection with mutants that inflicted less cell damage. However, we also identified mutants with normal damage potential but altered cytokine response, suggesting that fungal factors indeed modulate the immune response. From over sixty mutants in which damage potential and cytokine response appeared uncoupled in the initial screen or which induced cell type-specific host responses, six genes of interest were selected for the construction of homozygous deletion mutants for further investigations.
The phenotypical characterization of these *C. albicans* mutants is currently under way, and involves analysis of morphology and growth properties. Furthermore, we analyze the interaction of these mutants with different human epithelial cell lines and immune cells in more detail, including recognition by the host cells and activation of signaling pathways that contribute to cytokine production.
In summary, by using a large-scale screening approach we identified several candidate genes that might modulate epithelial cell responses to infection and might thus contribute to the organ-specific host responses observed *in vivo*.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**035/EKP**

**Aberrant DNA-binding of STAT1 in *Toxoplasma gondii*-infected macrophages**

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The intracellular parasite *Toxoplasma gondii* is able to establish chronic infections in mammals including humans which persist for the host's life. This may be facilitated by different immune evasion strategies, including a global inhibition of the interferon (IFN)-γ response in infected macrophages. The IFN-γ response, mediated by STAT1 (signal transducer and activator of transcription 1), is crucial for host defence against the pathogen. Previously, the activation and nuclear translocation of STAT1 were found to be unimpaired in *Toxoplasma*-infected macrophages. Here, we describe a refined analysis of STAT1 and its activities in infected murine macrophages.
Immunoblotting of mild detergent lysates from infected and non-infected macrophages revealed that phosphorylation of STAT1 at Tyr701 (pTyr701) which is required for activation of STAT1 was unimpaired in infected macrophages, thus confirming previous results. In contrast, phosphorylation of Ser727 (pSer727), which is required for full transcriptional activity of STAT1, appeared to be reduced after infection with *T. gondii*. Surprisingly, subcellular fractionation indicated increased amounts of both phosphorylated STAT1 isoforms as well as total STAT1 in the nuclei of infected macrophages. Subsequent comparison of mild detergent lysates treated or not with DNase showed a parasite-induced increase of Tyr701 and Ser727-phosphorylated STAT1 in DNase-treated lysates only. These results thus indicated an increased association of STAT1 with DNA in *Toxoplasma*-infected cells. We then assessed the binding of STAT1 to the gamma activated site (GAS) using electrophoretic mobility shift assay. Depending on the GAS sequence used, *T. gondii* inhibited formation of canonical GAS-STAT1 complexes. In contrast, a non-canonical GAS-STAT1 complex of lower electrophoretic mobility was consistently formed in infected macrophages independently of the GAS sequence used.
Together, our findings suggest that the infection with *T. gondii* results in an increased DNA-binding of STAT1 and induces a GAS-binding complex of different composition than the conventional GAS-STAT1 complex.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**036/EKP**

**Glycoshield regulation by the Ace2 signaling pathway in *Candida albicans***

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The transcription factor Ace2 is found in many fungal genomes and positively regulates expression of genes involved in cell separation and cell wall biogenesis. Distinct from its conserved function Ace2 was shown to regulate transcription of *PMT* genes encoding protein-*O*-mannosyltransferases in the human fungal pathogen *Candida albicans*. *C. albicans* mutants lacking either Ace2, the membrane sensor Msb2 or the MAP kinase Cek1 are unable to overcome defects in cell wall glycostructures. To investigate the function of Ace2 for regulation of *PMT* genes, genome-wide chromatin immunoprecipitation (ChIP) on chip analyses were used to explore binding of Ace2 to target sequences. The results indicate that Ace2 mainly localizes to promoter regions of genes encoding proteins, which function in regulation of gene expression, protein modification and cell wall organization. Direct binding to *PMT*-promoter regions could not be detected in any condition, suggesting that Ace2 regulates *PMT* transcription indirectly. Under glycostress conditions Ace2 binding to its own promoter was detected. Further we identified binding to the promoter region of *MSB2* and the promoter of the *CST20* gene encoding the Ste20

homolog protein kinase Cst20 an upstream component of the Cek1-MAP kinase pathway. Transcriptional analysis revealed Ace2 dependent upregulation of genes encoding Cek1 pathway components Msb2, Cst20, Hst7 and Cek1. These results indicate that under glycostress conditions, Ace2 functions as a downstream target of Cek1 promoting positive autoregulation as an adaptive mechanism to overcome impaired glycostructers.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**037/EKP**

**Click Beetle Luciferases as Reporters of Gene Expression in *Candida albicans***

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Worldwide more people die of fungal caused diseases then from tuberculosis or malaria. Inside these fugal species *Candida albicans* has a major importans because it is part of most peoples' microbiota. While superficial infections of the skin are easily cured, systemic ones in immunocompromised patients are often lethal. One major challenge during *C. albicans* treatment is its ability to grow in multimorphogenic ways (e.g. yeast and hyphae). Also its capability to colonize different plastic surfaces like catheters makes *C. albicans* the most significant fungal pathogen in hospitals. We have introduced a click beetle (CB) dual luciferase reporter system, which can be used to measure promoter activity as well as protein expression levels by simultaneously measuring red and green luminescence with practically no background. This system was successfully used to fuse sequences encoding a red and green luciferase with several promoters like *YWP1* and *HWP1*, genes, which are expressed in yeast- or hyphae-specific manner. These results show that it is possible to measure increasing and decreasing promoter activity over time. To make this system more reliable we established the luciferase half-life. In another approach we constructed a protein fusion of CB luciferases with Efg1, the main transcription factor responsible for hyphae formation through the PKA pathway. Promoter activity decreases during hyphae formation followed by protein activity with a 30 minutes delay. This reporter system can easily be used to screen for new substances that affect *C. albicans* growth forms.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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## FREE TOPICS

**038/FTP**

**Landfill leachate as "underappreciated" reservoir of bacteria harbouring co-resistance to antibiotics and heavy metals**

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**Introduction**: Resistance to antibiotics is a global health challenge and attention is currently focusing on the environment as reservoir of resistance. Metals found in polluted ecosystems can select for antibiotic resistance in bacteria. Leachates from municipal solid waste (MSW) dumpsites (landfills) are sources of metal input into the environment but leachates and leachate contaminated ecosystems have been minimally investigated as reservoir of bacteria harbouring co-resistance to heavy metals and antibiotics.
**Objectives**: This study investigated the incidence of co-resistance to metals and antibiotics in gram negative bacteria from leachates and leachate-contaminated surface and groundwater in Ibadan, Nigeria.
**Materials and Methods**: Leachate and leachate contaminated water samples were collected from three landfills in Ibadan, 2 leachate-contaminated streams and 5 hand-dug wells in residential houses around the landfills from June to August 2014. The concentrations of Cu, Zn, Pb, Cd and Ni in the samples were determined, and bacteria isolated on Mueller Hinton (MHA) and Eosine Methylene Blue Agar plates. Fifty gram negative bacteria were tested for tolerance to graded concentrations (100 µg/mL - 1100 µg/mL) of Zn, Cu, Pb and their mixture. Susceptibility to ertapenem (ETP 10 µg), cefpodoxime (CPD 30 µg), ciprofloxacin (CIP 10 µg), gentamicin (CN 10 µg), tetracycline (TET 30 µg), florfenicol (FFC 30 µg) and sulphamethoxazole/Trimethoprim (SXT 25 µg) were determined by disc diffusion. Correlation between heavy metals and antibiotics resistance was determined by Pearson correlation at 0.05 and 0.01 confidence levels.
**Results**: Concentrations of Cu and Zn in the samples were below the minimum, while the concentration of Pb, Cd and Cr are above the minimum recommended for discharged effluent and potable water in Nigeria (NESREA 2009). Forty (80%) isolates are resistant to at least one metal and antibiotic in combination. 31(62%) of these were resistant to CPD and 17(34%), 16(32%), 12(24%), 5(10%) and 3(6%) were resistant to ETP, TET, FFC, SXT, CN and CIP respectively. Metal MIC for these isolates ranged 200 µg/mL to ≥ 1100 µg/mL. Correlation was observed between ertapenem, florfenicol and copper resistance in Citrobacter sp.; between ciprofloxacin, gentamicin, tetracycline and Zn resistance among the pseudomonads, and between gentamicin and Zn resistance in Stenotrophomonas.
**Conclusion**: There is a prevalence of co-resistance to metals and antibiotics in bacteria isolated from leachates and leachate-contaminated samples in this study. There is significant correlation between resistance to selected metals and antibiotics suggesting a role for the metals in the development of antibiotic resistance. This suggests that leachates and leachate contaminated ecosystems deserve attention as reservoir of bacteria harboring co-resistance to metals and antibiotics.

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**039/FTP**

**Lipid kinases - a nexus of cell envelope biosynthetic pathways**

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Lipids are major constituents of biological membranes. They play an important role in many fundamental cellular processes in prokaryotes and eukaryotes. Membrane-associated proteins and integral membrane proteins are attached and inserted into the membrane according to the prevalent lipid environment. Diacylglycerol (DAG) and phosphatidic acid (PA), intermediates of phospholipid biosynthetic reactions, modulate membrane properties and directly influence the activity of membrane-standing proteins. In addition, they are thought to play a role in signal transduction processes as intracellular messengers. Polyprenol lipids, e. g. undecaprenyl phosphate, are universal carrier lipids that facilitate transport of hydrophilic molecules across membranes required for polymer biosynthesis, such as peptidoglycan, wall teichoic acid, capsule, and lipopolysaccharides in bacteria. Three (putative) lipid kinases are encoded in the *S. aureus* genome. The kinases that phosphorylate and provide these lipids to various biosynthetic pathways were expressed as recombinant proteins in *E. coli*, purified to homogeneity and characterized on the molecular level.

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**040/FTP**

**Antibiotics in drinking water, Austria 2014**

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This study reports on the first survey on antibiotics in drinking water in Austria. Testing for 19 antimicrobials was performed by an online solid phase extraction - liquid chromatography - high resolution mass spectroscopy method. Selection of sample sites for public drinking water focused on areas considered susceptible to contamination (100 samples) and on geographic population distribution (100 samples). At each site, a sample of 500 ml was collected once during the 2nd quarter and once during the 4th quarter of 2014. Sulfamethoxazole (SMX) was detected in 10 (5%) of the 200 drinking water samples tested. The 18 remaining antibiotics were not detected. Five samples from 2 sampling sites in Upper Austria (3 samples) and from 1 site in Lower Austria (2 samples) yielded SMX in concentrations above the limit of quantification (LOQ: 2.5 ng/L), with a median of 5.2 ng/L and a mean of 5.8 ng/L (range: 4.4 ng/L - 8.9 ng/L). Positive findings showed no significant seasonal variation. One site (2 pos. samples) was chosen based on population distribution, two sites (3 pos. samples) were risk based. Another 5 samples yielded SMX in concentrations of >=1 ng/L but below the LOQ. The concentrations measured in our study were generally low and risk of toxic effects with the current use of water is highly unlikely. However, even low-level concentrations of antibiotics in the environment could increase the rate at which pathogenic bacteria develop resistance to these compounds.

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**041/FTP**

**"CREDIBLE": A Versatile *In Vivo* System to Track Antigen and Drug Delivery and to Monitor the Distribution of Virulence Factors in Real Time**

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*Yersinia* outer protein M (YopM) is one of the virulence proteins of human-pathogenic *Yersinia*. Along with other Yops, it is usually transported into host cells *via* the type III secretion system (T3SS) upon infection. Additionally to the delivery by T3SS, YopM is able to penetrate plasma membranes autonomously. The protein transduction domain (PTD) responsible for the cell-penetrating ability of YopM is enclosed within two *N*-terminal α-helices (2αH). To investigate 2αH as a novel PTD of bacterial origin and a potential cargo transporter in more detail we have designed a Cre-mediated double reporter ('CREDIBLE') system. The transgene constructed includes two reporter genes, near-infrared fluorescent protein (iRFP) and luciferase. For both expression is dependent on Cre/loxP-recombination. We have demonstrated that the 'CREDIBLE' system is functional *in vitro* and both reporters are expressed upon recombination. Furthermore, we have generated transgenic mice using pronuclear microinjection of the transgenic DNA and 15 transgene founders have been identified. Mating of the offspring mice with PGK-Cre mice expressing Cre-recombinase has resulted in recombination events indicating that the 'CREDIBLE' reporter system is also functional *in vivo*. Therefore, the distribution of 2αH-Cre and other PTD-Cre fusion proteins administrated by different routes into these transgenic mice can be investigated in real time by employing non-invasive live optical imaging.
Additionally, the approach we describe here can be applied in studies dealing with various drug delivery systems (e.g. exosomes, targeted drug delivery etc.) to estimate and follow their efficacy *in vivo*. Furthermore, this system opens up possibilities to monitor bacterial or viral infections *in vivo* and, in particular, to investigate various virulence factors during infection in more detail (e.g. effector proteins injected by bacterial secretion systems, outer membrane vesicles etc.).

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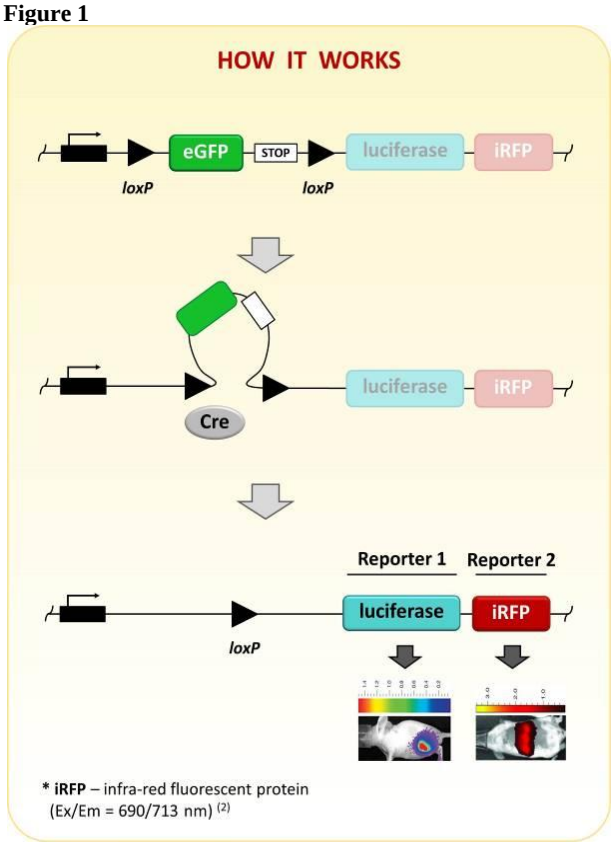
**Figure 1**

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**042/FTP**

**Screening of heroin for *Bacillus anthracis*-contamination**

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We describe a culture-based method suitable for isolating *Bacillus anthracis* and other live bacteria from heroin. This protocol was developed as a consequence of the bioforensic need to retrieve bacteria from batches of the drug associated with cases of injectional anthrax among heroin-consumers in Europe. This uncommon manifestation of infection with the notorious pathogen *B. anthracis* has resulted in 26 deaths between the years 2000 to 2013. Thus far, no life disease agent has been isolated from heroin during forensic investigations surrounding these incidences. Because of the conjectured very small number of disease-causing endospores in the contaminated drug it is likely that too few target sequences are available for molecular genetic analysis. Therefore, a direct culture-based approach was chosen here. Endospores of *B. anthracis* (Sterne) artificially spiked into heroin were successfully retrieved at 86-98% recovery rates.
Finally, 82 samples of heroin originating from the German heroin analysis program seized during the period between 2000 and 2014 were tested and found to be surprisingly poor in retrievable bacteria. Notably, while no *B. anthracis* were isolated from the drug batches, other bacteria were successfully cultured. The resulting methodical protocol is therefore suitable for analyzing heroin which can be anticipated to comprise the original microbiota from the drug's original source without interference from contaminations introduced by cutting.

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**043/FTP**

**Analysis of T3SS-independent Autonomous Internalisation of the Bacterial Effector Protein SspH1 from *Salmonella typhimurium***

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Bacterial pathogens including *Salmonella*, *Yersinia* and *Shigella* spp. express effector proteins of the LPX subtype of leucine-rich repeat (LRR) proteins that are translocated into the host cell via a type three secretion system (T3SS). Subversion of the host's immune response by LPX effectors of *Shigella* and *Salmonella* is mediated by a novel E3 ubiquitin ligase (NEL) domain. Previous studies identified the effector protein YopM of *Yersinia enterocolitica* as a novel bacterial cell-penetrating protein (Rueter *et al*., 2010). YopM's ability to translocate across the host cell plasma membrane independently of *Yersinia*'s type III secretion system (T3SS) is mediated by its two N-terminal α-helices. The SspH1 effector protein of *Salmonella typhimurium* shares significant homology in sequence and structure with YopM, which prompted us to investigate potential cell-penetrating abilities of this effector protein.
For this, we recombinantly expressed SspH1 in *Escherichia coli* and analysed a potential T3SS-independent translocation of the protein by cell fractionation of HeLa cells, immunofluorescence microscopy and FACS analyses. The functionality of the recombinant protein as an E3 ubiquitin ligase was determined using in vitro ubiquitination assays. Additionally, an effect of the recombinant protein on the expression of pro-inflammatory cytokines was analyzed by quantitative real time PCR.
In this study, surprisingly, we could show that the SspH1 effector protein of *Salmonella typhimiurium* is able to translocate into eukaryotic cells without a requirement for additional factors. Furthermore we could show that recombinant SspH1 is a functional E3 ubiquitin ligase that is able to reduce the expression of Interleukin-8 in IL-1β stimulated cells (Lubos *et al.*, 2014).
These results show that SspH1 is a novel bacterial cell-penetrating protein and, together with YopM, a hint for a general concept of T3SS-independent translocation by LPX effector proteins.

**Reference**

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**044/FTP**

**The antimicrobial efficiency of peracetic acid**

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Since the (bio-) terroristic attacks in 2001 in the United States peracetic acid (PAA) has seen a renaissance. The strong oxidizing agent is an all-purpose disinfectant including sporicidal efficiency. By establishing carrier assay models the efficacy of PAA has been tested against Bacillus spores, viruses and toxin which were air dried on carriers with hydrophobic flexible surfaces prepared from protective suit material. Especially a carrier assay was used where 10 µl of PAA were dispersed over an area of 2 cm2. Detergents were added to reduce the surface tension of PAA and thus to enhance the covering of the hydrophobic carrier surface.
Using 1% PAA against spores of Bacillus subtilis or 2% PAA against spores of B. thuringiensis and B. anthracis a high reduction of viable spores (between 5 to 6 log10 steps) could be observed within 3 to 5 min of PAA exposure. The spores from B. subtilis and B. anthracis were unable to germinate neither on solid nor in liquid media after that treatment. A few spores of B. thuringiensis which survived were only able to germinate in liquid medium. A low concentration of PAA (0.24%) in 40% ethanol was highly effective against the B. thuringensis spores after 10 min of exposure. Even a concentration of 0.05% or 0.1% PAA inactivated Vaccinia virus or Adenovirus, respectively, within a contact time of one minute. Regarding ricin the toxin protein proved to be the most resistant agent. 2% PAA and a contact time of 10 min were necessary to reach a high reduction of cytotoxicity.
In our study we investigated the PAA activity preferentially against biological substances which model, simulate or even belong to bio-hazardous agents. Therefore the bio-agents were fixed on carriers prepared from protective suits with a hydrophobic outer layer and exposed to a thin layer of PAA. Under these conditions PAA turned out to be a highly effective disinfectant even under organic load or low temperatures (≤10°C).

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**045/FTP**

**Notification of *E.coli* Enteritis as an example for the implementation of the new case definitions 2015 in North Rhine-Westphalia**

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**Background:** At the beginning of 2015 updated case definitions were released by the department for infectious disease epidemiology at the Robert Koch-Institute. In this context there was a modification in notifying cases within the category "*Escherichia (E.) coli* Enteritis" between the local and state public health level. The notification at local public health authorities according to § 7 (1) 13b IfSG is not affected by this change.
In the past generally serotyping and no further molecular diagnostics (e. g. PCR) for pathogenic factors were performed by laboratories. Since serotyping only provides information about the surface of the respective strain, a distinct classification into ETEC, EIEC, EPEC and EAEC was often not possible. Additionally, infections with these *E. coli* strains were mostly sporadic cases and thus, less relevant for epidemic prevention in Germany. Clusters of suspected cases of non-EHEC-infection are still notifiable according to § 6 (1) 2 IfSG.
**Aims**: The modification of notifying cases within the category "*E. coli* Enteritis" enables the public health authorities to focus on surveillance of EHEC cases causing more severe illnesses. At the same time the work at local public health authorities is simplified and infection prevention and control measurements can be implemented more effectively.
**Methods**: Evaluating the implications of this modification via analysing the notification data of EHEC and *E. coli* Enteritis compared to previous years on the state level.
**Results**: After the EHEC outbreak 2011 increased numbers of both EHEC and *E. coli* Enteritis were observed in North Rhine-Westphalia (NRW) most likely due to enhanced surveillance. Following the new case definition the NRW Centre for Health (LZG.NRW) expects a decrease in notified *E. coli* Enteritis cases on the state level in the future.
Since notification of *E. coli* Enteritis cases is no longer required between the local and state public health level, the investigation for EHEC cases on a local level could be intensified to obtain better data quality with higher significance.
**Summary**: Due to this new case definition the NRW Centre for Health expects an improvement in data quality compared to diagnostically inconclusive data from previous years. Furthermore, better analysis of these data may lead to improved infection prevention and more effective control measurements.

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**046/FTP**

**Genotyping and gene expression analysis of putative reference genes in Enterobacteriaceae**

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**Introduction**: *Enterobacteriaceae* play an important role as pathogens. Several virulence factors and related genes are used by *E. coli* to infect hosts or to form biofilms. To gain precise information about pathomechanisms quantitative real-time polymerase chain reaction (qPCR) is the method of choice. It enables a quantification of gene expression by relative quantification of the expression levels. Target genes are normalized to the expression levels of reference genes. However, the selection criteria of reference genes appear not to be systematic in the literature. Many publications lack information about the testing conditions and the calculation of the expression levels using qPCR analysis.
**Aim**: The aim of this study was to identify putative reference genes for normalization in qPCR experiments in biofilm studies. To receive precise and reproducible qPCR data we applied the Minimum Information required for the publication of qPCR Experiments (MIQE)-Guidelines by Bustin *et al.* 2009 (Clin. Chem.).
**Material/Methods**: A Venn-diagram analysis of literature data revealed that none out of 20 putative reference genes (*adk, arcA, csgA, fimH, fumC, gapA, gstA, gyrA, gyrB, idnT, proC, purA, recA, rpoB, rpoD, rpoS, rpsA, sdhA, uspA, yqaB*) was present in all Enterobacteriaceae. We focused on 9 *E. coli* pathotypes (incl. *EPEC, STEC, EAEC*) for further analysis. As a high number of samples (90 isolates of different E. coli pathotypes) was handled a semi-automatic workflow for high-throughput analysis was established. As diagnostic targets we have chosen *csgA* and *fimH*, which are associated to biofilm formation. The implementation of these guidelines should create the basis of precise and reliable qPCR data.
**Results**: A frequency analysis revealed that the gene idnT had the lowest prevalence in pathotypes (15% - 70%, $p < 0.05$) and adk was among those genes with the highest prevalence (100%, $p < 0.05$). Multifactorial correspondence analysis revealed that *fumC* and *idnT* contributed to the largest variation. Subsequently, we selected 20 isolates (biofilm forming and non-biofilm forming) for gene expression analysis and applied the gNorm algorithm for the selection of the most stable expressing genes.
**Summary**: In summary, we propose a set of putative reference genes, which can be used for gene expression analysis according to the MIQE-Guidelines.

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## GASTROINTESTINAL INFECTIONS (GIP)

**047/GIP**

***Arcobacter butzleri* induce inflammatory responses in gnotobiotic IL-10 deficient mice**

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**Background and objectives:** Acute gastroenteritis with abdominal pain and acute or prolonged watery diarrhoea has been described for humans infected with *Arcobacter* (*A.*) *butzleri*. Adhesive, invasive and cytotoxic capacities have been described for *A. butzleri in vitro*. So far, only limited information is available about the immune-pathogenic mechanisms of infection *in vivo*.
The aim of this study was to investigate the immune-pathological properties of *A. butzleri* in a well-established murine infection model.
**Methods:** Gnotobiotic IL-$10^{-/-}$ mice were orally infected with two different *A. butzleri* strains and clinical signs as well as fecal shedding were determined over time. At day 6 and day 16 post-infection apoptotic and proliferating cells, intestinal infiltration with immune cells and cytokine expression patterns were determined.
**Results:** Despite no overt macroscopic signs of disease, stable infection of gnotobiotic IL-10-/- mice with *A. butzleri* led to increased numbers of apoptotic cells, influx of immune cells and higher expression levels of pro-inflammatory cytokines in the intestine, depending on the respective *A. butzleri* strain.
**Summary and conclusion:** We here clearly show that *A. butzleri* is able to stably colonize the intestinal tract and induce apoptosis paralleled by induction of pro-inflammatory immune responses in the intestine of infected IL-$10^{-/-}$ gnotobiotic mice, pointing towards an immune-pathogenic potential of *A. butzleri in vivo.*
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**048/GIP**

**Human α-defensin-1 protects cells from intoxication with *Clostridium difficile* toxins A, B and CDT**

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*C. difficile*-associated diseases (CDAD), which frequently occur in hospitalized patients after prolonged treatment with antibiotics, include diarrhea and the severe form pseudomembranous colitis. In recent years, more severe forms of CDAD were described which are associated with hypervirulent strains of *C. difficile*. *C. difficile* secretes the exotoxins A (TcdA) and B (TcdB), which glycosylate and thereby inactivate Rho-GTPases in mammalian cells and are considered as the causative agents of CDAD. In the hypervirulent strains, the ADP-ribosyltransferase CDT was identified as a third toxin in addition to TcdA and TcdB. In target cells, CDT ADP-ribosylating G-actin, which destructs the actin cytoskeleton and increases the adherence of *C. difficile* to the intestinal epithelium via microtubule-based protrusions. Because the hypervirulent strains are more resistant against antibiotics and the toxins are the relevant virulence factors, novel pharmacological inhibitors against the toxins are needed.
Prompted by earlier results that a certain form of human α-defensin-1 (HNP-1), a peptide inhibitor, protected cultured epithelial cells from intoxication with TcdB (1), we discovered that α-defensin-1, but not ß-defensin-1, also inhibits the intoxication with TcdA and CDT in a concentration-dependent manner when applied prior to the toxin to the cells. This was determined by analysis of the toxin-induced cell-rounding, a specific and highly sensitive endpoint to monitor the intoxication. However, the inhibition of TcdA was less efficient compared to TcdB. Moreover, α-defensin-1, prevented the cytotoxic effects of all three toxins combined in the medium, which might mimic the situation after an infection with hypervirulent *C. difficile*. Currently, the molecular mechanism underlying the inhibitory effects of α-defensin-1 are investigated in human colon cells and a clinically relevant mini-gut organoid model. In conclusion, our results demonstrate that human α-defensin-1 not only exhibits the established microbicidal effects but also inhibits the three relevant toxins of *C. difficile* which contribute to CDAD.

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**049/GIP**

**Intoxication of cells with *Clostridium difficile* toxins A, B and CDT is impaired by bacitracin**

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Bacitracin is a cyclic polypeptide antibiotic which inhibits the cell wall synthesis of Gram-positive bacteria. As such, it has been applied against bacterial infections including pseudomembranous colitis, for which the most serious cause is an infection with *Clostridium (C.) difficile*. The causative agents of *C. difficile*-associated diseases (CDAD) are the exotoxins A (TcdA) and B (TcdB) which are secreted by the bacterium and glycosylate and inactivate Rho-GTPases in the cytosol of human target cells. In recent years, hypervirulent strains of *C. difficile* have been recognized, which were associated with more severe forms of CDAD and increased death rates. These strains were found to be more resistant towards the few antibiotics generally used for treatment of therewith associated diseases raising the need for novel pharmacological strategies against *C. difficile*-associated diseases. Moreover, the hypervirulent strains, such as the subtype BI/Nap1/O27, produce a third toxin in addition to TcdA and TcdB, the binary ADP-ribosylating toxin CDT which targets G-actin, resulting in actin depolymerization within target cells.
By performing cell intoxication experiments, we observed that bacitracin prevented intoxication of cultured epithelial cells with the isolated toxins TcdA, TcdB, and CDT in a concentration- and time-dependent manner. Intoxication was inhibited either when bacitracin was applied prior to the toxins or at the same time point, but not when applied afterwards to the cells. Although our previous results suggest an extracellular mode of action of bacitracin towards the three toxins, more detailed investigations to elucidate the precise underlying mechanism are underway. In conclusion, bacitracin might represent a suitable therapeutic against the hypervirulent *C. difficile* strains since it acts not only on bacterial growth but also inhibits the mode of action of the relevant toxins produced during the infection with the bacteria.

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**050/GIP**

**Genome sequencing of two novel EHEC/EAEC hybrid strains isolated from human infections**

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**Introduction**: The so far the highest number of life-threatening hemolytic uremic syndrome was associated with a food-borne outbreak in 2011 in Germany which was caused by an enterohemorrhagic *Escherichia coli* (EHEC) of the rare serotype O104:H4. Most importantly, the outbreak strain harbored genes characteristic of both EHEC and enteroaggregative *E. coli* (EAEC). Such strains have been described seldom but due to the combination of virulence genes show a high pathogenicity potential. To evaluate the importance of EHEC/EAEC hybrid strains in human disease, we analyzed the EHEC strain collection of the German National Reference Centre for Salmonella and other Enteric Bacterial Pathogens (NRC). Additionally to molecular methods, we here analysed the strains of interest by means of whole genome sequencing (WGS).
**Methods**: The search for EHEC/EAEC strains and their subsequent analysis included the following methods: PCR or Southern blotting for the detection of EHEC (such as *stx* and *eaeA*) and EAEC marker genes (such as *aatA*) as well as for aggregative adherence fimbriae genes (AAF), characterization of adherence pattern and cytotoxicity, analysis of antibiotic resistance profile, macrorestriction analysis / pulsed-field gel electrophoresis, multi locus sequence typing, *stx* sequence analysis and PacBio and Illumina MiSeq WGS.
**Results**: After exclusion of O104:H4 EHEC/EAEC strains, out of about 2400 EHEC strains sent to NRC between 2008 and 2012, two strains exhibited both EHEC and EAEC marker genes, specifically were *stx2* and *aatA* positive. Like the 2011 outbreak strain, one of the novel EHEC/EAEC, isolated from a patient with bloody diarrhoea in 2010, harboured *stx2*a, was serotyped as O59:H$^-$, belonged to MLST ST1136, and exhibited genes for type IV AAF. The second strain was isolated from a patient with diarrhea in 2012, harboured *stx2*b, was typed as Orough:H$^-$, and belonged to MLST ST26. No AAF genes corresponding to fimbrial types I to V were detected in this strain. WGS revealed a genome size of ~5.2 Mb for both strains and 3 to 4 plasmids of 7 to 124 kb carrying important virulence and adherence genes. The WGS data enabled us to confirm and newly detect virulence markers, to perform genoserotyping using the O-antigen gene cluster, to find genes coding for new potential adherence structures and to compare the core genome of the stains with stains of the same MLST sequence type, the outbreak strain EHEC/EAEC O104:H4, EHEC O157:H7 EDL933 and EAEC O44:H18 042.
**Conclusion**: So called mixed *E. coli* pathovars or hybrid strains have been seldom described and show a high virulence potential. We found two novel strains isolated from human disease cases in Germany in 2010 and 2012. Those strains belong to MLST sequence types and/or serotypes seldom associated with human disease and in addition to *stx2* harbour EAEC characteristics which further qualify them to cause severe disease.

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**051/GIP**

**Genome-wide patterns of homologous recombination after natural transformation of *Helicobacter pylori***

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The naturally competent bacterium *H. pylori* has a highly plastic genome. In addition to an extraordinarily high mutation rate, recombination is the major mode of genomic diversification in this organism. Our previous studies of genome diversification during chronic human infection have demonstrated that *H. pylori* imports very short fragments of foreign DNA into its chromosome. However, factors limiting horizontal gene transfer or regulating recombination-tract lengths have not been systematically elucidated on a whole-genome level. We used an *in vitro* system to elucidate genome-wide patterns of DNA imports after natural transformation. Due to the high sequence diversity between the *H. pylori* strains used as donor and recipient, import borders could be mapped with high precision. We performed single or multiple rounds of transformation and monitored genome evolution by analyzing recombination events within recombinant clones. In total, 41 recombinant genomes were sequenced (21 clones derived from the single transformation experiment and 20 clones obtained after multiple rounds of transformation). Import lengths ranged from less than 50 bp to more than 13,000 bp of contiguously replaced sequence. Clones isolated after repeated transformation contained numerous imports (mean no. of imports, 41.3 ± 24.1), leading to the allelic replacement of up to 8% of the genome sequence. Imports were found with similar frequencies in genes belonging to different functional categories. Importantly, and in contrast to previous reports, homeologous recombination of DNA derived from non-isogenic donor strains was not limited by restriction-modification systems of the recipient. This probably

explains the rapid genomic evolution of *H. pylori* especially during mixed infections supporting the successful co-existence with its human host.

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**052/GIP**

**Bacterial enteropathogens associated with diarrhoeal diseases in paediatric patients and pattern of drug susceptibility in Lagos, Nigeria**

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**Introduction:** Acute diarrhoea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries. One of the major challenges is the recent increase in the number of probable aetiological agents.
**Objective:** This study was embarked on to determine the prevailing bacterial enteropathogens associated with childhood diarrhoea and patterns of susceptibility to commonly used antibiotics.
**Materials and Methods:** A total of 160 faecal samples were collected from children (0 - 12 years) presenting with symptoms of gastroenteritis in three referral public health care centres in Lagos State. Samples were subjected to bacteriological analysis; isolates were identified by standard procedures and were subjected to antimicrobial susceptibility testing using standard methods.
**Results:** Out of the 160 faecal samples evaluated, 102 samples were positive for bacterial growth, made up of seven (7) bacteria genera. A total of 140 isolates were identified. Co-existence of two or more bacterial enteropathogens were identified in 22 faecal samples and a single enteric pathogen in 80 samples. *Enterobacter* spp. (47.1%) was found to be the predominant enteropathogen identified, followed by *Escherichia coli* (20%), *Klebsiella* spp. (11.4%), *Acinetobacter* spp. (8.6%), *Citrobacter* spp. (5.7%), *Salmonella* spp. (4.3%) and *Alcaligenes faecalis* (2.9%). Age group 3 - 5 years was at the high risk of diarrhoea in this study. Antimicrobial susceptibility testing revealed that all the bacterial isolates were 97.1% susceptible to gentamicin, followed by 94.3% pefloxacin,92.9% augmentin, while 62.9% of the isolates were least susceptibility to co-trimoxazole.
**Conclusion:** This study revealed wide range of enteropathogens associated with childhood diarrhoea with *Enterobacter* species being the most prevalent bacterial agent This information is essential to facilitate strategy of looking beyond routine identification of convectional diarrhoeaic agents of *E. coli* and *Salmonella* spp in cases of gastroenteritis for effective public health management and control of diarrhoeal disease in children.

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**053/GIP**

**Screening for a small molecule inhibitor targeting the biogenesis of outer membrane virulence factors in gram-negative Enterobacteriaceae**

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Despite the fact, that the enormous economic burden and individual suffering caused by gastrointestinal infections permanently persists in developing and newly industrialized countries, healthcare systems in First world countries underestimated its significance for a long time. The alarming prevalence of multidrug-resistant gram-negative bacteria, combined with a high epidemic potential of gastrointestinal pathogens, however, demonstrates the urgent need for new antibiotics and antiinfectives worldwide. 2,5 million deaths per year were actually caused by acute diarrheal infections. The most common causative agents of acute diarrheal infections, amongst others, are *Yersinia enterocolitica, Campylobacter jejuni*, *Salmonella spp., Shigella spp., Escherichia coli, Vibrio cholerae*, and *Clostridium difficile. The established treatment based on antibiotics is* mostly ineffective or may even have adverse side effects and result in prolonged shedding. In either way, antibiotic treatment also eradicates at least parts of the intestinal microbiome, and thereby disrupts colonization resistance, fosters overgrowth of pathogens and prolongs shedding times. Therefore, the development of future drugs should be focused on highly specific antiinfectives, which enable a direct pathogen-specific treatment. One very promising strategy is the inhibition of the biogenesis of outer membrane virulence factors. Due to the fact that many decisive virulence-associated outer membrane proteins (OMPs) of gram-negative enteropathogens are substrates of the periplasmic chaperone SurA exclusively, we developed a new assay format to determine SurA *in vitro* chaperone activity. Previous publications by Behrens *et al.*, 2001 and Buchner *et al.*, 1998 documented an assay to determine SurA *in vitro* chaperone activity with extremely limited sensitivity and minimal detectable concentration, which was not suitable for high throughput screening (HTS). We now developed a luciferase-based screening assay. This highly sensitive and robust test system has been validated extensively and now gives reliable output with an appreciable z-factor of > 0,6. In cooperation with the HZI Braunschweig (Germany) and the HZI Saarbruecken (Germany), we were able to screen over 7000 purified compounds and over 500 extracts of myxobacteria. During the ongoing screening period, the assay generated four validated primary actives, which corresponds to a positive hit rate of 0,05 %. Additionally, we developed an elaborate *follow-up* strategy to validate positive hits, which includes a well-established mouse infection model. We are looking forward to escalate our screening efforts and would like to use this abstract to invite all scientist who are interested in testing compound/natural extract libraries for an activity against the target structure SurA.

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**055/GIP**

**Elucidating the mechanism of Shiga toxin reduction in Enterohemorrhagic *E. coli* by the probiotic *E. coli* strain Nissle 1917**

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Enterohemorrhagic *E. coli* (EHEC), which are transmitted by contaminated food, have become a significant threat for humans as these pathogens can lead to the development of severe gastrointestinal disease and life threatening complications such as HUS. Since the large outbreak in Germany in 2011 a lot of research addressed pathogenicity of EHEC and the development of new treatment strategies. The most important EHEC virulence factor is Shiga toxin (Stx), an $AB_5$ exotoxin. Once secreted this toxin can bind with its B subunits to the globotriaosylceramide receptors (Gb3) of enterocytes and enter the cells by endocytosis. The A subunit has a specific N-glycosidase activity and cleaves an adenine base from the 28S rRNA of the ribosome by which the protein synthesis is blocked and the cells dye due to apoptosis. Treatment of patients with antibiotics is not recommended as this is linked to an increase of released Stx [1]. Previous studies with probiotics showed *E. coli* Nissle 1917 (EcN) to inhibit both growth of and Stx production by EHEC strains, which can only be traced back in part to the production of antibacterial operating microcins [2, 3]. Co-cultivation studies by Stefan Rund could show that EcN has strong Stx reducing effects on EHEC strains [3]. The next objective is to elucidate the underlying mechanism by examination of the cultivation media for substances secreted by EcN. Up to now the Stx production of EHEC strains was always analyzed in complex medium, which contains too many different substances for analysis. Therefore, we positively tested whether EHEC strains grow and produce Stx in a defined minimal medium. We could also verify that the microcin negative EcN mutant SK22D shows Stx reducing effects being co-cultivated with EHEC in minimal medium. In a next approach we will investigate whether EHEC

needs to be present in order to induce the production of Stx-reducing substances by EcN. Therefore EHEC will be incubated in EcN spent culture minimal medium and examined for the Stx production via a Stx-ELISA. This will be followed by a mass spectrometric analysis of the spent culture media. EcN has been proven to have Stx reducing effects on EHEC strains. Substances that induce this decrease could not be identified yet. Objective of this study is to perform cultivation studies in minimal medium and analyze it for possible Stx influencing substances. This approach could help to get a better understanding about the Stx regulation in EHEC by EcN.

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**056/GIP**

**Fast DNA-Microarray-Based Subtyping for the different alleles of Shiga Toxins 1 and 2 and comparison to conventional PCR detection**

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In humans Enterohaemorrhagic *Escherichia coli* (EHEC) can cause bloody diarrhoea (enterohaemorrhagic colitis) and haemolytic uremic syndrome (HUS) including renal failure, as they are able to produce different Shiga toxins. In general, the detection of the Shiga toxins is performed by ELISA of culture supernatants. An identification of the different variants of this toxin (stx1a, stx1c, stx1d, stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, stx2g) is epidemiologically and medically advisable, because this might yield information as well as on transmission paths and virulence as well as on prognosis and treatment indications. Molecular identification, according to a nomenclature proposed by Scheutz, 2012, can be achieved with a set of conventional PCR´s and the subsequent gel analysis.
As an alternative approach, the use of multiplex linear primer extension reactions was investigated using 22 primers simultaneously. During amplification, biotin-dUTP was incorporated for labelling. This was, followed by hybridisation to a specific DNA microarray, washing steps and staining with streptavidin-horseraddish conjugate and a precipitating dye. Stained arrays were scanned and analysed automatically allowing the identification of single and multiple stx variants within one experiment.
The assay was initially validated with 21 Shiga toxin-producing *E. coli* (STEC) reference strains that were previously tested by the complete set of conventional subtyping PCRs. These strains were selected to include all stx subtypes alone and in various combinations. The microarray results showed 100% concordance with the PCR results. For further validation of the microarray, 446 STEC field isolates of human and animal origin were screened identifying their Stx subtypes or even combinations of several subtypes within one isolate.
In summary, this oligonucleotide microarray based test represents an excellent, fast and economic diagnostic tool that provides some advantages over standard PCR-based subtyping. If necessary, the number of the spotted probes on the microarrays can be easily increased in order to cover additional targets such as novel alleles, other typing markers or resistance genes. Another option is the identification of stx subtypes by arrays that carry monoclonal antibodies rather than oligonucleotide probes. First experiments with a prototype allowed an identification of stx1, stx2a, stx2b, stx2c/2d and stx2e/2f and further efforts are underway.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**057/GIP**

***Characterization of the proteome of* Helicobacter pylori *VacA-containing vacuoles (VCVs), VacA intracellular trafficking and interference with calcium signaling in T-lymphocytes***

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*Helicobacter pylori* is a human gastrointestinal pathogen that colonizes about half of the world's population, with a notably high prevalence in the developing world. It has been linked to gastric diseases such as chronic gastritis, ulcers, and gastric cancer. *H. pylori* modulates its habitat, the gastric mucosa, using various secreted proteins and toxins, one of which is the vacuolating toxin VacA. VacA is located on the bacterial surface, or is secreted by the bacterium, binds to the plasma membrane of the gastric mucosa and is internalized via endocytosis. The toxin forms hexamers which create pores in the cytoplasmic or endosomal membrane, resulting in the swelling of these endosomal structures to form acidic VacA containing vacuoles (VCVs).
To begin understanding the function of VCVs we isolated VCVs from Jurkat E6-1 T-cells using a VacA-specific antibody coupled to magnetic beads and identified their proteome by mass spectrometry. As a background control we used a mutant of the VacA protein which is still internalized, but cannot form pores and therefore the sample does not contain VCVs. A second background control, without any form of VacA, was also examined to eliminate any unspecific binding to the mutant VacA. Using the mass spectrometry data we were able to identify the VCV specific proteins and thus the VCV proteome.
An interesting candidate protein in this VCV specific subset was the Inositol 1,4,5-triphosphate receptor type 3, ITPR3. The receptor is an essential part of the calcium signaling pathway leading to T-cell activation. Interestingly, this receptor usually locates to the endoplasmic reticulum and VacA has previously only been shown to localize to the mitochondria. Since VacA influences local T-cell activity through interfering with the phosphatase calcineurin and the calcium signaling transcription cascade, we further concentrated on one of the most important calcium signaling factors in the ER, the stromal interaction molecule 1, STIM1. We found that VacA strongly interferes with the movement of STIM1 to the plasma membrane localized calcium channel ORAI1 after calcium store depletion and thus inhibits the increase of cytosolic calcium in the Jurkat E6-1 T-cell line and CD4+ T-cells. VacA not only co-localized with STIM1 in the ER, but can also be found in the Golgi apparatus, as demonstrated by immunofluorescence microscopy. These are novel VacA target structures whose interactions need to be studied further to be fully understood.
Ref:
Characterization of *Helicobacter pylori* VacA-containing vacuoles (VCVs), VacA intracellular trafficking and interference with calcium signaling in T-lymphocytes
Kern *et al.* (submitted)

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**058/GIP**

**An in-depth analysis of a recurrent *Clostridium difficile* infection - adding more pieces to the puzzle**

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*Clostridium difficile* is a Gram-positive strictly anaerobically growing bacterium that is capable to form spores. *C. difficile* can colonize the human gut asymptomatically, but by misbalancing the normal microbiome with antibiotics there is a high risk of developing *C. difficile* infection (CDI). That is why *C. difficile* is a leading cause of antibiotic-associated and nosocomial diarrhea. CDI can range from mild diarrhea to pseudomembranous colitis even up to a toxic megacolon that is associated with high mortality (1). CDI has not only become an extreme burden for the healthcare system but moreover for the patients suffering from this infection. Despite initially successful treatment of the disease, about 20% to 30% of the patientswill have a recurrence within 1 to 3 months (2). In this study, we investigated a case of a recurrent CDI over 18 months with seven episodes of diarrhea. Interestingly, during some episodes more than one isolate of *C. difficile* could be identified in stool samples. Additionally, we included three isolates from asymptomatic periods. We performed a detailed phenotypical and molecular analysis of the 11 isolates to gain more insight into factors that might contribute to such a persistently recurrent CDI. Morphological characterization revealed that some isolates differed extremely in terms of cell shape, motility, sporulation capacity and toxin production. Molecular typing verified the presence of identical as well as different ribotypes (RT), including the hypervirulent 078/126 and a yet unknown RT. This indicates that the recurrent symptoms were caused by relapses as well as by reinfections. We used a whole-genome sequencing approach to search for differences between consecutive reisolates of the same RT, identify potential reinfections with strains of the same RT, and to test for potential recombination among isolates of different RTs. Relating antibiotic treatment, hospitalization and lifestyle habits over the course of time to the appearance of a respective RT might increase our understanding of recurrent *C. difficile* infections.

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**059/GIP**

**Evaluation of the new fluorescence-based PCR assay FluoroType® CDiff for the direct detection of *Clostridium difficile* and toxin B from stool specimens**

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**Questions**: The increasing incidence of severe nosocomial *Clostridium difficile* infections is a reason for concern in healthcare settings worldwide. Fast and reliable detection of toxin-positive *Clostridium difficile* (CDiff) is crucial for diagnosis and for prevention of outbreaks with this serious infectious agent. We evaluated the performance of the new FluoroType® (FT) CDiff (Hain Lifescience, Nehren, Germany) for the direct detection of *C. difficile* and toxin B directly from stool specimens. Results were compared to toxigenic culture and a CE-marked PCR test (GenoType (GT) CDiff) performed directly from stool specimen and from toxigenic culture.
**Methods**: 210 liquid or soft stool specimens with request for *C. difficile* analysis were examined with the FT CDiff. This new assay is based on fluorescence-labeled probes and detects the *tpi* gene (species *C. difficile*) and *tcdB* gene (toxin B). PCR amplification and detection by melting curve analysis is performed on the FluoroCycler® (Hain Lifescience). Evaluation of the melting curves is done by the Fluoro-Software®. All stool specimens were routinely tested with the C. DIFF CHEK™-60-EIA (Techlab, Blacksburg, VA) for the detection of the GDH-antigen. Direct toxin detection was performed with an EIA detecting toxins A and B (WAMPOLE™ C. difficile TOX A/B II, Techlab, Blacksburg, VA). Toxigenic culture was setup on CDiff-agar (bioMérieux, Marcy L`Etoile, France). Toxigenic *C. difficile*-isolates were confirmed by the GenoType CDiff (Hain Lifescience) for the differentiation of *C. difficile* (*tpi*-gene), *tcdA* and *tcdB* toxin genes, binary toxin genes *cdtA*/*cdtB*, deletions in the regulator gene *tcdC* and the moxifloxacin resistance-mediating mutation in the *gyrA* gene. DNA Extraction of the stool specimens was performed with the GenoXtract® (Hain Lifescience). All assays were performed according to manufacturer´s instructions.
**Results**: Using the FluoroCycler®96 for PCR and evaluation of the FT CDiff, out of the 210 stool specimens with suspected CDI, 59 samples were detected as toxin B DNA positive samples and 151 samples were determined as toxin B DNA negative. 49 of the 59 toxin B DNA positive samples were also determined as toxin A/B positive in the toxigenic culture. Eight of these ten discrepant samples could be confirmed as correctly positive by the GT CDiff. 149 of the 151 negative samples detected with the FT CDiff were also detected as negative samples in the toxigenic culture. The two discrepant samples could be confirmed as correct negative samples by the GT CDiff test. Sensitivity, Specificity, NPV and PPV of the FT CDiff were 100%, 98.7%, 100% and 96.6%, respectively.
**Conclusions**: The FluoroType® CDiff PCR assay evaluated showed rapid, sensitive and specific results for the detection of *C. difficile* and toxin B directly from stool specimens.

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**060/GIP**

**Influence of multiple strain infections on eukaryotic cells**

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*Helicobacter pylori* is a gram-negative bacterium colonizing the stomach of approximately 50% of the world's human population. *H. pylori* infections strongly correlate with chronic gastritis, duodenal ulcers and gastric carcinoma. In contrast to this high prevalence, however, only about 30% of the population develop these gastric pathologies.
Depending on the presence and functionality of the cytotoxin-associated gene A (CagA), *H. pylori* strains can be classified into the more pathogenic type I and less pathogenic type II strains. Type I strains are able to translocate CagA via the type IV secretion system into their host cells. Intracellular CagA toxin gets phosphorylated and induces changes in cell morphology as well as IL-8 secretion of the infected eukaryotic cell.
We performed infection assays with gastric adenocarcinoma cell lines using co-infections with more than one bacterial strain (type I and type II). Amounts of intracellular phosphorylated CagA were determined using western blot analysis. IL-8 secretion levels were quantified using enzyme-linked immunosorbent assays (ELISA). Compared to single strain infections these experiments showed a reduced induction of IL-8 as well as phosphorylation of CagA by the second infecting strain, indicating that CagA translocation into eukaryotic cells can be inhibited by multiple strain infection. The question, which aspects influence this mechanism, is subject of this research.
Our preliminary studies testing different *H. pylori* mutants in infection assays showed that the amount of CagA phosphorylation

was influenced by certain outer membrane proteins (OMP). The strongest effect was observable upon deletion of the OMPs HopQ or HopI. Therefore, infection experiments with different *H. pylori* wild-type strains either expressing *hopQ* type I or type II gene or lacking the *hopI* gene were performed and blocking efficiency was analyzed. We could show that different *H. pylori* wild-type strains exhibit varying blocking efficiencies. The variation seems to depend on the type of *hopQ* gene. However, this does not apply to the *hopI* deletion mutants. Here, depending on the genomic background CagA phosphorylation amounts varied but could still be reduced. These results present a first hint at the interplay between eukaryotic cells and *H. pylori* upon multiple strain infections. We could show that the ability of different bacterial strains to inhibit each other's CagA translocation into host cells seems to be genetically encoded, possibly related to OMPs. If the limited availability of cellular binding sites to these OMPs is influencing the process, this has to be investigated further.

**Reference**

Luisa F. Jiménez-Soto *et. al*, Dynamics of the Cag-type IV secretion system of *Helicobacter pylori* as studied by bacterial co-infections, Cellular microbiology (2013), doi:10.1111/cmi.12166

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**061/GIP**

**Analysis of the role of the conserved protein HP1473/HPP12_1451 of *Helicobacter pylori* in natural transformation, electroporation and conjugation**

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**Introduction**: The human gastric pathogen *Helicobacter pylori* is one of the most genetically diverse bacterial species known. The basis for this variation is its competence for natural transformation and its high recombination activity. In the process of natural transformation, a type IV secretion system, called ComB system, as well as an inner membrane pore, ComE3, and further proteins, such as ComH and DprA, are involved. However, the exact mechanism for natural transformation is currently not completely understood. In this study, we have investigated a gene encoding a protein with similarity to ComFC of *Bacillus subtilis* and CtsW of *Campylobacter jejuni* (HP1473/HPP12_1451) which is present in all strains of *H. pylori* and has been reported before to be essential for transformation (Chang et al., 2001).
**Methods**: Using a marker-free counterselectable gene deletion system (*rpsL* counterselection method), the gene *hpp12_1451* was deleted in *H. pylori* strain P12. Furthermore, a complemented mutant was constructed. The deletion mutant and the complemented mutant were tested with respect to natural transformation, electroporation and conjugation.
**Results**: The protein HPP12_1451 was found to be essential for natural transformation with plasmids integrating into the bacterial chromosome, as well as with self-replicating plasmids. The complemented mutant, constructed after previous insertion of *hpp12_1451* in a distant chromosomal locus (*moeB*), or as a shuttle plasmid, was transformable again. Furthermore, HPP12_1451 is needed for other mechanisms of DNA uptake, such as electroporation and conjugation. This indicates that HPP12_1451 is involved in a final combined pathway of DNA uptake. Additionally, we were able to show that the N-terminal region of HPP12_1451 alone is sufficient to restore natural transformation competence.
**Conclusion/Outlook**: Our data show that HPP12_1451 is not only essential for transformation, but also for electroporation and conjugation. Further experiments are required to determine what the molecular basis for this unexpected phenotype is, and which role HPP12_1451 plays for the process of colonization.

**Reference**

Chang, K.C., Yeh, Y. C., Lin, T. L., Wang, J.T. 2001.Identification of genes associated with natural competence in *Helicobacter pylori* by transposon shuttle random mutagenesis. Biochem Biophys Res Commun. 288(4):961-8

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**062/GIP**

**Chlorin e6-assisted photodynamic inactivation of *Helicobacter pylori* lab and patient strains**

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**Purpose**: The pathogenic bacterium Helicobacter pylori (HP) infects the stomach of more than half of the global population and is considered to be the main cause for chronic gastritis and gastric ulcers. Due to the increasing emergence of antibiotic resistance to conventional triple drug therapy, Photodynamic Inactivation of bacteria (PDI) can present a new approach to treat pathogenic microorganisms and chronic bacterial stomach infections. This study evaluates the inactivation of HP lab and patient strains using the photosensitizer Chlorin e6 (Ce6).
**Methods**: In-vitro experiments were performed with different strains (CCUG 38770, ATCC 43526, ATCC 43054) and HP isolated from patient specimen with a pre-phase treatment of antibiotics. HP was suspended in 0.9 % NaCl and incubated with Ce6-concentrations in the range of 0.1 - 1000 µM. The samples were irradiated for different times using two different types of high power LEDs with wavelengths of 405 nm and 660 nm. The optical power density was varied from 9 - 31 mW/cm². The quantification was performed by counting the number of grown colonies after recultivation.
**Results**: A 6-log reduction was achieved within 30 seconds of irradiation ($\lambda$ = 660 nm, E = 0.93 J/cm²) and a Ce6-concentration of 100 µM for the lab strains. A stronger decrease of the survivals was reached using the blue light source. After two seconds, on average of 90 % of the CFU of the lab strains were destroyed with both illumination systems, whereby some patient strains were significantly more resistant, depending on their prior therapy. A total inactivation was shown for all tested strains after an irradiation of three minutes using Ce6-concentrations in the range of 100 µM. The energy dose ranged from 0.27 to 0.93 J/cm². Furthermore, photoresistance, induced by PDI, could be excluded. In ex-vivo investigations, a complete inactivation of HP was realized on a mucosal model of mucin, Ce6 and HP using higher fluence rate.
**Conclusion**: In conclusion, PDI of Helicobacter pylori using Ce6 shows an efficient and desired elimination rate. The inactivation of HP strains obtained from patients who have failed standard antibiotic treatment is also very promising. Based on the results of these auspicious in vitro an ex vivo studies, PDI of HP has directly led towards clinical application, where medical partners are following a strategy for an in vivo therapy.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

**063/GIP**

***Influence of host factors on Shiga Toxin 2 expression* by Enterohemorrhagic *Escherichia coli* (EHEC)**

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**Introduction**: Enterohemorrhagic Escherichia coli (EHEC) are intestinal pathogens that can cause hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans. These severe diseases are linked to the expression of Shiga toxins (Stx) which are encoded on prophages integrated in the bacterial genome (stx1/stx2). *Stx* expression is tightly regulated and closely linked to bacterial SOS-response and prophage induction. Not all EHEC infected patients develop hemorrhagic colitis and only some progress to an HUS. The risk factors for developing HUS remain unclear as well as the signals triggering Stx production in the human gut.

**Objectives**: Our study aims to identify risk factors for the development of HUS in infected patients. *In vitro* experiments have shown that polymorphonuclear leukocytes (PMN) and reactive oxygen species (ROS) have an influence on Stx-production (Wagner et *al.* Infection Immunity 2001). Since data on this topic is scarce, the aim of our study is to validate these findings and investigate the effect of other host derived factors on *stx2* expression EHEC *in vitro*. In the next step, in vitro findings shall be tested *in vivo* (EHEC mouse infection model).

**Materials and Methods***:* To study *stx2* expression in response to various host derived stimuli, we use a set of well characterized *stx2* transcriptional reporter strains generated in our laboratory. The reporters carry genes for Gaussia luciferase (*gluc*) and *gfp* inserted into the *stx2* locus, thereby rendering the EHEC strain BSL2. Human PMNs were isolated using a density gradient protocol. In addition we established quantification assays for ROS production by PMNs using fluorescent dyes.

**Results**: We have developed a medium throughput assay to detect luciferase activity upon stimulation and co-culture with PMNs. Using this assay, we did not observe a robust activation of *stx2* expression by PMNs, despite expression was activated by $H_2O_2$, the major ROS produced by PMNs. Quantification of $H_2O_2$ released by activated PNMs suggested that this concentration range insufficient for *stx2* induction.

**Conclusion***:* The role of PNMs in the Stx2 activation remains unclear. Therefore, we plan to study the effect of phagocytosis of the bacteria on Stx2 expression using the *gfp*-reporter strains. Further experiments will also focus on the role of other host factors such as lysozyme, complement system and antimicrobial peptides.

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# GENERAL AND HOSPITAL HYGIENE (HYP)

**064/HYP**

**Promoting hygienic hand disinfection as an ongoing task: Results of the PSYGIENE cluster-randomized controlled trial to (re-)raise compliance of physicians and nurses based on psychological tailoring in a tertiary intensive care setting**

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**Introduction:** Insufficient use of psychological theory is one reason that conclusive evidence regarding hand hygiene promotion is scarce. In addition, compliance has been shown to be lower among physicians than among nurses. The PSYGIENE-project set out to draw on theoretical advances (Health Action Process Approach-HAPA) to optimise education and feedback interventions.

**Objectives:** To test whether psychologically tailored interventions lead to higher increases of hand hygiene compliance than usual care (German Clean Care is Safer Care-campaign).

**Methods:** In PSYGIENE, a project funded by the German Federal Ministry of Health (project-ID INFEKT-019), a cluster-randomized controlled trial was conducted on intensive care and hematopoietic stem cell transplantation units of Hannover Medical School, a tertiary university hospital. Clusters were defined by classifying wards as early/late adopters by 2008-12 compliance [1]. Tailoring targeted wards and was informed by problem-focused interviews with physicians and chief nurses (response rates: 100%) and a written survey which assessed HAPA-factors (physicians: 71%; nurses: 63%). The outcome was 2014 compliance observed by WHO-standards.

**Results:** In 2013, 15 education sessions for physicians (participation rate: 46%) and 39 for nurses (50%) and 12 feedback meetings with chief nurses (100%) were conducted. Overall, from 2013-14 compliance increased from 48 to 63% (physicians) and 56 to 67% (nurses). Increases on the 6 tailored wards was not greater than given usual care (10 vs. 13%, p=.126). This held both for physicians, among whom tailoring even led to a significantly lower increase than usual care among late adopter-wards (7 vs. 23%, p=.046), and nurses (10 vs. 11%, p=.590).

**Conclusion:** Compliance increased both in the tailored and the usual care-group. While explanations of this result (e.g. study design issues or insufficiency of psychological theories of population behaviour to explain organisational behaviour) remain speculative, the overall increase in compliance does stress behavioural strategies to promote hand hygiene compliance as a habit in need of self-monitoring [2] is an ongoing task in which to continuously (re-)invest.

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[2] von Lengerke T, Lutze B, Graf K, Krauth C, Lange K, Schwadtke L, Stahmeyer J, Chaberny IF. Psychosocial determinants of self-reported hand hygiene behaviour: a survey comparing physicians and nurses in intensive care units. J Hosp Infect in press

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**065/HYP**

**To screen or not to screen? MRSA screening at hospital admission in a low prevalence setting**

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**Introduction**: The current national guideline on MRSA in Germany (KRINKO 2014) recommends screening of patients with an increased risk for MRSA colonization at hospital admission (e.g. patients with known history of MRSA, patients hospitalized >3d within 12 months, etc.). Different regional studies in Germany showed that the expected screening rate for this targeted screening should be as high as 40-60% of all hospital admitted patients. In Baden-Wuerttemberg, reporting of MRSA screening rates and other data on MRSA to an external quality control institution is mandatory for all hospitals.
**Objective**: In 2014 surveillance data of our tertiary care hospital showed a very low MRSA incidence and a low incidence density of nosocomial MRSA cases, both compared to the national reference data (MRSA-KISS). The screening-rate at admission, however, was very low (3% and 5.4 %; 1st vs. 2nd half-year of 2014) compared to the external quality control benchmark data (mean 18.5% and 18.8%). This led us to the following questions: What would be an `adequate proportion´ of screened patients in our institution? And what is the benefit of an extended MRSA screening program in a situation when MRSA seems sufficiently controlled? To gain further data on our situation we conducted an admission prevalence study on MRSA.
**Methods**: In a 12-day period in January 2015 all patients admitted to our hospital (excluding newborns) were screened for MRSA. Patients were asked for written informed consent. Data on patients´risk factors were collected using a questionnaire based on the national guideline and a nose-swab was conducted. Nose-swabs were analyzed by a culture based identification method with chromogenic agar.
**Results**: 1,648 patients were admitted to the hospital in the study period. 1,230 patients could be included in the study (consent and screening result for MRSA available [75%]). 8 out of 1.230 patients were tested MRSA positive resulting in an overall admission prevalence of 0.65% (0-2.6% in individual departments). 1,228 questionnaires (74.5%) were returned. Analysis is pending.
**Conclusion**: This study found a low prevalence of MRSA at hospital admission in our institution concordant to our surveillance data. To define the adequate proportion of screened patients and establish a more targeted screening strategy based on 8 MRSA positive patients will presumably not be reliable enough. Furthermore, in a setting of quasi mandatory MRSA-admission screening this may not result in an acceptable screening-rate. But, increasing the MRSA screening rate at admission at least to the mean of the external quality institution benchmark will add workload and costs but its impact on the number of nosocomial infections or on nosocomial transmission events is questionable in a low prevalence setting. In a situation of limited resources shouldn´t these be invested in measures with proven benefit in terms of infection prevention?

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**066/HYP**

**Retrospective observational study to assess the improvement of microbiological correlates of gastrointestinal endoscope reprocessing over time within the quality assurance programme of the Bavarian Association of Statutory Health Insurance Physicians**

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**Introduction**: The hygiene laboratory at the Institute for Hygiene and Microbiology of the University of Wuerzburg participates in the quality assurance programme for reprocessing of gastrointestinal endoscopes of the Bavarian Association of Statutory Health Insurance Physicians.
**Aims**: We analysed whether microbiological correlates of reprocessing quality improved over time by comparison of two periods, i.e. 2002/2003 versus 2007/2008.
Materials and Methods: Microbiological analyses were conducted according to the standards provided by the Association (https://www.kvb.de/praxis/qualitaet/qualitaetssicherung/hygiene-in-der-endoskopie/). A database was developed in MS Access and microbiological records were validated retrospectively.
**Results**: A total of 336 tests including 36 follow-up investigations from 77 medical practices were analysed. 45 practices participated in both periods. The rate of objections declined from 75 % in 2002 to 5,7 % in 2008. The most frequently found genera were Pseudomonas, Staphylococci, Stenotrophomonas and Bacilli. With regards to the source of bacteria, genera from moist environments dominated by far in 2002, but lost their importance thereafter. From the routine questionnaire it became clear that the improved quality was associated to an increased number of coloscopes and coloscopies per practice. Logistic regression revealed only the use of non sterile water for the optic rinse system and the final washing of disinfected endoscopes as a risk factor. Interestingly, the reprocessing strategy (manual, automated) and the use of brushes did not have an impact on the microbiological quality.
**Summary and Conclusions**: The microbiological quality as a surrogate parameter for endoscope reprocessing quality has been markedly improved since the beginning of the campaign. The improvement of water handling by practices eliminated contamination with Pseudomonads. It is surprising that automated procedures and the use of brushes for canals had no impact. Their importance might be overestimated. However, it is more likely that sporadic microbiological testing has insufficient sensitivity to reveal risks associated with manual reprocessing and avoiding brushing.

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**067/HYP**

**Interaction mechanisms of technical plasmas with biological samples**

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**Introduction**: Plasma is the 4th state of matter and describes an ionized gas. Plasmas generated by dielectric barrier discharges (DBDs) are promising tools for biomedical applications [1] and several clinical studies regarding their efficiency for the treatment of skin infections are currently underway [2]. DBDs can be used to ignite cold plasma from ambient air, obviating the need for additional gas bottles. Furthermore, the resulting plasma emits only low-intensity UV radiation, which is preferable for the treatment of patients. DBDs generate large amounts of reactive oxygen and nitrogen species due the use of ambient air as the feed gas from

which the plasma is generated [3]. While clinical studies using DBD sources are underway, characterization of interactions between discharges and their biological targets are still lagging behind.

**Aims**: Employing different biological model substrates, such as RNase A as a model for highly stable proteins, the influence of technical plasmas on cellular components can be investigated. Observed chemical modifications give insights into the capability of plasma to affect biological targets, for example skin or bacteria, and enable a more in-depth risk assessment of medical plasma applications.

**Methods**: Enzyme activity was monitored after plasma treatment and the loss of activity correlated to the loss of protein structure monitored by Circular Dichroism (CD) spectroscopy. Chemical modifications induced by plasma treatment were observed by Raman and FTIR spectroscopy as well as mass spectrometry.

**Results**: RNase A activity was significantly reduced after plasma treatment especially when treatment occurred in a liquid environment. CD spectroscopy revealed protein denaturation. Raman spectroscopy and mass spectrometry revealed oxidation of thiol-containing amino acids in a time-dependent fashion as a main target for DBD treatment and similar results were reported for free cysteines [4]. Interestingly, methionines were the prime target for plasma-generated radicals, followed by cysteines. Here, disulfide bonds were broken by over-oxidation of cysteine residues to sulfonic acid. Additional experiments with other models indicated that oxidation of sulfur-containing amino acid residues presents a general mechanism of action.

**Conclusions**: Rapid and permanent inactivation of metabolic enzymes such as GapDH by thiol oxidation seems to contribute significantly to bacterial inactivation [5]. Efficient inactivation of RNase A indicates that DBD might also be used to efficiently handle other difficult-to-inactivate proteins such as prions.

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**068/HYP**

**Plasma sterilization as an innovative tool to inactivate *Bacillus subtilis* endospores for improved surface decontamination**

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**Question**: The process of sterilization is absolutely essential in medical settings and health care in order to assure hospital hygiene and facilitate safe surgical procedures, thereby preventing nosocomial infections and spreading of multi-resistant bacteria. Being the most resilient form of a biological system, bacterial endospores are extremely resistant against a broad spectrum of sterilization methods and therefore are commonly used as a bio-indicator in order to verify functionality of a decontamination process [1].

Many conventional sterilization methods suffer from disadvantages by either introducing damage to sensitive material, alteration of surface properties, or insufficient removal of potentially pathogenic biological material. The process of low-pressure plasma sterilization is a promising alternative to conventional sterilization methods as it is fast, efficient and gentle to heat-sensitive material, such as innovative medical plastics, due to low-temperatures operations (30-80°C) [2, 3]. In addition to the applied electrical field, plasma discharges contain a high degree of UV/VUV-radiation, as well as charged particles and free radicals, which exert detrimental effects on microorganisms by damaging genetic material, outer cell layers and proteins [2].

**Methods**: The double inductively coupled plasma reactor (DICP) is an innovative low-pressure plasma system, which allows homogeneous sterilization of large surface areas by effectively inactivating and removing bacterial spores within minutes [4].In this study we present novel insights into the key factors involved in spore inactivation by low pressure plasma sterilization using a DICP reactor.

**Results and Conclusion**: Particular proteins of the multi-layered spore coat -the first barrier to environmental influences - were revealed to be major factors contributing to spore resistance towards plasma treatment. With a systematic analysis of *Bacillus subtilis* spores lacking individual coat and crust layers we observed significant morphological differences in coat structures by AFM-analysis, affecting spore survival and resistant properties after treatment with low-pressure plasma and isolated plasma components. We identified spore-specific and general DNA repair pathways during spore germination, leading to a better understanding of the complex molecular mechanisms involved in the plasma sterilization process.

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**069/HYP**

**Hospital epidemiology of vancomycin-resistant *Enterococcus faecium* in an intensive care unit over a one year period**

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The last years witnessed an enormous spread of vancomycin-resistant *E. faecium* (VRE*fm*) strains worldwide. In our institution VRE*fm* is now the predominant nosocomial gram-positive multi-resistant pathogen.

To assess the epidemiological situation in more detail we have begun a project to analyze the distribution of VRE*fm* in an intensive care unit over a one year period (2014). We screened new patients on admission according to our MRSA schedule and each new VRE*fm* strain was kept for further analysis to determine the molecular relatedness. Because any new VRE*fm* strain will be kept at the Inst. for Clinical Microbiology and Hygiene strains from patients that were admitted with a known VRE history were also available for molecular characterization.

The focus on this ongoing study is to draw a complete picture of the epidemiological distribution of all VRE*fm* strains within intensive care unit over a one year study period.

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**070/HYP**

**Methicillin-resistant Staphylococcus aureus (MRSA) screening from patients in a Hospital in Saxony-Germany**

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MRSA is especially troublesome in hospitals and nursing homes, where patients with open wounds, invasive devices and weakened immune systems are at greater risk of nosocomial infections (NI). Patient screening upon hospital admission prevents the cohabitation of MRSA carriers with non-carriers and exposure to infected surfaces.

**Objective**: Determine the percentage of MRSA in hospitalized patients.

**Material and Methods**: We conducted a retrospective study from January to December 2014 among patients admitted at Oberlausitz-Kliniken gGmbH (Saxony, Germany) with specific comorbidity risk factors such as patients with a known history of MRSA, patients transfers from other health centers, patients with chronic skin lesions, patients from foreign hospitals and dialysis patients. The following data were collected: age, sex, inpatient unit and comorbidity risk factors. The health workers used moistened swabs (COPAN Transystems) to collect material from patients anterior nares, throat, inguinal und others. The swabs were inoculated directly onto BBL chromagar MRSA II (BD), Agar CNA (BD) and BH Infusion (Oxoid). All isolates of MRSA were identified on the basis of colony characteristics, identification and antibiogram by MicroScan WalkAway®System. If no growth was observed on the plate or in the broth after 48 hours, it was considered negative.

**Results**: A total of 845 patients at high risk of MRSA colonization were studied, 69 patients were MRSA positive. The mean age of patients was 71,69 years. MRSA patients were found in the following units: 66,7% Internal Medicine, 30,5% Surgery, 1,4% Pediatrics and 1,4% Intensive Care Unit. 39,2% were patients with chronic skin lesions, 36,2% were patients from other centers, 15.7% were patients with dialysis treatment and 8,7% were patients with a history of MRSA known.Four out of 69 MRSA positive patients were nosocomial MRSA cases (nosocomial in this setting means collecting samples later than day 3 of hospital stay). The mean age of patients was 73,5 years. These patients were found 50% in Internal Medicine Unit and 50% in Surgery Unit.

**Conclusions**: The percentage rate of MRSA positive patients in patients with a high risk was 7,7% and the percentage rate of nosocomial MRSA cases in MRSA positive patients was 5,8%. This also means that 94,2% of MRSA positive patients were positive upon admission. Therefore, the control of NI is a responsibility of a multidisciplinary team of Medical Units, Medical Laboratory, Microbiologists, Hygiene and Infection control. So, an active surveillance culture should be considered in patients at high risk for MRSA colonization in patients admitted in the hospital.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**071/HYP**

**Cases of *Clostridium difficile* diarrhea in a Hospital in Saxony-Germany**

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*Clostridium difficile* infection *(CDI)* is the primary cause of antibiotic-associated diarrhea and is a significant nosocomial disease.

**Objective**: Determine the percentage of cases of *Clostridium difficile* infection in hospitalized patients.

**Material and Methods**: We conducted retrospective study from January 2013 to December 2014 among patients admitted at Oberlausitz-Kliniken gGmbH (Saxony, Germany) with diarrhea. The following data were collected: age, sex and inpatient. The health workers used faeces container (Sarstedt) to collect stool material from patients. The diarrhea was defined as having three or more loose, watery stool passages during a 24-h period. The stool sample was diluted with sample dilution buffer (1:11) of Clostridium difficile Toxin A/B qualitative ELISA Test (RIDASCREEN®) and the test was performed according to manufacturer's instructions. The samples were considered positive if their extinctions were more than 10 % above the calculated cut-off (extinction for the negative control + 0.15). The samples were considered as equivocal and they were repeated, if their extinctions were within the rage 10% above to 10% below the cut-off and the samples were considered negative if their extinctions were more than 10 % below the calculated cut-off.

**Results**: From 2013 to 2014, a total of 2309 stool samples were analyzed from 1429 (702 patients in 2013; 727 patients in 2014) patients with diarrhea. 39 (5,5%) patients were positive for CD Toxin A/B in 2013, 2014 were positive 72 (9,9%) patients. Of the positive CDI cases were 46,84% women and 53,15% men and the mean age of patients was 72,26 years. Patients with CD Toxin A/B were found in the following units: 58,5% Internal Medicine, 24,3% Surgery, 15,3% Intensive Care Unit, 0,9% Pediatrics and 0,9% Gynecology/Obstetrics Unit. 111 out of 63 (56,7%; 58,97 2013 and 55,55% 2014) CD positive patients were nosocomial CDI cases (nosocomial in this setting means collecting samples later than day 3 of hospital stay). These patients were found 20,64% in Intensive Care Unit, 30,15% in Surgery Unit and 49,20% in Internal Medicine.

**Conclusions**: The percentage rate of CDI in patients with diarrhea in 2013 to 2014 was 7,7% and the total percentage rate of nosocomial CDI cases were 56,7%. CDI remains a significant nosocomial problem and the disease poses a serious threat to human, especially those with underlying morbidities. Thus, the correct treatment, and more important, the preventive measures are urgently required to combat this pathogen.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**072/HYP**

**Specific detection of *Legionella pneumophila* in water samples with qPCR, compared to conventional culture method**

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**Introduction:** Since the outbreaks in Ulm, 2010, and Warstein, 2013, *Legionella pneumophila* infections caused by cooling waters are a matter of great public health interest in Germany, too. The water-borne Gram-negative bacterium *Legionella pneumophila* is the causative agent of the Legionaires' disease, an atypical kind of pneumonia. The organism appears ubiquitous in natural and man-made aquatic environments. In Europe *Legionella* infections become a considerable public health problem. Contaminated drinking water and cooling water are often the source of *Legionella* infections. In Warstein first time waste water was identified as the source transmission and possibly infection in Germany. In case of an outbreak it is very important to detect the source of infection as fast as possible. The conventional culture plate method to detect *L. pneumophila* involves several disadvantages. It needs seven up to ten days for a certain result. While the long incubation period overgrowth of the present micro-flora can happen. Moreover "viable but non culturable" (VBNC) cells are not detected. Using quantitative real-time PCR (qPCR) would offer results within one single day and compensate the disadvantages of the culture method.

**Objectives:** In this master thesis qPCR and culture method had been compared for drinking, cooling and waste water. For every kind of water the detection limit for both methods should be determined by performing spiking experiments. Afterwards more than 50 samples of unknown concentration were tested by qPCR and culture method to give a statement about routinely handling and sensitivity of qPCR and the suitability of qPCR for cooling and waste water. Furthermore, a public health risk value in the qPCR unit (GU/L) should be defined for the different types of water.

**Methods**: Both methods were conducted according to ISO/TS 12869:2012 (qPCR) and DIN EN ISO 11731:2008-2 (culture method). The commercial, AFNOR standardized kits of Bio-Rad were used for DNA-Extraction (Aquadien™ kit) and qPCR (iQ-Check™ *L. pneumophila* Quanti kit, Bio-Rad; primers attaching the *mip* gene).
**Results**: The detection limit with qPCR is lower than 1.000 cells per litre for every kind of water. With culture method it was the tenfold value. 77 % of all samples were positive by qPCR, 45 % by culture method. Especially in waste water the yield of positive samples was higher with qPCR. The qPCR is suitable for the routinely handling. The public health risk value of *L. pneumophila* concentration in water samples is proposed at 5.000 GU/L for tap water and 25.000 GU/L for cooling and waste water.

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## NOSOCOMIAL INFECTIONS: OUTBREAKS AND SURVEILLANCE (HYP)

**073/HYP**
**Monitoring a *Clostridium difficile* outbreak in a hospital by comparison of four different methods**
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**Introduction:** *Clostridium difficile* infection is an urgent public threat and outbreaks increased markedly in hospitals in recent years. Ribotyping of strains has been the most widely used molecular tool to distinguish an outbreak from a coincidental accumulation. As ribotyping is time-consuming we investigated alternative methods for outbreak analysis.
**Goals**: We compared four different methods for typing of clostridium difficile strains to find out, which methods could be useful for the monitoring of hospital outbreaks.
**Material and Methods:** 14 *Clostridium difficile* bacterial isolates from different patients were collected in a cardiac surgery clinic within one month. The number of CDI in the hospital was much higher than the average of the previous month suggesting a *C. difficile* outbreak. Capillary gel electrophoresis-based PCR ribotyping was performed and resulting peak patterns were assigned to PCR ribotypes using the Webribo database. The results were compared to a subtyping dendrogram generated by MALDI-TOF mass spectrometry using Biotyper software. Random amplified polymorphic DNA (RAPD)-PCR was also performed. As RAPD-PCR is often used for subtyping Gram-negative bacteria, while the more recent method of Fourier transformation-Infrared Spectroscopy (FT-IR) was published for different Gram-positive isolates (e.g. *Corynebacterium ulcerans*), we added this to the investigative panel.
**Results:** The 14 isolates belonged to 8 different ribotypes. With RAPD-PCR 9 diverse gel patterns could be observed. The MALDI MS based approach showed a similar diversity in the relatedness dendrogram.
With FT-IR preliminary results allowed subtyping of 5 different groups. While RAPD-PCR and MALDI allowed no direct correlation with known ribotypes, the FT-IR spectra could be compared to database spectra to detect correlations to known ribotypes.
**Conclusions:** The subsumed evaluation of the four methods clearly showed that CDI threat in the hospital was more likely a coincidental accumulation than a confirmed outbreak. Even if the data for the alternative methods RAPD-PCR, mass spectrometry and FT-IR might be preliminary, it showed promising strength in differentiating the strains on a molecular level.

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**074/HYP**
**Resistance profiling and ribotyping of C. difficile isolates in Germany: Results of the PEG study 2013/2014**
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**Introduction**: *C. difficile* is the most abundant pathogen causing infections in hospitals. Due to the clinical importance, the appearance of hypervirulent strains and increased antibiotic resistance to macrolides and fluorquinolones *C. difficile* was focused for the first time as a part of the current Paul Ehrlich Gesellschaft (PEG) study.
**Methods**: A total number of 502 isolates generated from symptomatic patients were included. Strains were provided by 24 cooperating laboratories for hospitalized patients and by 23 laboratories for outpatients. Genotypic characterization using PCR ribotyping and antibiotic sensitivity testing using agar dilution testing were applied for all isolates.
**Results**: Hypervirulent ribotype 027 isolates were identified in most German regions except for the North (postal code 2). Highest abundance was detected for postal code region 4 and 5. Interestingly, also other ribotypes with hypervirulent genetic profiles were found in addition to 027. 027 subtyping using MLVA revealed polyclonality of 027 isolates in Germany and also in the same regions. Antibiotic resistance to clindamycin, levofloxacin, moxifloxacin and rifaximin was associated with ribotypes of high abundance in hospitals. However, all isolates were still susceptible to antibiotics used for *C. difficile* therapy as metronidazole, vancomycin and also fidaxomycin.
**Conclusion**: *C. difficile* epidemiology is a dynamic process characterized by epidemic spreading of ribotype 027 in Germany during recent years. Actually, antibiotic sensitivity testing is not obligate for *C. difficile* routine testing because all isolates are susceptible to the recommended drugs. However, German-wide surveillance is required to focus spreading of epidemic strains and the development of antibiotic resistance in the coming years.

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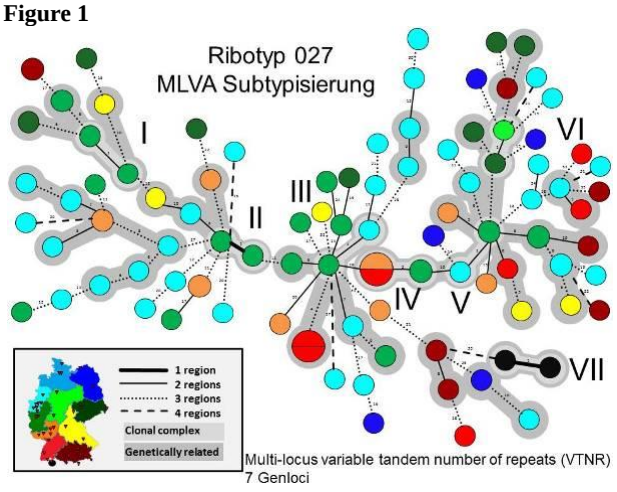
**Figure 1**



**075/HYP**

**ICU MDR bacteria screening for 2 months at a 600-bed tertiary care hospital**

**Intention, approach, results and conclusions**

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**Intention:** During 2014 we found a strong increase in patients colonized with vancomycin-resistant enterococci (VRE) in the two ICUs of the hospital (89 VRE colonized patients). The number of beds of these ICUs is in total 41; ICU1 = 20 beds/ICU2 21 beds. The two ICUs care to >3000 patients per year.
The screening regime performed 2014 and before included an universal screening for methicillin-resistant *Staphylococcus aureus* (MRSA) on admission to the ICU, and weekly MRSA controls during the ICU stay. If other multiple drug resistant (MDR) bacteria (e.g. VRE or multiple drug resistant gram negative rods (MRGN)) were found on bacteriologic testing, contact patients were examined. In cases where nosocomial transmission could not be ruled out, screening of the involved ICU was initiated.
**Approach**: Due to the observed increase in VRE colonization we discussed adjustments to the existing screening programs. For this purpose a two-month period was set (January to March 2015) to distinguish imported from nosocomial cases and to compare different screening approaches. The number of patients included was 431.
The chosen procedure for the study screening regime included
- Screening on admission to the ICU (MRSA, MRGN, VRE)
- Weekly control screening
- Screening on ICU dismissal

**Results**: The prevalence of MDR bacteria on ICU admission was 1) 2.1% for MRSA (9 patients), 2) 3.7% for 3MRGN (= MRGN with resistance to penicillins, cephalosporins and fluoroquinolones) (16 patients; 2.6% 3MRGN-*E. coli* (11 patients) and 1.1% 3MRGN-*Klebsiella spp.* (5 patients), 3) 10.0% VRE (43 patients).
The number of probable nosocomial transmissions during the study period was
1) 2× MRSA (+22%),
2) 2× MRGN-*E. coli* (+18%),
3) 17× VRE (+40%).
No infection occurred with the bacteria monitored during the study period. For ICUs, vancomycin-resistant enterococci were the MDR pathogens detected most often on newly admitted patients, and the MDR pathogens with the highest rate of nosocomial transmission.
**Conclusions**: - MRSA, MRGN and VRE have only little infectious potential in ICUs with a controlled hygiene regime.
- The rate of MDR bacteria colonization in ICUs is high, in spite of established hygiene structures.
- A ward adapted screening regime is necessary to discriminate the imported and nosocomial cases and should play an important role in evaluation of microbiological data and surveillance of nosocomial infections.

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**076/HYP**

**MRSA burden in the region - the Saarland Nursing Home Prevalence Study**

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**Introduction**: Colonization rates with methicillin-resistant *Staphylococcus aureus* (MRSA) in the elderly and long-term care population are increased, with the consequence of enhanced MRSA-associated morbidity and mortality. The German government fosters regional networks combatting the spread of antimicrobial resistance, and the State-funded Saarland network (MRSA$^{ar/netz}$) analyzes MRSA prevalence rates throughout acute and long-term care institutions for prevalence-adapted intervention strategies. In this line, we here report our results on a State-wide MRSA screening in elderly and nursing home facilities.
**Method**: Culture-based screening was performed with nasopharyngeal swabs, using selective media and molecular analysis. The evaluation was accompanied by an ascertainment of risk factors using a standardized questionnaire.
**Results**: Of the 136 registered nursing homes in the State of Saarland, 65 (47%) participated in this study. Of 2878 residents screened, 138 residents were found to be colonized with MRSA (prevalence, 4.8%). No Panton-Valentine leucocidin (PVL) positive MRSA was detected. Most of the isolates were attributable to spa sequence type t003 (Rhine-Hesse clone) or to the 'Saarland clone' t504. Risk factor analysis yielded ulcer / deep soft tissue infection, urinary tract catheter, and multiple MRSA eradications as multivariate risk factors.
**Discussion**: This is one of the largest MRSA nursing home prevalence studies ever performed. It shows that in our state, colonization with MRSA is common in this setting. These findings can be related to the recently published results on the MRSA prevalence rates in Saarland acute care hospitals (Herrmann et al., PLoS ONE, 2013), and to ongoing and planned studies in Saarland patients groups as well as in the general population. The consequent mapping of the MRSA burden throughout care institutions of an entire State allows for the development of infection control recommendations tailored to the needs of regional population.

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**077/HYP**

**The Saarland Nursing Home Prevalence Study on MDRO - Data from the MRSA Regional Network MRSA$^{ar/netz}$**

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**Introduction**: Elderly and long-term care patients are prone for colonization and subsequent infection with multidrug-resistant organisms (MDRO). The spread of antimicrobial resistance within health institutions is fostered by the German concerted antimicrobial resistance initiative, and the State-funded Saarland regional network (MRSA$^{ar/netz}$) analyzes the MDRO prevalence in its various health institutions. Here we report the respective rates of VRE and gramnegative MDRO in a recent study performed in long-term care facilities of our State.
**Method**: Culture-based screening was performed with anal swabs propagated on selective media, antimicrobial susceptibility testing, and genotyping. The MRGN definition of gramnegative MDRO according to the Commission for Hospital Hygiene and Infection Prevention (KRINKO) classification was used. The evaluation was accompanied by an ascertainment of risk factors using a standardized questionnaire.
**Results**: Of the 136 registered nursing homes in the State of Saarland, 54 (40%) participated in this study. Of 1899 residents screened, 353 residents were found to be colonized with MDRO (prevalence 18.6%). Of these, 266 were found to be positive for ESBL producing MDRO (14%), 275 were positive for MDRO according to MRGN classification grades (resistance to three or four antibiotic classes) (14%), and 22 were found to be colonized with VRE (1.2%). Multivariate risk factor analysis yielded for ESBL / MRGN revealed i.a. a shared bathroom, bed confinement, care level II, antacid use, previous ESBL, urogenital infection, infection of skin / deep soft tissue, diabetes, and male gender as independent risk factors. The VRE risk was significantly associated

with care level II, immunosuppression, infection of gastrointestinal tract, and previous hospitalization.
**Discussion**: To the best of our knowledge, this is the first point prevalence analysis so far encompassing this broad spectrum of MDRO (including VRE) in an entire State and comprising a large number of long-term care facilities. The here reported prevalence of 14/100 for gramnegative MDRO clearly illustrates the size of the problem. The risk factor analysis may provide a basis for rational recommendations for screening and patient care. In contrast, the relatively low prevalence of VRE may suggest that the implementation of specific interventions to control this notoriously difficult-to-restrict but less pathogenic organism may not be of first priority in this care population.

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**078/HYP**

**State-wide surveillance of Methicillin-resistant Staphylococcus aureus (MRSA) appearance in hospitals and spa-type analysis from blood cultures in North Rhine-Westphalia (NRW), 2011-2014**

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**Background**: MRSA is a threatening cause of nosocomial infections like bacteraemia. To tackle this problem, the achievements of EurSafety health-net in the German-Netherland border region were transferred to statewide network (MRE-Netzwerke NRW) of several participants of the healthcare sector.
**Aim**: Participating hospitals submit data, called "OEGD-Reports" to achieve a "quality and transparency seal". From that, no data about MRSA was published yet.
**Methods**: Within the framework of the "MRE-Netzwerke NRW, clinics used surveillance protocols from EurSafety health-net. They accumulated structure data and MRSA surveillance data, i.e. the number of nosocomial MRSA cases and bloodstream infections. Every participating hospital had to submit data for at least two years in succession. Percentages were calculated only if complete data were available. We analysed the data descriptively using Microsoft Excel ®. Between 2011 and 2014, 46 hospitals submitted data for at least two years.
**Results:** A choice of MRSA parameters is shown in table 1.
Between 2011-2014 the screening rate is increasing whereas the percentage of nosocomial MRSA cases is decreasing. Less than 12% of all MRSA cases were spa-typed. Thus limited information on distribution of spa-types is received. Blood culture's numbers are above the German average but decreasing since 2012.
**Summary**: This is the first attempt to claim and analyse MRSA surveillance data modeled on EurSafety Health-net. Although evidence based recommendations for MRSA-prevention is known since many years, number of MRSA cases still is high - indicating that strengthen of MRSA prevention is necessary . Incompleteness of submitted data reveals problems of clinics to specify basic MRSA surveillance data.
Data show that it's possible to collect state-wide surveillance data using the EurSafety health-net protocols. However, clinics have to improve their OEGD-reports.
If good data quality is reached, results of interventions could be seen in the network MRSA data. Good quality data is achievable. In MRE-Netzwerke NRW both is required: collecting and evaluating of statistic relevant data as well as intervention such as training in hand hygiene and other prevention measures.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

| Parameter | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|
| % nasal swabs/total of patients | 22.1 | 31.9 | 38,2 | 49,2 |
| % MRSA/total of patients) | 0.9 | 1.2 | 1,3 | 1,1 |
| % nosocomial MRSA cases/ MRSA cases | 18.4 | 13.1 | 12 | 8.6 |
| % blood cultures MRSA/ blood cultures s. aureus | 15.5 | 23.5 | 18.8 | 11.9 |
| % blood cultures s. aureus / total patients | 0,2 | 0,2 | 0,2 | 0,3 |
| % typed MRSA of all MRSA | 10,4 | 3.5 | 11.6 | 5.2 |

# INFECTION IMMUNOLOGY (IIP)

**079/IIP**

**Impact of antimicrobial effector mechanisms of in vitro generated murine neutrophils on the growth of *Anaplasma phagocytophilum***

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*Anaplasma phagocytophilum* is an obligate intracellular bacterium that replicates in neutrophil granulocytes. It is still a matter of debate to what extent neutrophils contribute to the antimicrobial control of *A. phagocytophilum*. To study this, we used in vitro generated murine neutrophils that were derived from immortalized progenitors. Wild-type neutrophils and neutrophils defective for their main antimicrobial effector mechanisms such as NADPH-oxidase (phox), inducible nitric oxide synthase (iNOS) and myeloperoxidase (MPO) were infected with *A. phagocytophilum*. However, the bacterial growth in the gene-deficient neutrophils was comparable to that in wild-type cells. This indicates that the antimicrobial effector mechanisms of neutrophils do not contribute to the control of *A. phagocytophilum* in vitro as it was observed in vivo. Whereas $gp91^{phox}$ and MPO expression remained unchanged, the infection led to an induction of iNOS. As interferon-γ (IFN-γ) is an important modulator of neutrophil function and IFN-γ was shown to contribute to the early control of *A. phagocytophilum* in vivo, infected granulocytes were stimulated with this cytokine. In this situation, the bacterial growth was significantly impaired. This indicates that there is a direct antibacterial effect of IFN-γ on *A. phagocytophilum* replicating in neutrophils. IFN-γ stimulation led as did the infection to an iNOS induction. To test whether the INF-γ effect was iNOS-dependent, iNOS-deficient neutrophils were infected with *A. phagocytophilum* and stimulated with IFN-γ. However, IFN-γ impaired the bacterial growth similarly in wild-type and iNOS-deficient neutrophils. Therefore, the direct anti-bacterial effect of INF-γ seems to be iNOS-independent.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**080/IIP**

**The alarmin S100A9 as a promising target for early non-invasive monitoring of acute lung injury**

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**Question**: The activation and local invasion of phagocytes are early events in antimicrobial responses of the human immune system. Activated myeloid cells release the proinflammatory alarmin S100A8/S100A9 at local sites of inflammation. Due to its favorable kinetics S100A8/S100A9 (calprotectin) is a very early local as well as systemic biomarker in a broad spectrum of infectious and inflammatory diseases.
The purpose of our study was to establish a S100A9 binding tracer for *in vivo* monitoring of the host immune response in inflammatory and infectious diseases.

**Methods**: We developed a S100A9 specific tracer coupled to the fluorescence dye Cy5.5 (CES271-Cy5.5) and tested the *in vivo* specificity of our new tracer in a model of irritant dermatitis. Intravenous injection of CES271-Cy5.5 was performed 24h after elicitation of inflammation. Specificity was assessed by performing blocking studies and using S100A9$^{-/-}$ mice.
We used LPS-induced lung injury as model of acute lung injury (ALI). Lung injury was elicited in Balb/c mice via intranasal application of either 10 or 50µg LPS. Optical imaging (OI) was performed at several time points after parallel LPS and CES271-Cy5.5 application. Systemic and local (bronchoalveolar lavage fluid) S100A8/S100A9 levels were analyzed and S100A9 immunohistochemistry of lung sections was performed.
**Results:** *In vivo* OI showed a significantly increased uptake of CES271-Cy5.5 in the inflamed ear as compared to the healthy ear, which was diminished in blocking studies as well as in S100A9-deficient mice.
In the lung, LPS dependent CES271-Cy5.5 accumulation was accompanied by a simultaneous increase of S100A8/S100A9 level ($p<0.001$). Our control tracer for possible early perfusion changes in LPS treated mice did not reveal any significant accumulation.
**Conclusions**: CES271-Cy5.5 is a non-peptidic S100A9 specific tracer that enables early diagnosis of ALI in mice.

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**081/IIP**

**Length and folding state governs the capacity of staphylococcal peptide toxins to attract leukocytes via formyl peptide receptors**

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Most staphylococci produce short α-type PSMs and twice as long β-type PSMs that are potent leukocyte attractants and toxins. PSMs are usually secreted with N-terminal formyl groups but are only weak agonists for the leukocyte formyl-peptide receptor (FPR) 1. Instead, the FPR1-related FPR2 senses PSMs efficiently and is crucial for leukocyte recruitment in infection. Which structural features distinguish FPR1 from FPR2 ligands has remained elusive. We hypothesized that short, formylated, unstructured peptides activate preferentially FPR1 whereas α-helical peptide structures favour activation of FPR2. To test this thesis we generated truncated versions of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylocooccus lugdunensis* β-type PSMs and analyzed if the proinflammatory and cytolytic properties depend on full peptide length or can be attributed to subdomains. Most of the N- terminal β-type PSM fragments retained their capacities to activate FPR2 indicating that these peptide parts can be functional without the C-terminal halves. Of note, the N-terminal parts exhibited less stable α-helical structures compared to the full-length peptides, which corresponded to diminished capacities to activate FPR2.
Instead, the N-terminal parts became potent FPR1 agonists thereby indicating that besides N-terminal formylation the peptides' folding state may be crucial for agonist recognition by FPR2 or FPR1. Our data should help to unravel the ligand specificities of two critical human pattern recognition receptors and they may be important for new antiinfective and antiinflammatory strategies.

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**082/IIP**

***Streptococcus pyogenes* triggers activation of the human contact system by streptokinase**

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**Introduction**: The human plasma contact system comprises the serine proteases coagulation factor XII (FXII) and XI (FXI), and plasma kallikrein (PK), as well as the non-enzymatic co-factor high molecular weight kininogen (HK). The cascade is initiated upon contact to unphysiological surfaces, e. g. bacterial surface structures, and regulates procoagulant and proinflammatory processes. Systemically activated it contributes to life-threatening complications during serious invasive infections (1). The exclusive human pathogen *Streptococcus pyogenes* is a causing agent of such conditions. One of its prominent virulence factors is the plasminogen activator streptokinase (SK), which is thought to play a key role in the progress from local to systemic infections (2). Providing uncontrolled activity of the broad-spectrum serine protease plasmin on the bacterial surface, SK enables the pathogen to overcome tissue barriers, fibrin clots, and degrades several immune peptides as well (3).
**Objectives/Materials & Methods**: We investigated the influence of streptokinase and plasminogen on contact system activation by *S. pyogenes* using an M49 wild type and its isogenic Δ*ska* mutant strain, comparing FXII/PK activity in normal and plasminogen deficient plasma. FXII/PK activity induced at the bacterial surface or by culture supernatants was measured using the specific chromogenic substrate S-2302 (Chromogenix). Moreover, we addressed a possible correlation between an invasive phenotype with the capability to trigger efficiently the contact cascade, by testing FXII/PK activity of 50 clinical *S. pyogenes* isolates from invasive and non-invasive infections.
**Results**: Our data clearly indicate that contact activation by *S. pyogenes* M49 is dependent on streptokinase and plasminogen. Further, our experiments revealed that not only surface-associated but also secreted streptokinase is able to induce plasmin activity, which in turn activates contact factors. This results in the processing of HK, and the release of bradykinin. In addition, we found that culture supernatants from invasive *S. pyogenes* strains induced more potently FXII/PK activity in plasma than supernatants from non-invasive strains.
**Summary**: The results give new insights into the mechanisms by which *S. pyogenes* triggers the human contact system and stresses the function of soluble and surface-located plasmin exploited as a virulence factor through the action of streptokinase.

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**083/IIP**

**12/15-lipoxygenase mediates protection from severe influenza A infection**

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**Question:** We have previously shown that glucocorticoid (GC)-treatment of monocytes does not generally suppress monocyte functions but induces a distinct anti-inflammatory phenotype in these cells. Moreover, treatment of inflammatory monocytes with GC leads to re-programming towards a specific population actively involved in resolution of inflammation. Gene analysis has shown

up-regulated expression of 12/15-lipoxygenase (12/15-LOX) in resting and activated monocytes treated with GC. Lipoxygenases generate lipid mediators that contribute to the resolution of inflammation. Our aim was to analyze the influence of GC-induced 12/15-LOX on monocytes ability to counteract severe influenza A infection *in vitro*.

**Methods:** Bone-marrow-derived monocytes were isolated from C57BL/6 wild-type (wt) and 12/15-LOX$^{-/-}$ mice, stimulated with GC for 2 days and subsequently infected with the H7N1influenza A virus FPV (A/fowl plague virus/Rostock/34) for 24h. Virus titers were determined using *Madin Darby canine kidney* (MDCK) cells incubated with the supernatants of virus infected monocytes from wt and 12/15-LOX$^{-/-}$ mice (plaque assay). Viral and pro-inflammatory gene expression was analyzed using quantitative-RT-PCR. Viral and host protein expression was examined by Western-Blot, Flow-Cytometry and ELISA.

**Results:** 12/15-LOX$^{-/-}$ monocytes showed significantly increased virus titers after 24h post infection with FPV as compared to wt monocytes. Surprisingly, in GC-treated monocytes from both strains, very low virus titers were detected. Expression of the viral proteins M1, M2 and NP was strongly upregulated in infected control 12/15-LOX$^{-/-}$ monocytes as compared to infected wt monocytes, whereas GC-treated monocytes from both mouse strains showed significantly lower protein expression. Analysis of pro-inflammatory cytokines revealed no secretion of IL-1β in infected monocytes from both wt and 12/15-LOX mice.

**Conclusions:** Our results clearly demonstrate a protective role for12/15-LOX as well as GC during influenza A infection of monocytes. Whether the protective role of GC-treatment is independent from 12/15-LOX or whether it is strong enough to mask the 12/15-LOX-induced effect in wt monocytes, has to be further analysed. Specific targeting of the 12/15-LOX-pathway could help to find new therapeutic trails for the treatment of viral infections.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**084/IIP**

**Regulation of immune response by cylindromatosis (CYLD) in experimental chronic *Staphylococcus aureus* infection**

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**Introduction**: The deubiquitinating enzyme CYLD plays an important role in the regulation of immune response and inhibition of tumor cell proliferation. CYLD negatively regulates the NF-κB pathway by removing K63-linked polyubiquitin chains from several signaling molecules. The immune response against *S. aureus* is primarily mediated by the NF-κB pathway. Therefore, we investigate the role of CYLD in a model of chronic murine *S. aureus* infection.

**Methods**: C57BL/6 wild-type and CYLD-deficient mice were infected intravenously with *S. aureus* ($2x10^7$) (n=18). The weight loss was monitored daily and bacterial loads in spleen , liver and kidney were determined on day 2, 5, 20 and 49 p.i. CFUs from IFN-γ stimulated bone-marrow derived macrophages (BMDM) were performed 24h p.i.

**Results**: Our preliminary data show that CYLD-deficiency significantly prevented body weight loss and improved pathogen control in liver and kidney. Furthermore, Cyld$^{-/-}$ BMDM macrophages were able to clear *S.aureus* better compared to WT BMDM suggesting that CYLD prevented an effective immune response in chronic *S.aureus* infection.

**Future plan**: Histopathological examination of liver, kidney, and spleen of the infected mice will be performed on days 2, 5, 20 and 49 p.i. Immune cell population in spleen will be determined by FACS. The cytokine levels in the serum and organs would be measured by CBA and RT-PCR respectively. In complementary in vitro studies we will determine the mechanisms of improved pathogen control in macrophages and DCs as well as the impact of CYLD on protective signalling pathways.

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**Figure 1**

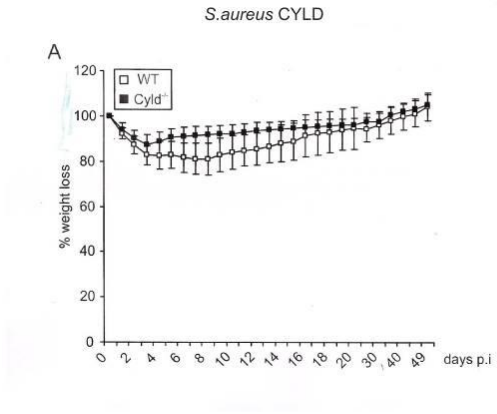
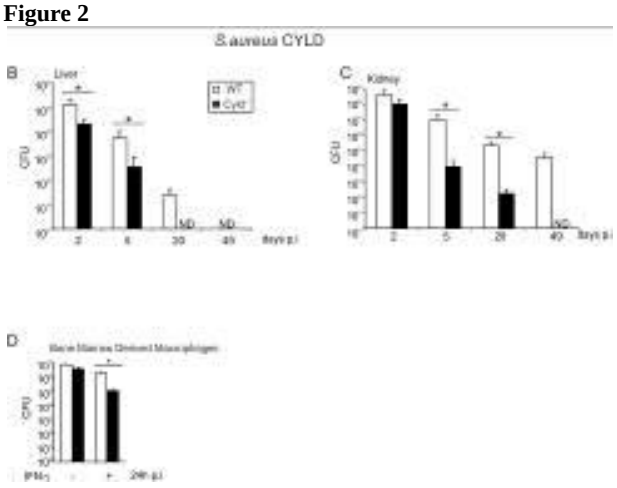



**Figure 2**

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**085/IIP**

**XIAP protects macrophages from cell death induced by cIAP1/2 inhibition.**

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XIAP is a member of the Inhibitor of Apoptosis Protein (IAPs) family of ubiquitin ligases. Via their RING domains IAPs function as ubiquitin E3 ligases in regulating a number of immune signalling pathways including TNF, TLR and NOD signalling. The IAPs are generally involved in activation of NF-kB from these receptors and are required to prevent cell death induced by their activation. Recent evidence suggests that of the three IAPs regulating these processes, cIAP1, cIAP2 and XIAP, in myeloid cells, XIAP appears to play a crucial and overlapping role in suppressing both inflammatory responses and cell death in response to activation of both TNF and TLR receptors. Humans deficient for XIAP suffer from XLP2, a severe inflammatory disease often triggered by infection, and inflammatory bowel disease too, but the causes behind these symptoms remain elusive. Here we show that using HoxB8 immortalized myeloid progenitors to generate macrophages and an IAP antagonist that preferentially targets cIAP1 and 2 (Birinapant) that XIAP deficient macrophages are highly sensitive to killing induced by Birinapant treatment when compared to wild-type macrophages. The increased sensitivity of XIAP$^{-/-}$ macrophages is not due to increased TNF production and is also RIPK1 dependent as it can be abrogated by treatment with RIPK1-Inhibitor Necrostatin, which effectively blocks TNF-production in both wild type and XIAP$^{-/-}$

macrophages. Furthermore, we show that wild-type HoxB8 progenitors are also highly sensitive to Birinapant killing and this could be correlated with XIAP expression, which is absent in the progenitor cells. These data show that as well as being required for NOD signalling, XIAP appears to be an important regulator of macrophage survival in situations of cIAP depletion.

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**086/IIP**

**YopM as a Bacteria-Derived Anti-Inflammatory Cell-Penetrating Peptide (CPP) for the Topical Treatment of Immune-Mediated Inflammatory Skin Diseases**

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Psoriasis is one of the most common immune-mediated chronic inflammatory skin disorders affecting approximately 2-3% of the population. It is considered as an incurable, life-long skin condition that affects all races, age groups and both sexes. A common disadvantage of all currently available drugs for treating this primarily cutaneous disease is that they need to be applied systemically. Previously, we identified the T3SS-dependent YopM of *Yersinia enterocolitica* as a bacterial cell-penetrating protein (CPP) capable of penetrating eukaryotic cells without a need for additional factors. Moreover, we showed that cell-penetrating rYopM efficiently down-regulates the transcription of several pro-inflammatory cytokines (e.g. TNF-α). Therefore, YopM might be applicable as a topical therapeutic agent for the treatment of psoriasis.
To investigate whether YopM might be functional as an immune-modulator for the treatment of psoriasis, we utilized the murine imiquimod (IMQ)-induced psoriasis model. In this model, daily topical application of imiquimod (IMQ), a TLR7/8 ligand and potent immune activator, on mouse back skin induces and exacerbates psoriasis. Here, we applied rYopM either topically or subcutaneously to groups of mice over a period of 10 days. Our results confirmed the 'self-delivering' abilities of YopM across the cutaneous barrier for topically applied rYopM, and indicated a remarkable dampening of overt inflammatory reactions. Furthermore, truncated rYopM variants were generated via site-directed mutagenesis to determine domains required for the penetration of epithelial barriers and for its anti-inflammatory activity. Their cell-penetrating and immunomodulatory capacities were characterized by different approaches including cell fractionation, FACS analyses and immunofluorescence microscopy. Candidates will then be tested in the IMQ-induced psoriasis model.
Taken together, our data indicate that epicutaneously applied YopM can penetrate across the cutaneous barrier in an IMQ-induced psoriasis mouse model and triggers remarkable anti-inflammatory effect. Therefore topical YopM treatment might be suitable for targeted therapy of immune-mediated inflammatory skin disorders.

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**087/IIP**

***Staphylococcus aureus* lipase 1 - a novel staphylococcal immunomodulatory factor?**

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*Staphylococcus aureus* is a common commensal but can also cause severe infections. This ambiguity can, at least partly, be explained by the multi-facetted interactions between the bacterium and the human immune system. *S. aureus* produces numerous virulence factors that interfere with different functions of the immune system. However, already in healthy humans an IgG response directed against most of the known secreted virulence factors can be observed and there is accumulating evidence that this specific immunological memory may provide partial clinical protection. An exception to this rule is *S. aureus* lipase 1 (Lip), an abundantly secreted lipolytic enzyme which is highly conserved in the bacterial species. Intriguingly, anti-Lip IgM and IgG antibodies are rare in healthy humans. The goal of this project is to improve the understanding of Lip's impact on human lymphocytes.
Using proliferation and apoptosis assays as well as functional characterization (measuring cytokines as well as lineage and activation markers) the effects of Lip on T and B cells are analyzed. First results indicate that Lip exerts a strong mitogenic effect on human T lymphocytes. Stimulation with the active enzyme induced proliferation in a large proportion of T cells, suggesting T cell activation independent of the T cell receptor specificity. This effect was abolished when using a nonfunctional S408A mutant; here, the proliferation indices were comparable to typical *S. aureus* recall antigens. Therefore, the T-cell-activating effect of Lip appears to be dependent on the enzymatic function. In contrast, the mitogenic capacity of other T cell mitogens like staphylococcal superantigens is dependent on their three-dimensional structure. The functional characterization of the Lip-responsive T cells as well as first apoptosis experiments with murine Lip-specific B cell hybridoma cells are currently under way.

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**088/IIP**

**ICU patients generate a pathogen-specific humoral immune response during sepsis**

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**Question:** Sepsis is the third leading cause of death in hospitalized patients in Germany. Diagnosis of the causative agent is difficult. Blood culture, the gold standard of pathogen diagnosis, is positive in less than half of the cases of clinical sepsis. Yet the identification of the causative agent is essential for a targeted antimicrobial therapy. In the present study we examined whether the ability of the patients to generate a highly specific humoral immune response towards the invading pathogen might support conventional sepsis diagnosis.
**Methods:** 54 patients with suspected sepsis were recruited in a prospective clinical trial. Serum antibody binding to extracellular proteins of the eleven most common sepsis pathogens was quantified using ELISA or a Simple Western Assay (ProteinSimple). Dynamics in antibody binding were assessed in 49 sepsis patients over two weeks as well as in twelve healthy subjects over two months.
**Results:** In healthy subjects, basal anti-bacterial IgG levels were highly variable, both between individuals and between the bacteria species. However, in each individual, these IgG levels were stable over at least two months. In contrast, sepsis patients frequently exhibited dynamic antibody profiles in the course of the disease. An increase of specific IgG could be detected in 40% of patients with a microbiological diagnosis (11/28). Notably, also in one third of sepsis patients without a suspected pathogen (7/21) there was an

increase of IgG binding to at least one of the tested bacterial species. In ten cases this increase was selective for one or two bacterial species, and this was in agreement with the microbiological diagnosis where available. However, in cases with an intraabdominal infection focus, an antibody response to more than one bacterial species was often observed.
**Conclusion:** Kinetic studies with multiple bacterial antigens revealed that sepsis patients can mount a specific antibody response to the invasive pathogen(s).

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**090/IIP**
**Adaptive immune response to membrane-bound lipoproteins of *S. aureus***
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L. Steil[3], J. Pané-Farré[2], S. Engelmann[2], M. Hecker[2], U. Voelker[3]
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*Staphylococcus aureus* is a frequent commensal but also a dangerous pathogen, causing many forms of infections ranging from mild to life-threatening conditions. Among its virulence factors are lipoproteins, which are anchored in the bacterial cell membrane. Lipoproteins perform various functions in colonization, immune evasion, and immunomodulation. These proteins are potent activators of the complex of innate immune receptors termed Toll-like receptors (TLR) 2 and 6. This study addressed the specific B-cell and T-cell responses directed to lipoproteins in human *S. aureus* carriers and non-carriers. 2D immune proteomics and ELISA approaches revealed that titers of antibody (IgG) binding to the *S. aureus* lipoproteins were very low. Proliferation assays and cytokine profiling data showed only subtle responses of T cells; three lipoproteins did not elicit proliferation. Hence, the robust activation of the innate immune system by *S. aureus* lipoproteins does not translate into a strong adaptive immune response. Reasons for this may be inaccessibility of lipoproteins for B cells as well as ineffective processing and presentation of the antigens to T cells.

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**091/IIP**
**Modulation of kinase signaling in macrophages by Mincle activation**
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The glycolipid Trehalose-6,6-dimycolate (TDM), also known as cord factor, is a major component of the mycobacterial cell wall and may play a dual role in infection. On the one hand TDM is a virulence factor of pathogenic mycobacteria, which inhibits phagosome maturation. On the other hand TDM is also recognized as a pathogen-associated molecular pattern (PAMP) that triggers the innate immune system. Our group and others have identified the C-type lectin receptor Mincle as the pattern recognition receptor for TDM and also for its synthetic analog Trehalose-6,6-dibehenate (TDB). After TDM recognition, downstream signaling is effected by means of Syk phosphorylation and activation of the Card9-Bcl10-Malt1 complex. Activation of the NFκB and NFAT pathway as well as the MAP-kinases leads to expression of transcription factors Egr1/2/3 and Cebpβ, followed by increased release of proinflammatory cytokines, for example G-CSF and IL-6, and enhanced expression of Mincle receptor. Phosphorylation of Syk, Erk and p38 is detectable in murine macrophages stimulated with TDM after 40 minutes and this protein activation is dependent on the Mincle receptor and the FcRγ chain. Another signaling cascade, which is triggered downstream of the Card9-Bcl10-Malt1 complex by TDM via Mincle, is the PKB/PI3K pathway.We could show PKB activation after stimulation of murine bone marrow macrophages via western blot. Blocking of PI3K or PKB by pharmacological inhibitors LY29402 or PKB inhibitor VIII led to reduction of G-CSF and IL-6 production. Therefore, we suggest that this pathway is important for regulation of the cytokine production. To confirm this presumption, knockout mice deficient in this pathway could be use.

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**092/IIP**
**Exploring human T cell responses to various *Staphylococcus aureus* antigens by transcriptome profiling**
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**Question:** Active vaccination relies on the core competence of the adaptive immune system to generate immune memory. In case of *Staphylococcus aureus*, there is evidence that such memory is established in healthy adults as reflected by the presence of a large repertoire of *S. aureus*-specific antibodies. However, their role in *S. aureus* infection is discussed controversially, as to date, all vaccination trials that aimed at inducing a protective antibody response have failed. The establishment of immune memory further based on the cognate interaction of T and B cells, but the role of human T cells in *S. aureus*-host interaction is poorly understood. We have recently characterized the T cell response against a panel of recombinant *S. aureus* antigens and estimate that up to 3% of human peripheral T cells are specific for *S. aureus*.
In this study we examined the human memory T cell response to *S. aureus* antigens using cytokine profiling as well as transcriptomics.
**Methods:** Peripheral blood T cells of ten healthy donors were stimulated with selected recombinant proteins of *S. aureus* to generate antigen-specific T cell lineages *in vitro*. After 10 days of cultivation, secreted cytokines were quantified and RNA was isolated from these cells to perform microarray-based transcriptome analysis.
**Results:** We were able to determine whether the T cell response specific for the tested antigens was dominated by a certain T cell subpopulation, e. g. Th1, Th2 or Th17. Stimulation with the *S. aureus* antigens Hla, Plc, Geh and Lip induced the secretion of Th1/Th17 cytokines, as it is typical for an anti-bacterial response. The global gene expression analysis confirmed and extended the observed cytokine profiles by revealing a strong induction of specific surface markers and transcription factors of the corresponding T cell subpopulations.
**Conclusions:** In summary, we have shown that microarray technology is suited to analyze the human memory T cell response to *S. aureus* using antigen specific T cell lineages. This offers the opportunity to characterize the quality of the immune response to potential *S. aureus* vaccine candidates *in vitro*.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**093/IIP**

**A20 deficiency in T cells enhances host immune response to bacterial infection**

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**Introduction**: The ubiquitin-editing enzyme A20/TNFAIP3 is an important negative feedback regulator of the NF-κB signalling pathway. Mice deficient for A20 die prematurely due to severe inflammation and cachexia caused by a persisting NF-κB activation. With respect to T cells, it has been shown that A20 deletion leads to an improved T cell-mediated control of tumour growth. Nevertheless, the role of A20 in T cells during infection remains unclear. Therefore, we use the bacterium Listeria monocytogenes (Lm), a model organism to study host T cell response, and investigate how A20 influences the course of listeriosis.
**Methods**: We generated mice in which A20 is specifically deleted in T cells (CD4-Cre $A20^{fl/fl}$) and challenged them with Lm. Bacterial loads in spleen and liver were determined, as well as flow cytometric analysis for further characterization was performed.
**Results**: Generation of CD4-Cre $A20^{fl/fl}$ mice revealed, unlike conventional A20 knockout mice, no severe autoimmune phenotype. Mice breed and develop normally. Upon challenge with Lm, CD4-Cre $A20^{fl/fl}$ mice exhibited improved bacterial clearance from infected spleen and liver compared to $A20^{fl/fl}$ control mice. Further analysis revealed an increased amount of Lm-specific T cells, producing more effector molecules such as IFN-γ and granzyme B.
**Conclusions**: We therefore propose that the absence of A20, which induces an increased NF-κB activation, leads to a hyper activation of T cells and consequently to a better pathogen control, due to an enhanced T cell response.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**094/IIP**

**Role of Dusp9 MAPK phosphatase in type I interferon (IFNs) production in plasmacytoid dendritic cells (pDC)**

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The innate immune recognition and reactivity to microbes is mediated mainly by Toll-like receptors (TLRs). Upon triggering, TLRs elicit activation of multiple downstream pathways, including the activation of the MAPK cascade. Activation of MAPK (ERK1/2, p38, JNK1/2) is achieved by phosphorylation of a Thr-X-Tyr motif. Dual specificity phosphatases (Dusp) regulate the MAPK activity by dephosphorylating both threonine and tyrosine. Plasmacytoid dendritic cells (pDC) are characterized by their ability to produce large amounts of type I IFN in response to TLR7 and TLR9; however, the molecular mechanisms behind this capacity are still unclear. By transcriptome analysis we observed the selective expression of Dusp9 in pDC and not in cDCs. High Dusp9 expression in pDC correlated with impaired phosphorylation of ERK1/2 upon TLR9 stimulation. Retroviral overexpression of Dusp9 in GM-CSF-differentiated cDC increased the production of IFNb and IL-12 upon TLR9 stimulation. Conditional deletion of Dusp9 in pDC was effectively achieved in Dusp9flox/flox:CD11c-Cre mice at the mRNA and protein level. However, the lack of Dusp9 in pDC did not restore ERK1/2 activation after TLR9 stimulation and had no effect on IFNb production. Taken together, our results suggest that expression of Dusp9 is sufficient to impair ERK1/2 activation and enhance IFNb expression, but despite selective expression in pDC not essentially required for high level IFN production by these cells.

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**095/IIP**

**Recombinant YopM as an auto-penetrating bacterial effector for the putative treatment of inflammatory bowel disease**

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Inflammatory bowel diseases (IBD; e.g. Morbus Crohn, Colitis ulcerosa) are chronic disorders associated with severe pathology in the gastrointestinal tract. The current immunosuppressive therapeutics are systemically applied and maintain serious side effects and complications including an increased risk for infections, insufficient control of cell proliferation or the onset of dormant infections such as e.g. TB. Moreover, the prohibitive cost of antibody therapeutics causes a heavy burden on the health system. Hence, novel less expensive strategies targeting only the site of inflammation would be highly advantageous. Microorganisms have developed intriguing strategies to bypass or undermine the immune system of their hosts. For this they often use secretion systems such as the type 3 secretion system (T3SS) which deliver a plethora of immune modulatory proteins into the targeted mammalian cells. While such virulence factors have always been regarded as **targets** for vaccines or pharmaceuticals, we consider them instead as valuable **tools** for the development of innovative treatment options. The *Yersinia enterocolitica* derived YopM T3SS effector protein has been identified as the first cell-penetrating effector protein of Gram-negative pathogens, which inhibits the expression of important pro-inflammatory cytokines such as TNFa, IL-12p35, IL-15, or IL-18. We showed in murine models of IBD that upon oral administration YopM passes the stomach and delivers itself inside cells of the intestine. Preliminary results indicate that oral treatment with rYopM is able to improve tissue histology and overt signs of inflammation. Recently, we found that DSS employed to induce acute DSS-colitis apparently hampers the efficacy of rYopM-uptake in vitro. Hence, the efficacy of rYopM will be assessed in a murine IBD-model without the presence of DSS.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**096/IIP**

***Leishmania major* induces distinct gene expression patterns in neutrophil granulocytes from resistant and susceptible mice.**

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**Question:** Experimental leishmaniasis is an excellent model system for analyzing genetic host factors which contribute to the outcome of infection. Resistance to *Leishmania* (*L.*) *major* as seen in C57BL/6 mice depends on the development of a L. major specific Th1 response, while Th2 differentiation in BALB/c mice results in susceptibility. There is growing evidence that the early microenvironment of the infected tissue delivers initial triggers for Th-cell differentiation. Neutrophil granulocytes are among the first leukocytes which arrive in the infected tissue and take up parasites. They have long been regarded as short-lived effector cells but are now recognized to also influence the development of adaptive Th-cell immunity. Thus we analyzed genetic differences in the interaction of granulocytes and *L. major* between susceptible and resistant mice.
**Methods:** We isolated granulocytes from polyacrylamide gel pellets which were implanted subcutaneously into C57BL/6 and BALB/c mice. Applying adherence to plastic surfaces and negative MACS selection using the macrophage cell surface marker F4/80 we depleted contaminating macrophages from the granulocyte cultures. We added infectious metacyclic L. major promastigotes at a ratio of 5:1 per granulocyte. Phagocytosis of *L. major* was similar between both mice strains. We employed microarray technology, real-time PCR and protein assays (cytometric bead assay).
**Results:** We found that interaction between *L. major* and granulocytes resulted in altered gene expression in granulocytes

from both strains of mice. While many genes were down regulated we also found considerable induction of gene expression including chemokines like MIP-1-alpha and beta in resistant and susceptible mice. Gene induction was found on the RNA and also on the protein level. Of special interest, we found 24 genes which were differentially regulated between BALB/c and C57BL/6 mice. Bioinformatical analysis revealed a cluster of genes, which are known to be regulated by type I interferon, which was more strongly induced in BALB/c mice.
**Conclusions:** Thus, our data indicate genetic differences in *L. major* induced gene expression in granulocytes between C57BL/6 and BALB/c mice. This could contribute to the early local microenvironment and thereby influence Th1/2 immunity and ultimately the outcome of infection.

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**097/IIP**

**Peripheral blood mononuclear cells of naïve blood donors secrete interferon-gamma and tumor necrosis factor alpha after exposition to whole cell chlamydial antigens**

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**Introduction:** Chlamydiae are obligate intracellular pathogens with a worldwide clinical and epidemiological relevance. *Chlamydia* (*C.*) *trachomatis* causes blinding trachoma, sexually transmitted diseases and poses a risk for tubal infertility. *C. pneumoniae* causes infections of the respiratory tract. *C. psittaci* and *C. abortus* are zoonotic species causing severe systemic infections both in humans and animals. Despite several well-defined animal models, cellular innate and adaptive immune response to chlamydial infections in humans is still poorly characterized. Key cytokines in clearance of infection as well as in immunopathogenesis are interferon-gamma (IFNg), mainly produced by T and NK cells, and tumor necrosis factor alpha (TNFa), secreted mainly by NK cells, monocytes and macrophages.
**Aims:** In previous work, we characterized humoral immune response against *C. trachomatis* and *C. abortus* using serological proteome analysis. In this work, we aim to analyze the human innate immune response to whole cell and recombinant antigens of the clinically relevant species *C. trachomatis*, *C. pneumoniae* and *C. abortus*.
**Material and Methods:** Peripheral mononuclear blood cells (PBMC) were isolated from whole blood samples of healthy blood donors via density gradient centrifugation. The serostatus of donors was investigated by a microimmunofluorescence test and a line immunoassay containing recombinant antigens. Viable or UV-irradiated, semi-purified elementary and reticulate bodies of *C. trachomatis*, *C. pneumoniae* and *C. abortus* were used as an antigenic stimulus for PBMC. Cells themselves as well as supernatants were analyzed via flow cytometry and cytokine ELISA.
**Results:** We found a strong secretion of IFNg and TNFa in PBMC of naïve donors, especially after stimulation with UV-inactivated and therefore non-infectious whole cell chlamydial antigens. *C. trachomatis* seemed to be the most effective stimulus. Moreover, chlamydial elementary bodies were more efficient stimuli than chlamydial reticulate bodies. Preliminary data suggest NK cells as the major source of IFNg production.
**Summary and Outlook:** We were able to induce species-dependent IFNg and TNFa production in PBMC of naïve donors. Further experiments are on the way to identify the cellular source of cytokines and compare our findings between PBMC obtained from naïve donors and those from people with active chlamydial infection. In addition, defined recombinant antigens including surface and virulence-associated antigens will be investigated for their potential to induce cellular immune response.

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## CLINICAL MICROBIOLOGY AND INFECTIOLOGY (KMP)

**098/KMP**

**Electrooptical analysis for determination of antibiotics synergetic activity**

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**Introduction**: Study of the adaptation of microbes to antibiotic action is an important problem that is of theoretical and applied significance. There are different approaches for investigation of antibiotic resistance. Differences in the biochemical activity of antibiotic substances widely used in chemotherapy. In order to prevent the emergence of resistant forms of microorganisms are used at the same time two or more antibiotics. The aim - is to assess changes in the electrooptical properties of the microbial cells in the combined use of kanamycin and tetracycline.
**Materials & methods**: All experiments were conducted by ELBIC EO analyzer at a wavelength of 670 nm.
**Objectives**: Obtaining the effect at the while the action of kanamycin and tetracycline on the electrophysical characteristics of Escherichia coli cells.
**Results**: Essential changes in the orientation spectra (OS) of cell suspensions incubated at different concentrations of kanamycin, were found to occur only in the first 5 frequencies of the orienting electrical field (10-1,000 kHz)). The maximum change in the intensity of the electrooptical signal occurred at a concentration of kanamycin of 10 μg/ml. Antibiotic concentration of 5 microg/ml caused no changes in OS. During the incubation of the cells with tetracycline (1.7, 2.5, 5.0 microg/ml) no changes in OS of the cell suspension were registered. Considerable changes in the intensity of the electrooptical signal occurred during the incubation of the cells with kanamycin (5 microg/ml) and tetracycline (1.7 microg/ml) simultaneously, which was due to the synergic action of these two antibiotics.
**Conclusion**: Thus, as found with the use of the electrooptical analysis, the joint action of kanamycin and tetracycline could increase their antibacterial effect. The results demonstrated the effectiveness of using electrophysical methods for the registration of microbial cells synergistic antimicrobial effect of antibiotics.

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**099/KMP**

**Microevolution of *S. pseudintermedius* isolated from one dog between 2008 and 2014**

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**Introduction**: The opportunistic pathogen *S. pseudintermedius* mainly causes purulent infections in dogs. Recurrent infections have been described in the past. In order to unravel the microevolution as well as the phenotypic diversity of this pathogen within one patient phenotypic and genotypic characterization was performed for methicillin-susceptible (MSSP) and methicillin-resistant *S. pseudintermedius* (MRSP) isolated from multiple wound infections of a dog during a seven-year time period.
**Material and Methods**: Between 2008 and 2014, S. *pseudintermedius* was isolated from 25 wound swabs of one patient. In total, 38 isolates (up to eight colonies / swab) were sequenced. Clonal relationship was determined based on the allele diversity of 1064 target genes (Ridom SeqSphere + 2.3.1).

Variability within isolates of distinct genotypes (gene content and SNPs [single nucleotide polymorphisms]) was analyzed (geneious 6.1.5). To display the phylogenetic relationship for isolates of each lineage neighbor-joining trees were built. The binding capacity to fibrinogen and fibronectin as well as biofilm formation was determined for eleven isolates.
**Results**: MLST+ typing revealed three distinct genotypes (I, II, III). All MRSP (n=21) belonged to genotype I. MSSP clustered into two genotypes II (n=15) and III (n=2). Within each of the two predominant lineages only minor variations were detected regarding the gene content and SNPs. Despite the low number of identified SNPs (MRSP-I n=26; MSSP-II n=27), an accumulation was observed over the time for MSSP-II.
While MRSP-I showed only weak adherence to fibrinogen, MSSP-III had a moderate and MSSP-II a strong binding capacity. Strong biofilm formation was observed for all MRSP. Isolates sharing the same genetic background displayed a comparable phenotypic profile.
**Discussion**: Sampling of 25 wound infections from one patient revealed two different successful genetic lineages. Interestingly, isolates sharing the same genetic background showed only minor genetic variation even though the strains were isolated over seven years. While mixed infections with MRSP-I and MSSP-II were determined twice, exchange of mobile genetic elements was not detected.
Phenotyping revealed opposing abilities for MRSP-I and MSSP-II regarding adherence to fibrinogen and biofilm formation, indicating that none of these tested mechanisms is essential for *S. pseudintermedius* to successfully infect dogs. The lack of phenotypic variability of isolates sharing the same genetic background is in accordance with the stable genome of these strains. A reasonable explanation for the lack of variability within the identified lineages might be recurrent auto-infections or a persistent infection rather than re-infections due to an external source.

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**100/KMP**
**Novel method for genotyping clinical herpes simplex virus type 1 isolates**
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**Background***:* Three distinct genotypes A, B and C of herpes simplex virus type 1 (HSV-1) have been classified on the basis of sequence analysis of US4 and US7, localized in the unique short (US) genomic region and encoding the glycoproteins G (gG) and I (gI). However, clinical manifestations have not been shown to be associated with different gG/gI genotypes.
*Objectives:* A novel HSV-1 genotyping method on the basis of polymorphism of the US2 gene, encoding a tegument-associated protein, was established and validated. Associations between the different US2 genotypes and clinical manifestations were examined.
**Study design***:* After amplification, US2 DNA fragments of HSV-1 were characterised by restriction fragment length polymorphism (RFLP) analysis using the enzyme *Eco*O109I. By this method, 465 clinical
HSV-1 isolates of the last four decades were analysed. The characteristic restriction fragment pattern was compared to those of US4-/US7-based genotyping.
**Results***:* All HSV-1 isolates were classified into three different US2 genotypes, 46.6% as genotype A, 23.2% as B and 30.2% as C. The frequency of genotype A was significantly increased in female compared to male patients with herpes labialis. Furthermore, the US2 and the US4/US7 genotypes A showed a highly significant correlation.
**Conclusions***:* The novel US2-based RFLP analysis is a suitable and easy to perform screening method for genotyping of clinical HSV-1 isolates. Further studies are required to demonstrate the clinical benefit of this HSV-1 genotyping assay.

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**101/KMP**
**Direct detection of *Borrelia burgdorferi*-DNA with PCR and a melting probe technology**
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**Objectives**: *Borrelia burgdorferi* is the infectious agent of Lyme disease. Due to sensitivity reasons PCR today does not play an important role in diagnosing Lyme borreliosis from whole blood, skin biopsies, synovial fluids or cerebrospinal fluid(CSF) specimens. We evaluated a commercial PCR-assay (FluoroType(FT) Borrelia, Hain Lifescience, Nehren, Germany) for the detection of *B. burgdorferi*-DNA.
**Methods**: *B. burgdorferi*-DNA from 500µl of specimen was isolated with an automated device GenoXtract 12 and the GXT NA Extraction Kit (Hain Lifescience). PCR and detection run on a Fluorocycler (FC) 12 real time thermocycler including software based data evaluation (FluoroSoftware, Hain Lifescience).
**Results**: In total 160 samples were investigated.The validation consisted of three parts:68 specimens run in parallel to routine diagnostic (18 aspirates, 40 csf, 4 EDTA whole blood, 6 ticks) and were compared to an "in house TaqMan-assay" modified as published byCourtney et al., 2004. 23 ticks stored for up to some months in a freezer an pre-characterized with the LC-mix Borrelia (TibMolbiol, Berlin, Germany) were tested in parallel. 69 samples derived from DNA strain collections including different Borrellia species and other bacteria were analyzed for specificity testing.
From 68 routine samples 52 were congruent negative and 12 were congruent positive, two were false positive and two were invalid due to inhibition. From all tested ticks 10 were congruent negative, 11 were congruent positive, and two were false positive. Specificity testing showed detection of all *Borrelia*-species. Some *Borrelia*-species showed a characteristic Tm peak shift. No other "non-*Borrelia"*-spirochetes and other tested bacteria showed cross reactivity.
**Conclusions**: The FluoroType Borrelia for the direct detection of *Borrelia*-DNA from ticks and clinical specimens showed high sensitivity values compared to other PCR assays. Special probes enable melting curves with specific Tm - values for target characteristic polymorphisms. Combination of a full set of instrumentation, software and chemistry provides high handling and data interpretation

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**102/KMP**
**Host receptors for *Staphylococcus aureus* wall teichoic acid**
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Cell wall glycopolymers (CWGs) of Gram-positive bacteria are major surface determinants which play a key role at the interface of bacterial and host cell interaction. In *Staphylococcus aureus* we could demonstrate that a CWG termed cell wall teichoic acid (WTA) is an important non-protein adhesin that governs attachment to different host cell types. Thus, WTA-receptor interaction plays a key role in *S. aureus* colonization and infection. Interestingly, asymptomatic nasal colonization is a major risk factor for *S. aureus* infections. The mechanisms responsible for colonization are still not well understood and involve several factors on the host and the bacterial side. We recently identified a WTA receptor on nasal epithelial cells that plays a role in *S. aureus* adhesion to nasal surfaces. The WTA receptor is termed SREC-I and is a type F-scavenger receptor that binds WTA in a charge dependent manner. Especially under shear stress conditions, the WTA/SREC-I interaction facilitated *S. aureus* adhesion to nasal

epithelial cells by modulating the initial contact of bacterial- and host cells. Furthermore, we were able to demonstrate an important role of this WTA-receptor interaction in a "state of the art" cotton rat *in vivo* model of nasal colonization. Based on our results, we postulate a reservoir of *S. aureus* cells in the inner nasal cavity as a source for continuous colonization, which is at least partially maintained by WTA dependent adhesion to epithelial cells. Most importantly, inhibition of WTA mediated adhesion abrogated nasal colonization in the animal model. In addition, we identified a WTA receptor on endothelial cells and we have evidence for a considerable impact of WTA/receptor interaction on *S. aureus* blood stream infections. Therefore, we propose targeting of this glycopolymer/host-receptor interaction as a novel strategy to prevent or control *S. aureus* nasal colonization and infection. This novel approach could have a considerable impact as it directs the attention to bacterial glycopolymer/host-receptor interactions, a so far neglected field with a huge potential for therapeutic interventions.

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**103/KMP**

**Validation of the new fluorescence-based PCR assay FluoroType® MRSA 2.0 for the direct detection of MRSA, mecA and mecC from clinical specimens**

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**Questions**: We present the first validation results of the new FluoroType (FT) MRSA assay (Hain Lifescience, Nehren, Germany) for the direct detection of MRSA in clinical swab specimens. The FT MRSA test is based on HyBeacon fluorescence-technology and is performed on the FluoroCycler (Hain Lifescience). The detection of MRSA is enabled by the simultaneous detection of S. aureus specific sequence within the staphylococcal cassette chromosome mec (SCCmec) and the methicillin resistance mediating *mec*A or *mec*C genes.
**Methods**: 273 patient swab specimens from nose, throat, skin and wound were collected for the validation. The results of the FT MRSA 2.0 test were compared to culture (CHROMagar MRSA, CNA blood agar, thioglycollate broth) and to a CE-marked PCR test (FT MRSA 1.0). For DNA isolation the Spherolyse® Extraction Kit (Hain Lifescience) was used. The swabs were inoculated in Spherolyse buffer, and then inoculated on CHROMagar MRSA, CNA agar and into a thioglycollate broth (all Becton Dickinson, Heidelberg, Germany). The thioglycollate broth was incubated for 24 h and inoculated on CNA agar and CHROMagar. The Spherolyse-lysis buffer was vortexed for 10 min, and afterwards inoculated at 95°C for 15 min. 6 µl of the DNA lysate was used for FluoroType MRSA-PCR. S. aureus-colonies were identified by MALDI Biotyper (Bruker Daltonics,Bremen). MRSA detection from cultured MRSA colonies was realized by a PCR-based assay (GenoType MRSA 3.0, Hain Lifescience).
**Results**: Of 273 specimens collected, 47 specimens were MRSA-culture positive. 45 were positive with the FT MRSA tests. For 2 specimens the FT MRSA assay showed a positive result, while culture was negative. Sensitivity, specificity, negative predictive value and positive predictive value were 95.7%, 99.1%, 99.1% and 95.7%.
**Conclusions**: The new FluoroType MRSA method (Hain Lifescience) showed an excellent sensitivity and specificity for the detection of MRSA in clinical swab specimens. The turnaround time of the FT MRSA assay is approximately 20 min for sample set up and DNA isolation and 2.15 hours for amplification and detection.

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**104/KMP**

**Copper on surfaces and various disinfections in tubes act as antimicrobial substances**

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Pathogenic bacteria remain viable on touch surfaces and in water pipes. Contaminated surfaces play a role as a pathway for transmission and increase infection rates primarily in hospitals and dental clinics. Bacteria survive in water hoses and even under dry conditions on stainless steel surfaces. Surfaces and hoses can therefore serve as reservoirs of pathogens which may be infectious and may present a serious threat for public health. Therefore, the uses of copper containing surfaces gain increasing attention for applications achieving antimicrobial effects as well as of high efficient decontaminating substances in water tubes.
In this study we analyzed bacterial resistance to disinfections in tubes to identify sensitive as well as resistant naturally occurring species and we investigated the sensibility of various indicator bacteria as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* on copper based surfaces.
In water tubes we found various disinfections sensible bacterial species which are mainly integrated in biofilms but also resistant bacteria being able to form biofilms by themselves. The use of various disinfections may reduce bacterial resistance formation.
On copper surfaces our results revealed an antimicrobial effect with a reduction of at least two $\log_{10}$-units of the cultivable bacteria. The use of antimicrobial surface materials such as copper may constitute a way to minimize the risk of bacterial emergence and spread. However, even when antimicrobial surfaces are used, surfaces are exposed to the surrounding environment and become contaminated; henceforth cleaning should be carried out periodically.

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**105/KMP**

**High efficacy of a novel bacterial DNA dependent RNA polymerase inhibitor against various chlamydial species**

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**Introduction:** Doxycycline and azithromycin are the recommended substances for treating respiratory and urogenital tract infections with *Chlamydia* spp. Although an eradication of the bacteria is observed in most cases, treatment failures are reported in up to 8 % of genital chlamydial infections. In former studies we could show impaired efficacies of these antibiotics under physiologically relevant low oxygen concentrations, which might explain in parts these observations. Therefore we are seeking to develop a new drug against *Chlamydia* spp. for clinical use.
**Methods:** In this study we tested the efficacy of a novel compound targeting the bacterial DNA dependent RNA polymerases against *C. trachomatis* serovars D and L2 as well as *C. pneumoniae* strain CWL029 under different oxygen concentrations (2 and 20% $O_2$). *In vitro* cell culture, minimal inhibitory concentration ($MIC_{99}$) and recoverable *Chlamydia* spp. were measured after infected cells were treated by the new compound. Recoverable inclusion forming units (IFUs) were determined applying compound concentrations from 0.125 to 1.5µg/ml.
**Results***:* Treatment with the novel compound had the same effect in both oxygen concentrations for *C. trachomatis* serovar L2 (MIC=0.5 µg/ml); *C. trachomatis* serovar D (MIC=1 µg/ml) and *C. pneumoniae* strain CWL029 (MIC=0.5 µg/ml). Adding the compound 0, 4 and 8 hour post infection, we observed the same effect on *Chlamydia* spp. eradication. We did not observe a

significant difference in the efficacy of the compound between 20% and 2% $O_2$ at any concentration of the compound. The observed recoverable IFUs were dependent on the dose of the novel compound as it was confirmed by Spearman rank correlation coefficient.
**Conclusion:** The novel bacterial DNA dependent RNA polymerase inhibitor exhibits high efficacy *in vitro* against intracellular growing *C. trachomatis* and *C. pneumoniae* under 20% and 2% $O_2$. Further studies are under way to test the efficacy of the novel compound in a recently established *in vivo* mouse model to demonstrate the potential clinical use.

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**106/KMP**

**Characterization of the Atl-dependent staphyloccocal internalization by human host cells**

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*Staphylococcus aureus* and the coagulase-negative *Staphylococcus epidermidis* are major human pathogens that are responsible for a variety of infections. The ability of the staphylococcal cells to be internalized by host cells thereby hiding from the host immune system and antibiotic treatment is considered one of the most critical pathogenicity factors in persisting and recurrent infections. *S. aureus* internalization by host cells is mediated by the fibronectin (Fn)-binding proteins FnBPA and FnBPB, integrin a5b1, and Fn acting as a bridging molecule. However, we recently identified an alternative internalization mechanism that involves the major autolysin Atl (AtlA from *S. aureus* or AtlE from *S. epidermidis*) and the heat shock cognate protein Hsc70 as host cell factor [1]. Because *S. epidermidis* lacks the FnBPs, the Atl-mediated mechanism might represent the sole internalization mechanism in *S. epidermidis* or in coagulase-negative staphylococci in general. To further characterize the Atl-dependent internalization mechanism, we performed flow cytometric internalization assays. Pharmacological inhibition of actin, microtubules, clathrin, Src kinase, and PI3 kinase significantly and dose-dependently reduced the internalization of *S. epidermidis* O-47 and the *fnbA*/*fnbB S. aureus* deletion mutant DU5883 by EA.hy926 endothelial cells delineating the importance of these factors. Moreover, preincubation of the EA.hy926 cells with anti-Fn, anti-a5b1, and anti-Hsc70 antibodies resulted in decreased internalization of *S. epidermidis* and *S. aureus* DU5883. Additionally, the involvement of Fn, the integrin a5b1 and Hsc70 could be confirmed and visualized by confocal laser-scanning microscopy (CLSM) that demonstrated a colocalization of these molecules with entering *S. epidermidis* O-47 and *S. aureus* DU5883 cells. In control experiments, colocalization was also observed with the wild type *S. aureus* 8325-4 mainly representing the FnBP-mediated internalization mechanism. In conclusion, we studied the Atl-dependent internalization mechanism in *S. epidermidis* and *S. aureus* DU5883 and identified Fn, the integrin a5b1 and Hsc70 as host cell factors that contribute to the internalization process and actin, microtubules, Src- and PI3 kinase signaling as well as clathrin as intracellular mediators of the bacterial entry. The characterization of the FnBP-independent staphylococcal internalization mechanism may open new avenues for treatment or prevention of chronic and recurrent infections especially with coagulase-negative staphylococci.

Reference
1: Hirschhausen N, Schlesier T, Schmidt MA, Go¨tz F, Peters G, et al. (2010) A novel staphylococcal internalization mechanism involves the major autolysin Atl and heat shock cognate protein Hsc70 as host cell receptor. Cell Microbiol 12: 1746-1764.

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**107/KMP**

**A food-grade bacterium turns virulent: molecular analysis of the first *Staphylococcus condimenti* isolate causing a port catheter infection**

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**Objective**: Usually, bacteria being used during food production are believed to possess no relevant pathogenic potential for humans. Here we report on the first case of a port-associated infection caused by *S. condimenti*, a species closely related to non-pathogenic *S. carnosus*. In order to test the hypothesis that *S. condimenti* could possess traits which might account for its pathogenicity, the clinical isolate was further analyzed using phenotypic and molecular methods.
**Methods**: We used Next Generation Sequencing (NGS) to compare the genome of *S. condimenti* and *S. carnosus.* Over 2 million paired-end (2x151bp) reads were obtained by sequencing on an Illumina MiSeq instrument. The reads were assembled using SPAdes. The resulting assembly has an N50 of 269,8 Mb and consists of 2.59 Mb in 57 contigs. 99.63% of the contigs are covered at least 20x. For annotation RAST was employed. Genome comparison was performed using ACT Artemis. Unaligned sequences were analysed by BLASTn and BLASTp.
**Results**: Although genetic identity between *S.condimenti* and *S.carnosus* is very high we could identify several loci solely present within the *S.condimenti* genome that share high nucleotide or amino acid identity with virulence associated genes from other members of the staphylococcus genus. The *S.condimenti* genome comprises not only genes for iron acquisition (*isdA, -B, -C* and *-E*), but also genes for capsule formation (*CapA*) and the *ica*-locus. In addition to these genetic features, additional phenotypic traits (e.g. a reduced lysozyme susceptibility) associated with virulence clearly separates *S.condimenti* from *S.carnosus.*
**Conclusion**: Overall genetic identity between *S.condimenti* and *S.carnosus* is about 92,95%. Identified differences between *S.condimenti* and *S.carnosus* pointed to loci associated with virulence in other staphylococcal species. The impact of these findings with regard to the role of *S.condimenti* during infection and the general aspect of food safety in immuno-compromised patients has to be further evaluated.

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## FOOD MICROBIOLOGY AND HYGIENE (LMP)

**108/LMP**

**An update on status of Shiga-toxigenic *E.coli* as a neglected foodborne pathogen , India**

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**Introduction**: Shiga-toxin producing *Escherichia coli* (STEC) are food borne pathogens of worldwide importance but there is a scarcity of data on STEC isolation from India. Therefore, we conducted an epidemiological and environmental study covering a large geographic area in north India which is a major milk-producing and animal rearing area. A hospital based surveillance for STEC causing bloody diarrhea and HUS was also carried out.
**Material and Methods**: Ruminant stool samples (n=650) were collected from 59 dairies. Meat samples (n=450) were collected from local abattoirs and the main slaughter house of the region. Six hundred human cases of diarrhoea and HUS were screened. Isolates were characterized for the virulence gene profile and serogroups, and molecularly typed by multilocus variable number tandem repeat analysis (MLVA).

**Results**: Overall, 12.3% of animal stool samples and 6.3% of mutton samples (n=160) were positive for STEC .STEC were also isolated from 1.7% and 1.6% of watery (n=290) and bloody (n=310) stool specimens. Animal stool isolates were significantly more prevalent from hilly areas (P<0.05) than plain areas. Prevalence of virulence genes wasas follows: *stx*1 (83.5%), *stx*2 (67.1%), *eae* (10.7%), *hly* (55%), *etpD (6.4%), espP* (23%) and *katP* (1.4%), *saa* (44.2%), *toxB* (20.7%), *efa*1 (5%) and *iha* (36%). Five new serogroups (O55, O33, O173, O165 and O136) were found from India. Four isolates of serogroup O103 were found in mutton and stool samples of cattle and humans. One of these harbored a 7 virulence genes (*stx*1, *stx*2, *eae*, *hly*, *saa*, *toxB*, *efa*1) which is a matter of concern. One isolate belonging to serogroup O104 was isolated from a mutton sample. MLVA suggested potential transmission of STEC from contaminated meat and bovine sources. Prevalence of s*tx*2 gene was significantly higher in animal stool isolates than in meat or human isolates (P<0.05), suggesting the virulence potential of STEC isolates present in fecal reservoirs of animals which can pose a public health threat. We did not isolate O157 STEC in spite of the immunomagnetic separation method signifying either the absence or presence in very low numbers of this serogroups in our region.
**Conclusions:** This study demonstrates the presence of STEC which carry a large repertoire of virulence genes, and potential transmission of STEC, from contaminated mutton and animal stools, in north India.

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**109/LMP**

**Safety assessment of *Staphylococcus carnosus* strains for their use as meat starter cultures**

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Particular *Staphylococcus carnosus* strains are commonly used as starter cultures for fermented meat products. Starter cultures are added in high numbers therefore, the strains have to be confirmed as safe for the consumer, before they can be used in foods. One standard to assess the safety of starter cultures is the Qualified Presumption of Safety (QPS) concept of the European Food Safety Authority. This includes the presence of acquired antibiotic resistance determinants as well as the production of toxins and biogenic amines.
In this study, 40 different *S. carnosus* strains were analyzed for their resistance against 17 antibiotics, using the agar disc diffusion test recommended by the Clinical and Laboratory Standards Institute. Ten strains were classified as resistant or intermediate against cefotaxime, chloramphenicol, oxacillin or trimethoprim/sulfamethoxazole. Of these, only two strains showed resistance to more than one antibiotic. Additionally, the strains were examined for the antibiotic resistance genes *blaZ*, *mecA* and *tetK* by PCR. *blaZ* alone was found in four strains, *blaZ* and *tetK* in combination in three other strains, while none of the strains was positive for *mecA*. Moreover, none of the PCR-positive strains showed a resistance phenotype to the corresponding antibiotic.
None of the tested strains was PCR-positive with primers targeting the typical staphylococcal enterotoxin genes (*sea-see*, *seh*), the exfoliative toxin gene (*eta*) and the toxic shock syndrome toxin gene (*tst-1*). Two strains showed β-hemolysis of human blood cells and therefore have to be excluded as starter culture.
None of the 26 remaining antibiotic-sensitive and non-toxigenic strains produced the biogenic amines cadaverine, putrescine and histamine as shown by HPLC-analysis. However, 11 of these produced phenethylamine in concentrations of 2.6 - 15.0 µg/ml.
Although *S. carnosus* is generally recognized as safe, the results of this study indicate that safety risks, such as antibiotic resistance and biogenic amine production, are quite common among strains of the species *S. carnosus.* Consequently, each strain should be analyzed individually before it is applied as starter culture in meat products.

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**110/LMP**

***In vivo* system for the investigation of the internalization of enterohemorrhagic *E. coli* in lettuce**

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Enterohemorrhagic *Escherichia coli* (EHEC) O157 present a serious threat to human health. While extensive research has been conducted under laboratory conditions, the mode of interaction between EHEC and leafy greens *in vivo* is not fully understood yet. In this study the internalization of EHEC in batavia lettuce by the rhizosphere as well as the phyllosphere was investigated under greenhouse conditions. For this purpose lettuce plants were inoculated with suspensions of the Shiga toxin-negative non-motile sorbitol-fermenting *E. coli* O157:H$^-$ strain 431/97 by irrigation of the soil or the leaves. The suspensions had viable counts of $3.0 \times 10^1$ and $2.3 \times 10^6$ colony forming units (cfu)/mL. At different time points after inoculation samples were drawn from roots and leaves. The root samples were analyzed with attached soil without disinfection. One part of the leaf samples was disinfected with gentamicin to detect only internalized *E. coli* O157:H$^-$ strain 431/97, while the other remained untreated. The colony counts were determined on sorbitol Mac Conkey agar and the resulting isolates were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MALDI biotyping). The inoculation of the rhizosphere resulted in declining bacterial counts of $10^6$ to $10^2$ cfu/0.25 g lettuce roots with attached soil over the growth period. In the leaf samples no internalized *E. coli* were detected. Contrarily, after inoculation of the plants *via* the phyllosphere, bacterial counts of $10^2$ cfu/0.25 g lettuce leaves were determined in disinfected leaf samples. It is thus hypothesized that these EHEC could either enter stomata or were integrated in biofilms. These results indicate that while EHEC may survive in high numbers in the soil, the main route of entry into lettuce plants might indeed be over the leaves, which constitute the edible part of the plant.

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**111/LMP**

**Quantification of total bacterial count in poultry meat using real-time PCR**

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**Background:** The real-time PCR based methods are highly admired and widely applied in the microbiological analysis of food. Due to their sensitivity and rapid nature of assays such molecular-biological methods are considered as suitable alternatives for cost- and time-consuming culture-based methods. However, few studies were performed using real-time PCR to quantify the total bacterial count in food, which is important for predicting their shelf-life and spoilage.
**Objective:** The objective of this study was to develop a real-time PCR based method using a new primer set that amplifies conserved sequences on *rpoB* gene of a wide range of spoilage associated bacteria to determine the total bacterial count in poultry meat.

**Methods:** The spoilage associated bacteria in poultry meat were identified by culturing the bacteria from poultry meat samples onto different selective agar and applying a direct nucleotide sequence analysis based on 16S r*RNA* PCR fragments. A new primer set was developed using consensus sequences on *rpoB* gene, a protein-coding gene which has only one copy in the genome of all bacteria. A standard curve was developed by preparing a mixed culture of bacteria and photometric measurement of their growth. Setting a serial dilution from the mixed culture, the bacterial count of each dilution level was calculated by culturing 1 ml of each dilution level onto Plate Count agar. Simultaneously, a DNA isolation procedure was performed for each dilution level. A standard curve was created automatically by real-time PCR software, comparing the Ct values resulting from amplification of DNA isolates described above and the bacterial count of each dilution level ($Log_{10}$ CFU/g; $R^2$=0.98). Extracting DNA from poultry meat with different bacterial load using a universal DNA isolation kit and determining their bacterial count using standard plate count technique (DIN 10161:1984), a quantitative real-time PCR assay was developed using the new primer for amplification of a 174 bp PCR fragment.
**Results & Discussion:** The bacterial species identified in this assay associated with poultry meat spoilage were comparable to those reported previously in the literature. According to the post-PCR melting curve analysis and electrophoretic separation of PCR products, the primer set could successfully amplify the targeted region on *rpoB* gene. The comparison of Ct values obtained from real-time PCR analysis have shown a good correlation coefficient ($R^2$ = 0.83, n = 30) with the $Log_{10}$ CFU/g resulting from plate count method. Respectively, the comparison of GU obtained from standard curve analysis by real-time PCR with the $Log_{10}$ CFU/g has shown a correlation coefficient of 0.76.
The results of this study indicate that a rapid quantification of total bacteria in poultry meat by real-time PCR based on amplification of conserved regions on the *rpoB* gene of bacteria can be implemented in the microbiological food analysis.

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**112/LMP**

***Pseudomonas aeruginosa* - Determination of Contamination in Water Flow Meter using GreenLight® System**

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**Background:** The demand of rapid methods for determining bacterial load for quality control has increased in the last years. A new rapid approach is presented by the GreenLight® 930 series of instruments developed in cooperation by Luxcel Biosciences® and Mocon Inc®. GreenLight® is an assay that can relate oxygen depletion to microbial load using an oxygen sensor located at the base of each vial (15 ml). The signal is measured within microseconds of fluorescence decay from the sensor. Normally, the vials are filled with a specific nutrient broth, with proper proportions to the sample, to promote the rapid growth of bacteria. According to the manufacturers' data this system is capable of determining the Total Viable Count (TVC) within 1-16 hours depending on the initial bacterial load.
**Methods:** A rapid assay based on the GreenLight® system was developed to determine *Pseudomonas aeruginosa* in water flow meters comparing the results with the traditional plate count method. On the basis of CFC-, CN (Merck) and Pseudalert®250 (Idexx) broth the system was tested to detect *Pseudomonas aeruginosa* in water samples. The GreenLight tubes were filled with 9 ml selective broth and 1 ml of dilution from $10^5$ to $10^0$ CFU/ml *Pseudomonas aeruginosa* (DSM 939). The water samples were mixed with specific culturing broths and were transmitted into the Greenlight® with an incubation temperature of 37 °C and a threshold of 32 µs. Simultaneously, the cfu of samples were determined by using a traditional plate count technique. The non-target organism control was performed with a mixture of *Salmonella Enterica* (DSM 17420), *Enterococcus hirae* (DSM 3320) and *Escherichia Coli* (DSM 17420).
**Results and conclusions:** The results of samples of the three culture broths show good correlations (CN: R2 = 0.98, Pseudalert® R2 = 0.99, CFC: R2 = 0.99) with the bacterial load in the range of $10^1$ to $10^5$ CFU/ml. The non-target microorganisms show no influence on the results. The time to threshold shows differences in time duration depending on the bacteria count and the different broths. For example, the sample ($10^2$ cfu/ml) with Pseudalert reached the threshold after 20h and the sample with CN-broth after 12h.
Using pre-determined criteria such as fail and pass, the system can be a suitable rapid method for determination of Pseudomonas aeruginosa or Pseudomonas spp. in the field of quality control of water flow meters.

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**113/LMP**

**Survival of pathogenic *Escherichia coli* on seeds and impact of aggregative adherence fimbriae on colonization**

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**Introduction:** The latest and largest *Escherichia coli* O104:H4 outbreak in Germany in 2011 shifted the focus of epidemic research towards enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC). During the tracing of the outbreak, contaminated fenugreek seeds as vehicle came into the focus of investigation. It is believed that a contaminated batch of seeds from Egypt, grown and distributed by a company in Lower Saxony, was responsible for the 2011 outbreak.
The outbreak strain LB226692 was classified as a hybrid of EAEC and EHEC due to its abundance of virulence factors of both pathotypes like the Shiga toxin-encoding prophage ($stx_2$) from EHEC or the possession of aggregative adherence fimbriae (AAF), which are typical for EAEC. Of these AAFs, five distinct types are known so far. The strain LB226692 expresses AAF type I, whereas the prototypic EAEC strain 55989 expresses AAF/III fimbriae. This unusual combination of virulence factors, Stx2 and AAF/I, was rarely observed before and may contribute to the pathogenicity of this particular strain.
**Material and methods:** Fenugreek seeds were incubated with wild type strains of diarrheagenic *E. coli* carrying the five known fimbrial gene clusters and three control strains carrying no *aaf* clusters, respectively. Long-term survival was evaluated by sampling the survival rate of each strain after certain time points in order to analyze a possible advantage of the outbreak strain due to its combination of virulence factors.
To analyze and compare the contribution of the five different AAF variants to bacterial adhesion to plants, the corresponding fimbrial gene clusters were also cloned into an expression vector with inducible promoter and introduced in an isogenic *E. coli* K-12 strain background. The adhesive properties on small fenugreek seedlings were evaluated.
**Results:** The outbreak strain did not exhibit a significantly increased ability to survive on fenugreek seeds under the tested conditions. In fact, *E. coli* K-12 displayed the highest survival rates at all tested time points.
The five different *aaf* gene clusters were successfully expressed in an isogenic *E. coli* K-12 strain background. Differential effects of bacterial adhesion to fenugreek seedlings were observed upon expression of the different AAF variants.
**Discussion:** Under the assumption that the vehicle of infection during the outbreak were seeds or seedlings, it is remarkable to see that there seems to be no advantage in survival for the outbreak strain LB226692, at least under the conditions tested. The impact of the different AAF types on short term adhesion and colonization of seedlings will be discussed.

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# MICROBIAL PATHOGENICITY (MPP)

**114/MPP**

**A Streptococcal NRAMP homologue is crucial for the survival of *Streptococcus agalactiae* under low pH conditions.**

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**Introduction**: *Streptococcus agalactiae* or Group B *Streptococcus* (GBS) is a commensal bacterium of the human gastrointestinal and urogenital tracts as well as a leading cause of neonatal sepsis, pneumonia and meningitis. Hence, GBS encounters pH environments that vary from the acidic pH of the vagina and intracellular endocytic compartments to the near-neutral pH of amniotic fluid, fetal lung or human blood. Metal ion homeostasis in streptococci is fundamental to express adhesins for interacting with host surfaces, to resist host defenses and to grow under environmental conditions with a limited supply of essential metal ions. Several studies in eukaryotes identified a group of proteins (Natural Resistance-Associated Macrophage Protein, NRAMP) that function as divalent cation transporters for $Fe^{2+}$ and $Mn^{2+}$ and confer on macrophages the ability to control replication of bacterial pathogens. Genome sequencing predicted potential NRAMP homologues in several prokaryotes. Therefore, while mammalian NRAMPs are involved in the defense against microbial pathogens, bacterial NRAMPs may play a role in pathogenesis. The available literature on bacterial NRAMPs is limited and no streptococcal homologues have been identified.
**Objectives**: To characterize the role of the NRAMP gene in the survival and growth of GBS under low pH conditions
**Materials and Methods:** A mutant library generated by transposon mutagenesis was screened for mutans failing to grow at low pH. One of our mutants harboured a chromosomal integration interrupting a $Mn^{2+}$/$Fe^{2+}$ion transporter gene of the NRAMP family (*mntH*). This gene was further characterized by sequence alignment, growth experiments at pH 5 in presence and absence of $Mn^{2+}$and/or $Fe^{2+}$, and promotor analysis of the *mntH* gene. Functional assays were carried out in mixed cultures with lactobacilli, adherence assays, biofilm formation assays, and determination of the intracellular survival of the NRAMP mutant inside acidic macrophage compartments, as well as the ability of the mutant to tolerate oxidative stress.
**Results**: Here we describe for first time, a pH-dependent NRAMP $Mn^{2+}$/$Fe^{2+}$ transporter in GBS, designated MntH, which confers resistance to reactive oxygen species (ROS) and is crucial for bacterial growth and survival under low pH conditions. Survival of GBS *mntH* mutant in THP-1 macrophages was severely diminished in comparison to the wild-type strain. We also could show that the *mntH* gene is important for the coexistence of GBS with acidogenic lactobacilli and in the ability of GBS to attach to host extracellular matrix proteins.
**Conclusion:** Our investigation identifies MntH as an important colonization determinant for GBS in the female vagina. It helps bacteria to adapt the harsh acidic environments and seems to play an important role in oxidative stress resistance and virulence.

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**115/MPP**

**EPEC secreted protein B (EspB) induces cell death in macrophages**

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Diarrhoea is the second leading cause of death in children and is often caused by Enteropathogenic *Escherichia coli* (EPEC). These bacteria subvert signaling pathways of the targeted host cells. To deliver effector molecules, EPEC express a Type Three Secretion System, a syringe-like nanomachine that consists of a basal body, a filament, and two pore forming proteins in the host cell membrane, EspB and EspD. Interestingly, EspB was found recently in the host cell cytosol where it interacts with myosins to contribute to the attaching and effacing phenotype. In addition, the protein was found in the bacterial supernatant where no function has been described so far. Here we show that recombinantly expressed and purified EspB (rEspB), which corresponds to secreted EspB, induces cell death in monocytes and macrophages.
To investigate the role of secreted EspB in infection, monocytic and epithelial cell lines were incubated with rEspB and examined by cytotoxicity assays, live-cell imaging, confocal microscopy, cell fractionations, and flow cytometry. Using these techniques, we showed that rEspB is a self-delivering, autopenetrating effector in several eukaryotic cell lines. Furthermore, rEspB exerted a cytotoxic effect specifically towards monocytic THP-1 cells as well as primary monocyte-derived macrophages. rEspB treated THP-1 cells developed a necrotic phenotype, including membrane and nuclear swelling. The treatment of THP-1 cells with rEspB further led to a disfunction of mitochondria. An inhibitor study revealed that cytotoxicity is exerted via a member of the necrotic signaling pathway and including a caspase. When THP-1 cells were differentiated to macrophages, reduced amounts of radical oxygen species were produced, indicating that rEspB drastically damages THP-1 cells.
We conclude that EspB is not only part of the T3SS arsenal but also exhibits cytotoxic properties towards macrophages most likely to reduce the immune response during infection in the gut.

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**116/MPP**

**Phobalysin, a new small b-pore forming toxin from *P. damselae* subsp. *damselae***

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*Photobacterium damselae* subsp. *damselae* (*Pdd*) is an autochthonous member of aquatic ecosystems. It is an important pathogen of marine animals, which may also cause a hyper-aggressive variant of necrotizing fasciitis in humans. Most of the reported infections in humans have their primary origin in wounds exposed to salt- or brackish water, inflicted, for instance, during handling of fish. Due to ocean warming, *Pdd* and related organisms, like *Vibrio vulnificus,* are globally spreading and the number of case reports is steadily increasing. Initially, we found that highly hemolytic strains of *Pdd* are more virulent. Further, it was found that they bear pPHDD1, a transferable plasmid encoding damselysin, previously shown to function as a phospholipase D. This led to the identification of a novel pore forming toxin which

we termed phobalysin P (PhlyP), for "photobacterial hemolysin encoded on a plasmid". In co-culture experiments, *Pdd* led to rapid permeabilization of epithelial cells, which could be largely attributed to PhlyP. PhlyP formed stable oligomers and small membrane pores. It caused massive efflux of $K^+$, no significant leakage of lactate dehydrogenase but entry of vital dyes. Attack by PhlyP provoked rapid loss of cellular ATP, attenuated translation, and provoked profound morphological changes in epithelial cells. Unexpectedly, hemolysins promoted the association of both Gram-positive and -negative bacteria with target cells. Use of cellular inhibitors and bacterial strains deficient in pili-formation indicated the involvement of an active, cell-dependent mechanism that is super-imposed on conventional adhesion. Similar observations with other hemolysins, target cells and bacteria suggest that this represents a common effect of membrane damaging toxins.

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**117/MPP**

***Mycobacterium abscessus*, an emerging pathogen in cystic fibrosis patients, degrades the *Pseudomonas* quinolone signal**

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**Introduction**: The opportunistic pathogen *Pseudomonas aeruginosa* is one of the major causes of nosocomial infections, and colonizes the lungs of cystic fibrosis patients. It regulates its virulence via a complex quorum sensing (QS) network including *N*-acylhomoserine lactones and the alkylquinolones (AQs) 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal, PQS) and 2-heptyl-4(1*H*)-quinolone (HHQ) as signal molecules [1].
A PQS cleaving enzyme from *Arthrobacter* sp. Rue61a despite its low catalytic efficiency, nevertheless reduces the production of key virulence factors when added exogenously to cultures of *P. aeruginosa* [2].
With *Rhodococcus erythropolis* BG43, the first strain able to degrade both PQS and HHQ, as well as the respiratory electron transport inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO), was isolated recently [3]. Homologues of its genes involved in AQ degradation are conserved in many *Mycobacterium abscessus* strains. *M. abscessus* is an emerging pathogen causing pseudotuberculous lung disease in patients with cystic fibrosis [4].
**Objectives**: Assessing the potential of an emerging cystic fibrosis pathogen to interfere with QS and virulence of *P. aeruginosa.*
**Methods**: Biotransformation assays, heterologous expression of candidate genes, HPLC analyses.
**Results**: Desalted cell free extracts of *M. abscessus* DSM 44196 are able to rapidly degrade PQS and HHQ. PQS conversion does not require cosubstrates, whereas conversion of HHQ takes place in the presence of NADH. Additionally, the cell-free extracts of *M. abscessus* supplemented with NADH are able to convert the antibiotic HQNO synthesized by *P. aeruginosa* into PQS, transiently forming an intermediate with the mass of a hydroxylated form of HQNO.
Expression of candidate genes in *E. coli* revealed the following pathway for degradation of the QS signal molecules HHQ and PQS: HHQ is first oxidized to PQS by the monooxygenase AqdB in an NADH dependent reaction. Then cleavage of PQS is catalyzed by the dioxygenase AqdC. The resulting *N*-octanoylanthranilic acid is hydrolyzed into octanoate and anthranilic acid.
**Conclusion**: Our findings tentatively suggest that *M. abscessus* strains due to their ability to inactivate the AQ signals PQS and HHQ may have the potential to interfere with *P. aeruginosa* QS and hence reduce the synthesis of virulence factors.

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**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**118/MPP**

**Mechanism of inhibition of TNF-mediated apoptosis by *Chlamydia trachomatis***

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*C. trachomatis* is an obligate intracellular bacterial pathogen that is recognized as the leading cause of bacterial sexually transmitted disease and preventable blindness worldwide. It has an intracellular biphasic life cycle, in which the elementary body (EB), the infectious form, develops into the replicative form, called the reticulate body (RB), upon uptake into human host cells. Replication occurs within a specialized vacuole known as the inclusion. It is well accepted that *C. trachomatis* can inhibit apoptosis induced by extrinsic stimuli including tumor necrosis factor (TNF). However, the question about the precise molecular mechanism remains unanswered and in part controversial. Here we investigate the molecular mechanisms of apoptosis inhibition by *C. trachomatis* studying TNF-mediated apoptosis in infected human epithelial cells.
It is well accepted that the binding of TNF to the TNF receptor-1 (TNFR1) induces the oligomerization of receptor complexes and the recruitment of TRADD. Additionally, RIPK1, TRAF2 and IAPs associate and form the so-called complex 1, resulting in ubiquitination of RIPK1 and activation of the NF-kB survival pathway. Internalisation of TNFR1 can be linked to the formation of a death inducing signaling complex, known as complex II, which includes FADD and caspase-8. Activation of caspase-8 results in the activation of caspase 3 and 7 and, ultimately, in apoptosis.
HeLa cells infected with *C. trachomatis* were resistant to TNF-mediated apoptosis. This inhibition was seen even when IAP-function was blocked, either by knock-down of individual IAPs or by complete inhibition using a chemical inhibitor (Smac-mimetic). Signaling analysis determined that apoptosis-induction was blocked by *Chlamydia* upstream of the activation of caspase-8. In contrast, over-expression of FADD in infected HeLa cells, which leads to formation of the complex II, induced apoptosis that was not blocked by *Chlamydia*. Importantly, *Chlamydia*-infected cells showed a reduced internalization of TNFR1 upon ligand binding. Thus, *Chlamydia* specifically blocks the induction of apoptosis by TNF acting at an early step. This mechanism alters the response of *Chlamydia*-infected cells to the local immune reaction and may enhance the chance of survival of the bacteria.

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**119/MPP**

**Impact of two putative oligosaccharyltransferases on biofilm formation in *Vibrio cholerae***

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Recent studies link the O-glycosylation in bacteria to essential and beneficial roles such as protection of glycosylated proteins against proteolytic degradation, protective immunity, cell adhesion and motility and cell-cell interactions. *Vibrio cholerae* encodes two putative oligosaccharyltransferases involved in O-glycosylation (O-OTases), which are integral inner membrane proteins and contain conserved Wzy_C signature domain. For one of these enzymes O-OTase activity has been recently demonstrated in a heterologous expression system in *E. coli*, showing that it has a

relaxed glycan and target specificity. However, in *V. cholerae* the glycan substrate, target proteins as well as the physiological function of these enzymes remain unknown. Here we show that single or double deletion of these O-OTases alters initial attachment of bacterial cells to abiotic surface and results in enhanced biofilm formation of *V. cholerae*. We hypothesize that O-glycosylation might be a feedback mechanism controlling biofilm formation by reducing attachment efficiency. Indeed, our experiments indicate that the activity of the O-OTases affect the secretion of adhesive proteins in the biofilm matrix, which mediate cell-cell or cell-surface adhesion.

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**120/MPP**

**Proteolytic control of flagella motility and biofilm formation in *Vibrio cholerae***

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*V. cholerae*, known to be the causative agent of the severe secretory diarrheal disease cholera, is a natural inhabitant of the aquatic ecosystem. For colonization of the human host and biofilm formation, flagella dependent motility plays an important role. In example, recent studies have shown that motility of the bacteria is important for the penetration of the intestinal mucosal layer within the host. The synthesis of the polar flagellum is tightly regulated, whereas the late genes are regulated by the $\sigma^{28}$-factor (FliA). Interestingly, penetration through the mucus results in breakage of the flagellum resulting in secretion of the anti-$\sigma^{28}$-factor FlgM, which in turn allows liberation of FliA. We demonstrated that absence of FlgM allows FliA to bind the RNA polymerase but also gets accessible for proteolysis. Within this study, we identified the relevant cytosolic proteases for this posttranslational control step. Interestingly, further phenotypical characterization of the protease mutants revealed changes in biofilm formation and swarming behavior. Currently, we try to elucidate the impact of this so far overlooked posttranslational control mechanism via proteolysis for different steps along the *V. cholerae* lifecycle.

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**121/MPP**

**The metabolic gene *gbuA* is essential for inducing QS-regulated virulence factors at low population density and nutrient limitation in *Pseudomonas aeruginosa***

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The ubiquitous bacterium *P. aeruginosa* employs the same set of quorum sensing (QS)-regulated virulence factors for attacking organisms of different kingdoms like other microbes, plants, and humans, indicating that growth conditions, especially nutrient availability, rather than specific host factors are controlling QS in opportunistic infections. We investigate the QS-dependent expression of virulence factors with a model system consisting of a bacterial co-culture with *P. aeruginosa* and the opportunistic pathogen *Aeromonas hydrophila* using chitin as sole growth substrate [Jagmann et al. (2010). *Env Microbiol* 12: 1787-1802]. In this model system growth of *P. aeruginosa* is dependent on the QS-regulated parasitic exploitation of the chitinolytic properties of *A. hydrophila*. *P. aeruginosa* possesses three QS systems, two of which are mediated by N-acyl-homoserine lactones as signal molecules, and one of which is mediated by 2-alkyl-4(1H)-quinolones (AQs), mainly 2-heptyl-4-quinolone (HHQ) and 2-heptyl-3-hydroxy-4-quinolone (Pseudomonas Quinolone Signal: PQS). During parasitic growth with *A. hydrophila*, the QS-regulated virulence factor pyocyanin is crucial for growth of *P. aeruginosa*. The production of pyocyanin is thereby already induced at a low population density as a consequence of limited nutrient availability.

To identify genes that are involved in QS-regulated virulence factor production, transposon mutants of *P. aeruginosa* were screened in co-culture with *A. hydrophila* for a reduced production of pyocyanin.

We identified the gene *gbuA* (PA1421) that encodes a guanidinobutyrase (GbuA) catalyzing the conversion of 4-guanidinobutyrate (4-GB) to 4-aminobutyrate and urea and that is regulated by GbuR. *GbuA* and *gbuR* deletion mutants did not produce pyocyanin in co-cultures with *A. hydrophila* and showed reduced pyocyanin production in single cultures. Transcriptional promoter-*lacZ* fusions revealed that in a *gbuA* mutant transcription of the operon *pqsABCDE*, which is involved in the AQ QS system, and of two operons required for pyocyanin biosynthesis, *phzA1G1* and *phzA2G2*, were reduced during growth in both single and co-cultures. Addition of the QS signals HHQ and PQS, which are produced by PqsABCD, as well as overexpression of PqsE restored pyocyanin production in a *gbuA* mutant in co-culture. The effect of *gbuA* deletion on pyocyanin production in single cultures could be enhanced by the addition of 4-GB. These results suggest that the intracellular accumulation of 4-GB leads to a reduced transcription of genes of the AQ system and, thus, a reduced transcription of genes for pyocyanin biosynthesis.

The metabolic context of 4-GB formation and degradation is currently under investigation. Our results indicate that nutrient availability can dominate QS-dependent virulence factor production via a so-far unexplored metabolic pathway.

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**122/MPP**

**The TCS08 of *Streptococcus pneumoniae* regulates the expression of the adhesin PavB**

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**Introduction:** The human pathogen *Streptococcus pneumoniae* (pneumococci) possess 13 Two-Component Regulatory Systems (TCS), crucial for bacterial fitness and virulence. Traditionally, these systems consist of a sensor Histidine Kinase (HK) and an output, the Response Regulator (RR). The TCS08 encoding genes are located downstream of *pavB*, a gene encoding the matrix binding protein and adhesin PavB (PneumoccalAdherence andVirulence FactorB) (1). Hence, the interaction of the TCS08 proteins and its effect on the regulation of PavB and other pneumococcal surface proteins was evaluated in this work.

**Objectives:** To assess the impact of the TCS08, its HK and RR, respectively, on the expression of surface proteins with a special emphasis on PavB.

**Methods:** Mutants in *tcs08*, *rr08*, and *hk08* were generated by allelic replacement. Affinity chromatography was employed to purify MBP- or His-tagged HK08 and RR08. Phosphotransfer profiling and electrophoretic mobility shift assay (EMSA) were conducted to assess 1) the interaction between the recombinant HK08 and RR08, and 2) the RR08 and the promoter regions of *pavB* or other potential target genes.

**Results:** Immunoblot analysis indicated a dramatically increased expression of the PavB protein for the *hk*08-mutant, while the *rr08*- or the *tcs08*-mutant (deficient for RR08 and HK08) showed PavB levels similar to the wild-type. The phosphotransfer assays with HK08 and its cognate RR08 suggests autophosphorylation of RR08 and a phosphatase activity of HK08 resulting in dephosphorylated

RR08. The EMSA with purified RR08 and a *pavB* promoter DNA-fragment illustrated binding of non- and phosphorylated RR08 to the promoter region of *pavB*.
**Conclusion:** These data demonstrate that the TCS08 regulates *pavB*-gene expression. The results further suggest that non-phosphorylated RR08 functions as a repressor while the phosphorylated RR08 acts as an activator for the expression of PavB.

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**124/MPP**
**The *Acinetobacter baumannii* trimeric autotransporter adhesin Ata and its linkage to adhesion, invasion and pathogenicity**
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*Acinetobacter baumannii* is an emerging nosocomial pathogen causing difficult-to-treat, severe infections worldwide. However, very little is known about virulence factors of *A. baumannii*. We hypothesised, that the recently identified trimeric *Acinetobacter baumannii* autotransporter adhesin (Ata) is an important virulence factor and therefore investigated Ata-mediated adhesion to different targets, invasion into host cells and virulence *in vitro* and *in vivo*.
*A. baumannii* ATCC 19606 and ATCC 17978 (WT) and respective isogenic *ata* deletion mutants (D*ata*) were generated and analysed in infection assays. In the *Galleria mellonella in vivo* infection model, 10% of WT *A. baumannii* ($10^5$ CFU) infected caterpillars survived 24 h post infection, whereas 50% of infected larvae with Δ*ata* ($10^5$ CFU) were still alive after 5 days. To monitor Ata-mediated virulence on human cells, LDH release of infected HUVECs were determined after 24 h. Here, 38% of WT and 22% of D*ata* infected HUVECs showed cell death, respectively.
Given the fact that adhesion is an early and crucial step during infection, binding to extracellular matrix proteins und human cells was investigated. Bacterial adhesion was quantified by fluorescence microscopy and densimetric image analysis, determining relative fluorescence units of CD31-Alexa647 conjugated primary human endothelial cells (HUVEC) and CFSE stained bacteria, as well as by amplifying bacteria specific genes to calculate genomic equivalents using qRT-PCR. Deletion of *ata* led to a statistical significant decreased adhesion (up to seven-fold) to collagen and laminin as well as HUVECs under static conditions. To analyse adhesion under blood stream infection conditions, HUVECs were infected with *A. baumannii* in laminar flow chambers under shear stress. Compared to static experiments, differences of WT and D*ata* adhesion were similar, confirming the previous results, but total binding rates decreased five-fold. However, when employing an *ex vivo* dynamic infection model using human umbilical cord veins, bacterial binding rates were six-fold higher in dynamic infected umbilical cord veins compared to the flow chamber model. Invasion into HUVECs was analysed *in vitro* by determination of intracellular bacteria after gentamycin treatment. Interestingly and in contrast to the adhesion assays, invasion into HUVECs was not significantly different between WT and D*ata*.
Our results indicate that Ata mediates adhesion to ECMs and human host cells *in vitro* and in the *ex vivo* umbilical cord vein infection model under dynamic flow conditions. Accordingly, virulence of *A. baumannii in vitro* and *in vivo w*as dependent on Ata. Thus, Ata critically regulates virulence in *A. baumannii* and might represent an attractive target for new therapy approaches in the future.

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**125/MPP**
**UafA of *Staphylococcus saprophyticus* is a glycosylated protein**
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**Introduction:** *Staphylococcus saprophyticus* is a gram-positive and coagulase-negative pathogenic staphylococcus causing urinary tract infections in young women. Some of its surface proteins have been characterised in the recent past. These include the uro-adherence factor A (UafA) which shows binding to uro-epithelial cells. We found out *S. saprophyticus* strains 7108 and ATCC 15305 bind to fibronectin and additionally to smooth surfaces like glass and plastic when UafA is expressed on the cell surface. Now we wanted to find out if UafA is a glycosylated surface proteins.
**Methods**: In the wild type strains of *S. saprophyticus* two genes for a glycosylation machinery were located, gtfA and gftB. We replaced a part of gtfA with an ermB-cassette. A protein preparation of the wild type, the uafA knock out and the gtfA knock out was made.
The overnight cultures were incubated at 37 °C. The bacteria pellets were washed and resuspended in PBS buffer. Lysostaphin, DNase and AEBSF were given to the samples and they were incubated for 4 h at 37 °C. The samples were centrifuged and the supernatant was used for an SDS gel electrophoresis. The gel was stained using the GLYCOPRO staining kit by Sigmal-Aldrich according to the protocol.
**Results**: The protein samples of *S. saprophyticus* 7108 wild type, 7108 gtfA knock out, 7108 sdrI knock out and 7108 uafA knock out were separated on a SDS gel. Then the gel could be stained with the GLYCOPRO kit. In the wild type and the ΔsdrI lane a band with a size more than 260 kDa could be stained. In contrast there was no visible band in the lane of ΔuafA and ΔgtfA.
**Discussion and outlook**: UafA seems to be a glycosylated protein and in a gtfA knock out either UafA is not glycosylated or this protein is even not expressed on the bacterial cell surface. Further should be investigated if the glycosylation is necessary for the binding of UafA to glass and plastic.

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**126/MPP**
**Thymidine-auxotrophic *Staphylococcus aureus* small colony variants: *In-vivo* mutation of *drm* (phosphopentomutase) promotes growth under low-thymidine and thymine conditions**
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Thymidine-dependent small colony variants (TD-SCVs) of *Staphylococcus aureus* are associated with chronic and recurrent infections. Using comparative whole genome sequencing of a clinical *S. aureus* isolate and its corresponding TD-SCV, we here, in addition to a mutation in *thyA*, identified a so far unrecognized non-synonymous mutation in *drm*, encoding for phosphopentomutase of the pentose phosphate cycle. The mutated *drm* allele enhanced growth of TD-SCVs at low external thymidine concentrations, presumably by increasing the pool of deoxyribose-1-phosphate, feeding thymidine salvage pathways. Thus, our finding provides evidence for a novel strategy of TD-SCVs to compensate for the loss of thymidilate synthase function.

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**127/MPP**

**Interplay of global regulators of *Staphylococcus aureus* during the chronic osteomyelitis**

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*Staphylococcus aureus* is a major human pathogen that can cause chronic and difficult-to-treat infections. *S. aureus* persistence in host tissue is linked to the bacterial ability to change to small colony variants (SCVs), which are adapted phenotypes for long-term intracellular persistence. The switching process between the aggressive wild-type phenotypes and persisting SCVs is very dynamic, but the underlying mechanisms are largely unknown. Our recent data from cell culture experiments indicated that regulatory mechanisms are involved in this dynamic switching process that involve downregulation of the quorum-sensing system *agr* and upregulation of the stress-related transcription factor SigB during bacterial persistence (Tuchscherr L et al. PLoS Pathogens 2015). In this work we investigated, whether these dynamic adaptation processes can be measured in vivo, as well. We have established a hematogenous murine osteomyelitis model with the *S. aureus* strain 6850 that develops to chronicity over the course of 2 month and closely resembles the human disease (Horst S et al. Am J Pathol 2012). Firstly, we tested 6 other *S. aureus* strains in this model and identified two clinical isolates that caused chronic osteomyelitis with bone deformation such as strain 6850, whereas the other strains tested also persisted in bone tissue without causing deformation processes. During persistence all tested *S. aureus* strains developed SCVs. Further on, we analysed the role of the global *S. aureus* regulators Agr and SigB in bacteria recovered from bone tissues of mice during the infection process. With all trains tested we found that *agr* is silenced and that the SigB-system is highly upregulated during persistence. These results could be reproduced with *S. aureus* isolates recovered from patients` specimens of chronic osteomyelitis Our findings clearly demonstrate that silencing of *agr* and high *sigB* expression is a common feature of chronic infection processes.

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**128/MPP**

**YadA-dependent interaction of *Yersinia enterocolitica* with vitronectin**

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One of the major virulence determinants of *Yersinia enterocolitica* (Ye) is the Yersinia adhesin A (YadA). YadA is the prototype of trimeric autotransporter adhesins (TAA) and has multiple functions such as mediating adhesion to host cells as well as extracellular matrix components and soluble serum factors. It is known that YadA mediates serum resistance by direct interaction with the complement regulatory factors (CRFs) factor H, C4bp und C3 (reviewed in 1).Here we show that Ye can also bind the serum glycoprotein vitronectin (Vn) and that this binding depends on the serotype of Ye and more specifically on the presence of a short stretch within the head domain of YadA. We also found that binding of Vn to YadA leads to a reduction of YadA-mediated adhesion to epithelial cells and consequently to a reduction of type-three-secretion mediated translocation of effector proteins into host cells. Additionally, upon binding of Vn we observed a reduction of terminal complement complex formation on the surface of Ye. In a systemic mouse infection model, Vn-deficiency resulted in reduced susceptibility to *Yersinia* infection with reduced bacterial counts in the spleen. Our findings demonstrate that the interaction of Ye with Vn affects multiple functions of YadA and that subtle differences within YadA may determine the entire protein interaction network of Ye and thus Ye virulence.

Reference

1. Muehlenkamp M. et al., *IJMM*, **2015**, 305(2):252-258

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**129/MPP**

**Organ microbiology using human umbilical cords allows to analyse adherence of *Bartonella henselae* and *Acinetobacter baumannii ex vivo***

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Adhesion to host cells is the first step in interaction of pathogenic bacteria with their respective hosts. Experimental approaches elucidating bacterial adhesion have been performed in the past mainly by using methods of cellular microbiology. However, *in vitro* infections of cell monolayers reflect the *in vivo* situation only partially. The *ex vivo* infection of human organ systems might represent an attractive method to overcome these methodical limitations.
We infected fresh human umbilical cords with *Bartonella henselae* or *Acinetobacter baumannii* under dynamic flow conditions mimicking the *in vivo* infection situation of human endothelium. For this purpose, an *ex vivo*-human vessel infection assay has been set up. Methods for quantifying endothelium-adherent wildtype and adhesin (*Bartonella* adhesin BadA, *Acinetobacter* adhesin Ata) deficient bacteria have been established by (i) determining the ratio of relative fluorescence units of CFSE-labelled bacteria versus anti-CD31-Alexa647 stained endothelial cells, (ii) real time PCRs allowing to calculate genome equivalents of bacteria and host cells (*B. henselae*, *glyA*; *A. baumannii*, *rpob*; human cells, *hmbs*) and (iii) fluorescence microscopy. Bacterial adherence strictly correlated with the expression of BadA or Ata, respectively, and adherence rates were ~2-fold higher when infecting human umbilical cords compared with cell-culture based infection models. Our organ infection model allows to dissect the biological function of trimeric autotransporter adhesins (BadA, Ata) in the natural course of human infections and might open the door to new infection models using complete human organs instead of cell culture infection models ("organ microbiology").

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**130/MPP**

**Set up and MR-PET imaging of a *S. aureus* vascular graft infection model in mice**

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*Staphylococcus aureus* readily binds to the surface of implanted devices forming thick biofilm layers, this causes rare but serious vascular graft infections. These polymer-associated infections are difficult to detect and very tough to eradicate with antibiotics. These infections usually result in the removal of the foreign material and are associated with a high morbidity and mortality. Thus, it is essential to have a representative *in vivo* model, which closely mimics the situation in patients, in order to study the underlying mechanisms of these infections and find better detection methods in order to treat the patient as fast as possible.
The aim of this study was to create a mouse model to realistically study vascular graft infections and to combine this with MRI and PET imaging to allow early detection and non-invasive follow-up of the biofilm formation.
A Teflon catheter was inserted into the right carotid artery of mice to act as a vascular graft; subsequently flow velocities in the carotid arteries and inflammation were measured. Mice were infected intravenously via the tail vein with *S. aureus*. 10 days after infection flow velocities in the carotid arteries and inflammation were measured again and 14 days post infection mice were sacrificed and organs were analyzed for bacterial load. Additionally, 26 clinical *S. aureus* strains from patients with vascular infections and 33 *S. aureus* strains collected from nasal swabs were tested for their capacity to form biofilm *in vitro*. A few strong and weak biofilm producers were selected to analyze their biofilm formation *in vivo*.
This murine model showed that after infection with a high dose of *S. aureus*, the mice develop a sepsis followed by biofilm formation on the catheter; this was confirmed using electron microscopy. Analysis of the different strains showed that the ability to form biofilm *in vivo* was unrelated to the capacity to form biofilm *in vitro* and unrelated to the level of infection they caused in the mice. MRI imaging showed that the flow velocity in the right carotid artery is strongly reduced after infection and MR-PET revealed that there is a high level of inflammation specific to the site of the catheter after infection. Combining these two imaging modalities could be a valuable tool for the early detection and non-invasive follow-up of vascular graft infections.

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**131/MPP**

**Analysis of the interaction of *Bartonella* adhesin A with fibronectin domains and endothelial cells**

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*Bartonella henselae* causes cat scratch disease and vasculoproliferative disorders in humans. Expression of *Bartonella* adhesin A (BadA) is crucial for bacterial autoagglutination, adhesion to host cells, binding to extracellular matrix proteins and proangiogenic reprogramming of infected host cells. BadA belongs to the class of trimeric autotransporter adhesins (TAAs) and is modularly constructed consisting of a head, a long and repetitive neck-stalk and a membrane anchor. The head region seems to be crucially involved in binding to endothelial cells whereas the stalk region might play a decisive role in binding to extracellular matrix proteins such as fibronectin. Here, we analysed the interaction of the BadA stalk with fibronectin in greater detail. For this purpose, fibronectin-fragments were produced by enzymatic digestion of human fibronectin and BadA-binding peptides were detected by immunoblotting. We found that a 50 kDa fragment of fibronectin bound specifically to BadA; the amino acid sequence of this protein fragment, however, has still to be determined. The 50 kDa fragment also enhanced the binding of *B. henselae* to the surface of human endothelial cells significantly (~2 fold) and, therefore, probably mediated the interaction of *B. henselae* with endothelial cells via BadA bridging. Infection studies using BadA deletion mutants and binding analysis using recombinantly expressed neck-stalk elements may identify the exact BadA fibronectin binding motive.

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**132/MPP**

**Staphylococcus aureus long-term persistence in cystic fibrosis airways is characterized by increased expression of SodM and iron transporters and down-regulation of sugar acquisition proteins**

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The airways of cystic fibrosis (CF) patients exhibit high amounts of viscous mucous that impede ciliary clearance and facilitate chronic bacterial infection. *Staphylococcus aureus* is one of the earliest pathogens that colonizes this niche. Even after several years, the same *S. aureus* clone can be isolated from the airways of an individual patient. Using clonal first and late *S. aureus* isolates from CF patients, determined by multilocus sequence typing, *spa*-typing and pulsed-field gel electrophoresis, we studied adaptation mechanisms present after long-term persistence. One strain pair was chosen for analysis of cytoplasmic and cell-surface associated proteome using 2D-gel electrophoresis and a gel-free approach, respectively. Five additional strain pairs were used to verify proteomic data by qRT-PCR and functional assays.
The proteomic analysis revealed multiple changes in protein abundance. 27 intracellular identified proteins and 48 out of 253 cell-surface associated proteins were expressed differently comparing both isolates. Interestingly, one of the two staphylococcal superoxide dismutases, SodM, was more abundant in the late isolate. In addition, three proteins of the sugar uptake system (FruA, TreP, CcpA) were lower abundant, whereas four proteins involved in iron acquisition (IsdA, IsdE, SirA, Fur) were higher abundant in the long-persisting clone. qRT-PCR confirmed the respective gene expression pattern for both isolates. Examining five additional strain pairs, sugar and iron up-take genes were similar expressed in many but not all traits. However, the up-regulation of *sodM* could be detected in four other late isolates. On functional level, higher expression of SodM as well as higher abundance of iron acquisition proteins was verified for the deeper analyzed isolates.
As the CF airways are characterized by high amounts of oxidative stress generated partially by neutrophils, up-regulation of the oxidative stress defense protein SodM seems to be beneficial for bacterial long-term persistence. Lower abundance of sugar uptake proteins, but higher expression of iron acquisition genes might represent adaption to the sugar-rich, but iron-restricted

environment of the CF airways. In summary, our project provides insights into several putative adaptation mechanisms that facilitate the long-term persistence of *S. aureus* in the hostile environment of CF airways.

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**133/MPP**

**The α-hydroxyketone LAI-1 regulates LqsS- and LqsT-dependent phosphorylation signaling and gene expression of *Legionella pneumophila***

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The environmental bacterium *Legionella pneumophila* is the causative agent of Legionnaires´ disease, a life-threatening pneumonia. For cell-cell communication the facultative intracellular bacteria employ the autoinducer molecule LAI-1 (3-hydroxypentadecane-4-one). LAI-1 is produced and detected by the Lqs (*Legionella* quorum sensing) system, comprising the autoinducer synthase LqsA, the sensor kinase LqsS and the response regulator LqsR (all encoded in the *lqs* cluster), as well as the "orphan" LqsS homologue LqsT [(1)]. Lqs-regulated processes include pathogen-host cell interactions, production of extracellular filaments and natural competence. LqsS and LqsT are autophosphorylated by [γ-$^{32}$P]-ATP, and phosphorylation signaling through the sensor kinases converges on LqsR [(2)].
Further biochemical analysis revealed that LAI-1 decreases the rate and level of LqsS/LqsT autophosphorylation in a dose-dependent manner. Enantio-pure (*S*)- or (*R*)-LAI-1, as well as the putative LAI-1 precursors, (*S*)- or (*R*)-amino-LAI-1, reduced phosphorylation of both LqsS and LqsT. In contrast, (*S*)- or (*R*)-CAI-1 (3-hydroxytridecane-4-one; cholera autoinducer-1), as well as (*S*)- or (*R*)-amino-CAI-1 increased phosphorylation of LqsS, while the compounds reduced autophosphorylation of LqsT. LAI-1 did neither affect the stability of phospho-LqsS or phospho-LqsT, nor the dephosphorylation of phospho-LqsS or phospho-LqsT by LqsR. Finally, a transcriptome analysis revealed that LAI-1 regulates components of the *rsmYZ*-dependent gene expression pathway in *L. pneumophila*.

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**134/MPP**

**Title: Role of HIF-1α in Infections with *Streptococcus pneumoniae* at the Blood-Brain Barrier**

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The blood-brain barrier (BBB) is a physiological barrier formed by endothelial cells (ECs), supported by astrocytes and pericytes of the brain microvasculature, which protects the brain from circulating toxins and pathogens yet transporting essential nutrients such as glucose and iron. In brain infections such as meningitis circulating pathogens gain access to the central nervous system by breaching the BBB and infecting the brain parenchyma. Mechanisms underlying the transfer of meningeal pathogens across the BBB are still poorly understood. Based on our previous reports showing a general activation of hypoxia inducible factor (HIF)-1α in bacterial infections, we hypothesized that the activation of HIF-1α leading to secretion of vascular endothelial growth factor (VEGF), is involved in the invasion process of pathogens across the BBB. To test our hypothesis, we performed pneumococcal infections of brain ECs in vitro in and obtained permeability of the endothelial monolayers to dextrans of various molecular sizes and to pathogens. We further performed confocal immunofluorescence analysis to visualize the localization of the S. pneumoniae on ECs followed by expression analysis of several components of EC cell junctions (claudins, occludins) by qRT-PCR and Western blotting. Our results demonstrate an increase in paracellular permeability of endothelial monolayers to dextrans of various sizes. Correspondingly, bacterial counts confirmed migration of bacteria across such monolayers. Immunofluorescence analysis showed localization of pneumococci predominantly at the cell-cell junctions, qRT-PCR analysis showed an upregulation of HIF-1α and VEGF. Western blot analysis showed a downregualtion of VE-cadherin, critical adherens junctions member and also upregulation of HIF-1α upon infection in vitro. These results suggest a paracellular route for pneumococcal transmigration potentially involving HIF-1 activation in ECs. Our results from oxygen consumption kinetics analysis showed that S. pneumoniae infection leads to rapid reduction of oxygen concentration in ECs in cell culture. Furthermore, in vivo analysis of human and murine brain tissue samples also showed upregulation of HIF-1α in the brain vessels in meningeal infections indicating HIF-1α as a potential mediator of vessel permeability. To determine the functional role of HIF-1α in the transfer of pneumococcal pathogens, we performed loss-of-function experiments using echinomycin, a potent HIF-1α inhibitor. Our results indicate a protective effect of echinomycin on vessel permeability upon infection, which were confirmed by siRNA knockdown of HIF-1α in human/murine brain endothelial cells. Expression for cell-junction-associated proteins and monitoring of the endothelial electrical resistance using CellZscope is currently underway in addition to permeability studies in vivo upon infection in EC-specific HIF-1 knock-out mice.

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**135/MPP**

**Role of virulence factors, immune system and microbiota derived colonization resistance in *Yersinia enterocolitica* infection**

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*Yersinia enterocolitica* (*Ye*) expresses a number of virulence factors like the adhesin Yad A and a type-III-secretion system that both contribute to effective colonization, invasion and abscess formation in lymphoid tissues after orogastral infection.The gastrointestinal (GI) tract harbors a dense and complex microbial community which is important for the maturation of the host immune system and may confer colonization resistance (CR) against enteric pathogens like *Ye*.
The aim of our project is to investigate the trilateral interactions between *Ye*, intestinal microbiota and host immune response. We want to shed light on the alterations of microbial composition in the murine GI tract during *Ye* infection and the consequences for further pathogen colonization, overgrowth of certain bacterial species and the shaping of host immune response. Furthermore, we want to find out which constituents of the commensal microbiota or metabolites contribute to CR against *Ye* and to evaluate the role of *Ye* virulence and fitness factors in this interplay. In first experiments we could show that mutant strains of *Ye*, lacking certain virulence factors, are unable to establish intestinal colonization in the presence of a commensal microbiota, but are highly virulent in germfree (GF) mice. It is not clear whether these effects are due to direct interactions with intestinal commensals or due to host inflammatory response. Co-infection experiments via the orogastric route using 1:1 mixtures of *Ye* wildtype and mutant strains will allow us to investigate the role of virulence factors in the presence of the commensal microbiota and under GF conditions. Microbiome and metagenome analyses, followed by computation of statistical correlations between present taxa will enable us to predict possible relations and interactions between *Ye* and commensal bacteria. Furthermore, we will try to shed light on the involved immune mechanisms by performing oral infection experiments with normally colonized or GF Myd88 knockout mice.

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**136/MPP**

**Role of proteins of the OMP biogenesis machinery for virulence of *Yersinia enterocolitica***

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The trimeric autotransporter adhesin Yersinia adhesin A (YadA) and the type Ve autotransporter Invasin (Inv) are important pathogenicity factors of the human pathogen *Yersinia enterocolitica* (*Ye*). During infection YadA and Inv are mediating the binding to host cells which is then followed by the translocation of Yersinia outer proteins (Yops) via a type III secretion system. The biogenesis of the outer membrane proteins (OMP) YadA and Inv depends on the β-barrel assembly machinery (BAM) (1, 2). The BAM-complex consists of the essential proteins BamA and BamD and the non-essential proteins BamB, BamC and BamE. The unfolded OMP are guided through the periplasm to the BAM-complex with the help of the periplasmic chaperones DegP, Skp and SurA. The BAM-complex then inserts the OMP into the lipid bilayer (3).
The aim of this study is to find out, how factors involved in OMP biogenesis contribute to *Ye* virulence. We will therefore investigate if the deletion of the non-essential proteins BamB, BamC, BamE and of the periplasmic chaperones DegP, Skp and SurA affect *Ye* growth behaviour, cell morphology, outer membrane integrity and composition, virulence and adhesion and invasion to host cells.

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**137/MPP**

**Post invasion events after infection with *Staphylococcus aureus* are strongly dependent on the host cell type**

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Host cell invasion and intracellular persistence are major features of *Staphylococcus aureus*. Recent work has demonstrated that the host cell invasion of non-professional phagocytes essentially contributes to infection development. After invasion the intracellular metabolically active bacteria can induce host cell activation and death but they can also persist for long time periods, which might be a reservoir for persistent infections. All infection and post invasion-effects are dependent on the interplay between different factors from the bacterial and host cell side. In this study we performed a comparative analysis of different well-characterized *S. aureus* strains in their interaction with a wide variety of host cell types.
We compared *S. aureus* invasion in different types of human cells, including lung epithelial cells, endothelial cells, keratinocytes, fibroblasts and osteoblasts by infecting them with different *S. aureus* strains. After removing all extracellular staphylococci the number of intracellular bacteria was determined, cell inflammation was measured by RT-PCR, and cell death was analyzed by propidium iodide staining. Using A549 cells, cytoplasmically expressing yellow fluorescent protein fused to cell wall-targeting domain, phagosomal escape of bacteria was investigated. Proteomic analysis was performed on all strains to explain differences in bacterial invasion and cytotoxicity.
Endothelial and epithelial cells showed the highest capacity to take up bacteria. Big differences were notable between primary human osteoblasts and the osteoblast cell line. Cytotoxicity following host cell invasion was strongly strain and host cell dependent. Cytotoxic strains showed a high expression of alpha-toxin. All bacterial strains but Cowan1 were able to escape into the cytoplasm, fitting with the observed virulence of these strains. A fast inflammatory reaction was detectable in all host cells. Primary human osteoblasts showed the highest signal which remained high over days. Although there were cell type specific differences in host cell invasion, inflammation and cytotoxicity, *S. aureus* was able to persist in all cell types up to 7 days.
The results from all cell culture experiments were entirely dependent on the chosen *S. aureus* strain and host cell type. Importantly the immortalized cell lines did not sufficiently reflect postinvasion and postinfection events as seen in primary cells. Therefore, cell line based *in vitro* infection models should always be critically analysed. Despite all cell types being able of degrading intracellular bacteria *S. aureus* always managed to resist degradation and persist intracellularly at low numbers. This indicates that this is an important infection strategy for *S. aureus*.

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**138/MPP**

**Analysis of the assembly of bacterial type III secretion systems**

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**Introduction**: Many pathogenic gram-negative bacteria use type III secretion systems (T3SS) to secrete effector proteins into target host cells. These proteins are able to modulate host immune responses or can lead to the uptake of bacteria into non phagocytic cells and by this lead to severe infections and cause enteric diseases. T3SS are composed of over 20 different proteins and build a membrane spanning multi-megadalton complex. Although progress concerning the structure and composition of T3SS is ongoing and gives deeper and deeper insights into the mechanism of action, the question how these systems assemble and thereby enable a functional secretion remains unclear.
**Aim**: In this project, the assembly pathway of *Salmonella enterica* serovar Typhimurium SPI-1 T3SS is examined in detail.
Material and Methods:
We use a plasmid based *in vivo* photo-crosslinking system to find signature crosslinks for several protein-protein interactions between different T3SS components. In this method, the synthetic amino acid para-Benzoyl-phenylalanine (pBpa) is incorporated at specific positions of the target protein. After UV irradiation the benzophenon group of pBpa reacts to nearby C-H bonds and thereby links interacting proteins covalently. Interaction partners are identified by Western blot or mass spectrometry. The presence of signature crosslinks is tested in different genetic backgrounds, to assess the dependence of a specific interaction on the presence of other T3SS components.
**Results**: Preliminary results show different crosslinks for several proteins of the T3SS. For the export apparatus component SpaP we were able to proof the presence of multimers inside of the complex. Furthermore we could show that these SpaP-SpaP interactions are independent of all other T3SS components and thereby proof the central role of SpaP in T3SS assembly.
Summary:
We have established an *in vivo* photocrosslinking-based approach to study the details of T3SS assembly at native conditions.

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**139/MPP**

**Reprogramming of Myeloid Angiogenic Cells by *Bartonella henselae* leads to microenvironmental regulation of pathological angiogenesis**

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The contribution of myeloid cells to tumor microenvironments is a decisive factor in cancer progression. Tumor associated macrophages (TAMs) mediate tumor invasion and angiogenesis through matrix re-modeling, immune modulation and release of pro-angiogenic cytokines. Nothing is known about how pathogenic bacteria affect myeloid cells in these processes. Here we show that *Bartonella henselae*, a bacterial pathogen causing vasculoproliferative diseases (bacillary angiomatosis) reprograms human Myeloid Angiogenic Cells (MACs), a pro-angiogenic subset of circulating progenitor cells, towards a TAM-like phenotype with increased pro-angiogenic capacity. *B. henselae* infection resulted in inhibition of cell death, activation of angiogenic cellular programs and induction of M2 macrophage polarization. MACs infected with *B. henselae* incorporated into endothelial sprouts and increased angiogenic growth. Infected MACs developed a vascular mimicry phenotype *in vitro* and expression of *B. henselae* adhesin A (BadA) was essential in inducing these angiogenic effects. Secretome analysis revealed that increased pro-angiogenic activities were associated with the creation of a tumor-like microenvironment dominated by angiogenic-inflammatory cytokines and matrix re-modeling compounds. Our results demonstrate that manipulation of myeloid cells by pathogenic bacteria can contribute to microenvironmental regulation of pathological tissue growth and suggest parallels underlying both bacterial infections and cancer.

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**140/MPP**

**Characterization of different capsular polysaccharide synthesis clusters in *Staphylococcus saprophyticus***

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Among staphylococci *Staphylococcus saprophyticus* is the only species that is typically uropathogenic and an important cause of urinary tract infections in young women. It was previously shown that the polysaccharide capsule of *S. saprophyticus* reduces adherence to human uroepithelial cells and mediates resistance to complement-mediated opsonophagocytic killing by human neutrophils. The capsule gene cluster of *S. saprophyticus* ATCC 15305 has a size of 13,467 bp and consists of 13 open reading frames lying on a staphylococcal cassette chromosome. The putative gene products show homology to proteins involved in synthesis, transport and polymerization of capsular polysaccharides in other staphylococci but also in distantly related bacteria. The capsular polysaccharide architecture of strain ATCC 15305 has been solved previously. Antibodies against this structure failed to

react with other strains of S. saprophyticus suggesting that more than one capsular serotype exists in this species.
We sequenced the genome of two encapsulated *S. saprophyticus* strains 9325 and Bo45 and found many differences in the capsule gene clusters in comparison to ATCC 15305. The capsule gene clusters of these three strains only share sequence similarities to *cap*ABCD and *cap*M. The location of the cluster in the genome of 9325 is also different from Bo45 and ATCC 15305. The results support the hypothesis that capsular polysaccharides in *S. saprophyticus* are diverse and that several serotypes exist. A *cap*ABCD mutant of 9325 displayed a complete loss of encapsulation shown in Maneval's stain. The loss of encapsulation led to a strong increase of biofilm formation and binding properties to glass and plastic revealing a high impact of capsular polysaccharides on surface properties in *S. saprophyticus*.

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**141/MPP**

**The eukaryote-like Ser/Thr signaling system in chlamydiae**

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**Question:** Eukaryote-like Ser/Thr kinases and phosphatases are involved in the regulation of bacterial cell division, stress responses and cell wall biosynthesis. In *Chlamydiaceae*, a functional cell wall has not been detected but ring-like shaped peptidoglycan structures were found and ß-lactams block cell division. Analysis of the eukaryote-like Ser/Thr signaling system in *Chlamydiaceae* contributes to understand regulatory processes within the chlamydial lifecycle.
Main focus of this study is the characterization of three eukaryote-like Ser/Thr kinase homologs (PknD, Pkn1 and Pkn5) and one putative corresponding Ser/Thr phosphatase (Cpn0397) from *Chlamydia pneumoniae* and their regulatory functioning within chlamydial cell wall biosynthesis and cell division.
**Methods:** Native purifications of full-length kinases (including catalytic, transmembrane, and extracellular domains) and the phosphatase were performed via affinity chromatography. [$\gamma$ $^{33}$P]-ATP as well as D7 and staurosporine inhibitors were used in *in vitro* kinase assays. Kinase mutants were generated via site-directed mutagenesis. With the help of a Ser/Thr phosphatase assay system dephosphorylation activity of Cpn0397 was tested.
**Results:** Phosphorylation activity of full-length kinases PknD and Pkn1 was observed *in vitro* and first insights of their regulatory function within cell wall precursor biosynthesis were achieved. The secreted kinase Pkn5 showed no phosphorylation activity in line with a study on the *Chlamydia trachomatis* homolog. The corresponding phosphatase Cpn0397 was capable of dephosphorylating the synthetic phosphopeptide RRA(pT)VA *in vitro*.
**Conclusions:** Our results indicate a functional eukaryote-like Ser/Thr signaling system in chlamydiae, where Ser/Thr kinase activity might be associated with the regulation of chlamydial biosynthesis of cell wall precursors.

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**142/MPP**

**The zinc-metalloprotease ProA of *L. pneumophila* and its effects in human lung tissue infections**

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*Legionella pneumophila*, a Gram-negative, rod-shaped bacterium is the main causative agent of Legionnaire´s disease, a severe form of an atypical pneumonia. The bacteria replicate intracellularly in protozoa, and in human alveolar macrophages [Steinert *et al.*, 2002; Diederen, 2008]. The zinc-metalloprotease ProA, which is the main soluble extracellular protein, is a major virulence factor of *L. pneumophila*. The 38 kDa protein is secreted via the type-II secretion system and is homologous to other proteases like elastase of *Pseudomonas aeruginosa,* and thermolysin of *Bacillus proteolyticus* [Thompson *et al.*, 1981; Black *et al., 1990*]. As a key player of *L. pneumophila* virulence, it causes cytotoxic effects against eukaryotic cells, tissue necrosis after intradermal injection and acute pulmonary damage after intranasal inoculation [Conlan *et al.*, 1986; Williams *et al., 1987;* Rechnitzer *et al.*, 1989]. In order to further characterize the impact of ProA on pathogenesis, we performed systematic analyses using recombinantly produced ProA, and a ProA-deficient mutant. In an *ex-vivo* human lung tissue model we could show that deleting *proA* results in reduced tissue damage. Similarly, recombinant ProA caused serious disintegration of the tissue architecture. Interestingly, the ProA-negative mutant also had a replication defect within human lung tissue, but not within the macrophage-like cell line THP-1, confirming that it is important for the extracellular pathogenicity of wild type bacteria.

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**143/MPP**

**The NAIP-NLRC4 inflammasome activates caspase-1 in response to the T3SS needle protein of *Burkholderia pseudomallei***

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**Background:** The NAIP-NLRC4 inflammasome is critical for defence against bacterial infection and is activated in response to proteins from pathogens that reach the host cytosol. Whereas murine NAIP5/6 detects flagellin, NAIP1 and NAIP2 sense needle and rod components from bacterial type III secretion systems (T3SS), respectively. Activation of caspase-1 leads to the processing and secretion of IL-1β and IL-18 and is responsible for the induction of pyroptotic cell death, which serves as a host defence mechanism to restrict intracellular bacterial growth. We recently identified the T3SS inner rod protein BsaK of *B. pseudomallei*, the causative agent of melioidosis, as an early activator of the NLRC4 inflammasome. This study aimed to characterize the role of the T3SS needle protein BsaL and its cytosolic receptor NAIP1 in inflammasome assembly and caspase-1 activation.
**Results:** Single and double mutants of the rod and needle protein from *B. pseudomallei* showed a similar bacterial growth, but diminished secretion of the T3SS translocator BipD and effector BopE compared to the wild-type. As previously shown for BsaK deletion of BsaL also failed to activate caspase-1 in primary murine

macrophages, revealed strongly reduced IL-1β secretion and pyroptosis, and higher intracellular bacterial numbers during early but not during late infection. Challenge of mice with the BsaL mutant by the intranasal route resulted in lower IL-1β levels and reduced neutrophil influx. These results were accompanied by decreased pulmonary bacterial loads and dissemination to distant organs, cytokine production as well as lethality of mice.
**Conclusion:** Our results indicate that in addition to the *B. pseudomallei* T3SS rod protein BsaK, the needle protein BsaL plays a major role in the early activation of caspase-1, pyroptosis and IL-1β secretion in macrophages and for *in vivo* virulence of *B. pseudomallei*.

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**144/MPP**

**Pertussis toxin regulates specific host cell signaling pathways for promoting invasion and translocation of *E. coli K1 RS218* in human brain-derived microvascular endothelial cells**

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The exotoxin Pertussis Toxin (PTx) is a major virulence factor of the Gram-negative bacterium *Bordetella pertussis* which causes whooping cough. Especially in infants severe complications such as encephalopathies are observed which can lead to neurological disorders. These sequelae have been associated with PTx which is capable of disrupting the integrity of the blood-brain-barrier (BBB) as shown in differentin vitromodels although the molecular mechanism is still not understood. As a result, bacterial secondary infections of the central nervous system are facilitated, above all by the pathogenic *Escherichia coli*(E. coli) strain *E. coli K1 RS218*.
In this study HBMEC and TY10 cells were used in a two-compartment tissue culture model to investigate the cellular and molecular effects of PTx such as invasion and translocation rates of meningitis-causing *E. coli K1 RS218*(O18:K1). qRT-PCR of pro-inflammatory cytokines and pathogen binding receptors after PTx (200ng/ml) application and/or *E. coli K1 RS218* infection as well as western blot studies and confocal imaging were performed to investigate molecular changes in signaling, protein transcription, and protein localization.
We could show that incubation of HBMEC and TY10 cells with PTx increases the invasion and translocation rates of *E. coli K1 RS218* which are not caused by direct cytotoxic effects of the bacteria or PTx itself. Instead we found an up-regulation of the pathogen-binding host receptors gp96 on the transcriptional level. Additionally, PTx is able to activate signaling cascades which are described to be essential for *E. coli K1 RS218* invasion and translocation. Pull-Down assays and Western Blot studies showed a significant activation of STAT3 and Rac1. Furthermore co-immunoprecipitations and confocal imaging revealed a reduced interaction of VE-Cadherin with ß-Catenin after application of PTx. This reduced interaction might be induced via inhibition of ERK1/2 by PTx, since application of the ERK1/2 inhibitor U0126 decreased the interaction of VE-Cadherin with ß-Catenin.
In summary, we show that PTx acts at several key points to increase the permeability of the BBB ranging from up-regulation of pathogen binding receptors to activation/inhibition of several signal cascades responsible for actin rearrangements and destabilizing adherence junctions.

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**145/MPP**

**In vivo role of the mFPR2 receptor in *S. aureus* infection**

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Virulence of the emerging Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) and other highly pathogenic *S. aureus* depends on Phenol-Soluble Modulin (PSM) peptide toxins, which combine the capacities to attract and lyse neutrophils and play a role in phagosomal escape of *S. aureus.* We have previously shown that PSM sensing by the human formyl-peptide receptor 2 (FPR2) leads to leukocyte activation and chemotaxis. However, mice have several potential FPR2 orthologs and it has remained unclear how FPR2 affects the course of *S. eureus* infections.
Here we demonstrate that the mouse mFpr-rs2 receptor (now referred to as mFPR2) is the functional FPR2 ortholog. It senses PSMs at nanomolar concentrations and initiates recruitment of leucocytes in response to infection with CA-MRSA *in vivo*.
Use of mFPR2 knockout mice show that neutrophil activation and chemotaxis through PSM peptides and culture filtrates of highly pathogenic CA-MRSA occured only in neutrophils of wild type mice and not in neutrophils of mFPR2 $^{-/-}$. Moreover in an *in vivo* peritonitis model we could show that FPR2 knock out leads to strongly reduced monocyte and neutrophil immigration after *S. aureus* infection. In contrast, no difference in leukocyte immigration between wild type and mFPR2$^{-/-}$ mice was observed after infection with an isogenic PSM deletion mutant.
Thus, the innate immune system uses FPR2/mFPR2 to sense highly virulent bacterial pathogens. Targeting FPR2 may help to manage severe infections induced by *S. aureus*

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**146/MPP**

**Virulence gene regulation in *Escherichia coli* ABU 83972 by H-NS: A novel approach for the dissection of local and higher-order nucleoprotein complex dependent repression mechanisms**

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**Introduction**: In the natural habitat of a bacterial species, the expression of horizontally acquired genes in an individual cell is much more likely to result in a reduced than in an increased fitness when compared to an isogenic wild type population. A regulatory mechanism that results in repression of heterologous genes under normal growth conditions, but allows for the activation of these genes under survival conditions could apparently constitute an evolutionary advantage for a bacterial species. Members of a class of abundant bacterial host proteins that bind DNA in a rather unspecific manner, the so called nucleoid-associated proteins (NAPs), were found to play an important role in transcriptional repression of horizontally acquired DNA. The first NAP that was identified to selectively silence horizontally acquired DNA in *Escherichia coli* (*E. coli*) was H-NS. In contrast to binding to operator sites at gene promoters, xenogenic silencing is believed to require H-NS polymerisation and the formation of large DNA-H-NS nucleoprotein complexes. Both mechanisms are difficult to distinguish by classical methods. Here, we used a novel approach to dissect the H-NS dependent regulation mechanisms on the *hlyCABD* operon and the potentially transcriptionally linked *cnf*-1 gene, which are localized within a horizontally acquired genomic island and important virulence factors of uropathogenic *E. coli*. We analyzed the expression of *hlyC* and *cnf-1* by means of reporter gene fusions in wild type and *hns* knock out strains. In addition, we

used a set of strains with *dps* promoter-*yfp* fusion modules within and outside of the genomic island in order to test, if H-NS mediated repression of genes in this island is dependent on larger nucleoprotein complexes, or rather occurring directly at the promoter level.
**Results and discussion**: *Expression of hlyCABD* and *cnf-1*. The *hlyCABD-cnf-1* determinants are most likely co-transcribed from a single promoter in wild type cells, as judged by the similar YFP expression patterns of *cnf-1::yfp* and *hlyC::yfp*. Expression of *hlyC* was apparently repressed by H-NS, as judged by the elevated YFP production in this mutant. Interestingly, YFP production was not elevated when *yfp* was used to replace *cnf-1*. Repression mechanism. The *dps* promoter module was much more active in the pathogenicity island sequence context downstream of *hlyCABD-cnf-1* than in the chromosomal backbone of wild type *E. coli* ABU 83972. In contrast to what is known for *E. coli* K-12, we observed a reduction of *dps* promoter activity in the absence of H-NS. The reduction in *dps* promoter activity was not uniform, which suggests that H-NS-dependent higher order nucleoprotein structures are involved in the regulation of genes in this genomic island.

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**147/MPP**
**"Molecular and functional characterization of the ser/thr protein kinase PknB and phosphatase Stp of *Staphylococcus aureus*."**
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*Staphylococcus aureus* is one of the most common causes of community and nosocomial infections giving rise to life-threatening conditions including bacteremia, endocarditis, sepsis, or toxic shock syndrome. The activity of many metabolic proteins and virulence factors are controlled by phosphorylation and dephosphorylation via kinases and phosphatases.
PknB (Stk) is a eukaryote-like serine/threonine kinase, which acts in signal transduction of *S. aureus* through reversible phosphorylation of target proteins. PknB and its cognate phosphatase Stp are involved in central metabolic processes like cell wall metabolism, purine synthesis, glycolysis and importantly affect also *S. aureus* virulence.
At present, several putative phosphorylation targets of PknB are identified. We aim to investigate the role of PknB and Stp on central metabolism as well as its interplay with classical two-component systems.
Western Blot analysis revealed a strong impact of PknB and Stp at the phosphoproteome of *S. aureus* suggesting that both proteins are global protein regulators with overlapping target protein specificity. Next, these targets are to be identified using phosphoproteomics and protein interaction studies. Eventually, we intend to reconstruct the regulatory network surrounding the counteraction of PknB with the phosphatase Stp.

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**148/MPP**
**The *agr*-system of *Staphylococcus saprophyticus* is functional**
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**Introduction**: The *agr*-System is a well-studied quorum-sensing system and responsible for the growth phase dependent regulation of virulence factors in staphylococci. In addition, it has been recognized as important regulator of biofilm formation. For *S. saprophyticus*, the existence of an *agr* has been described, but the functionality has not been investigated so far. Here we describe the influence of the *agr* on several known virulence factors in *S. saprophyticus*.
**Material & Methods**: We constructed an isogenic *agr*-Knock-out mutant via allelic replacement with an *erm*B cassette and investigated the physiological significance of the *agr*-system. Activity of the D-serine deaminase was measured by detecting the generated pyruvate with 2,4-DNPH; activity of the urease was measured with Nessler reagent detecting the formed ammonia. Binding to polystyrol and biofilm formation was analyzed in micotitre plates. Adherent bacteria were stained with crystal violet and measured with an ELISA reader. In addition, real-time quantitative reverse transcription PCR (qPCR) was performed to determine the transcript amounts of known virulence factors at different times. The influence of the *agr* on surface proteins was investigated by total protein isolation and subsequent SDS-gel analysis.
**Results**: The *agr* mutant showed decreased primary attachment and biofilm formation. qPCR and SDS-PAGE analysis showed that the MSCRAMM proteins UafA und SdrI were strongly downregulated in the mutant strain. These Proteins are known to influence primary attachment and may also contribute to intercellular binding. The Δ*agr*-mutant also showed highly increased D-serine-deaminase activity, but qPCR revealed that there was no influence of *agr* on *dsd*A transcription. The activity of the urease was twofold higher in the mutant strain. Also the surface-associated lipase Ssp seemed to be positive regulated by the *agr*-system.
**Discussion**: The *agr*-system of *S. saprophyticus* thus appears to have an important impact on the expression of surface proteins UafA and SdrI, which leads to a different phenotype in primary attachment and biofilm formation. Furthermore, the enzymes D-serine deaminase and urease as well as the surface-associated lipase Ssp seems to be influenced by the *agr*.

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**149/MPP**
**The sensor kinase PA4398 regulates swarming motility, biofilm formation and virulence in *Pseudomonas aeruginosa* PA14**
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**Introduction**: *Pseudomonas aeruginosa* is an important opportunist human pathogen causing a wide variety of acute and chronic infections. Due to its high intrinsic resistance to a wide range of antibiotics, the secretion of numerous virulence factors (e. g. siderophores, exotoxins, proteases and type III secretion effectors), and the ability to form robust biofilms, treatment of *P. aeruginosa* infections still remains difficult. Moreover, flagellum- and type IV-pili-mediated swimming, swarming and twitching motility enable *P. aeruginosa* to rapidly colonize new environments including the human host. All these virulence-associated processes are regulated by a sophisticated regulatory network including two-component systems.
**Objectives**: In this study, we studied the histidine kinase PA4398 of *P. aeruginosa* PA14 in more detail, which has been identified previously during an extensive screening of the Harvard PA14 mutant library for motility and biofilm defects [1].
**Methods and Results**: In order to get a detailed insight into the regulatory cascade of the *P. aeruginosa* PA14 sensor kinase PA4398, a knockout mutant was constructed and its function in virulence-associated processes and virulence investigated. The PA14 PA4398 mutant exhibited a distinct reduction of 80 % in swarming motility while the production of biofilm mass was increased approximately 2-fold in comparison to the respective PA14 wild-type. In accordance with these phenotypes LC-MS analyses quantified an elevated c-di-GMP level of 50 % in the mutant strain. Moreover, global trancriptome studies of PA4398 swarmer cells, using microarrays, demonstrated a differential expression of genes encoding virulence factors. In particular, genes that are associated with the iron-scavenging molecules pyoverdine and pyochelin, which are supposed to be involved in swarming [2],

were down-regulated compared to the respective wild-type and thus provide a possible explanation for the swarming defect of PA14 PA4398 [3]. Furthermore, a reduced *in vivo* virulence could be observed for the PA4398 mutant in a plant, insect and acute murine lung infection model. The molecular mechanisms involved in this virulence phenotype were analyzed by the means of gene expression analyses using RT-qPCR.

**Summary**: In this study, we demonstrate that the sensor kinase PA14 PA4398 is involved in the regulation of virulence-related phenotypes such as swarming motility, biofilm formation and virulence.

**Reference**

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[3] Strehmel J *et al.*, 2015. Appl. Environ. Microbiol. 81: 1274-1285.

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**150/MPP**

**Degradation of bioactive secondary metabolites of *Pseudomonas aeruginosa* by environmental and clinical bacteria**

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**Introduction**: *Pseudomonas aeruginosa*, a ubiquitous bacterium that can be found in different environments, is a major opportunistic pathogen in lung infections in cystic fibrosis (CF) patients, and in nosocomial infections especially in immunocompromised patients. It produces a large array of bioactive 2-alkyl-4(1*H*)-quinolone (AQ) secondary metabolites. Whereas 2-heptyl-3-hydroxy-4(1*H*)-quinolone (the *Pseudomonas* quinolone signal, PQS) acts as quorum sensing signal molecule and significantly contributes to the regulation of virulence factor production, 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) interferes with quinone-dependent respiratory cytochromes. HQNO exhibits antimicrobial activity on Gram-positive bacteria, contributing to the ability of *P. aeruginosa* to become a dominant species in CF lung infections. HQNO moreover has been shown to induce the formation of persistent small-colony variants of *Staphylococcus aureus* (reviewed in [1]).

**Objectives**: Assessing the potential of environmental and clinical bacteria to detoxify or even degrade HQNO and other AQs.

**Methods and Results**: In whole-cell biotransformation experiments, we observed that HQNO at subinhibitiory concentrations is degraded by environmental strains of *Arthrobacter* sp. and *Rhodococcus erythropolis.* Identification of some metabolites formed by *Rhodococcus* by HPLC and MS analyses suggests that degradation involves hydroxylation and *N*-oxide reduction steps, forming PQS as an intermediate [2], prior to ring cleavage. *Bacillus subtilis* converted HQNO to a still unidentified derivative, whereas cell suspensions of *P. putida* and *Corynebacterium glutamicum* did not transform HQNO under the conditions tested. Most interestingly, *Mycobacterium fortuitum* and *M. abscessus*, an emerging pathogen in CF patients, transform HQNO via a pathway that might be similar to that of *R. erythropolis*.

**Conclusion**: The respiratory inhibitor and antibiotic HQNO and related AQs can undergo biotic conversion. Among the bacterial species tested, members of the *Actinobacteria* appeared to be the most efficient degraders. Future studies will be required to find out whether in bacteria such as *M. abscessus*, which co-exist with *P. aeruginosa* in some infections, this ability contributes to their competitiveness.

**Reference**

[1] Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M (2011) FEMS Microbiol Rev 35: 247−274.

[2] Mueller C, Birmes FS, Niewerth H, Fetzner S (2014) Appl Environ Microbiol 80: 7266−7274.

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**151/MPP**

**Characterization of symptomatic and asymptomatic enterohemorrhagic *Escherichia coli* isolates of serogroup O91 focusing on virulence properties *in vitro***

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**Introduction**: Enterohemorrhagic *Escherichia coli* (EHEC) are foodborne pathogens which can cause severe intestinal disorders, e.g. gastroenteritis, which may expand to an enterohemorrhagic enteritis with bloody diarrhea. Some patients even develop a severe complication leading to a hemolytic-uremic syndrom (HUS). The bacterial virulence factors responsible for human disease are various: the Locus of Enterocyte Effacement (LEE) enables a tight association of the bacteria to the gut epithelium, production of the Shiga toxin and subsequent uptake into cells lead to inhibition of the ribosomes and cell death. Furthermore, EHEC strains are capable to express a plasmid-encoded EHEC-hemolysin, damaging the lipid membrane of eukaryotic cells.

Besides these dramatic actions on the human host, some EHEC strains do not cause symptoms in certain hosts. Some patients asymptomatically carry EHEC bacteria but potentially infect other people, which are in close contact, like in families, nurseries, day-care centers and hospitals. Interestingly, asymptomatic carriage is common in the natural reservoir of EHEC bacteria, which are mainly cattle but also sheep and goat.

**Aims**: We want to elucidate the mechanism(s) underlying the symptomatic and asymptomatic outcome of colonization by EHEC strains of the same serotype. Therefore we investigated closely related EHEC O91 isolates, which caused either symptomatic disease or asymptomatic carriage by means of genotypic and phenotypic characterization *in vitro*.

**Materials and Methods:** We focused on eight human EHEC O91 isolates from diarrhea, HUS or asymptomatic carriers. To assess the virulence potential of the strains we measured the amount of Shiga toxin in supernatants by ELISA. Further, the cytotoxicity of supernatants towards Vero cells was determined, taking into account other factors like the EHEC-hemolysin. We also compared the adhesion characteristics to epithelial cells in cell culture.

**Results**: EHEC isolates of serogroup O91 produced different amounts of Shiga toxin and showed a dissimilar cytotoxicity towards Vero cells. In addition the adhesion characteristics to epithelial cells are discriminable. However, a correlation of the phenotypic characterization of the strains *in vitro* and the health status of the corresponding patient is not always possible.

Discussion and Outlook

Our results show that EHEC isolates of serogroup O91 from symptomatic or asymptomatic cases exhibit different phenotypic traits regarding virulence properties *in vitro*, which cannot be unambiguously correlated with the clinical outcome of infection. A careful comparison of phenotypes of symptomatic and asymptomatic isolates of individual serotypes, e.g. O91:H21, O91:H14, and O91:H- will indicate whether asymptomatic carriage may result from reduced expression of virulence factors.

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**152/MPP**

**Analysis of cellular uptake of an ETEC-derived putative exported protein**

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Tumor necrosis factor-alpha (TNF) plays a key role in the cytokine regulation of the immune system. Upon detection of pathogen-associated molecular patterns, the pro-inflammatory TNFα signaling and NF-κB pathways are activated. The NF-κB signaling cascade is a key regulator of the innate immune response to pathogen infection and mediates the transcription of genes involved in the pro-inflammatory host-response.
Many bacterial pathogens have evolved strategies to subvert the host immune response. NF-κB and mitogen-activated protein kinase (MAPKs) pathways are targeted by extracellular pathogens to inhibit important signaling cascades of the immune system. Enterotoxigenic *Escherichia coli* (ETEC) can cause diarrheal disease, a significant problem in developing countries. How ETEC subverts the host immune response is not yet well understood. It was reported that ETEC secrets a heat-stable protein that blocks NF-κB signaling normally induced by TNFα, interleukin-1, or flagellin (Wang *et al.*, 2012). It was also reported that ETEC supernatants modulate directly the NF-κB pathway by preventing the polyubiquitination of IκBα without affecting its phosphorylation. Furthermore the data suggest the ETEC factor uses a clathrin-dependent endocytosis pathway (Wang *et al.*, 2012). By using transposon mutagenesis of ETEC H10407 a candidate for the secreted factor was found in gene ETEC_1754, which encodes a putative exported protein (PEP). The ability of TNFα to induce IκBα degradation was reduced when HCT-8 cells were treated with purified PEP. To investigate the cellular uptake of PEP in more detail, its clathrin-dependent endocytosis and intracellular transport were investigated using immunofluorescence microscopy, cell fractionation, and fluorescence-activated cell sorting (FACS) in the presence or absence of commonly used endocytosis inhibitors.

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**153/MPP**

**Characterization of the role of Pls glycosylation in *Staphylococcus aureus* pathogenesis**

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Protein glycosylation in bacteria is often linked to bacterial virulence factors, i.e. adhesins and invasins. However, the occurrence and importance of protein glycosylation in the major human pathogen *Staphylococcus aureus* remains largely unknown. To study the impact of protein glycosylation in *S. aureus*, we analysed lysostaphin lysates of the methicillin-resistant *Staphylococcus aureus* (MRSA) strain COL by SDS-PAGE and periodic acid-Schiff's staining that specifically stains glycosylated proteins. We detected four glycosylated surface proteins (molecular masses: >300, ~250, ~165, and ~120 kDa). The ~250 and ~165 kDa proteins were identified as plasmin-sensitive protein Pls by mass spectrometry. Pls is a serine-rich surface protein, which is encoded by the staphylococcal chromosome cassette (SCC)*mec* type I in MRSA strains. In a search for potential glycosyltransferases involved in Pls glycosylation, we found two open reading frames downstream of *pls*, which we termed *gtfC* and *gtfD*. In addition two further glycosyltransferase genes were found downstream of the *sdrCDE* locus referred to as *gtfE* and *gtfF*. We showed that all four glycosyltransferases contribute to Pls glycosylation and demonstrated that glycosylation occurs at serine residues of the C-terminal SD-repeat region of Pls. Furthermore we could identify the modifying carbohydrates as *N*-acetylhexosaminyl residues. Pls is known to prevent *S. aureus* adherence to host factors and also its internalization by host cells probably acting by sterical hindrance. Functional characterization indicated that these traits are not due to the glycosylation of Pls. In a further search for the function of the Pls sugar modifications, we investigated, if they are involved in elevating *S. aureus* resistance towards antibiotics or if they are able to protect Pls against proteolytic cleavage and degradation. Our recent results indicated that the sugar moieties are not involved in these features either. However, in biofilm and aggregation assays, we could observe stronger biofilm formation and cell aggregation of strain Newman expressing Pls that is glycosylated by all four glycosyltransferases in comparison to strain Newman expressing Pls glycosylated by only GtfC/GtfD or GtfE/GtfF or expressing non-glycosylated Pls.
In conclusion, we identified Pls as a *S. aureus* surface glycoprotein, identified glycosyltransferases involved in its glycosylation, and found a role for the modifying sugars, when *pls* is expressed in strain Newman, i.e. biofilm formation and intercellular adhesion. Further studies are on the way to exactly define the function of Pls sugar modification.

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**154/MPP**

**Serotype 3 pneumococci interact with human matricellular thrombospondin via the sortase-anchored Hic protein**

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**Introduction:** *Streptococcus pneumoniae* (pneumococci) evade the innate immune defence by their recruitment of the fluid phase complement inhibitors Factor H and vitronectin. In most pneumococcal serotypes these human proteins bind to the non-covalently anchored choline-binding protein PspC. Serotype 3 pneumococci express only the PspC-like protein Hic, which is covalently anchored to the peptidoglycan and represents therefore a non-classical member of the PspC family. Similar to the choline-binding protein PspC the sortase-anchored Hic protein binds vitronectin and Factor H. The matricellular glycoprotein human thrombospondin-1 (hTSP-1) is secreted mainly by activated thrombocytes and interacts with a wide range of other matrix molecules. A recent study identified the pneumococcal adhesins PavB and classical PspC of *S. pneumoniae* as hTSP-1 binding proteins (Binsker et al., 2015). In this study was assessed whether the PspC-like adhesin Hic of serotype 3 pneumococci also acts as a hTSP-1-binding protein. Interestingly, Hic contains no repeating sequences compared to TSP-binding proteins PavB and PspC from *S. pneumoniae* and Atl from *Staphylococcus aureus* (Kohler et al., 2014).
**Methods**: Heterologously expressed protein domains of Hic were produced and their binding to TSP-1 was investigated by surface plasmon resonance and ELISA. Recombinant *Lactococcus lactis* exposing the full length Hic protein on their surface were analyzed for their ability to recruit soluble hTSP-1 to the bacterial surface.
**Results:** Binding studies with fragments of Hic identified the PspC-like protein Hic as hTSP-1 binding protein of serotype 3 pneumococci. Moreover, competitive ELISA experiments with immobilized Hic fragments and soluble hTSP-1 in the presence of increasing molar ratios of vitronectin showed a dose-dependent inhibition of hTSP-1-binding. Furthermore Hic-expressing *L. lactis* showed a significant increase in hTSP-1 recruitment compared to control lactococci harboring an empty vector.
**Conclusion:** Taken together, we present here an additional pneumococcal adhesins interacting directly with hTSP-1 and underline the importance of matricellular hTSP-1 as target for pneumococcal adherence during an infection process as hTSP-1

has been shown to act as a molecular bridge during adherence of Gram-positive bacteria (Rennemeier et al., 2007).

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**155/MPP**

**Is biofilm formation of uropathogenic *E. coli* a predisposing factor for catheter-associated urinary tract infection?**

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**Question**: Prolonged catheterisation with urinary catheters often develops into a catheter-associated urinary tract infection (CA-UTI). Biofilm formation of *E. coli* is an important mechanism to colonize biotic and abiotic surfaces. Biofilm formation as a virulence trait of uropathogenic *E. coli* (UPEC) can contribute to persistent infection as well as to colonization of urinary catheters and thus to CA-UTI. In this study, we investigated whether biofilm formation of UPEC isolates from CA-UTI was enhanced relative to UPEC from non-catheter-associated UTI. We also addressed the question whether biofilm formation can be correlated with the expression of individual biofilm-associated factors in pooled human urine.
**Methods**: To determine whether increased biofilm formation may be a predisposing factor of UPEC to cause CA-UTI, we investigated biofilm formation of UPEC isolates from (i) patients with catheter-associated urinary tract infection, (ii) patients with non-catheter-associated UTI as well as from fecal *E. coli* isolates from healthy volunteers. We compared biofilm formation under static and under flow conditions in minimal medium (MM) and in pooled human urine. The expression of biofilm-associated factors, i.e. antigen 43 and poly-β-1,6-N-Acetyl-D-glucosamine (PGA) was analyzed by immuno (dot) blot. Curli adhesin and cellulose expression was tested by Congo Red or calcofluor staining to possibly correlate their expression with biofilm formation.
**Results**: UPEC strains were significantly more likely to form biofilm than fecal isolates under static growth conditions in MM as well as in pooled human urine. CA-UTI isolates had no superior ability to form biofilms than UPEC from non-catheter-associated UTI. Consequently, biofilm formation cannot be considered a predisposing factor of CA-UTI isolates. Interestingly, biofilm formation in urine was significantly weaker and less frequent than in MM. Ag43 expression could be associated with biofilm formation in MM, but not in urine. PGA expression did not correlate with the ability to form biofilms. Biofilm assays under static versus flow conditions led to inconsistent results.
**Conclusions**: Our data confirm that the outcome of biofilm assays markedly depends on the growth medium and biofilm assay used. Interestingly, biofilm formation in pooled human urine was reduced relative to standard laboratory media. This questions the use of laboratory media to analyze virulence traits, especially biofilm formation, of UPEC.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**156/MPP**

**Regulation of colibactin synthesis in *Escherichia coli***

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**Introduction and Aims**: Colibactin is a bacterial virulence factor that is produced by extraintestinal pathogenic *E. coli* (ExPEC) and commensal strains of the phylogenetic lineage B2. Infection with colibactin-producing ($Clb^+$) *E. coli* leads to the induction of double-strand breaks in mammalian cells, which, as a consequence, results in cell cycle arrest and megalocytosis [1]. The polyketide colibactin is synthesized by multiple enzymes that are encoded on the 54-kb colibactin (*clb*) island. In order to study the function of colibactin and to prevent the cytopathic effect on mammalian cells caused by $Clb^+$ *E. coli*, we are investigating the expression regulation of this island.
**Methods and Results**: Our results revealed that the two-component system (TCS) BarA/UvrY is involved in the regulation of *clb* gene expression. The BarA/UvrY TCS is part of the carbon storage regulator system (Csr) and controls the expression CsrB and CsrC, two small non-coding RNAs which inhibit the function of CsrA, a global RNA-binding regulatory protein [for review: see 2]. Reduced CsrA function results in a strongly altered colibactin-mediated cytopathic effect. A putative CsrA binding motif was identified within the 5´UTR of the gene *clbQ* and interaction of purified CsrA and ClbQ RNA was shown *in vitro* by means of RNA electric mobility shift assay (EMSA) experiments. The direct influence of CsrA on ClbQ synthesis was verified by the analysis of a combination of various *clbQ* reporter gene fusions.
Moreover, we found that a small LuxR-type regulator, ClbR, is encoded on the *clb* island. The deletion of the respective gene, *clbR*, in *clb* encoding *E. coli* abrogates megalocytosis of mammalian cells. Our luciferase-based reporter gene and DNA EMSA studies suggest that ClbR acts as a transcription activator of *clb* gene expression.
**Discussion**: Colibactin can function as a virulence factor of ExPEC. Therefore, a better understanding of colibactin expression regulation will help us to elucidate the role of colibactin during ExPEC pathogenesis. Furthermore, our research on colibactin gene regulation may allow us to identify possible targets to interfere with the expression of this potent bacterial cyto- and genotoxin.

**References**

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**157/MPP**

**Determinants of the polar localization of the host cell targeting type VI secretion system 5 in *Burkholderia thailandensis***

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Bacteria possess an elaborate subcellular organization and the localization of proteins to a particular site within the bacterial cell is central to many cellular processes. An increasing number of studies show that the mechanisms underlying the localization of proteins are highly diverse and complex. However, the majority of these studies analyzed housekeeping proteins and the positioning of complex virulence factors, such as secretion systems remain largely elusive. The type VI secretion system (T6SS) is a widespread cell envelope spanning secretion apparatus, which is employed by bacteria to deliver toxins and effector proteins into other bacteria or host cells. *Burkholderia thailandensis* is a Gram-negative rod shaped bacterium, which harbors multiple T6SSs. Of these, the T6SS-5 is a major virulence factor that targets host cells and the T6SS-1 targets other bacteria. In a previous study, we showed that the subcellular localization of the T6SS-1 and T6SS-5 is distinctly different: the T6SS-1 localizes in a nearly random manner along the cell length while the T6SS-5 is found at the pole of the bacterial cell. This finding suggests that the localization of the T6SS-1 and T6SS-5 is of functional importance. The aim of this study was to gain an insight into the localization mechanisms of the T6SS-5 by analyzing the role of the nucleoid, cytoskeleton and peptidoglycan -which are involved in the spatial organization of the bacterial cell- in the polar positioning of the T6SS-5. Using a GFP reporter fusion to ClpV-5, the ATPase of the T6SS-5, we showed that the T6SS-5 does not reach the pole by nucleoid

occlusion as the induction of DNA condensation by chloramphenicol did not affect the positioning of ClpV-5-sfGFP to the pole. Instead, we found that the treatment of *B. thailandensis* with A22, an inhibitor of the cytoskeleton protein MreB, caused a diffuse and non-specific localization of ClpV-5-sfGFP within the bacterial cell. In addition, treatment with D-cycloserine, a cyclic analog of D-alanine that blocks peptidoglycan synthesis, did not impact localization of ClpV-5-sfGFP to discrete foci but the precise positioning to the pole was impaired. Furthermore, our data suggest that the negative curvature of the pole does not play a role as a localization cue for the T6SS-5. Altogether, the results indicate that the localization of the T6SS-5 virulence factor relies on specific interactions with core cellular components of the bacterial cell, which will be investigated in more detail in future studies.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**158/MPP**

**Role for the Novel Locus *comEB* in eDNA-dependent *Staphylococcus lugdunensis* Biofilm Formation**

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*Staphylococcus lugdunensis* is an emerging human pathogen, however very little has been known about the mechanisms underlying its pathogenicity, such as biofilm formation. To elucidate the genetic basis of biofilm formation in *S. lugdunensis*, we performed transposon (*Tn*917) mutagenesis using the biofilm-forming clinical isolate a19263. One mutant (mut12) had a significantly reduced biofilm-forming capacity and carried a *Tn*917 insertion within the competence gene *comEB*. Site-directed mutagenesis and subsequent complementation with a functional copy of *comEB* in the clinical isolate *S. lugdunensis* w701 verified the importance of *comEB* in biofilm formation. Competence is known to induce lysis-dependent and -independent mechanisms of DNA release. Extracellular DNA (eDNA) has been demonstrated to be an important structural component of bacterial biofilms acting as a cohesive agent that glues the bacterial cells together. We therefore hypothesized that the disruption of *comEB* may lead to diminished amounts of eDNA within the mutant biofilm. Quantification of eDNA in the biofilms with real-time PCR confirmed our hypothesis. Using high-resolution images and 3D data obtained via confocal laser scanning microscopy (CSLM), we further verified the impact of the *comEB* mutation on the biofilm integrity, thereby confirming the loss of eDNA as the cause of lowered biofilm production in the *comEB* mutant. In an effort to determine, if lysis-dependent mechanisms are involved in *comEB*-mediated DNA release, we analyzed the autolytic activity and the expression of the *atlL* and *aaL* autolysin genes of the strains by using a Triton X-100-induced autolysis assay, zymographic analysis and RT-PCR, respectively. None of the assays revealed a significant difference among the w701 wild type, its *comEB* mutant or the complemented mutant suggesting an alternative mechanism. Reduced amounts of eDNA in the *comEB* mutant biofilms may also be due to elevated levels of the *S. lugdunensis* thermonuclease NucI. However, RT-PCR did not reveal significant differences in the expression of *nucI* among the w701 wild type, its *comEB* mutant or the complemented mutant either. Therefore, we suggest a role for the previously uncharacterized *comEB* gene in staphylococcal biofilm formation via stimulating lysis-independent DNA release. Further experiments are needed to elucidate the exact mechanisms underlying the *comEB*-induced lysis-independent DNA release subsequently leading to elevated biofilm formation.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**159/MPP**

**Characterization of the *barAB* regulon in *Staphylococcus epidermidis***

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**Introduction**: Due to the ability to form multilayered biofilms *S. epidermidis* is a leading cause of nosocomial infections. Cell-to-cell adhesion within biofilms is mediated by the polysaccharide intercellular adhesin (PIA), which is synthesized by the *icaADBC* gene products. The regulation of PIA expression is mediated by a complex regulatory network including the negative regulator IcaR, the alternative sigma factor $\sigma^B$, the *agr* quorum sensing system, the regulator SarA as well as the newly characterized regulatory locus *barAB* (*yabJ*/*spoVG*). In this study we characterized the *barAB* regulon by investigation of the transcriptome in different isogenic mutants of the *barAB* locus.
**Materials and Methods**: Differences in RNA expression were compared between the laboratory strain *S. epidermidis* 1457 and the deletion mutants *S. epidermidis* 1457*barA*, 1457*barB* and 1457*barAB*. The cDNA libraries were prepared using the Illumina TruSeq Stranded mRNA Library Prep Kit. RNA-Sequencing data were generated on the Illumina MiSeq system (v3 chemistry). Data analysis was performed using CLC Genomics Workbench software 7.5.
**Results**: RNA sequencing confirmed that in mutants with single deletion of *barA* or *barB* the corresponding gene was transcribed as also observed for the gene products by western blotting. In the *barA*- *barB*- and *barAB*-mutants 47, 154 and 586 genes were differentially regulated, respectively. Interestingly, only 18 gene loci were differentially regulated in all three mutants. 471 gene loci were only differentially regulated if both *barA* and *barB* were inactivated. In the *barA* mutant a slight but significant increase of *icaR* transcription and a corresponding decreased *icaADBC* transcription was observed. Interestingly in the *barB* mutant *icaADBC* transcription was strongly decreased independent of *icaR* transcription, which was further decreased in the double mutant. Within the differentially regulated gene loci, the global regulator $\sigma^B$, the *agrBDCA* gene locus, as well as *sarA* displayed also a decreased transcription in the *barAB* double mutant.
**Conclusion**: The genes *barA* and *barB* are regulatory genes interacting with the regulatory network of *icaADBC* transcription and can repress *icaADBC* transcription independent of the negative regulator IcaR. The deletion of both genes resulted in strong transcriptional changes with 586 differentially expressed genes. Thereby, in the *barAB* mutant a wide variety of other regulators were influenced, indicating a function as a global regulator.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**160/MPP**

**Biofilm-forming capability and virulence of a relP, relQ mutant is significantly reduced in *Staphylococcus aureus***

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**Introduction**: The stringent response is one of the most conserved regulatory mechanisms in bacteria, characterized by the rapid synthesis of (p)ppGpp and involved in a multitude of processes such as stress response, antibiotic tolerance, biofilm formation and virulence. *Staphylococcus aureus* possesses three different (p)ppGpp-synthases: the bifunctional RSH (RelA/SpoT homolog) which consists of a synthase and a hydrolase domain as well as two truncated (p)ppGpp synthases, named RelP and RelQ. While the former is activated by amino acid starvation the latter two were

shown to react to cell wall stress. Both are activated upon vancomycin or ampicillin treatment. A relP, relQ double mutant is significantly impaired in its ability to survive antibiotic-induced cell-wall stress (Geiger 2012).
**Aim**: Within the recent study we intend to deepen our knowledge about relP, relQ mutant strains of *S. aureus* with respect to biofilm-forming capability and virulence in an animal model.
**Material and Methods**: Biofilm assays were performed to quantify the absolute amount of biofilm in different *S. aureus* strains.
To examine *in vivo* relevance of RelP and RelQ we used a kidney abscess model and a muscle abscess model. In case of the muscle abscess model, some groups of animals received vancomycin treatment (15 or 30 mg/kg body weight per day). Mice were infected, weight checked for five days, animals sacrificed and organs taken. Tissue was homogenized, diluted and CFU/organ measured.
**Results**: Compared to the wild type, biofilm amount was significantly decreased in relP, relQ mutants of strain HG001 but only partially in USA300.
The kidney abscess model revealed that animals infected with the wild type were healthier than animals infected with relP, relQ double mutant following the infection. The relP, relQ mutant displayed a lower CFU/kidney than the wild type.
**Discussion**: The extent to which the biofilm forming capability was impaired in the relP, relQ mutant varied, depending on the strain. Thus the chemical composition of biofilms of the strains analysed (either PIA- of protein-based) seems to play a pivotal role for that mechanism and needs further elucidation.
The kidney abscess experiments suggest an impaired virulence of the relP, relQ mutant in an animal model. The evaluation of the muscle abscess experiments is still under way and we are interested in whether the vancomycin treatment leads to a further decrease in the survival ability of the relP, relQ mutant.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**161/MPP**

**Nuclear export of anti-inflammatory *Yersinia* effector YopM is mediated by DEAD box helicase DDX3 and controls phosphorylation of nuclear Ribosomal S6 Kinase 1**

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YopM is an effector protein of *Yersinia enterocolitica, Y. pseudotuberculosis* and *Y. pestis* that becomes translocated into target cells by the bacterial type three secretion system and strongly contributes to *Yersinia's* pathogenicity. After its translocation into target cells YopM enters to the nucleus. Here we identified the DEAD-box helicase DDX3 as a novel interaction partner of YopM. Both, knockdown of DDX3 and inhibition of the nuclear exportin CRM1 caused accumulation of YopM in the nucleus. Increasing the level of YopM in the nucleus by blocking its nuclear export further enhanced nuclear RSK1 phosphorylation. Transcriptome analysis of *Y. enterocolitica* infected human macrophages revealed suppression of inflammatory mediators by YopM.
These data indicate that YopM associates with DDX3 to exit the nucleus via the CRM1 export pathway. The thereby enabled nucleocytoplasmic shuttling of YopM is instrumental for controlling the phosphorylation of nuclear RSK1 and reveals a strategy of *Yersinia* to modulate nuclear activity of RSK in host cells. As a consequence the expression of genes for inflammatory mediators and -pathways is downregulated.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**448/MPP**

***Citrobacter rodentium* NleB blocks TRAF3 K63-linked ubiquitination to inhibit interferon-b production**

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**Introduction**: Many bacterial pathogens utilize a type III secretion system (T3SS) to inject virulence proteins (effectors) into host cells to subvert various biological functions. Effector subversion of pro-inflammatory host responses is well studied, but less attention has been given to the potential inhibition of host interferon (IFN) signaling.
**Objectives**: Type I IFNs are important both to maintaining intestinal homeostasis and to responding to pathogen infection. Pathogens have evolved strategies to interfere with host type I IFN production. A recent study found both that IFN-b is induced by enteropathogenic *E. coli* (EPEC) infection and that the EPEC T3SS effector NleD inhibits IFN-b induction. IFN expression is known to be important to limiting *Citrobacter rodentium* infection, but whether *C. rodentium* T3SS effectors inhibit host IFN-b induction is unclear. We screened *C. rodentium* strains bearing deletions in individual T3SS effectors to determine the extent to which this pathogen might inhibit the host IFN-b response.
**Materials and Methods**: To determine if *C. rodentium* T3SS effectors inhibit the host type I IFN response, we monitored the survival of a recombinant vesicular stomatitis virus (VSV). Since TRAF3 is critical to IFN signaling, we also monitored effector-mediated inhibition of the TNF receptor (TNFR)-associated factor 3 (TRAF3) ubiquitination in RAW264.7 cells.
**Results**: Supernatants from cells infected with *C. rodentium* AE*escN* inhibited VSV to levels similar to those induced by LPS treatment. By contrast, supernatants from cells infected with WT *C. rodentium* did not inhibit VSV-GFP growth. These data suggested that a T3SS-effector inhibits the production of a host factor involved in virus inhibition. We then infected HeLa cells with *C. rodentium* strains lacking individual T3SS effector genes and screened the cell supernatants for anti-viral activity. D*nleB* inhibited virus replication most significantly. By monitoring TRAF3 activity in *C. rodentium*-infected cells, we also revealed the selective impact of NleB on K63-linked TRAF3 ubiquitination.
Conclusion: The T3SS effector NleB inhibits host IFN-b production by reducing the extent of the activation-associated K63-linked TRAF3 ubiquitination.

**Presentation:** Monday, September 28, 2015 from 15:00 – 17:00 in the industrial exhibition.

# MOLECULAR EPIDEMIOLOGY OF MICROORGANISMS (MSP)

**162/MSP**

**Predominance of sequence type 8 Staphylococcus aureus among isolates from free-living rodents trapped in Thuringia and Mecklenburg-Western Pomerania**

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**Introduction**: To date, approximately 3,000 different multi-locus sequence types have been identified for the important opportunistic pathogen Staphylococcus aureus (www.mlst.net). However, only a limited number of these sequence types (e.g. ST8) is frequently reported for isolates of different geographic and host origin, including methicillin-resistant and -susceptible variants. Here we report on the prevalence, genetic composition and background of S. aureus isolated from the nose of free-living small rodents trapped at different sites in Mecklenburg-Western Pomerania and Thuringia, including one methicillin resistant strain (MRSA) harboring mecC.

**Material and Methods**: In a pilot study, 100 rodents were trapped by the network "Rodent-Borne Pathogens" in 2011-2013 at different sites in Mecklenburg Western Pomerania and Thuringia as part of a multiple-pathogen screening study in wild rodents from Germany. All animals were frozen at -20 °C after trapping. For dissection, the animals were thawed and complete noses were removed under sterile conditions. Nose tissue was homogenized and subsequently cultured in an S. aureus enrichment medium for 48 hours and plated on mannitol salt agar dishes in serial dilutions. Then, all morphotypes that could be distinguished visually were subcultured on blood agar plates. S. aureus identity was confirmed by gyrase gene-specific PCR. All isolates were initially subjected to spa typing, and next-generation sequencing (Illumina, MiSeq®) was performed for all 29 S. aureus isolates representing diverse origins and trapping sites. Further data analysis was carried out by use of Ridom SeqSphere plus®.

**Results**: S. aureus was isolated from 29 of 100 rodents belonging to five different species. The majority of the S. aureus isolates (24/29) belonged to sequence type ST8-t211. Analysis of altogether 2332 targets based on MLSTplus (n=1811 loci) together with the accessory gene content (n=521 loci) revealed a very close relationship among these isolates from different federal states, trapping sites and host species. Two isolates belonged to sequence type ST130-t843, including one mecC-positive MRSA, harboring a complete blaZ-mecC-mecR1-mecI structure (class E mec complex) as well as the recombinase genes crrA1 and ccrB3 and associated loci (e.g. arsenic resistance operon) described for S. aureus LGA251. One further S. aureus isolate was assigned to ST88-t2311, harboring prophage L54a and genes encoding the LukD/E leukotoxin. Two further isolates belonged to ST890-t1736 and -t1773.

**Conclusion**: S. aureus ST8 strains, regardless if methicillin resistant or not, seem to be well established in wildlife and companion animals, as well as in hospitals and the human community. The success of certain lineages - denominated as extended host spectrum genotypes (EHSG) - in occupying multiple ecological niches and host species needs to be further evaluated.

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**163/MSP**

**Population structure of *Legionella* spp. from environmental samples in Gabon,2013**

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**Question:** Aquatic environments are the most important source for *Legionella* spp. infections such as Legionnaires' disease and Pontiac fever [1]. The reservoirs of *Legionella* spp. are not well known in sub-Saharan Africa. The aim of this study was to identify geographical areas with an increased risk for exposure to *Legionella* spp., and to describe the population structure of *Legionella* spp. from different water sources in a cross sectional study in Gabon in 2013.

**Methods:** Fresh water samples (n=200) were cultured on *Legionella* selective agar; species were confirmed by MALDI-TOF and 16s RNA gene sequencing and serogroups were identified by agglutination test. The population structure of *Legionella pneumophila* was assessed by multilocus sequence typing (MLST).

**Results:** In total, 23 of 200 samples were contaminated with *Legionella* spp. (11.6%) with a median concentration of 11 CFU/ml. Samples from hot water systems were more often tested positive than cold water samples (OR=21.9, 95% CI: 7.7-62.1, $p<0.001$). Eleven samples contaminated with *Legionella* spp. were collected from hospital settings (n=11, 47.8%). Open water bodies (i.e. rivers, lakes) were not contaminated with *Legionella* spp. Isolated *L. pneumophila* mainly belonged to serogroups 2-14 (n=19) and MLST sequence type ST1, ST75 (and related STs) and ST1911.

**Conclusion:** The frequent detection of *Legionella* spp. in water samples from hospital settings including a dental clinic could pose a risk for legionellosis in hospitalized patients in the studied areas in Gabon, particularly if patients have comorbidities. These results support other studies from South Africa with similar findings [2]. Both pandemic lineages (ST1, ST75) and local lineages (ST1911) were present in our setting.

**References**

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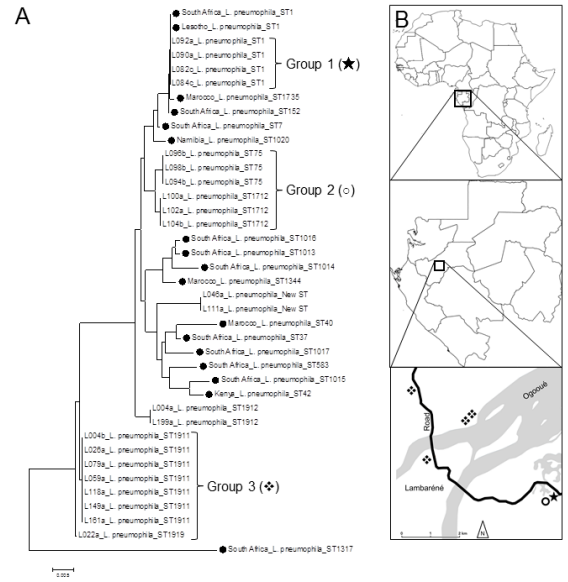
**Figure 1**




**164/MSP**

***Streptococcus tigurinus* - a distinct species or a pathotype of *S. oralis*?**

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**Background**: *Streptococcus tigurinus* is a very recently discovered oral pathogen, which is able to cause infective endocarditis, meningitis and spondylodiscitis. Except the type strain and three additional strains from the original publications no other *S. tigurinus* strains have been reported so far. *S. tigurinus* belongs to the mitis-group of streptococci with *S. oralis* and *S. infantis* as close relatives. As the strain collection of the National Reference Centre for Streptococci in Aachen contains 23 proven *S. oralis*/*S. infantis* isolates from cases of infective endocarditis, it was our intention to search among those for *S. tigurinus*, amplifying and sequencing housekeeping and virulence-associated genes.
**Method**: DNA was extracted from 21 *S. oralis* and two *S. infantis* blood isolates from endocarditis patients and all four *S. tigurinus* reference strains, including type strain AZ_3a (DSM 24864). Housekeeping genes such as 16S rRNA-gene, *gdh*, *groEL*, and *sodA* were amplified and sequenced. Sequences were compared *in silico* with reference strain genomes of related species available in the NCBI-database. The presence of three virulence genes present in AZ_3a and partly mediating adhesion in endocarditis were checked using self-designed PCR-assays. These genes were *rib*, coding for an M-like protein, *cshA*, coding for a fibronectin-binding protein, and an integrase of a transposable element. Furthermore the *gtfR* gene was analyzed, as it plays an important role in adhesion of *S. oralis* but is not present in all strains.
**Results**: Although *S. oralis* and *S. infantis* strains showed heterogeneity in housekeeping genes, no unique or typical sequence could be addressed to a *S. tigurinus*-like cluster. The *rib* and the integrase genes were only present in the *S. tigurinus* type strain but not existent in the other three strains demonstrating heterogeneity even within *S. tigurinus*. The *cshA* gene was present in all *S. tigurinus* strains while the *gtfR* gene could not be detected. In contrast to *S. tigurinus*, the *gtfR* gene was present in 37% and the *cshA* gene in 32% of the *S. oralis* strains. Interestingly, both genes were positively associated with an odds ratio of 20, p=0.013. This was also confirmed by phylogenetic analyses based on the 16S-rRNA-gene, resulting in two subclusters for *S. oralis*. One subcluster consisted mainly of strains carrying both genes, the other mainly of strains without these genes, indicating different pathotypes within the *S. oralis* group.
**Conclusions:** None of the analyzed strains matched the reference strain *S. tigurinus* in housekeeping genes or virulence instrumentation. All four *S. tigurinus* strains were heterogeneous somewhat challenging the species description. Phylogenetic analyses and the concordance in presence of *cshA* and *gtfR* in *S. oralis* indicate clusters within this species. Instead of describing new species or subspecies we recommend dividing *S. oralis* into pathotypes like *E. coli*.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**165/MSP**

**A longitudinal study to determine intra- and inter-individual variability of pulsed-field gel electrophoresis (PFGE) patterns in vancomycin-resistant enterococci (VRE)**

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**Introduction**: Vancomycin-resistant enterococci (VRE) are a cause of increasing concern in Germany, and a frequent cause of bacteremia in high-risk patients at the University Hospital Cologne.
**Objectives**: To better understand the local epidemiology of VRE an active surveillance program was established in the hematology department and the medical ICU. We analysed the intra- and inter-individual variability of PGFE patterns in isolates collected over time.
**Patients and Methods**: Weekly screening using direct culture of rectal swabs or stool specimens on selective media was performed on inpatients of the medical ICU as well as on admission of patients to the hematology department if prolonged neutropenia and an inpatient stay of more than 2 weeks were expected. Patients who were found colonized with VRE were followed until discharge by weekly control samples. From January to August 2014, patients were screened and all VRE isolates were collected prospectively. Patients with 3 or more VRE isolates obtained at different sampling times were included. VRE isolates were characterized by PFGE for intra- and inter-individual strain variability over time. Reference VRE strains representative of multilocus sequence type (MLST) 17 and 203, respectively, were analysed for comparison.
**Results**: 16 patients met our inclusion criteria. Up to 13 VRE isolates of one individual patient [mean, 6 (range 3-13) isolates/patient] were obtained over a mean observation period of 55 days [range 20-111 days].
Patients were assigned to 4 different groups based on stability of PFGE patterns over time: In 6 patients we observed one single pulsotype each with up to one band difference (Group 1). Three patients showed 2 distinct pulsotypes each (Group 2). One patient showed at least 5 distinct pulsotypes (Group 3). The remaining 6 patients presented with 1 or 2 separate pulsotypes showing up to 8 subtypes (i.e., 1-7 band differences) within each pulsotype over time (Group 4).
When comparing PFGE patterns inter-individually in 14 out of 16 patients most isolates corresponded to 2 major pulsotypes (A and B). Three patients displayed pulsotype A, 8 patients showed pulsotype B, and 3 patients had both types. Of note, the reference strain of MLST type 17 corresponded to PFGE pattern A, whereas the reference strain of MLST type 203 closely resembled pulsotype B.
**Conclusion**: Multiple variations in PFGE patterns suggest a high intra-individual variability of VRE strains colonizing the gastrointestinal tract in a subset of patients. Co-colonization with distinct pulsotypes may occur. By inter-individual comparison of PFGE patterns the majority of isolates corresponded to 2 major pulsotypes/MLST types. However, extensive variations in intra-individual PFGE patterns over time make transmission and outbreak analysis among VRE colonized patients a real challenge, requiring further investigation.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**166/MSP**

**Molecular epidemiology and antibiotic susceptibility of *Vibrio cholerae* outbreak strains in Ghana, 2011-2014**

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**Question:** With more than 20,000 people affected in the year 2014, Ghana experienced one of its largest Cholera outbreaks in more than a decade. In order to detect causative newly emerging strains or simultaneous outbreaks involving multiclonal strains, outbreak isolates are characterized, subtyped and compared to previous epidemics in 2011 and 2012.

**Methods**: For 92 *V. cholerae* isolates from the years 2011, 2012 and 2014 the serotype, biotype, antibiotic susceptibility (ampicillin, chloramphenicol, ciprofloxacin, gentamycin, tetracycline sulfamethoxazole/trimethoprim, nalidixic acid) and the presence of *ctx*A were determined. For a subgroup of 45 isolates pulsed-field gel electrophoresis (PFGE) analysis, multilocus sequence typing (MLST) and multilocus-variable tandem repeat analysis (MLVA) has been performed.

**Results:** 89 isolates (97 %) were identified as *ctx*A (classical type) positive *V. cholerae* O1 biotype El Tor, with 88 strains belonging to serotype Ogawa and one strain belonging to serotype Inaba. Three (3%) isolates are cholera toxin negative non-O1/non-O139 *V. cholerae*. While only sulfamethoxazole/trimethoprim resistance is detectable in 2011, 95% of all 2014 strains show resistance towards sulfamethoxazole/trimethoprim, ampicillin and reduced susceptible to ciprofloxacin. All subtyped O1 strains belong to MLST sequence type 69. PFGE analysis revealed 11 pulsotypes with two main clusters, which could be further distinguished by MLVA into 22 genotypes and 3 clonal complexes (CC), whereas each CC consists of strains from one of the three outbreak years. Apart from the outbreak clusters additional non-related genotypes circulate during each outbreak period.

**Conclusions:** This analysis suggests an endemic reservoir of *V. cholerae* in Ghana with distinct annual outbreak clusters accompanied by the occurrence of genetically distant genotypes. Rapidly emerging widespread multidrug resistance might result in prolonged clinical symptoms and fecal shedding, which in turn increases the likelihood of increased *V. cholerae* transmission.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**167/MSP**

**The development of surface reaction mechanisms and methods of analysis in early phases ofmolecular encroachment pathways.**

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In the early stages of molecular encroachment that is the actual invasion of alien molecules into the invaded body there are several stages of reactions between the reacting surfaces that can be examined.

**Aim**: The aim of the investigation is to measure and analyse the surface reactions at the point of initial molecular invasion.

**Methods**: The methods employed are those of optical microscopy at an advanced level with calibration techniques and mathematical analysis of photographs following small qauntity invasion volumes.

**Materials**: Human vascular epidermal samples are examined both before during and after introduced invasion with both bacterial RNA and integrin derivatives. Results: The results are found from advanced mathematical methods and visual interpretation to show that the epidermal and immediately subdermal reactions are important stages in molecular encroachment pathways.

**Conclusions**: The mechanisms of surface reactions are dependent on many factors which can be varied by consideration of the invading entity and the altering of these early surface reactions can alter the later effectiveness of methods to counter the invading entity.

Dr Shane Lawrence University of Cambridge and Sci - Tech (South) June 2015.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

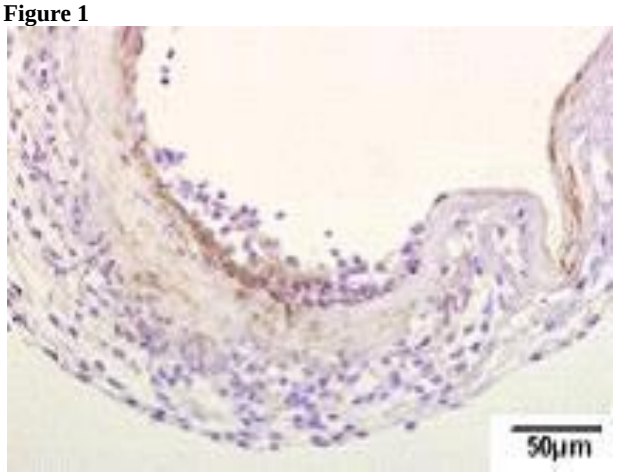
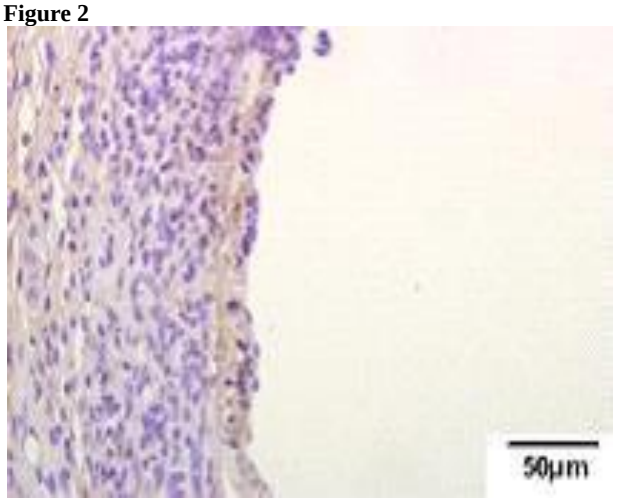



**Figure 2**




**168/MSP**

**Multi-locus-sequence typing of ESBL-producing *E. coli* in a German university hospital**

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The increasing prevalence of extend-spectrum-beta-lactamase (ESBL) producing Gram-negative bacteria is a serious threat for current healthcare settings. The objective of this study was to investigate the molecular epidemiology of ESBL-producing *E. coli* at the University Medical Center Goettingen (UMG). A total of 313 ESBL-producing *E. coli* isolates were collected during a six-month period in 2014. The frequency of ESBL producers on all *E. coli* isolates was found to be 8,2%. Samples were subjected to

multi-locus-sequence-typing (MLST) according to the Achtman scheme. *E. coli* isolates, which were obtained from the same patient within a short time period, and which showed identical sequence types were considered to be follow-up isolates and excluded from statistical analysis. The worldwide dominating *E. coli* sequence type 131 occurred with a frequency of 24% in the collective. The remaining 76% belonged to 51 different sequence types. The most prevalent non-ST131 *E. coli* were ST101 (5%), ST58 (5%), ST10 (4,4%), ST38 (4,4%), ST410 (3,8) and ST453 (3,1%). An analysis of the temporal and spatial distribution of all sequence types revealed that the majority of sequence types displayed a normal distribution. However, *E. coli* ST131 isolates showed a non-randomized distribution with three major clusters of 1-3 weeks within the six month collection period. Moreover, this study identified seven cases, where patients were colonized or infected simultaneously with two distinct ESBL-producing *E. coli* of different sequence types.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**169/MSP**

**Definition and Evaluation of a MLST$^+$ Scheme for Typing of *Enterobacter cloacae* complex**

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**Introduction**: Currently six species (*Enterobacter cloacae, asburiae, hormaechei, kobei, ludwigii* and *nimipressuralis*) are member of *Enterobacter cloacae* complex (ECC). Several of them are isolated as nosocomial pathogens causing sepsis, pneumonia, urinary tract infections, and postoperative peritonitis. In addition, antibiotic resistant strains have been increasingly reported worldwide. Because phenotypic methods frequently fail to determine the correct species, a genotypic method based on *hsp60* gene polymorphisms was already established to differentiate among the ECC members.
**Objectives**: To enable further subtyping of the ECC for infection control purposes, we developed a genome-wide typing scheme based on gene-by-gene comparisons (MLST$^+$ approach).
**Materials & Methods**: We first determined the ECC population diversity based on MLST data of *E. cloacae* using the Bayesian population analysis (BAPS). Subsequently, MLST$^+$ target genes were defined with representative isolates of the ECC based on genome sequence data by Ridom SeqSphere$^+$ software (Ridom GmbH, Muenster, Germany). Finally, we evaluated the MLST$^+$ scheme with genome sequences of 28 clinical ECC isolates and seven additional reference strains.
**Results**: BAPS analysis generated eleven ECC groups. Preliminary creation of the MLST$^+$ scheme based on available NCBI reference genomes sequences representative for six BAPS groups resulted in 2626 MLST$^+$ targets by using the target definer with relaxed parameters (≥ 70 % similarity and 100 % overlap) reflecting the high diversity among the ECC members. Of the MLST$^+$ targets, ≥ 94.2 % were present in all clinical isolates and additional reference strains. MLST$^+$ based grouping of strains was concordant to both, *hsp60* derived ECC groups and BAPS groups. Moreover, two pairs of strains with highly similar (< 2 alleles differ) MLST$^+$ genotypes were determined indicating transmission events. One addition pair of strains with an identical genotype was derived from the same patient from different specimens at different time points. The remaining isolates differed by > 13 alleles.
**Conclusion**: This MLST$^+$ scheme already enables a reliable differentiation within the ECC. Future investigations we will add the missing representatives of the BAPS clusters to achieve finally a pan-ECC MLST$^+$ typing scheme useful for sub-speciation and elucidation of transmission routes.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**170/MSP**

**Consecutive analyses of colonized patients involved in an outbreak with KPC-2 producing Enterobacteriaceae in a German hospital**

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**Objectives**: From 1 October 2013 to 30 September 2014, 132 patients with KPC-2 producing bacteria were detected in a hospital in Germany. Most patients showed rectal colonization with mainly KPC-2 producing *Citrobacter freundii* and *Klebsiella oxytoca*. Only a few symptomatic infections were reported. In August 2014, an additional consecutive screening for KPC-2 producing bacteria was initiated to further investigate the extent of spread of the $bla_{\text{KPC-2}}$ gene in gram-negative bacteria within the human gut.
**Methods:** Patients initially screened positive for KPC-2 producing bacteria and hospitalized during the study period (n=13) were consecutively tested for the presence of KPC producers up to 5 times over a period of 10 days. Rectal swabs were cultivated on selective chromogenic media and colonies of different colour or colony morphology (n=295) were further analysed. Species identification and antimicrobial susceptibility testing were performed. Beta-lactamase genes were identified by PCR and sequencing. Transferability of resistance was tested by broth mate conjugation. Demographic data, prescription of antibiotics and duration of hospital stay were collected from medical records.
**Results**: The 13 patients (3 male; 10 female, median age 80 years) initially identified as colonized with KPC-2 producing bacteria received different antibiotics after admission and in the consecutive screening period due to their underlying diseases; one received a perioperative antibiotic prophylaxis. Our screening revealed that 8 of 13 patients were still positive for different KPC-2 producing bacterial species/genera: *C. freundii, Escherichia coli, K. oxytoca* and *Klebsiella pneumoniae*. These patients received more often beta-lactam antibiotics than the KPC-2 negative patients. All 8 patients carried at least 2 different KPC-2 positive bacterial genera. PFGE analyses showed that 5 patients carried at least 2 different KPC-2 producing *E. coli* strains. Semiquantitative analysis of the bacterial growth on the selective agar plates revealed an increase of KPC-2 producing *E. coli* in 5 patients from day 1 till day 10 during consecutive screening. The $bla_{\text{KPC-2}}$ gene was found to be located on a conjugative plasmid (ca. 60 kb, IncI1). All identified KPC-2 producers were resistant to penicillins, cephalosporins, carbapenems, gentamicin, cotrimoxazole and ciprofloxacin.
**Conclusion**: Our study confirms the presence of at least 2 KPC-2 producing species per patient. The increase of the proportion of KPC-2 producing *E. coli* and presence of different *E. coli* strains and other species indicate a transfer of the $bla_{\text{KPC-2}}$ gene between the bacteria in the gut. Antimicrobial treatment may select for KPC-2 producing isolates and may trigger horizontal gene transfer. This demonstrates the importance of molecular analyses of carbapenem-resistant bacteria to evaluate the epidemiological context.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**171/MSP**

**Molecular characterization of carbapenem-resistant gram-negative bacteria from a Bulgarian hospital**

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**Introduction**: From September 2014 to January 2015, 72 carbapenem-resistant isolates (50 *Acinetobacter* spp., 14 *Proteus mirabilis*, 5 *Escherichia coli*, 1 *Enterobacter cloacae*, 1 *Providencia rettgeri*, 1 *Pseudomonas aeruginosa*) were identified in a hospital in Sofia, Bulgaria. The *Acinetobacter* spp. were isolated mainly from respiratory specimen, the Enterobacteriaceae were mainly from urine samples. In January 2015, all isolates were sent to the Robert Koch Institute for further molecular characterization.
**Methods**: *Acinetobacter* species identification was performed by PCR and sequencing of the *rpoB* gene. Antimicrobial susceptibility testing for all 73 isolates was performed by microbroth dilution and Etest. Beta-lactamase genes were identified by PCR and sequencing. Bacterial strain typing was performed by enzymatic macrorestriction and subsequent pulsed field gel electrophoresis (PFGE).
**Results**: Species identification of *Acinetobacter* isolated revealed the presence of 47 *A. baumannii*, 1 *A. seifertii*, 1 *A. radioresistens*, and 1 *A. pittii*. These isolates produced either carapenemases OXA-23 and/or OXA-72. Bacterial strain typing of all *A. baumannii* showed that the presence of 10 different strains belonging mainly to the international clone 1 (IC-2).
By carbapenemase PCR screening of the remaining isolates we identified NDM-1 in 1 *P. rettgeri*, 1 *P. mirabilis* and in the 5 *E. coli* isolates. VIM-2 was found in *P. aeruginosa* and VIM-1 in 13 *P. mirabilis* isolates. Only the *E. cloacae* isolate did not produce a carbapenemase and showed only increased MIC for imipenem (1mg/l) and meropenem (32mg/L). PFGE analyses revealed identical macrorestriction patterns for the 5 NDM-1 *E. coli* and the 13 VIM-1 producing *P. mirabilis* isolates. These *E. coli* isolates co-harbored beta-lactamases CMY-4 and CTX-M-15 and could be assigned to sequence type ST101. The VIM-1 producing *P. mirabilis* isolates co-harbored CMY-99 and SHV-12.
**Conclusion**: Our study confirmed the presence of carbapenemase producing bacteria in Bulgarian hospitals. For OXA producing *A. baumannii* the occurrence of different strains over the full period of time may indicate a permanent presence in the hospital environment or an import of distinct strains from outside and subsequent clonal transfer within the hospital. Furthermore, the repeated occurrence of one VIM-1 producing *P. mirabilis* and one NDM-1 producing *E. coli* strain is of concern since only few therapeutic options are available in case of infections. Intensified hygiene measurements and surveillance are necessary to control the further spread of these bacteria.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**172/MSP**

**Characterization of the plasmidome of chicken commensal *E. coli* isolates and APEC by plasmid MLST**

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**Introduction**: Antimicrobial drug resistance and virulence in *bacteria* are often mediated by plasmids. The majority of plasmids have the potential for conjugative transfer thus contributing to the emergence of new resistant and/or virulent bacterial strains. Here, we investigate the plasmidome of 120 commensal *E. coli* isolates from healthy chickens and APEC living in different forms of animal husbandry as well as different geographic regions to provide an insight into the plasmid population dynamics.
**Methods**: The plasmids of 120 chicken commensal *E. coli* and APEC isolates were assigned to the corresponding incompatibility (Inc) groups by PCR-based replicon typing according to Carattoli *et al.* (2005). On the basis of the identified Inc groups the plasmids were characterized by plasmid MLST (pMLST, http://pubmlst.org/plasmid/). Finally, phylogenic relationships of the plasmids were analyzed.
**Results**: By PCR-based replicon typing we could identify five strains with plasmids belong to Inc group HI2, 25 strains belonging to Inc group I1 and 104 strains belonging to group IncF. Our results show that none of the incompatibility groups was exclusively restricted to either commensal *E. coli* or APEC strains. Nevertheless, subtyping by pMLST and thus more detailed plasmidome characterization was still possible.
**Discussion**: The results of this study will help to analyze the prevalence, variability and phylogenetic relationship of plasmids in chicken-associated *E. coli* isolates. Correlation of our results of the plasmidome profiling with phenotypic traits like antimicrobial drug resistance and virulence properties may enable a better understanding of conversion from commensal to pathogenic behavior and the spread of virulence and resistance determinants.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**173/MSP**

**Spatial clustering of rabies virus genomes using affinity propagation clustering**

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Rabies is one of the oldest known zoonosis, caused by rabies virus an important species of the genus Lyssavirus. More than 55.000 people die of rabies in the world every year. So far, the spread of rabies virus is analyzed on regional levels, since a global phylogenetic clustering and classification system is not yet available. We therefore constructed a database containing all available sequenced rabies samples. A phylogenetic tree calculated by Maximum Likelihood method of 230 full genome sequences suggests a space-dependent clustering. The same analysis was conducted using the N-genes derived from the full genome sequences. An almost identical dendrogram concerning structure and bootstrap support was obtained. The availability of a large number of N-gene sequences with known origins (N=1,448) increases the spatial resolution. Our analyses revealed two limitations of this approach: (i) the analysis of large datasets results in highly complex dendrograms. Therefore, the definition of spatial clusters, as done with the full genome sequences, is ambiguous. (ii) The clustering of phylogenetic trees by visual inspection could lead to different results due to a lack of criteria for cluster definition.
Affinity propagation clustering is a mathematical method that uses the phylogenetic distance matrix to allocate the included sequences to generic clusters. In addition, the optimal number of clusters is calculated. We applied this method to the distances matrices derived from the full genome and from the N-gene sequences. The resulting cluster structures and the composition of each cluster strongly corresponds to the dendrogram structures seen in the Maximum Likelihood-based dendrogram. The observed spatial clustering could thus be confirmed. Affinity propagation clustering also facilitates analysis of large datasets since it defines one exemplary cluster member for each cluster.
In conclusion, affinity propagation clustering is a fast and objectified method for grouping sequences. We applied this method in order to validate evidence for spatially-dependent clustering of rabies virus sequences.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

# NGS GENOME ANALYSIS AND BIOINFORMATIC TOOLS (MSP)

**174/MSP**

**Complete genome sequence and DNA methylation profile of *Campylobacter coli* BFR-CA-9557**

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T. Lingner[4]
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*Campylobacter species* are the most prevalent bacterial pathogen causing acute enteritis worldwide. In contrast to *Campylobacter jejuni* about 5% of *Campylobacter coli* strains exhibit susceptibility to restriction endonuclease digestion by *Dpn*I cutting specifically 5'-G$^{m}$ATC-3' motifs. This indicates significant differences in DNA methylation.

The goals of the study was to analyze the methylgenome of a *C. coli* strain susceptible to *Dpn*I digestion, to identify its methylation motifs/restriction modification systems (RMS) and compare them to related organisms like *C. jejuni* and *Helicobacter pylori*.

50 *C. coli* isolates of different origin were analyzed using 5'-G$^{m}$ATC-3' specific isoschizomer digestion assays. One positive tested strain, BFR-CA-9557, was sequenced on SMRT cells using PacBio RS sequencing technology followed by PacBio Modification and Motif Analysis.

Using one SMRT cell the complete genome of *C. coli* BFR-CA-9557 (1 single contig: 1.72 Mb, average reference coverage: 500.8, 1851 genes, 1797 CDS, 44 tRNAs, 1 tmRNA, 9 rRNAs) was sequenced. The G+C content was 31.4%. 45,882 6-methylated adenins (ca. 2.7%) and 1,789 4-methylated cytosins (ca. 0.1%) have been detected. The genome contains a CJIE1-like element prophage and a pCC42yr-homologue plasmid.

11 different methylation motifs corresponding to 9 distinct recognition sequences (4 with partner motif, 2 palindromic) have been identified. 10 of these were m6A motifs and only one was a potential m5C methylation motif. Only 2 motifs correspond to known restriction modification motifs. Characteristic for this methylgenome was the very high fraction of methylation - mostly >99%.

In comparison to *C. coli* BFR-CA-9557 only 5 dominant methylation motifs have been identified in *C. jejuni*. All 5 have been associated with known restriction modification systems (RMSs). *C. coli* BFR-CA-9557 has only one of these, but 5 orfs could be assigned to putative type I RMS, 6 orfs to type II RMS and 4 orfs to type IV RMS. In accordance with the *Dpn*I prescreening RMS IIP, methylating GATC motifs, is expressed in *C. coli* BFR-CA-9557. RMS IIP is also found in *H. pylori*. The remaining methylation motifs are specific for *C. coli* BFR-CA-9557 and have been neither detected in *C. jejuni* nor in *H. pylori*. One outstanding finding was the detection of the possible m5C methylation motif GGB, which was not described before.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**175/MSP**

**"Coxomics": Enhancing the *Coxiella* genome with Omics data**

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**Introduction:** Up to date, there are more than twenty *Coxiella burnetii* whole genome sequences available. Some of them were annotated using different techniques and bioinformatics tools, the most recent once with the NCBI Prokaryotic genome annotation pipeline. Still none of these annotations reflect the recent effort in the reannotation of the seven reference strains and its new findings. To address this issue and to further refine the reference genome Nine Mile 493, different RNA-Seq experiments were conducted and additional omics data sets were integrated.

**Material and Methods:** The current available whole genome sequence data for *Coxiella burnetii* (22 strains) were analysed according to current database information of genome and replicon completeness, genes and proteins and the precision of bioinformatics tools for assembly and annotation.

Based on an earlier developed genome annotation pipeline (combining multiple gene-calling programs with similarity-based methods and synteny information of closely related species) including published data from proteome and transcriptome experiments (stranded RNA-Seq, short and long ncRNAs, 5' Tagged RNA-Seq), the *Coxiella* genomes were further refined.

**Results:** Compared to the existing data in the NCBI RefSeq database, our re-annotation revealed 9 new protein coding genes and 28 putative non-coding RNAs, removed 57 spurious ORFs, changed 34 pseudogenes into functional genes transcribed by programmed ribosomal frameshifting, corrected the start of 113 genes, confirmed 410 multi-gene operons, structurally annotated nearly 1,200 promotor regions and about 1,000 3' untranslated regions, confirmed the existence of 883 proteins as well as functionally annotated 86 former hypothetical proteins.

**Conclusions:** The relevance of re-annotation was impressively demonstrated with the studied *Coxiella burnetii* omics data. Beside the value in verification of genes, also functional aspects are very important for the understanding of pathogen characteristics including the interaction with different hosts. Therefore it will be a useful approach to publish genome sequences in the future only with the application of new and validated assembly and annotation tools. It is also the fundamental resource for comparative genomics resulting in a correct pan-, core and dispensable genomes.

In addition, curation and updating of existing datasets is also a meaningful task to achieve a validated and reliable basis for current and future scientific research on this query agent.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**176/MSP**

**Reproducible Analysis of DNA Amplification Experiments with an Open Source Software Environment**

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**Introduction**: There is an ever-increasing number of applications, which use quantitative PCR (qPCR), digital PCR (dPCR) or melting curve analysis (MCA) to elicit fundamentals of biological processes. Moreover, quantitative isothermal amplification (qIA) methods has come into use in diagnostics. Several software solutions have been proposed for the analysis of qPCR, dPCR, qIA or MCA experiments. However, most of them are either tied to a specific task (e.g., qPCR data) or closed source software with little control over the analysis algorithms

**Aim**: We argue, among others, that R is an excellent foundation for reproducible and transparent data analysis in a highly customizable cross-platform environment. However, for novices it is often challenging to master R or learn capabilities of the vast number of packages available.

**Material/Methods**: We started to build a comprehensive suite for the analysis of the various data types. Our software is based on the open source, cross-platform statistical computing language R. Our approach is useful for tracking numerical errors easy debugging and user-side code inspection. In addiation, we implemented selected functionality in the graphical user interface (GUI) and integrated development environment ***RKWard*** [1]. For rapid prototyping of ***RKWard*** GUI plugins we used the ***rkwarddev*** package.

**Results**: Here we show workflows for the analysis of qPCR, qIA, MCA or dPCR experiments based on our framework. The software is distributed as open source software and can be used to build non-monolithic blocks with freedom to perform highly customized

analysis procedures. Our software is targeted at users who develop novel assays or users who wish to analyze raw and unprocessed data from commercial systems. The complete analysis pipeline consisting of statistical procedures, raw data preprocessing, analysis, plots and report generation are implemented for increasingly demanded reproducible research [2-5].
**Summary**: Our analysis relies entirely on R packages available from public repositories. Additionally, we provide information related to standardized and reproducible research.

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**177/MSP**

**Comparative whole genome analysis of three *Salmonella diarizonae* isolates from a single patient causing a fatal endovascular graft infection**

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**Introduction:** Most cases of human salmonellosis are caused by *S. enterica* subsp. *enterica*, whereas infections of humans caused by other subspecies are very rare. Strains of *S. enterica* subsp. *diarizonae* (*S. diarizonae*) are often isolated from cold-blooded animals. Little is known about the virulence capabilities and underlying molecular mechanisms of *S. diarizonae*. Although *S. diarizonae* possess the *Salmonella* Pathogenicity Island 2 (SPI-2) that is required for intracellular survival and systemic infection, *S. diarizonae* was attenuated in a mouse model of systemic infection compared to *S.* Typhimurium[(1)].
**Methods:** The first *S. diarizonae* isolate was obtained from the stool of a 77-years old male patient suffering from advanced rectal cancer with acute diarrheal symptoms. After 7 months, the patient presented with a pseudoaneurysm due to an infected dacron aortobifemoral bypass that required surgical intervention. *S. diarizonae* was isolated from the excised graft and of stool 4 days later. The patient died due to the progressive infection. After serological characterization and pulsed-field gel electrophoresis (PFGE) of all strains, the genome of the first isolate was sequenced and *de novo* assembled using Pac Bio technology. All strains were sequenced using an Illumina MiSeq which reads were mapped on the Pac Bio reference. Intracellular replication of all strains was quantified in RAW264.7 macrophages. Furthermore, activity of a fluorescent SPI-2 reporter was quantified *in vitro* and in bacteria isolated from macrophages using flow cytometry.
**Results:** The identical antigenic formula (60:r:z) and a similar PFGE pattern indicated a clonal origin of all strains. However, whole genome sequencing (WGS) revealed 4 single nucleotide polymorphisms (SNPs) in the first isolate, one within the RNA chaperone Hfq and the others in metabolism-associated genes. Interestingly, there were marked differences in the plasmid content of the 3 isolates. Further characterization revealed that all isolates were attenuated for replication within mouse macrophages and SPI-2 activation was significantly lower in *S. diarizonae* compared to *S.* Typhimurium.
**Discussion:** The presence of SNPs leading to mutations in the first isolate and lack thereof in the other isolates points towards variability within a clonal population rather than host-directed evolution. There was no impact of these mutations on virulence in a macrophage infection model. Quantification of SPI-2 activation revealed a defect in SsrAB-dependent gene expression in *S. diarizonae.* This may explain the reduced capability for intracellular replication of *S. diarizonae*. However, immunosuppression due to multimodal therapy of advanced rectal cancer and/ or advanced rectal cancer may favor systemic *S. diarizonae* infection with fatal outcome.

**Reference**
(1) Katribe *et al*. (2009) J. Bacteriol. 191:2843-50

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**178/MSP**

**Comparative Analysis of the Gut Microbiome in Multiple Sclerosis and Parkinson's Disease Patients**

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**Introduction**: The etiopathogenesis of many progressive neurological disorders, such as multiple sclerosis (MS) and Parkinson's disease (PD), is still not fully elucidated even though an increasing number of neuropathologic, genetic and clinical trials have been performed during the last decades. As non-Mendelian diseases, both MS and PD have also attracted the identification of various environmental risk factors, including infectious agents, toxins, smoking, and decreased vitamin D levels. However, one of the largest body surfaces that profoundly interacts with the environment has long been neglected: the gut.
Aims: With the growing interest in Next Generation Sequencing (NGS) techniques, and the observation that antibiotic eradication of the gut microflora led to amelioration of disease course in the animal model of MS, we established the MIBI-MS-PD consortium, an interdisciplinary group of microbiologists, clinical neurologists, and neuroimmunologists, to prospectively study the role of the gut microbiome in etiopathogenesis of progressive neurological disorders.
**Material & Methods**: For proof of concept, we selected MS as a model neuroinflammatory disease with neurodegenerative features (with subgrouping according to the clinical course, i.e., relapsing remitting MS (RRMS) and secondary progressive MS (SPMS)), and PD as a model primary neurodegenerative disorder. In the pilot study, stool samples of 46 patients were included (PD: n=23; RRMS: n=9; SPMS: n=14), and subjected to NGS (Illumina MiSeq system) subsequent to 16S rDNA amplification using primers for the V1-V3 region. Forward and backward fragments were quality controlled and assembled with the mothur program suite using the MiSeq SOP or with the usearch program. OTUs were assigned to taxonomic lineages with the SILVA database and association of species with diseases was analyzed by the indicator species function of mothur or in R. Of identified species we selected a subgroup for which qPCR was done on stools and association of species with disease groups was reassessed with R.
**Results**: The NGS analyses revealed valuable general information on the bacterial composition and diversity of the gut microbiome in MS and PD patients. The disease groups differed in microbial composition as well as in diversity as assessed e.g. by Shannon index. Several species identified by the indicator species function also showed significant differences in the qPCR validation step.
**Summary**: We report first data gained by our newly established MIBI-MS-PD consortium. Preliminary results indicate that the gut flora of patients with these diseases differ significantly in composition. Follow-up projects are ongoing and might shed light on the role of the gut microbiome in etiopathogenesis of progressive neurological disorders.

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**179/MSP**

**MinION™-Sequencing- A new approach for rapid diagnostics in the field?**

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Rapid detection and identification is essential for the diagnosis of bacterial or viral infections especially in case of fatal diseases caused by dangerous pathogens. In stationary diagnostics Whole-Genome-Sequencing (WGS) of microorganisms combined with bioinformatics has become a key technology for the reconnaissance of unusual disease outbreaks. The feature of this non-selective method is its potential to enable the identification of unknown agents, where selective diagnostic tests failed to detect any target organism. For the reconnaissance of outbreak scenarios the Bundeswehr Institute of Microbiology employs a rapidly deployable mobile diagnostic unit with capabilities for molecular detection. The institute is currently evaluating novel sequencing capabilities for unknown pathogens as part of its growing diagnostic toolbox.
With the appearance of Nanopore sequencing, e.g., the MinION™ - device from Oxford Nanopore Ltd, this technique reached dimensions of a hand-held-device. In combination with a laptop as control and evaluation unit sequencing has become mobile and site-independent. Using cloud-based "on-the-fly" base-calling, first results can be obtained within 2 hours.
In this study we present a proof-of-concept approach for the use of the MinION™ - device as an extension of the molecular diagnostic capability within the framework of a rapidly deployable laboratory. During a field exercise at a German military training ground, which was focused on vector-borne diseases, specimens from ticks and rodents were investigated. For the identification of pathogenic bacteria, like *Rickettsia* or *Borrelia*, non-selective NGS-based 16S rDNA sequencing was performed and their results analyzed. Spiked samples were used as positive-controls.
We reliably obtained genome sequences of several pathogens. In the investigated samples, we could discriminate closely related bacteria, but were not able to detect any *Rickettsia* or *Borellia* specific sequences. This negative result could be confirmed by specific PCR subsequently.
Our proof-of-concept confirms that the MinION™ sequencing technology enables the on-the-spot identification of unknown organisms. Due to the limited data output the actual used system is not suitable for in-depth-investigation of 16S amplicon sequencing of metagenomes. For use in remote areas further improvements are needed to unbind the base-calling from "the cloud". However, equipped with a suitable communication link, the device is a reasonable complement to our deployable lab configuration and supports a rapid identification of unknown pathogens.

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**180/MSP**

**Single nucleotide polymorphism (SNP) analysis of bovine and human enterohemorrhagic *E. coli* (EHEC) and atypical enteropathogenic *E. coli* (aEPEC) strains of sequence type complex STC29**

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Multilocus sequence typing (MLST) of EHEC and aEPEC strains of the four most important non-O157 Serotypes (O26, O103, O111, O145) revealed that these strains represent a single sequence type complex (STC) - STC29. Within ST16, ST21, ST29 and ST113, the major sequence types of STC29, the mentioned serotypes cluster together. Furthermore both, aEPEC and EHEC, can be designated to the same STs. Hence STC29 displays the close relationship between aEPEC and EHEC and their connected evolutionary background, even though they were characterized as different serotypes by now.
For this reason we have chosen 100 strains of STC29 for whole genome sequencing. The strains were isolated from human patients (n=54) and cattle (n=64), and belong to both pathotypes aEPEC and EHEC. The maximum common genome (MCG) of the 100 genomes was defined by identifying a set of conserved genes occurring in every of the considered genomes. The following single nucleotide polymorphisms (SNP) analysis of 3.710 orthologous genes revealed >30.000 SNP-sites. On the basis of these SNPs maximum spanning trees (MSTs) were generated to highlight the different characteristics of each strain (ST, pathotype and host).
Clusters formed within the SNP-site based MST represented the single seuqnece types of an allele based MST. This underlines that the MLST of seven housekeeping genes is a powerful method to display the relationship of *E. coli* strains. For the representation of the pathotype within this MST no tendency was recognizable, underlining the similarity between EHEC and aEPEC strains and their likely equal evolutionary background. For the host the strains originated from no cluster formation was revealed, which rejects a host specificity of the strains and therefore supports the evidence for zoonotic transmission of EHEC and aEPEC strains.

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**181/MSP**

**Genome-wide Analysis and Comparison of EHEC for Improved Strain Typing**

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**Question**: Enterohaemorrhagic *E. coli* (EHEC) are important human pathogens which cause bloody and non-bloody diarrhoea. Due to their ability to cause large outbreaks and systemic complications, such as haemolytic uraemic syndrome (HUS), EHEC infections create a high public health impact. Current routine EHEC diagnostics mainly relies on the detection of *stx* and the intimin-encoding *eae* genes and focuses otherwise on the most common serotypes responsible for the majority of disease cases O157:H7, O26:H11, O103:H2, O111:H8 and O145:H28, the so-called "big five". Due to the focus on these serotypes, rapid identification of other EHEC variants, e.g. the O104:H4 outbreak strain in 2011 was severely impaired in many routine diagnostic labs. Consequently, there is an urgent need for comprehensive and reliable diagnostic tools for all clinically relevant EHEC serotypes.
A reliable risk assessment of EHEC isolates requires the determination of discriminatory marker combinations which allow unambiguous discrimination of EHEC variants with the potential to cause disease in humans. To achieve the goal to differentiate between highly pathogenic, HUS-inducing *E. coli,* other EHEC strains and even non-pathogenic Stx-producing *E. coli*, we apply a genome wide approach and compare all publicly available EHEC genomes with those of other pathogenic or non-pathogenic *E. coli*.
**Materials & Methods**: Publicly available *E. coli* genome sequence data from the RefSeq and sequence read archive (SRA) databases have been retrieved, assembled, and analysed. After *de novo*-assembly of the reads with SPAdes, the draft genome sequences were analysed with SeqSphere+, ssGeneFinder and Gegenees.
**Results**: 57 EHEC and 89 other diarrheagenic *E. coli* genome sequences, respectively, have been collected from the SRA and assembled with SPAdes. Following the assembly, these genomes were subjected to whole genome-based phylogenetic analyses incl. rMLST. Furthermore the allele content and SNP variants were investigated.

**Conclusion**: The combined results are a promising start to screen for genomic markers with a high discriminatory power to improve EHEC detection. These markers can then be used as targets for real-time PCR or LAMP assays. Additionally the gathered genome information can be used to address further questions concerning the variability of genomic regions, regulation of virulence genes and insights into outbreak population dynamics and bacterial evolution.

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**182/MSP**

**Staphylococcus aureus adaptation potential: clues from genomic analysis and bioinformatics**

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Staphylococcus aureus is not only clinical important, it its multiresistant form it is one of the main challenges in hospital treatment of chronic and severe infections. We systematically collect and analyse a large collection of different S.aureus strains with the aim to improve
therapeutic strategies and general understanding of its interaction with the human host as well as its general adaptation potential. We will present recent results in this area, comparing different approaches and bioinformatics tools:
(1) Metabolic modelling (YANA suite of programs) comparing the metabolism in different S.aureus strains under changing nutrient scenarios.
(2) Systems biological study of different protein complexes, highlighting here again strain specific changes and different tools to identify changes in protein complex composition
(3) Regulatory transcription factors important for environmental adaptation and tools to model this dynamic adaptation of S.aureus strains, e.g. to redox stress.
(4) Specific wall structure and resulting evolutionary adaptation speed.
We compare these approaches and their insights with a general overview on strain richness of S.aureus and the impact of this on its adaptation potential and virulence.

**References**

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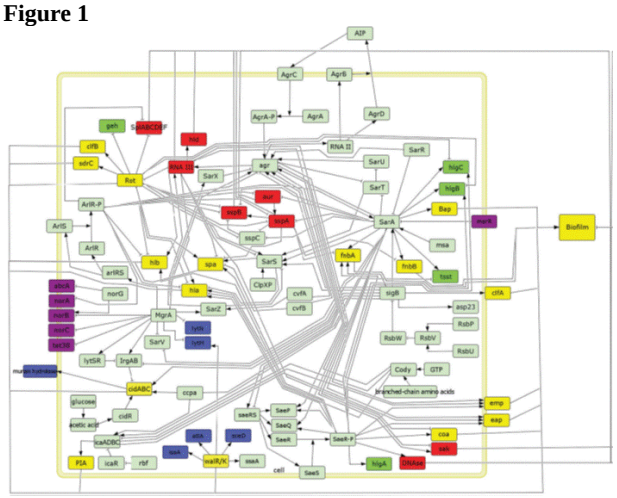
**Figure 1**



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**183/MSP**

**Comparative genomics-based analysis of different growth rates of *Coxiella burnetii***

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A recently developed Acidified Citrate Cysteine Medium (ACCM) allows considerable growth of the highly infectious obligate intracellular bacteria *Coxiella burnetii* in the absence of host cells. This is an important development since *C. burnetii* could previously only be studied with difficulties using in vivo models, which largely impeded the understanding of the molecular mechanisms behind its virulence and overall pathogenicity. Nevertheless, the axenic growth of *C. burnetii* in ACCM differs widely, with some strains presenting excellent growth and expanding 1,000 fold (reference strain Nine Mile), whereas some strains grow moderately, about 100 fold or grow weakly (only 2 fold expansion) or not at all. Here, we present the results of our in silico analysis to identify a possible molecular background and the genetic reasons for the divergent growth behaviour between strains Nine Mile phase 1 (RSA493), K (Q154) and Priscilla (Q177).
In a first step a tRNA abundance and mutation analysis was done. Secondly, the tRNA loci and gene contexts were investigated because there are quite a lot of known genome rearrangements in *C. burnetii* which could led to changes in translation efficiency.
Afterwards, the pan-, core- and dispensable proteome of the three strains was constructed using cluster of orthologous groups computed based on previously reannotated genomes. These clusters were then investigated at three different levels: presence/absence of orthologous genes, paralogs and variations in highly conserved genes.
The analysis revealed a promising candidate that likely has a relevant influence on the strains' axenic growth. Q154 and Q177 have a catalase which degrades $H_2O_2$ producing $O_2$, most likely considerably increasing the medium's oxygen concentration, which is negatively correlated with axenic growth performance, while this protein is truncated in RSA493. To elucidate this hypothesis further more studies will include strains which show no growth in ACCM.

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# ANTIMICROBIAL RESISTANCE AND DRUGS, INFECTION PREVENTION (PRP)

## 184/PRP

**Susceptibility to ceftobiprole in blood and respiratory isolates collected from hospitalized patients prior to the introduction of this group 5 cephalosporin: results of the PEG study**

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**Introduction and Purpose**: Empirical treatment of hospital-acquired pneumonia (HAP) has increasingly been threatened by methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug resistant Gram-negative pathogens. In contrast, empirical treatment of community-acquired pneumonia (CAP) is primarily impeded by antimicrobial-resistant pneumococci. Ceftobiprole, recently approved for the treatment of HAP (non-VAP) and CAP in Europe, is active against a broad-spectrum of Gram-positive and Gram-negative pathogens, including MRSA and *Pseudomonas aeruginosa*. The objective of this study was to evaluate the ceftobiprole susceptibility among a total of 1,246 respiratory tract and blood isolates of *S. aureus*, *Streptococcus pneumoniae*, Enterobacteriaceae species and *P. aeruginosa*.
**Methods**: Isolates were collected in 25 laboratories across Germany (n=21), Switzerland (n=3) and Austria (n=1) in 2010. Minimum inhibitory concentrations (MICs) were determined using the microdilution method according to the standard ISO 20776-1 and interpreted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Two-thirds of the isolates were obtained from respiratory specimens and one third from blood. There were 544 intensive care unit (ICU) isolates and 702 non-ICU isolates. The share of MRSA in *S. aureus* was 16%. Among pneumococci, 18.5% showed reduced susceptibility to penicillin. An extended-spectrum β-lactamase (ESBL) phenotype was confirmed for 18.4% of the *E. coli* and 16.7% of the *K. pneumoniae* isolates.
**Results**: $MIC_{50/90}$ values of ceftobiprole for methicillin-susceptible *S. aureus* (MSSA) and MRSA were 0.5/0.5 mg/L and 2/2 mg/L, respectively. All pneumococci were inhibited at 1 mg/L ceftobiprole. The activity of ceftobiprole against *E. coli* and *K. pneumoniae* was similar to that of ceftriaxone, but ceftobiprole showed superior activity against AmpC-producing Enterobacteriaceae. $MIC_{50/90}$ values of ceftobiprole for ceftazidime-susceptible (4/16 mg/L) and ceftazidime-resistant *P. aeruginosa* (16/>32 mg/L) were comparable to those of cefepime (4/8 mg/L and 32/>32 mg/L, respectively).
**Conclusion**: The findings suggest that ceftobiprole may represent a suitable option for the empirical treatment of HAP (non-VAP) and CAP.

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**Figure 1**

Table: Susceptibility of isolates to ceftobiprole

| Organism / phenotype (n) | Cumulative % of isolates inhibited at MIC (mg/L): ≤0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | ≥64 | %S |
|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* (188) | 21.8 | *83.0* | 84.6 | *98.4* | 100[a] | | | | | 98.4 |
| MS (158) | 25.9 | *98.7* | 99.4 | 100 | | | | | | 100 |
| MR (30) | | | 6.7 | *90.0* | 100[a] | | | | | 90.0 |
| *S. pneumoniae* (254) | *94.9* | 98.8 | 100 | | | | | | | 98.8 |
| PS (207) | *100* | | | | | | | | | 100 |
| PN (47) | *72.3* | *93.6* | 100 | | | | | | | 93.6 |
| *E. coli* (179) | *79.9* | 81.6 | 82.7 | | | | 83.8 | 84.9 | *100* | 79.9 |
| ESBL negative (146) | *96.6* | 98.6 | 100 | | | | | | | 96.6 |
| ESBL positive (33) | 6.1 | | | | | | 12.1 | 18.2 | *100* | 6.1 |
| *K. pneumoniae* (108) | *79.6* | 82.4 | | 84.3 | | | | 85.2 | *100* | 79.6 |
| ESBL negative (90) | *95.6* | 98.9 | | 100 | | | | | | 95.6 |
| ESBL positive (18) | | | | 5.6 | | | | 11.1 | *100* | 0 |
| *K. oxytoca* (44) | 43.2 | *68.2* | 77.3 | | | | | | *100* | 43.2 |
| *Enterobacter* spp. (89) | *74.2* | 77.5 | 78.7 | 82.0 | 89.9 | *93.3* | 94.4 | 95.5 | 100 | 74.2 |
| *Serratia* spp. (74) | *85.1* | *91.9* | 95.9 | 97.3 | | 98.6 | | 100 | | 85.1 |
| *Citrobacter* spp. (26) | *84.6* | *92.3* | | | | | | | 100 | 84.6 |
| Proteeae (43) | *76.7* | | 79.1 | | 81.4 | 83.7 | | 88.4 | *100* | 76.7 |
| *P. aeruginosa* (241) | | 0.8 | 5.0 | 29.0 | *57.7* | 77.6 | 87.1 | *90.0* | 100 | NE |
| CAZ-susceptible (191) | | 1.0 | 5.8 | 36.1 | *68.1* | 88.5 | *96.3* | 97.9 | 100 | NE |
| CAZ-resistant (50) | | | 2.0 | | 18.0 | 36.0 | *52.0* | 60.0 | *100* | NE |

[a] Etest© MICs were 3 mg/L (resistant) for one isolate and 1-1.5 mg/L (susceptible) for two isolates; Abbreviations: %S, % susceptible; MS, methicillin-susceptible; MR, methicillin-resistant; PS, penicillin-susceptible; PN, penicillin-non-susceptible; ESBL, extended spectrum β-lactamase; CAZ, ceftazidime; NE, not evaluable as EUCAST has not defined a species-related breakpoint yet; MIC50/90 values are given in italic.

## 185/PRP

**Admission Prevalence of third generation cephalosporin-resistant Enterobacteria (3GCREB) - a cross-sectional study in 6 university hospitals**

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**Introduction:** This admission prevalence survey is part of the multicenter study ATHOS (antibiotic therapy optimisation study). ATHOS aims at collecting prevalence and incidence data for nosocomial carriage of multi-drug resistant organisms (MDROs) and to intervene in the inpatient and outpatient setting.
**Objectives:** The aim of this admission prevalence survey was to assess the rectal carriage of third generation cephalosporin-resistant enterobacteria (3GCREB) in patients on hospital admission and to perform risk factor analyses for 3GCREB carriage.
**Methods:** In 2014, we recruited adult patients within 72 h of admission to non-intensive care units in six German university hospitals. We obtained rectal swabs that were screened for 3GCREB. Each patient was asked to answer a short questionnaire on potential risk factors for colonisation with MDROs. Univariable and multivariable risk factor analyses were performed on preliminary data to identify those factors that were associated with 3GCREB prevalence.
**Results:** Of the 4376 patients included, 417 patients were 3GCREB carriers (admission prevalence of 9.5%). Most isolates were *Escherichia coli* (76.8%). Surprisingly, 42.2% of all 3GCREB isolates were additionally resistant to fluoroquinolones. Five patients (1.2%) were colonised with carbapenemase-producing enterobacteria. Multivariable analysis associated the following risk factors with 3GCREB colonisation: centre, previous MDRO colonisation (OR = 2.1, p<0.001), antibiotic use (OR=2.1, p<0.001), travel outside of Europe (OR=2.9, p<0.001), occupational animal contact (OR=1.3, p=0.033) and management of gastroesophageal reflux disease (GERD) (OR=1.2, p=0.010).
**Conclusion:** To our knowledge, this is one of the largest admission prevalence surveys of 3GCREB in Germany. Interestingly, occupational animal contact, medical management of GERD and the specific centres to which the patients where admitted proved to be additional risk factors for 3GCREB colonisation on hospital admission. Whether information present on admission will be useful to improve prediction of nosocomial colonisation and infection as well as target infection control measures and therapy needs to be determined.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**186/PRP**

**Antimicrobial Drug Delivery by Bacterial Effector Protein-Derived Cell-Penetrating Peptides (CPPs)**

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Intracellular pathogens constitute a challenge for conventional antimicrobial therapies, since intracellular levels of therapeutic drugs are difficult to achieve. Drugs and therapeutic molecules generally lack a membrane penetrating capability and are not able to easily translocate into the cytoplasm. Attempts to bind these drugs to polymers usually resulted in degradation and/or loss of activity. As cell-penetrating peptides (CPPs) are capable to mediate the internalization of biologically active molecules through plasma membranes they might also be employed to translocate antimicrobials into infected host cells.
In this study, we characterized the alpha helical tandem PTD (2αH) and single PTDs (α1H and α2H) derived from the *Y. enterocolitica* effector protein YopM, which previously showed cell-penetrating ability. These CPPs, as well as the well-described Tat peptide derived from HIV, are investigated for their capability to deliver exemplary antimicrobial agents, such as siRNAs to inhibit viral replication or antibiotics to target intracellular bacteria.
We showed that YopM-derived peptides possess penetration ability to the same extent as the Tat peptide. Additional uptake mechanism studies revealed that both endocytosis and direct penetration are employed for translocation of the compounds indicating that multiple entry pathways are involved, a shared feature with many CPPs.
Upon covalent conjugation with CPPs, siRNA molecules were efficiently delivered into living cells. Moreover, CPPs delivering siRNA were shown to reduce viral replication.
Additionally, for the first time to our knowledge, CPPs were conjugated to an antibiotic. The CPP-antibiotic conjugates were delivered into infected cells and reduced the load of intracellular pathogenic bacteria such as *E. coli* K1, *Salmonella*, and *Shigella*, as demonstrated by invasion assays.
Taken together, these data underline the potential of CPPs as delivery vehicles for antimicrobial agents. This might open the possibility for a remarkable new tool for the treatment of infectious diseases caused by intracellular pathogens.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**187/PRP**

**MRSA decolonization in outpatients without topical antibiotics - a retrospective study**

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The majority of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) develops endogenously. Therefore the periinterventional decolonization therapy has become an important component of preventive infection measures. Mupirocin is still used as standard for nasal decolonization. However, mupirocin resistance and limitations of substance availability require evaluation of new treatment concepts.
Using the nasal topical antiseptic polihexanide 0.1 % (3x/d) without mupirocin, the effectiveness of MRSA decolonization treatment has been investigated in dermatologic outpatients at the University Hospital of Muenster. In addition, standardized topical and systemic therapy measures have been used in addition to the nasal treatment.
Within an observation period of 15 months, 63 patients presented to the outpatient department; 42 (66.7 %) of these persons were tested positive for MRSA and 27 (64.3 %) of them followed the protocol of decolonization. These patients were examined with regard to localization of colonization, presence of inhibiting factors for decolonization, the molecular pattern and classification (*spa*-typs) such as the effectiveness of the topical treatment. A successful decolonization was achieved in 81.5 % (n=22). In a post-decolonization period of six months no relapses were reported. A successful antiseptic decolonization was performed in approximately one third (n=7; 31.8 %) of MRSA-carriers in case of absence of inhibiting factors for eradication and colonization of only one localization. An additional systemic antibiotic was used in 15 patients (68.2 %). In case of a single nasal colonization with MRSA one topical eradication run with the mupirocin free antiseptic polihexanide 0.1 % was sufficient for a successful decolonization.
In conclusion, mupirocin-free eradication in dermatologic outpatients with MRSA colonization is possible. In times of increasing antibiotic resistances polihexanide shows little adverse effects and proves as a simple and successful alternative to current standard methods with topical antibiotics.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**188/PRP**

**Susceptibility of multidrug-resistant pathogens from German hospitals to fosfomycin, 2013: results of the PEG study**

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**Introduction and Purpose**: The treatment of infectious diseases has increasingly been threatened by the emergence and dissemination of multidrug-resistant (MR) pathogens, like methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase-producing or carbapenem-resistant Enterobacteriaceae. Fosfomycin (FOS) has been shown to play a role in the management of infections caused by MR bacteria. The objective of this study was to evaluate the susceptibility to FOS among a total of 405 MR staphylococci (*S. aureus*, *S. epidermidis*) and 125 MR Gram-negative (MRGN) bacteria of the family Enterobacteriaceae.
**Methods**: Isolates were collected in 22 laboratories across Germany from October to December 2013. Minimum inhibitory concentrations (MICs) were determined using the microdilution broth method according to the standard ISO 20776-1 and interpreted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints for FOS (version 5.0, 2015). The collection of pathogens included isolates of the following MR bacterial groups: MRSA (n=94), MR *S. epidermidis* (MRSE, n=311), 3MRGN *Escherichia coli* (ECO, n=59), 3/4MRGN *Klebsiella pneumoniae* (KPN, n=40), 3MRGN *K. oxytoca* (KOX, n=8), 3/4MRGN *Enterobacter cloacae* (ECL, n=14) and 3MRGN *Proteus mirabilis* (PMI, n=4).
**Results and Conclusion**: The MIC distributions of FOS are depicted in the Table. Overall, susceptibility to FOS was very high (>95%) among MRSA and 3MRGN ECO. Moreover, susceptibility was seen in >85% of 3/4MRGN KPN and approximately 80% of MRSE isolates. In contrast, FOS susceptibility tended to be lower among MRGN isolates of the remaining enterobacterial species, though the numbers of tested isolates were very low. The results confirm that FOS may play a role as therapeutic option against MR staphylococci and Enterobacteriaceae.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

Table: Distributions of FOS MICs and % of FOS-susceptible (S) isolates

| Organism / phenotype (n) | Number of isolates inhibited at MIC (mg/L): | | | | | | | | | %S |
|---|---|---|---|---|---|---|---|---|---|---|
| | ≤1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | ≥256 | |
| MRSA (94) | 31 | 37 | 17 | 5 | 2 | 1 | 1 | | | 98.9 |
| MRSE (311) | 99 | 31 | 48 | 31 | 13 | 25 | 2 | 2 | 60 | 79.4 |
| 3MRGN[1] ECO (59) | 28 | 21 | 6 | 2 | | | | 1 | 1 | 96.6 |
| 3MRGN KPN (36) | | | | 3 | 16 | 13 | 1 | | 3 | 88.9 |
| 4MRGN[2] KPN (4) | | | | | 1 | 2 | 1 | | | (75.0) |
| 3MRGN KOX (8) | | | | | 1 | 3 | 2 | 1 | 1 | (50.0) |
| 3MRGN ECL (13) | | | | 1 | 2 | 3 | 3 | 3 | 1 | 46.2 |
| 4MRGN ECL (1) | | | | | | 1 | | | | (100) |
| 3MRGN PMI (4) | | | | | | | | 1 | 3 | (0) |

[1] 3MRGN, Gram-negative rods showing resistance (categories resistant and intermediate) to 3 of the following 4 antibacterial drug classes: ureidopenicillins (piperacillin), cephalosporins of groups 3 and 4 (cefotaxime and/or ceftazidime), carbapenems (imipenem and/or meropenem), fluoroquinolones (ciprofloxacin);
[2] 4MRGN, Gram-negative rods showing resistance to all 4 antibacterial drug classes

## 189/PRP

**Occurrence of multidrug-resistant isolates (3MRGN, 4MRGN) among Gram-negative rods obtained from patients in German hospitals, 1995-2013: results of the PEG study**

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**Introduction and Purpose**: The treatment of infectious diseases caused by Gram-negative bacteria has increasingly been threatened by the emergence and dissemination of multidrug-resistant (MR) pathogens, like extended-spectrum β-lactamase-producing or carbapenem-resistant strains. The Commission for Hospital Hygiene and Infection Prevention (KRINKO) of the Robert Koch-Institute has proposed a clinically related definition of MR Gram-negative (MRGN) rods which takes account of non-susceptibility (NS, intermediate or resistance category) to four groups of bactericidal antibiotics that are used as first line monotherapy in patients with serious infections: ureidopenicillins, group 3 and 4 cephalosporins, carbapenems and fluoroquinolones. MRGN rods showing NS to three of the four antibiotic classes are designated 3MRGN and those being NS to all four classes 4MRGN. The objective of the present study was to document temporal variations in the dissemination of 3MRGN and 4MRGN among five major members of the Enterobacteriaceae family (*Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae*, *Proteus mirabilis*) as well as *Pseudomonas aeruginosa* and the *Acinetobacter baumannii* group in Germany between 1995 and 2013.

**Methods**: Susceptibility data of clinical isolates collected in 21, 20, 21, 21, 21 and 22 laboratories that participated in the surveillance studies conducted by the Paul Ehrlich Society (PEG) in 1995, 1998, 2001, 2007, 2010 and 2013, respectively were analyzed. MICs of antibacterial agents were determined by the broth microdilution method according to the standard ISO 20776-1 and interpreted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) species-related clinical breakpoints (version 5.0, 2015). The criteria for classifying isolates as 3MRGN or 4MRGN are given in Table 1.

**Results and Conclusion**: Susceptibility data of 11,601 Gram-negative rods were analyzed. Results are summarized in Table 2. Overall, rates of 3MRGN and 4MRGN increased in Germany between 1995 and 2013, but differences in the dissemination of 3MRGN and 4MRGN between various species are evident. 4MRGN are still rare among Enterobacteriaceae, but have become widespread among A. baumannii sensu stricto (almost 30% in 2013).

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

Table 1: Criteria for multidrug resistance in Gram-negative rods (R=resistant or intermediate, S=susceptible)

| Antibacterial drug class | Indicator compound(s) | Enterobacteriaceae | | *P. aeruginosa* | | *A. baumannii* | |
|---|---|---|---|---|---|---|---|
| | | 3MRGN[1] | 4MRGN[2] | 3MRGN[1] | 4MRGN[2] | 3MRGN[1] | 4MRGN[2] |
| Ureidopenicillins | Piperacillin | R | R | Only one antibacterial class in vitro active | R | R[3] | R[3] |
| Cephalosporins of groups 3 and 4 | Cefotaxime and/or ceftazidime | R | R | | R | R[3] | R[3] |
| Carbapenems | Imipenem and/or meropenem | S | R | | R | S | R |
| Fluoroquinolones | Ciprofloxacin | R | R | | R | R | R |

[1] 3MRGN (multidrug resistant Gram-negative rods showing resistance to 3 of the 4 antibacterial drug classes; [2] 4MRGN (multidrug resistant Gram-negative rods showing resistance to 4 of the 4 antibacterial drug classes; [3] EUCAST breakpoints have not been defined – *A. baumannii* group isolates were considered resistant to ureidopenicillins and cephalosporins

**Figure 2**

Table 2: Prevalence of 3MRGN and 4MRGN in Germany, 1995-2013

| Species / phenotype | Year | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1995 | | 1998 | | 2001 | | 2007 | | 2010 | | 2013 | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| *E. coli* | 516 | | 541 | | 492 | | 513 | | 505 | | 529 | |
| 3MRGN | 5 | 0.97 | 4 | 0.74 | 12 | 2.44 | 40 | 7.8 | 75 | 14.85 | 59 | 11.15 |
| 4MRGN | 1 | 0.19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. mirabilis* | 174 | | 176 | | 182 | | 188 | | 144 | | 189 | |
| 3MRGN | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.06 | 1 | 0.69 | 4 | 2.12 |
| 4MRGN | 5 | 2.87 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1.39 | 0 | 0 |
| *E. cloacae* | 178 | | 177 | | 199 | | 219 | | 188 | | 177 | |
| 3MRGN | 3 | 1.69 | 5 | 2.82 | 15 | 7.54 | 20 | 9.13 | 14 | 7.45 | 13 | 7.34 |
| 4MRGN | 0 | 0 | 2 | 1.13 | 1 | 0.5 | 1 | 0.46 | 0 | 0 | 1 | 0.56 |
| *K. pneumoniae* | 238 | | 181 | | 211 | | 216 | | 262 | | 267 | |
| 3MRGN | 3 | 1.26 | 4 | 2.21 | 9 | 4.27 | 21 | 9.72 | 31 | 11.83 | 36 | 13.48 |
| 4MRGN | 2 | 0.84 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1.91 | 4 | 1.5 |
| *K. oxytoca* | 90 | | 88 | | 128 | | 129 | | 123 | | 121 | |
| 3MRGN | 0 | 0 | 6 | 6.82 | 3 | 2.34 | 10 | 7.75 | 10 | 8.13 | 8 | 6.61 |
| 4MRGN | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0.78 | 0 | 0 | 0 | 0 |
| *P. aeruginosa* | 600 | | 594 | | 582 | | 612 | | 609 | | 646 | |
| 3MRGN | 25 | 4.17 | 9 | 1.52 | 14 | 2.41 | 31 | 5.07 | 38 | 6.24 | 27 | 4.18 |
| 4MRGN | 12 | 2.0 | 3 | 0.51 | 19 | 3.26 | 0 | 0 | 46 | 7.55 | 35 | 5.42 |
| *A.-baumannii*-group | n.t. | | n.t. | | 136 | | 145 | | 181 | | 155 | |
| 3MRGN | n.t. | | n.t. | | 23 | 16.91 | 24 | 16.55 | 19 | 10.5 | 17 | 10.97 |
| 4MRGN | n.t. | | n.t. | | 4 | 2.94 | 6 | 4.14 | 19 | 10.5 | 24 | 15.48 |
| *A. baumannii* | n.t. | | n.t. | | n.i. | | n.i. | | 94 | | 84 | |
| 3MRGN | n.t. | | n.t. | | n.i. | | n.i. | | 13 | 13.83 | 8 | 9.52 |
| 4MRGN | n.t. | | n.t. | | n.i. | | n.i. | | 19 | 20.21 | 24 | 28.57 |

n.t., not tested in years 1995 and 1998; n.i., not identified to species level in years 2001 and 2007

## 190/PRP

**Antimicrobial Drugs and Resistance: A Survey amongst the German General Population**

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**Introduction:** Selection and transmission of multidrug resistant organisms (MDRO) are complex issues involving hospitals, ambulatory care, travel, livestock, pets and more. So far, antibiotic (AB) stewardship approaches have usually been focusing on one small segment. RAI (*Rationaler Antibiotikaeinsatz durch Information und Kommunikation*) is an eastern German network project of veterinarians, primary care physicians, clinicians, infection control specialists as well as communication and design experts tackling the problem from multiple perspectives and developing a multifaceted information and communication intervention concerning AB usage and resistance.

The first project phase is dealing with evaluation of barriers and specification of target groups. Therefore, multiple qualitative and quantitative analyses are performed. Amongst others, the German general population was addressed.

**Objective:** To get insights into knowledge, experience and behavior of German Non-Prescribors concerning AB drug use and MDRO.

**Methods:** A telephone-based interview was conducted via an opinion research centre (TNS Emnid). A sample of 1004 persons, representative for the German population, was surveyed. General sociodemographic data were included and weighted. The questions addressed various aspects of MDRO and AB drug intake.

**Results:** The question "Who or what gets resistant towards ABs?" was answered right ["Bacteria"] by only 24% with a clear difference between levels of graduation. On the other hand only 1% stated not to know the expression "AB resistance" with nearly all such statements coming from the eastern part of Germany. 58% of people do not believe in the influence of their own behaviour on the development of MDRO (68 vs. 54% in East vs. West Germany). 66% saw the focus for prevention of an increasing

MDRO problem in the hospital hygiene but only 32% in AB utilisation by the patient.
8% answered the question: “Did you ever take an AB without prescription?” with “yes” with a strong tendency towards younger people (17% in the group of 14 to 29 years).
37% have been seeing a physician because of an infection during the last year. Of these, 59% received an AB prescription (22% of all attendees). 13% did not take the AB as prescribed.
20% stated to know at least one person with MDRO problems with a higher rate in West Germany (21 vs. 15%).
**Conclusion:** Whereas nearly all people know the expression "AB resistance", knowledge about development of MDRO is sparse. Surprisingly, many people have been taking AB without prescription, especially in the young generation. On the other hand, stated AB prescription by physicians was high. Hence, this survey highlights intervention points on both sides - physician and patient.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**192/PRP**
**Retrospective evaluation of an automated MRSA-screening programme at a University Hospital**
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**Introduction**: Due to their size and complexity, tertiary care hospitals cannot reliably ensure screening for MRSA in every admitted patient. Nevertheless, algorithms guiding the selection of patients to screen for MRSA are hampered by poor sensitivity and specificity.
**Aim**: To retrospectively evaluate the efficacy of an automated screening programme at a tertiary care hospital.
Materials and Methods: At the University Hospital Wuerzburg, screening for MRSA colonization on is based on an electronic questionnaire elicited at admission, which is transformed into a score. Depending on the score, a laboratory request for noncultural MRSA screening is automatically generated. The questionnaire covers hospital stay in the past 12 months, nursing home residence, nursing service level 3, indwelling urinary catheter, PEG, tracheostoma, referral within the hospital, venous ulcers, emergency admission, and age. In addition, hospital hygiene guidance requests physicians and nurses to consider MRSA risk factors issued by KRINKO.
**Results**: The records of 7,989 patients, screened between October 2011 to May 2015, were analyzed. MRSA was confirmed by culture in 3,2% of cases. The highest likelihoods for MRSA were found in patients from nursing homes (95/1898, 5%, odds ratio 1.9, p=0) and those with nursing service level 3 (35/472, 7.4%, OR 2.4, p=0). The score applied allowed prediction of MRSA carriage with a positivity rate of >7% in patients within the highest score group (> 75 points). However, even with very low score values the prevalence of MRSA was higher than those recently reported at the same hospital (3.1% versus 2.3%/1.7% as published in Elias et al. *BMC Infectious Diseases* 2013, **13**:111), which is probably due to selection of patients with high probability of MRSA rather than a rise in carriage prevalence
Summary: The automated process reduces user bias. The score applied in the routine programme increases the likelihood of MRSA detection compared to recently published data from the same hospital.
**Conclusions**: The observational study provides evidence for the effectiveness an automated selection procedure, which may serve as an example for other large hospitals. However, a controlled study is necessary to reliably assess the contribution of above risk factors.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**193/PRP**
**Characterization of a cryptic RND efflux pump in Acinetobacter baumannii**
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**Introduction**: Multidrug resistance in Acinetobacter baumannii is often associated with overexpression of resistance-nodulation-division (RND) efflux pumps, especially AdeABC, that exhibit broad substrate specificity. Two further RND pumps have been described in this species; AdeIJK and AdeFGH. Through data mining published A. baumannii genomes, we identified an uncharacterized RND pump (A. baumannii ATCC 17978 locus_tag A1S_2660) showing 49% identity to the predominant RND pump AcrB of E. coli. Using a reporter system, we detected weak expression of this cryptic pump when growing in the presence of carbapenems. However, induced overexpression of this pump was toxic for A. baumannii ATCC 17978.
**Objectives**: The objective of this study was to characterize expression of A1S_2660 in the AdeABC-deficient isolate NIPH 60.
**Methods**: To overexpress the pump, the gene-encoding A1S_2660 was cloned in-frame to an IPTG-inducible lac-promoter. A control out-of-frame (OOF) construct was generated. Expression was determined by qRT-PCR using transformed NIPH 60 cultures supplemented with 0.01, 0.1, 1, and 10 mM IPTG. Survival kinetics were performed recording the relative growth determined at hourly intervals for 4 hours; after 2 hours IPTG was added. Susceptibility testing to acriflavine, β-lactams, benzalkonium chloride, chloramphenicol, deoxycholic acid, erythromycin, ethanol, fluoroquinolones, gentamicin, metal salts, nalidixic acid, novobiocin, organic dyes, sulbactam and triclosan was performed by disc diffusion or agar dilution and ethidium accumulation was measured for the NIPH 60 transformants.
**Results**: The addition of 0.01 mM IPTG resulted in a 6-fold increased expression of the pump compared to control. However, adding 0.1 -10 mM IPTG, an expression maximum was reached by 200-300-fold. Survival kinetics revealed no impact on the growth rate regardless of IPTG concentration. Therefore all subsequent experiments were performed with 0.1 mM IPTG. No change in the susceptibility to any of the tested antimicrobials was detected between the overexpressing transformant and the OOF transformant. Similarly, no difference in Ethidium accumulation was observed.
**Conclusion**: Although overexpression of the cryptic A1S_2660 RND efflux pump was induced and no toxic effect on the growth rate was detected, we did not observe any difference in susceptibility to any of the tested compounds in the AdeABC-deficient isolate NIPH 60. Therefore this pump may be involved in other cellular processes.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**194/PRP**
**Emergence of NDM-1-producing *Acinetobacter pittii* in Germany**
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**Introduction:** *Acinetobacter pittii* is increasingly associated with antimicrobial resistance and clinical outbreaks. Of particular concern is the development of carbapenem-resistance which is typically mediated through acquisition of an OXA-type β-lactamase or the New Delhi metallo-β-lactamase 1 (NDM-1).
**Objectives:** The aim of this study was to characterize two carbapenem-resistant *A. pittii* isolated from the same hospital in Germany.
**Methods:** Species identification was confirmed by *gyrB* multiplex PCR. Relatedness of the isolates was investigated by rep-PCR based DiversiLab and MLST (Pasteur scheme). Antimicrobial

susceptibility was tested by microbroth dilution and carbapenem MICs were confirmed by Etest. Carbapenem-resistance genes were identified by WGS and Sanger sequencing. Transfer of carbapenem resistance was attempted by transformation of plasmid DNA into *A. pittii* and *A. baumannii* reference strains SH024 and ATCC 17978, respectively.

**Results:** Both isolates were identical by rep-PCR and WGS identified them as ST119 which has also been described in Japan and Australia. Both isolates were resistant to all tested β-lactams including carbapenems, but retained susceptibility to aminoglycosides, fluoroquinolones, tetracycline and tigecycline (Table 1). Sequence analysis revealed the presence of $bla_{\text{NDM-1}}$ encoded on the transposon *Tn125*. Transformation experiments were unsuccessful.

**Conclusion:** This study identified NDM-1 encoded on *Tn125* in carbapenem-resistant *A. pittii*. Furthermore, these data suggest the dissemination of an international clone of *A. pittii* which was first described in Japan. We recommend molecular typing of carbapenem-resistant *A. pittii* in order to better understand the clonal spread of this species.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

Table 1: Minimal inhibitory concentrations of *A. pittii* isolates.

| Antimicrobial agent | MIC [mg/L] |
|---|---|
| ampicillin | >16 |
| mezlocillin | >64 |
| piperacillin | >64 |
| meropenem | >8 |
| imipenem | >8 |
| ertapenem | >4 |
| ceftazidime | >16 |
| gentamicin | ≤2 |
| tobramycin | ≤2 |
| amikacin | ≤8 |
| moxifloxacin | ≤0.5 |
| ciprofloxacin | ≤0.5 |
| levofloxacin | ≤1 |
| colistin | 4 |
| tetracycline | ≤4 |
| tigecycline | ≤1 |

**195/PRP**

**Occurrence of multidrug-resistant isolates among Gram-positive pathogens obtained from patients in German hospitals, 1995-2013: results of the PEG study**

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**Introduction and Purpose**: Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE) have been involved in increased morbidity and mortality of hospitalized patients. The objectives of this study was (i) to investigate the occurrence of clinical isolates of MRSA, PRSP and VRE in the time period 1995-2013, and (ii) to determine the current prevalence of resistance to antibacterial agents used for therapy of infections caused by these pathogens.

**Methods**: Susceptibility data of clinical isolates collected in 21, 20, 21, 22, 21, 21 and 22 laboratories that participated in the surveillance studies conducted by the Paul Ehrlich Society (PEG) in 1995, 1998, 2001, 2004, 2007, 2010 and 2013, respectively were analyzed. Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method according to the standard ISO 20776-1 and interpreted by European Committee on Antimicrobial Susceptibility Testing (EUCAST) species-related clinical breakpoints (version 5.0, 2015).

**Results**: Susceptibility data of 10,316 Gram-positive cocci were analyzed. The rate of MRSA increased from 9.9% in 1995 to 22.5% in 2007, but decreased to 14.3% in 2013 (Table). Of the 94 MRSA obtained in 2013, 57.4%, 69.1% and 87.2% were resistant to clindamycin, erythromycin and ciprofloxacin, respectively, but only 6.4% and 4.3% were resistant to doxycycline and gentamicin, respectively. PRSP isolates (MIC >2 mg/l) were rare during the study period (max. 0.7%), (Table). However, among the 379 strains isolated in 2013, 41 (10.8%) showed reduced susceptibility to penicillin. Among these 41 isolates, 22 (53.7%) and 20 (48.8%) were resistant to doxycycline and erythromycin, respectively. The rate of VRE among *Enterococcus faecalis* (VREfs) did not exceed 1.3% during the study period, while the rate of VRE among *E. faecium* (Efm) was about 5% between 1995 and 2001 and then increased to 17.8% in 2013 (Table). All 51 VREfm isolates obtained in 2013 were resistant to ampicillin and ciprofloxacin, and 9 (17.6%) and 23 (45.1%) showed high-level resistance to gentamicin and streptomycin, respectively. All MRSA, PRSP and VRE were susceptible to linezolid.

**Conclusion**: The prevalence of MRSA is on the decline in Germany since 2010, as has been observed in other European countries. This may be the result of effective infection control methods implemented in hospitals. The prevalence of PRSP and VREfs was at a very low level throughout the study period. In contrast, the increasing prevalence of VREfm isolates is alarming and requires research to find effective countermeasures.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**

Table: Prevalence of MRSA, PRSP and VRE by year of study

| Species / phenotype (n) | Number (%) of isolates per year | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1995 | 1998 | 2001 | 2004 | 2007 | 2010 | 2013 |
| *S. aureus* (4,443) | 637 | 596 | 636 | 660 | 631 | 625 | 658 |
| MRSA (745) | 63 (9.9%) | 81 (13.6%) | 119 (18.7%) | 134 (20.3%) | 142 (22.5%) | 112 (17.9%) | 94 (14.3%) |
| *S. pneumoniae* (1,657) | -[1] | - | 272 | 289 | 310 | 407 | 379 |
| PRSP (6) | - | - | 2 (0.7%) | 0 (0%) | 1 (0.3%) | 3 (0.7%) | 0 (0%) |
| *E. faecalis* (3,054) | 530 | 509 | 471 | 445 | 390 | 341 | 368 |
| VREfs (9) | 0 (0%) | 1 (0.2%) | 1 (0.2%) | 6 (1.3%) | 0 (0%) | 0 (0%) | 1 (0.3%) |
| *E. faecium* (1,162) | 59 | 64 | 94 | 173 | 213 | 272 | 287 |
| VREfm (151) | 3 (5.1%) | 3 (4.7%) | 4 (4.3%) | 26 (15%) | 28 (13.1%) | 36 (13.2%) | 51 (17.8%) |

[1] Isolates of *S. pneumoniae* were not included in the studies performed in years 1995 and 1998; MRSA, methicillin-resistant *Staphylococcus aureus*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; VREfs, vancomycin-resistant *Enterococcus faecalis*; VREfm, vancomycin-resistant *Enterococcus faecium*

**196/PRP**

**Investigation of the mechanism involved in tigecycline resistance in *Enterococcus* spp.**

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**Introduction**: Tigecycline (TGC) represents one of the last-line therapeutics to combat multi-drug resistant bacterial pathogens including VRE and MRSA. The National Reference Centre for Staphylococci and Enterococci at the RKI received 79 TGC-resistant *E. faecium* and *E. faecalis* isolates in recent years. The precise mechanism of how enterococci become resistant to TGC remains undetermined. As part of an ongoing investigation this abstract is to describe examinations of efflux pumps and their contributions to TGC resistance in clinical isolates of *Enterococcus* spp.

**Methods**: High and low level TGC-resistant strains were analyzed with respect to genome and transcriptome differences by means of

whole genome sequencing and qRT-PCR. Genes of interest were cloned and expressed in *Listeria monocytogenes* for functional analyses including determination of the minimum inhibitory concentration (MIC) to TGC.
**Results**: As analyzed by microbroth dilution assays, varying levels of TGC MICs exist for the 27 strains investigated. Comparative genome analyses of three isogenic strains, showing different levels of TGC resistance, revealed the MFS efflux pump TetL and the ribosomal protection protein TetM as possible drug resistance proteins. Subsequent qRT-PCR confirmed the up-regulation of the respective genes compared to the isogenic TGC-sensitive strain. A correlation of gene copy number and level of resistance could be inferred from further analyses. Eventually, expression of both *tet*(L) and *tet*(M) in *L. monocytogenes* unequivocally demonstrated the potential to increase TGC MICs upon acquisition of the loci.
**Discussion**: Our results indicate that increased expression of two determinants, a tetL-encoded MFS-pump and a tetM-encoded ribosomal protection protein, is capable of conferring TGC resistance in some enterococcal strains. However, as not all TGC-resistant isolates investigated in this study were tested positive for *tet*(L), alternative TGC resistance mechanisms are suspected and will be addressed in future experiments.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**197/PRP**

**Complete sequence of a plasmid from a methicillin-resistant *Staphylococcus aureus* harbouring putative virulence genes in addition to antimicrobial and heavy metal resistance genes**

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**Questions:** During previous studies, methicillin-resistant *Staphylococcus aureus* (MRSA) with elevated apramycin minimum inhibitory concentrations of ≥32 mg/L have been detected in cattle, pigs and food of poultry origin. All of them harboured the apramycin resistance gene *apmA*. This gene was mainly located on multiresistance plasmids conferring resistance to seven classes of antimicrobial agents. The aim of this study was to sequence one of these multiresistance plasmids completely and analyse it for its structure and organisation.
**Methods:** Plasmid pAFS11, originating from a MRSA isolate obtained from a case of bovine mastitis, was chosen for sequence analysis. Sequencing was performed using the Illumina Hiseq 2000 (Berry Genomics Company, Beijing, China). Gap closure between the different contigs was done by PCR and sequencing of the amplicons. Sequence analysis and annotation was performed using Artemis (https://www.sanger.ac.uk) and the blast and ORF Finder tools from NCBI (http://www.ncbi.nlm.nih.gov).
**Results:** Plasmid pAFS11 had a size of 49,192 bp. Besides the apramycin resistance gene *apmA*, the plasmid harboured two copies of the *erm*(B) gene (conferring combined resistance to macrolides, lincosamides and streptogramin B), the *aadD* gene (kanamycin and neomycin resistance), the *tet*(L) gene (tetracycline resistance) and the *dfrK* gene (trimethoprim resistance). The *apmA* gene was located upstream of one of the two copies of the *erm*(B) gene. The two *erm*(B) genes were found 5,790 bp apart from each other and were in the same orientation. The remaining three resistance genes, *aadD*, *tet*(L) and *dfrK*, were detected on a 6,388-bp segment which was bracketed by two copies of IS*431* located in the same orientation. Another truncated *rep* gene, the cadmium resistance operon *cadDX* and an IS*257*R1-like transposase were found upstream of the right-hand IS*431* copy. Downstream of the left-hand IS*431*, the copper resistance genes *copA* and *mco* as well as a complete *ica*-like gene cluster, were detected. This *ica*-like gene cluster was composed of four genes which showed only limited homology to other staphylococcal *ica* genes and is supposed to mediate biofilm formation. Upstream of the *ica*-like gene cluster an IS*257* element, a *parA* gene and another *rep* gene were located.
**Conclusions:** The co-localisation of five different antibiotic resistance genes conferring resistance to seven classes of antimicrobial agents together with heavy metal resistance genes and an *ica*-like gene cluster - whose role in biofilm formation is currently under investigation - on the same plasmid is alarming. With the acquisition of this plasmid, antimicrobial multiresistance and potential virulence properties may be co-selected and can be acquired via a single horizontal gene transfer event.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**198/PRP**

**A new antibiotic kills pathogens without detectable resistance**

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D. E. Hughes[3], S. Epstein[6], M. Jones[7], L. Lazarides[7]
V. A. Steadman[7], D. R. Cohen[3], C. R. Felix[3], K. A. Fetterman[3]
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Antibiotic resistance is spreading faster than the introduction of new compounds into clinical practice, causing a public health crisis. Most antibiotics were produced by screening soil microorganisms, but this limited resource was overmined by the 1960s.
Synthetic approaches have been unable to replace this platform. Uncultured bacteria make up approximately 99% of all species in external environments, and are an untapped source of new antibiotics. We developed several methods to grow uncultured organisms by cultivation in situ or by using specific growth factors. Here, we report a novel antibiotic, teixobactin, discovered in a screen of uncultured bacteria. Teixobactin inhibits cell wall synthesis by binding to a highly conserved motif of lipid II, precursor of peptidoglycan, and to lipid III, precursor of wall teichoic acid. We failed to obtain any mutants of Staphylococcus aureus or Mycobacterium tuberculosis resistant to teixobactin. The properties of this compound suggest a path towards developing antibiotics that are largely free of resistance.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**199/PRP**

**Morphological effects and molecular targets of cell wall antibiotics in *Chlamydia***

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**Background and Significance:** β-lactams, which are one of our most widely used group of antibiotics block cell wall biosynthesis and kill free-living bacteria through cell lysis. The effects of β-lactams beyond blocking penicillin binding protein-catalyzed biosynthesis steps of the bacterial cell wall are still not fully

understood. Resistance to β-lactams can be bypassed by the addition of non-antibiotic β-lactamase inhibitors.
Harboring rudimentary cell wall biosynthesis and cell division machineries *Chlamdiaceae* are ideal model system to gain mechanistic insight into the mode of action of β-lactams. In these obligate intracellular pathogens, causing ocular, respiratory and sexually transmitted diseases, β-lactams and the β-lactamase inhibitor clavulanic acid inhibit cell division and induce a viable but non-infectious persistent stage, termed aberrant bodies (AB).
**Objectives:** We studied effects of β-lactams such as penicillins, cephalosporines and monobactams as well as of β-lactamase inhibitors clavulanic acid, tazobactam and sulbactam on the chlamydial life cycle in cell culture and aimed to identify the underlying targets on a molecular level.
**Methods:** Effects of β-lactams and β-lactamase inhibitors on *Chlamydia pneumoniae* and *Chlamydia trachomatis* were analyzed in cell culture using fluorescence-microscopy based assays. Additionally, we screened proteins of the chlamydial cell wall biosynthesis and processing machinery (penicillin-binding proteins, AmiA and NlpD) in single enzyme activity tests to identify molecular targets of the compounds.
**Results:** Here, we show that treatment with different variants of β-lactams and β-lactamase inhibitors results in varying numbers and morphological types of ABs. Of interest, clavulanic acid shows higher activity against *C. trachomatis* (MIC ≤1 µg/ml) than *C. pneumoniae* (MIC ≥32 µg/ml) and targets, on a molecular level, penicillin-binding protein PBP3. The monobactam aztreonam inhibits PBP3 as well as carboxypeptidase activity of dual functioning cell division amidase AmiA *in vitro*.
**Conclusions:** Our data help to gain a deeper insight into molecular events during β-lactam-induced persistence and contribute to develop new anti-infective strategies against persistent infections.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**200/PRP**
**Regulation of an efflux pump that mediates antimicrobial peptide resistance in *Candida albicans***
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**Introduction:** The yeast *Candida albicans* is a harmless colonizer of the oral cavity and the gastrointestinal and urogenital tracts in most healthy people, but it can also cause serious infections, especially in immunocompromised patients. Humans secrete saliva containing different antimicrobial peptides such as histatins in order to protect the oral mucosa from bacteria, fungi and other invaders. Unlike many other antimicrobial peptides that disrupt the cell membrane, histatin 5 acts within the cell and causes mitochondrial damage, which ultimately leads to cell death. *C. albicans* can tolerate the presence of low levels of histatin 5, because it prevents its intracellular accumulation by transporting it out of the cell *via* the Flu1 efflux pump. The expression of several other efflux pumps, which mediate antifungal drug resistance, is regulated by members of the zinc cluster transcription factor family. Gain-of-function mutations that result in hyperactivity of these transcription factors are a frequent cause of efflux pump overexpression and drug resistance in clinical *C. albicans* isolates.
**Hypothesis:** We hypothesized that *FLU1* expression might also be controlled by a zinc cluster protein and that *C. albicans* could develop histatin 5 resistance by acquiring activating mutations in such a transcription factor.
**Methods:** We used a comprehensive library of *C. albicans* strains expressing artificially activated forms of all 82 predicted zinc cluster transcription factors of this fungus to identify regulators of *FLU1* expression. As cells lacking *FLU1* are also hypersusceptible to mycophenolic acid (MPA), we screened the library for strains with increased MPA resistance. To investigate whether the increased MPA resistance conferred by hyperactive ZnTFs was caused by *FLU1* upregulation, the ZnTFs were introduced into a reporter strain expressing *GFP* under the control of the *FLU1* promoter. The fluorescence of the cells was quantified by flow cytometry.
**Results:** Four of the hyperactive transcription factors conferred MPA resistance in a *FLU1*-dependent manner, and the corresponding strains overexpressed *FLU1*. Among these transcription factors was Mrr1, which also regulates the expression of the multidrug efflux pump *MDR1*. Several fluconazole-resistant, clinical *C. albicans* isolates that have acquired gain-of-function mutations in *MRR1* exhibited increased MPA resistance and upregulated the *FLU1* promotor. Introduction of these activating *MRR1* mutations into the wild-type *C. albicans* reference strain SC5314 also resulted in MPA resistance as well as *FLU1* upregulation.
**Conclusions:** These results indicate that antimycotic therapy may select for strains that have developed drug resistance and at the same time an increased ability to withstand a natural host defense mechanism.

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**201/PRP**
**Nasal carriage of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* among patients admitted to two healthcare facilities in Algeria**
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**Introduction:** *Staphylococcus aureus* is a remarkably versatile pathogen able to cause several infections, produce a large arsenal of virulence factors, escape the defenses of the human organism and survive in harsh conditions. However, *S. aureus* is also a commensal organism which can colonize several sites without causing infection in its host. This colonization plays a key role in the spread of this pathogen and constitutes a risk condition for subsequent infection.
**Objective:** To evaluate nasal carriage rate and variables associated with *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted in two healthcare facilities.
**Patients and Methods:** A 27-month epidemiological descriptive study with molecular typing of MRSA isolates, was conduced on patients at admission into the Frantz-Fanon nephrology department and Amizour hospital in Bejaia, Algeria.
**Results:** *S. aureus* was isolated from 159 (26%) of the enrolled patients. Methicillin-susceptible *S. aureus* (MSSA) was isolated from 150 (24.5 %) patients, and MRSA was isolated from nine (1.5 %). Cancer and previous hospitalization were associated with a significantly higher frequency of nasal *S. aureus* carriage among the patients admitted to the general hospital and the nephrology department, respectively. MRSA isolates were heterogeneous with respect to their Staphylococcal Chromosomal Cassette (SCC) *mec* type, sequence type (ST) and toxin genes (*pvl* and *tst1*) content. Four isolates were attributed with the ST80-MRSA-IV clone which is known to be predominant in Algeria.
**Conclusions:** This is the first assessment of *S. aureus* and MRSA nasal carriage and associated variables in Algeria. Our findings provide also a picture of the MRSA strains circulating in community in this geographic area. They can be useful as a guide for implementing screening and control procedures against *S. aureus*/MRSA in the Algerian healthcare facilities.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**202/PRP**

**Susceptibility of 4MRGN *Enterobacter cloacae* strains to newly isolated bacteriophages**

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**Introduction:** *Enterobacter cloacae* is an important nosocomial pathogen, accounting for a considerable proportion of hospital-acquired septicemias, pneumonias, urinary tract infections, and postsurgical peritonitis cases. Of particular concern are multiresistant strains (4MRGN) that leave the clinician only little therapeutic options. The scarce prospects of newly introduced antibiotics in the future, has led to an emerging view of an old concept, namely the use of bacteriophages (phages) to treat bacterial infections.
**Aim:** In this study we isolated and characterized novel phages with therapeutic potential against multi-resistant *E. cloacae* (4MRGN).
**Material and Methods:** Hospital waste water was pre-incubated with current multiresistant strains from the *E. cloacae*-complex and phages were subsequently isolated by the double layer plaque assay. Purified phages were tested against a range of eight multiresistant and nine sensitive *E. cloacae* strains as potential hosts. Plaque morphology, transmission electron microscopy as well as genomic fingerprinting was used for phage characterization.
**Results:** In total five different phages could be isolated with lytic behaviour against *E. cloacae*. All tested bacterial strains were susceptible to at least one phage. The host spectrum of each phage varied and ranged between one and three bacterial strains. Defined mixtures of multiple phages as a cocktail enabled an efficient lysis of all bacterial strains without the development of resistance. Morphologic and genomic characterization of phages revealed that they belonged either to the group of T4-like or T7-like phages.
**Summary:** Combinations of viruses from various phage families constitute a stable "Achilles heel" for 4MRGN *E. cloacae* and could assist antimicrobial treatment of nosocomial infections.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**203/PRP**

**Daptomycin - A viable therapeutic option for VRE-UTI in Indian medical settings?**

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**Inroduction**: The proportion of nosocomial UTIs due to VRE organism increased from 5.56% to 27.86% (fivefold increase, p value <.0001) at our centre in India. VRE are difficult to treat due to multiple drug resistance. Teicoplanin, linezolid, quinpristin &dalfopristin etc. are optional reserved compounds for the treatment of upper and/or bacteremic VRE-UTI. These agents have their own limitations and there is a conspicuous need of new and better alternatives for the treatment of these multidrug resistant isolates.
**Aim and objective:** Data on the efficacy of daptomycin in treatment of VRE causing UTI is scarce from India. Therefore, we aimed to evaluate in vitro activity of daptomycin against hospital acquired enterococci isolated from urine specimens with the special reference to its role in the management of VRE-UTI with the comprehensive analysis of the complete clinic-epidemiological profile of the patients and risk factors.
**Material and methods:** A total of 140 non-repetitive urinary isolates of enterococci recovered from hospitalized patients with UTI over a period of six months (January 2013 to June 2013) were included prospectively. All strains were identified by colony characters, morphology on Gram's staining and conventional biochemical reactions, confirmed by MALDI-TOF and screened for vancomycin, teicoplanin and daptomycin susceptibility by E-test (*Bio Merieux, New Delhi, India)*and for amoxicillin (10µg), high level gentamicin(120µg), ciprofloxacin (5µg), tetracycline (30µg), nitrofurantoin (300µg) and linezolid(30 µg)by Kirby Bauer disc diffusion method. SPSS 20 was used to analyze the results and P<.05 was considered significant.
**Results:** On speciation, majority of isolates were *E. faecium* (72.21%) followed by *E. faecalis* (24.31%). 27.8% strains were recovered from urology unit followed by 11.43%, 10%, 7.85% and 5% from gastroenterology, hepatology, neurosurgery and nephrology specialties respectively. 27.8% were VRE and antimicrobial resistance profile of VRE isolates showed that resistance was most frequent for ciprofloxacin (94.8%), teicoplanin (84.6%), high level gentamicin (82%) and amoxicillin (82%). Daptomycin was active against 87.1% VRE isolates. Interestingly, nitrofurantoin and linezolid also expressed in vitro activity comparable to daptomycin (76.92% and 100%sensitivity respectively). $MIC_{50}$and $MIC_{90}$ for VRE isolates for daptomycin was 4mg/L and 32mg/L respectively.Resistance to vancomycin was 1.9 fold higher in *E. faecium* than *E.faecalis* strains. All *E. faecalis* strains were susceptible to daptomycin, however, 83.65% of *E. faecium* strains demonstrated resistance.
**Conclusion:** In conclusion, daptomycin shows excellent in-vitro activity against *E. faecalis*. However, 16.35% of *E. faecium* were found to be non-susceptible to daptomycin. Therefore, more clinical and microbiological data is required to elucidate the role of daptomycin to treat UTI caused by VRE.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**204/PRP**

**Novel KPC-23 carbapenemase identified in a *Klebsiella pneumoniae* isolate from Germany**

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**Introduction**: Carbapenemase-producing Enterobacteriaceae are a major cause of difficult-to-treat nosocomial infections worldwide. In the species *Klebsiella pneumoniae* KPC carbapenemases are of major importance. Variants other than KPC-2 or KPC-3 are only rarely reported.
**Methods**: Shotguncloning experiments were performed to obtain the complete sequence coding for the novel KPC β-lactamase. The novel gene $bla_{KPC-23}$ and the $bla_{KPC-3}$ and $bla_{KPC-2}$-genes were heterologously expressed in *Escherichia coli* Top 10, followed by comparative MIC studies by microdilution, Etest and disc diffusion in isogenic strains expressing KPC-23, KPC-3, KPC-2 and no betalactamase at all.
**Results**: *K. pneumoniae* strain 12052 was recovered from a urine culture of a hospitalized patient in Germany in 2014. The modified Hodge test, a combined disk test with boronic acid and a PCR for $bla_{KPC}$ were positive. After the complete $bla_{KPC}$ gene was obtained by shotgun cloning, the β-lactamase was assigned as KPC-23. The comparative MIC studies showed that $bla_{KPC-23}$ conferred resistance to carbapenems to a similar extent as $bla_{KPC-3}$. The MICs for cefotaxime, ceftazidime and cefepime of the clone expressing the novel KPC-23 were, however, significantly increased compared to the clone expressing KPC-3 or KPC-2. The $bla_{KPC-23}$-gene was found to be situated in a Tn*4401a* transposable element.
**Discussion**: The novel KPC-23 has a similar activity against carbapenems but increased acitivity against oxyimino cephalosporins. The observation of a novel KPC variant emphasizes the ongoing evolution in this β-lactamase family which underlines the importance of surveillance of β-lactamase-expressing Enterobacteriaceae.

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**205/PRP**

**Multi-center survey of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in German hospitals**

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I. Sobottka[9], I. Fenner[10], S. Ziesing[11], I. Burckhardt[12]
L. von Mueller[13], A. Hamprecht[14], I. Tammer[15], N. Wantia[16]
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**Question**: Aim of this study was to determine the prevalence and molecular epidemiology of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Germany.
**Methods**: From November 2013 to April 2014 *E. coli* and *K. pneumoniae* isolates non-susceptible to carbapenems were collected from clinical samples taken in 20 hospitals throughout Germany. The isolates were tested for the presence of carbapenemases by PCR and phenotypic methods and typed by multilocus sequence typing. Data including a previous hospitalization abroad were collected.
**Results**: Carbapenemases were detected in 24 isolates from 22 patients accounting for 0.047 cases per 1000 admissions. Carbapenemases included OXA-48 (n = 14), KPC-2 (n = 8) and NDM-1 (n = 2). Except of two *K. pneumoniae* strains with ST101 all OXA-48 producing strains belonged to different clones, whereas half of KPC-2 producing *K. pneumoniae* belonged to ST258 and both NDM-1 producing strains were of type ST11. Compared to carbapenem-susceptible controls, patients with carbapenemase-producing differed by a significantly higher proportion of males, wound samples and more reports of a previous stay abroad.
**Conclusions**: This multicenter study demonstrated a still low prevalence of carbapenemase-producing *E. coli* and *K. pneumoniae* from clinical samples in Germany. OXA-48 was more frequent than KPC-2 and NDM-1 and showed a multiclonal background.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**206/PRP**

***In vitro* activity of recombinant chimeric bacteriophage endolysin HY-133 against *Staphylococcus aureus* small-colony variants (SCVs) compared to their corresponding wild types**

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**Introduction**: The formation of small-colony variants (SCVs), a naturally occurring *Staphylococcus aureus* subpopulation frequently isolated in chronic, persistent and relapsing infections, complicates the therapy of *S. aureus* infections. Recombinant chimeric bacteriophage endolysins were shown to be highly active against *S. aureus* and exhibit no disadvantages such as the emergence of resistance. Here, we compared the *in vitro* activity of endolysin HY-133 against clinical wild type (WT)-isolates and their clonally identical SCVs.
**Methods:** Antistaphylococcal activity of the bacteriophage endolysin HY-133 (Hyglos GmbH, Bernried, Germany) was evaluated by the broth microdilution method in accordance to CLSI guidelines. We determined both the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for 12 representative methicillin-susceptible *S. aureus* (MSSA) clinical WT isolates, for their corresponding SCVs and *S. aureus* reference strain ATCC 29213. For all of these isolates, the direct colony suspension method was performed as recommended by CLSI for staphylococci. Additionally, we analysed the activity of HY-133 under exponential growth conditions.
**Results**: With direct colony suspension method, values for MIC and MBC were found to be identical. Namely, $MIC_{50}$ and $MBC_{50}$ values were both 0.12 mg/L for WT and 0.25 mg/L for corresponding SCVs. SCV and WT shared the same $MIC/MBC_{90}$ values of 0.5 mg/L. Inoculation of exponential growth cultures after 3 h of incubation in liquid medium also revealed identical values for MIC and MBC. $MIC/MBC_{50}$ values were 0.25 mg/L for WT isolates and 0.5 mg/L for corresponding SCVs. $MIC/MBC_{90}$ values were found to be equal to $MIC/MBC_{90}$ of direct colony suspension method, namely 0.5 mg/L for both WT isolates and SCVs. There were no considerable differences in activity of HY-133 between the WT strains and their corresponding SCVs, even under different growth conditions.
**Conclusions:** This study revealed not only a high bactericidal activity of HY-133 against *S. aureus* WT strains, but also against their clonally identical SCVs under different growth conditions. Although SCVs are characterized by altered cell walls and known to show decreased susceptibilities to several antibiotics, HY-133 eradicates SCVs in the same extent as WT isolates.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**207/PRP**

**Comparison of Vitek and microbroth dilution for susceptibility testing of piperacillin/tazobactam in ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae.***

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**Question**: False susceptible results for piperacillin-tazobactam (TZP) using Vitek 2 were reported previously and linked to expression of the Class D beta-lactamase OXA-1. Recently the use of TZP for treatment of infections caused by ESBL-producing bacteria has been suggested. Aim of this study was to determine the rate of very major errors for TZP susceptibility testing by Vitek 2 using the newly introduced AST-N223 card in a collection of ESBL-producing *E. coli* and *K. pneumoniae*.
**Methods**: A total of 86 clinical ESBL-positive strains of *E. coli* (n = 72) and *K. pneumoniae* (n = 14) with an MIC ≤ 4 mg/l for TZP

determined by semi-automated susceptibility testing (Vitek 2) were collected. Each strain was tested for susceptibility to TZP by microbroth dilution according to EUCAST guidelines as the reference method and again by Vitek 2 using the AST-N223 card. We performed bacterial counts on every third inoculum suspension. Furthermore, these isolates were characterized by PCR and sequencing of *bla* genes, particularly $bla_{CTX\text{-}M}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV}}$ and $bla_{\text{TEM}}$ .

**Results**: The majority of our ESBLs studied were CTX-M-positive (CTX-M-15: 40,7%, CTX-M-1: 23,3%, CTX-M-14: 10,5 % and CTX-M-9: 5,8 %). Ten strains (11,7 %) contained $bla_{\text{OXA-1}}$, nine in combination with $bla_{CTX\text{-}M\text{-}15}$ (90 %) and one with $bla_{\text{CTX-M-1}}$ (10 %). The susceptibility rate for TZP in the reference method was 96,5% and the $MIC_{50}$ and $MIC_{90}$ values of TZP were 2 and 4 mg/l respectively. The essential agreement was 98,8% with no very major error, one major error and one minor error.

**Conclusion**: There were no very major errors using EUCAST breakpoints in a collection of ESBL-producing *E. coli* and *K. pneumoniae* in the AST-N233 card, even if $bla_{\text{OXA-1}}$ was present. The essential agreement between AST-N233 and microbroth dilution was good.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**208/PRP**

**Functional analysis of penicillin binding protein 3 from *Chlamydia pneumoniae***

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**Background and Significance:** Intracellular *Chlamydiaceae* do not need a stabilizing envelope, and a functional peptidoglycan cell wall has not been detected in these pathogens. Nevertheless, a recent study revealed the presence of circularly shaped peptidoglycan-like structures. Moreover, the cell wall antibiotic penicillin inhibits cell division resulting in persistence.

**Objectives:** Analyzing potential penicillin-target enzymes, we focused on the chlamydial homolog of an essential monofunctional transpeptidase that is involved in cell division of *Escherichia coli* called penicillin binding protein 3 (PBP3) or FtsI.

**Methods:** The functionality of PBP3 from *Chlamydia pneumoniae* was analyzed using (i) complementation studies in *E. coli* PBP mutants and bacterial-two hybrid studies probing interactions with *E. coli* cell division proteins, (ii) activity assays with the purified enzymes using lipid II substrate and the inhibitor penicillin, and (iii) active site mutagenesis studies.

**Results:** Chlamydial PBP3 restored cell division in a filamenting *E. coli* PBP3 mutant and interacted with *E. coli* cell division proteins FtsK, FtsN, FtsQ, and YmgF. Despite its ability to complement transpeptidase functions in *E. coli*, purified PBP3 did not cross-link cell wall precursors but did have DD-carboxypeptidase activity. The penicillin-sensitive PBP3 had an unusual potential active site comprising three SxxK motifs.

**Conclusions:** Our findings help to clarify the role of PBPs in the development of beta-lactam induced formation of aberrant chlamydial bodies.Further research is needed to understand long-term chlamydial infection and orchestration of cell division in minimal genome bacteria.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**209/PRP**

**Direct and Fast Detection of Carbapenemase Genes with an Oligonucleotide Microarray using RPA (Recombinase Polymerase Amplification) for Amplification and Labelling**

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A rapid detection of antimicrobial resistance genes, especially of carbapenemase genes, in native patient samples might become crucial for infection control and prevention, surveillance and for epidemiological purposes. Furthermore, it may have a significant impact on the selection of an appropriate initial treatment and this might be of great benefit for ICU patients. Isothermal molecular identification methods could become a suitable tool for this task, especially in point-of-care settings, because these methods combine high speed with excellent sensitivity and specificity. Furthermore, they can be used in any environment without a need for trained technicians or sophisticated laboratories.

For this purpose, an RPA (Recombinase Polymerase Amplification) based assay was developed. RPA is a rapid isothermal amplification method, which takes place within less than ten minutes at a temperature range between 37°C and 42°C. Therefore, RPA does not need a complex thermal cycling instrumentation. The RPA reaction uses three core proteins. The first enzyme is a recombinase that binds to primers and forms filaments, which are able to recombine to homologous DNA. The second enzyme, a single-stranded DNA binding protein, prevents dissociation of the primers by binding to the displaced DNA strand. The third core enzyme is a strand-displacing polymerase, which opens the DNA double helix and amplifies the DNA starting from the 3’ end of the bound primers.

In a first set of experiments with this assay, various low copy number DNA samples of different reference strains containing the carbapenemase genes *bla*KPC, *bla*VIM, *bla*NDM and/or *bla*OXA-48 alone and in various combinations were analyzed. In a first step, the bacterial DNA exponentially amplified using RPA. Labeling was performed by the use of 5´-biotin-coupled primers. Afterwards, labelled amplicons were hybridized without further purification and specifically detected with an oligonucleotide microarray (Braun et al. 2014). The sensitivity of the entire assay was determined using dilution series of reference DNA samples from different strains. First experiments with RPA in which the target genes were tested in single- and multiplex reactions showed a good sensitivity and specificity. The assay was able to detect as few as approximately 10 genome equivalents per sample.

The sensitivity and speed of the isothermal amplification strategy allows a future development of a point-of-care device for the detection of clinically important carbapenemase genes in native patient samples.

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**210/PRP**

**Targeted photoinduced killing of bacteria utilizing maltohexaose-conjugated photoprobes**

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The recent report of the WHO, which predicts a post-antibiotic era in which minor infections or injuries display a serious problem, underlines the demand of the development of novel antimicrobial treatment strategies[1]. Photodynamic therapy constitutes an alternative antibacterial treatment, in which the affected tissue is targeted with a suitable photosensitizer and irradiated with appropriate light.

Fluorescence- and F-18 labeled maltohexaose-based probes have been recently described as selective and highly sensitive imaging agents targeting a bacteria-specific transport pathway[2,3]. Based on this we aimed to target axially substituted Si(IV) phthalocyanines with such polysaccharides in order to yield a novel targeted photosensitizer for treatment of bacterial infections. First candidate is the targeted photosensitizer AGA156.

By using fluorescence microscopy, flow cytometry and quantitative analysis of the number of colony forming units (CFU) we were able to monitor effectiveness of labelling and survival of bacteria after treatment with AGA156 and redlight exposure.

For evaluation of photoinactivation capabilities of AGA156, *E. coli* ATCC® 52922™ and *S. aureus* USA300 were selected as examples for gram-positive and gram-negative bacteria. Both species were clearly labelled with the red fluorescence of the phthalocyaninate (1h incubation). After irradiation flow cytometric analysis revealed an inactivation for *S. aureus* but not for *E. coli*. An extended incubation time (up to 24 h) of *E. coli* with AGA156 did also not lead to a significant photobactericidal effect. A quantitative analysis of the CFU revealed that after 1h of irradiation more than 99 % of staphylococci were inactivated, whereas no significant effect could be observed for *E. coli*. Irradiation of mixtures of AGA156 treated *S. aureus* and *E. coli* leads to selective inactivation of the gram-positive species.

In summary, we have developed a versatile toolbox for the construction of targeted phototriggered antibacterial substances. The selective activity against gram-positive pathogens has been demonstrated *in vitro*. Our next experiments will focus on disruption of biofilms, toxicity towards eukaryotic cells and a broader spectrum of gram-positive and gram-negative species.

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**211/PRP**

**Discovery of a new sactipeptide produced by Staphylococcus spec.**

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**Introduction:** The global rise and rapid spread of drug-resistant bacteria cause growing concern for the successful treatment of bacterial diseases. It is therefore imperative to search for novel antibiotics [1]. The ongoing optimization of sequencing techniques and bioinformatic tools has highly contributed to facilitate this search via genomic data analysis. In combination with conventional moleculobiological methods antibiotic biosynthesis gene clusters can be identified and characterized.

**Aim:** The aim of this study was to identify a putative novel substance produced by a clinical *Staphylococcus* isolate which had shown antimicrobial activity in previous experiments [2].

**Material and Methods:** The genomewas sequenced by sequencing-by-synthesis technology, assembled with SOAPdenovo,and analyzed with various bioinformatics programs. The composition of a putative antibiotic gene cluster was further investigated by PCR, inverse PCR and sequencing of the obtained products.

**Results:** Genomic data analysis initially revealed the existence of a part of a sactipeptide gene cluster. Sactipeptides are ribosomally synthesized and posttranslationally modified bacteriocins whose common features are intramolecular thioether bridges, in which the α-carbon atom of an amino acid is crosslinked with the sulphur atom of a cysteine residue. The formation of these bonds is mediated by radical S-adenosylmethionine enzymes. Only four sactipeptides have been discovered and investigated up to now [3,4]. The data obtained so far shows, that the gene cluster is composed of a sactipeptide precursor, a radical SAM enzyme, two proteases, a transcriptional regulator and an ABC transporter. Current investigations focus on finding further components of the gene cluster. Future experiments will be directed towards the investigation of the importance of the different components regarding the biosynthesis of the sactipeptide, elucidation of the peptide structure and the mode of action.

**Summary:** A new sactipeptide biosynthesis gene cluster was identified via genomic data analysis of a recently sequenced clinical *Staphylococcus* isolate. Upcoming investigations will primarily focus on the characterization of the sactipeptide in relation to the composition of the gene cluster, biosynthesis, structure, and mode of action.

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**212/PRP**

**Knock-outs of the pIP501 transfer genes to decipher the *Enterococcus faecalis* Type IV Secretion System**

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Increasing antibiotic resistances in pathogenic bacteria and their spread among each other through conjugative type IV secretion systems (T4SSs) present a serious threat for human health worldwide. Our research focuses on the putative transfer factors of the T4SS from broad-host-range plasmid pIP501, which is often present in nosocomial pathogens, such as *Enterococcus faecalis* and *Enterococcus faecium* strains. This multiresistance plasmid encodes 15 putative transfer genes in a single operon. The corresponding proteins TraA-TraO are proposed to form a T4SS multiprotein complex. The *tra* operon is negatively autoregulated by the relaxase TraA. Based on protein-protein interaction studies we were able to show a first model of the $T4SS_{pIP501}$. Postulated key factors of the conjugative transfer complex are two ATPases, TraE and TraI/TraJ, the first putative two-protein T4S coupling protein, the muramidase TraG, the putative channel factors, TraL and TraM and the surface factor TraO [1]. The functions of most of the transfer proteins and the mechanism of the conjugative T4SS are not known in detail. To elucidate the role of the pIP501 *tra* genes in T4S in Gram-positive pathogens we generated a number of single *tra* knock-out mutants in *E. faecalis* harbouring pIP501. The first knock-out mutants that we generated are the deletion mutants, *E. faecalis* pIP501Δ*traE* and *E. faecalis* pIP501Δ*traG*. As expected, biparental matings showed that the peptidoglycan-degrading enzyme, TraG and the ATPase TraE are essential for pIP501 conjugative transfer. We were able to recover wild type transfer rates of the knock-out mutants by complementing them with the wild type *tra* gene in trans [2, 3]. To get more detailed information about the function of TraG we tested whether one of the peptidoglycan-degrading domains (CHAP or SLT) is sufficient to complement the *E. faecalis* pIP501Δ*traG* mutant. Results on Δ*traG* complementation and the phenotypes of the other Δ*tra* deletion mutants will be presented. These studies will bring us a big step forward to decipher the pIP501 conjugative transfer machinery which would represent the first solved T4SS from a Gram-positive pathogen.

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**213/PRP**

***In vitro* activity of ceftobiprole against methicillin-resistant *Staphylococcus aureus* harbouring *mecC* gene**

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**Introduction**: Ceftobiprole recently became available. It is a broad-spectrum cephalosporin with high affinity for PBP2a and, therefore, it shows activity against methicillin-resistant *Staphylococcus aureus* (MRSA). A novel mecA homologue called mecC has been described in staphylococci isolated from animal and human infections. The alternative penicillin-binding protein encoded by mecC has only a 63% identity to PBP2a and it is unstable at 37ºC or higher. Furthermore, its affinity for beta-lactam antibiotics including new generation anti-MRSA cephalosporins has not sufficiently been investigated.

**Objectives**: The aim of our study was to evaluate if ceftobiprole could also be a therapeutic option against infections caused by these emerging *Staphylococcus aureus* harbouring *mecC*.

Strains and Methods: To assess the in vitro efficacy of ceftobiprole against *mecC*-positive MRSA, we performed susceptibility testing by agar diffusion (E-test) of a collection of clinical and veterinary *mecC*-MRSA at different temperatures. All the strains were previously identified and typed by microarray hybridization.

Results: All except one *mecC*-MRSA isolates investigated were interpreted as susceptible to ceftobiprole according to the clinical breakpoint of <=2 µg/ml established by EUCAST when incubated at 37ºC. By lowering the incubation temperature to 30ºC and prolonging the incubation time to 24 hours we observed in almost all strains slightly higher MICs, sometimes one half-dilution step over this breakpoint

**Conclusions**: Ceftobiprole appears to be active also against *mecC*-MRSA. However, the results of susceptibility tests showed significant temperature-dependent variations that should be further evaluated. More in vitro and clinical studies need to be done before ceftobiprole could be considered a valid therapeutic option against *mecC*-MRSA infections.

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**214/PRP**

**Elicitation of Secondary Metabolism in Sponge-Associated Actinomycetes**

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Actinomycetes (phylum Actinobacteria) are prolific producers of diverse secondary metabolites possessing various bioactivities including antimicrobial, antiviral, antiprotozoal and anticancer effects. With a vast number of metabolites already been discovered, efforts are now being laid to prevent the rediscovery of known ones by exploring the actinomycetes of un-explored or under-explored habitats and subjecting them to various manipulations. A large fraction of the biosynthetic gene clusters in actinomycetes remain silenced or unexpressed under conventional laboratory conditions. In this work, the effect of the chemicals γ-butyrolactone, glycerol, triclosan and N-acetyl-D-glucosamine (under rich and poor nutrition conditions) in eliciting secondary metabolism in five marine sponge-derived actinomycetes (*Nocardiopsis* sp. D53, *Streptomyces* sp. D92, *Streptomyces* sp. D56, *Streptomyces* sp. D49 and *Streptomyces* sp. D86) in solid and liquid fermentation conditions was assessed. Based on the comparison of HPLC profiles and bioactivity tests against indicator organisms, elicitation, enhancement and repression of metabolites were observed in these actinomycetes upon treatment with chemical elicitors.
Further, 194 extracts generated from the study above, were assessed for the inhibition of *Staphylococcus epidermis* RP62A biofilms. The extract from *Streptomyces* sp. D86 and *Streptomyces* sp. D56 grown in liquid state fermentation displayed potent biofilm inhibition. Identification of the active principle from these extracts which inhibit the biofilm formation forms the future perspective. Such compounds could serve as potential drugs against device-related infections caused by staphylococcal biofilms.

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**215/PRP**

**Evaluation of drug efficacy against intracellular- replicating *Francisella tularensis***

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*Francisella tularensis* is the causative agent of the zoonotic disease tularemia. The facultative intracellular gram-negative bacterium naturally occurs in the northern hemisphereand is classified by the CDC as a category A agent, due to its high pathogenicity and low infection dose .. one of its highly virulent subspecies, *Francisella tularensis tularensis*, can cause mortalities up to 60% in untreated patients and resiatnces against common antibiotics have been already observed.Therefore, the development and testing of new anti-*Francisella* treatments is of major importance.
After infection *Francisella tularensis* is able to proliferate in the cytoplasm of infected host cells, e.g. epithelial cells and especially macrophages. In order to measure the effectiveness of medical countermeasures during this intracellular profliferation, a standarizable system is needed. . Macrophages (J774 and PMA-differentiated THP-1) were infected with *Francisella tularensis* and extracellular bacteria were killed by incubation with gentamycin subsequently. After 24h the efficacy of drug treatment against intracellularic *Francisella* was assessed by determing the bacterial load of the cells using two different immunofluorescence techniques. Besides fluorescence microscopy flow cytometry was used to gain a semi-automated analysis system. Since *Francisella tularensis* gamma-glutamyl- transpeptidase (gGT) is an essential enzyme for intracellular growth, we tested additionally gGT inhibitors for their anti-*Francisella* function and could show a dose dependent reduction of intracellular *Francisella* proliferation. Thus, we established a useful standardized system for the verification of the efficacy of candidate drugs against *Francisella tularensis* during the intracellular phase of its life cycle.

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**449/PRP**

**Identifying risk factors for multidrug-resistant pathogens in urinary tract infections: a retrospective study.**

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**Background:** Multidrug-resistant (MDR) pathogens are a growing problem in the treatment of urinary tract infections (UTI). Especially Healthcare-associated (HCA) UTIs are under suspicion to be associated with MDR pathogens. Only few data are available to identify patients at risk for MDR infections. **Objective:** Identifying Healthcare-associated risk factors for MDR pathogens in UTIs.
**Methods:** A retrospective case-control study of emergency department (ED) patients with UTI and positive urine culture at admission between January 2013 and June 2015. Patient characteristics were analyzed by presence of MDR pathogens and a logistic regression was performed.
**Results:** 146 patients had a positive urine culture and were eligible for the study. In 16 of 146 (11%) cases an MDR pathogen was identified as cause of UTI. Logistic regression analysis calculated three variables associated with MDR in UTI patients: residence in nursing homes, male sex and renal transplantation, whereas recent hospitalization within 30 days and an indwelling catheter were not. The c-statistic of a Receiver Operating Characteristic analysis was 0.795. For >=1 risk factor the sensitivity is 93.8%, specificity 46.2% and negative predictive value 98.4%.
**Conclusion:** Our study identified residence in nursing homes, male sex and renal transplantation, but not recent hospitalization within 30 days and indwelling catheter, as risk factors for MDR pathogens in UTI patients.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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## MICROBIOTA, PROBIOTA AND HOST (PWP)

**216/PWP**

**Chronic infection by *Pseudomonas aeruginosa* is associated with a global change in the microbiome of the lungs in CF patients: A new avenue to design diagnostics biomarkers.**

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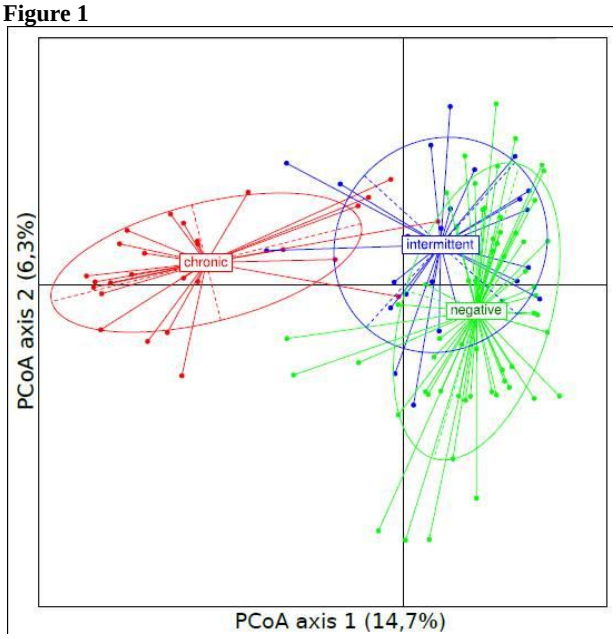
Although lung has long been considered sterile, evidence from previous studies suggests the existence of a microbiome in healthy lungs. Chronic lung disease in CF patients was demonstrated to be correlated to changes in that microbiota and those alterations of the microbiota may be important in pathophysiology of CF. One of the main pathogen in CF is *Pseudomonas aeruginosa* and chronic infection by this pathogens can occurs early in the life of the patient. However, little is known about the microbial causes of this chronic infection and cultures associated with precipitating

antibodies assays are the only available tools to diagnose the status of the infection.
In this cross-sectional study, we analyzed 115 sputum samples from 36 patients. Samples are classified as showing negative (n=62), intermittent (n=25) or chronic (n=28) infection by *P. aeruginosa* based on cultures results and precipitating antibodies titers. The microbiota was then explored by a 16S amplicons sequencing with Illumina MIseq.
Our results showed a strong difference in alpha diversity between chronically infected patients and the two other cohorts. No difference in alpha-diversity was observed between samples from negative and intermittent patients. We also quantified the global bacterial burden by qPCR and no differences were observed between the three classes. Finally, a beta diversity analysis based on Bray-curtis distances showed that chronically infected patients exhibit a significantly different microbiome than negative and intermittent patients regarding the structure of the microbiome (cf figure 1.). We found 23 OTUs showing significant link with the establishment of the infection. As expected, one of those OTUs is *P. aeruginosa* itself but we also noticed a decrease during the infection of other abundant OTUs (classified as *Streptococcus sp.*, *Veillonella sp.*, *Prevotella sp.*, *Haemophilus sp.* and *Neisseiria sp.*). In this study, we were able to show that changes occur during the process of infection by *P. aeruginosa*. In patient intermittently infected by the pathogen, we did not observed a major change in the structure of the microbiome or in the diversity linked to a small increase of the relative abundance of the pathogen. However, during the establishment of the infection, the important over-growth of the pathogen is linked to a global decrease of the other bacteria, especially the other abundant OTUs present in the negatively infected patients indicating a competition occurring between *P. aeruginosa* and those "commensal" bacteria. The abundance of those OTUs can be used as biomarkers to monitor the evolution of the infection and help to build a stronger diagnosis associated with culture and precipitating antibodies.

Supported by a grant from Gilead and the German Center for Lung Research.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**




**217/PWP**

**Genetic stability of the probiotic *Escherichia coli* strain Nissle 1917 (EcN) - yet another safety aspect of Mutaflor**

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EcN, which has GRAS status, interferes in vitro with adhesion, replication and Shiga-toxin (Stx) production in EHEC strains (Rund et al. 2013; Reissbrodt et al. 2009). Since *stx* genes are usually encoded by λ-prophages and antibiotic resistant genes are present on a conjugative pESBL-plasmid in EAHEC strains from the 2011 outbreak in Germany, we tested EcN for becoming a host of λ-phages and of the pESBL-plasmid. For that purpose *stx*-phages from various EHEC/EAHEC (EDL933 O157:H7, 1530/99 O26:H11, TY3456 O104:H4, 4392/97 O145:H25) were isolated and mixed with either EcN or *E. coli* K-12 strains (MG1655, DH5α, HB101), plated in soft agar and phage plaques subsequently counted. In contrast to *E. coli* K-12 strains, there were never any phage plaques observed on EcN, neither was DNA of the corresponding λ-phage in or Stx-production by EcN detectable. The outer membrane protein LamB is the most important receptor for λ-phages. The role of the different C-terminal LamB sequence in EcN and the *E. coli* K-12 strains for the observed λ-phages-resistance of EcN is under investigation.
Similarly, after coincubation with EcN or the *E. coli* K-12 strains and the 2011 EAHEC strain TY3730 transconjugants were observed at a high ratio for *E. coli* K-12 strains harbouring plasmid pESBL but up to 4 x $10^6$ - fold lower numbers of transconjugants for EcN. Interestingly, the isogenic microcin-negative EcN mutant SK22D showed a 180-fold higher conjugation rate than the EcN wild type. Obviously, the production of the two microcins H47 and M are only partly responsible for the very low transmission rate of the pESBL plasmid from EAHEC strain TY3730 into EcN. Also the conjugal transfer of the kanamycin-resistance mediating plasmid R1drd16 from *E. coli* into EcN was 347- to 1729-fold less efficient compared with *E. coli* K-12 strains. These results stress the fact, that EcN deserves GRAS status, also because of its high genetic stability.

Rund et al. (2013) Antagonistic effects of probiotic *Escherichia coli* Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7. Int J Med Microbiol 303: 1-8.

Reissbrodt et al. (2009) Inhibition of growth of Shiga toxin-producing *Escherichia coli* by nonpathogenic *Escherichia coli*. FEMS Microbiol Lett 290: 62-69.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**218/PWP**

**The differential cellular immune response to probiotic *E.coli* Nissle 1917 compared to EPEC is modulated by miRNAs.**

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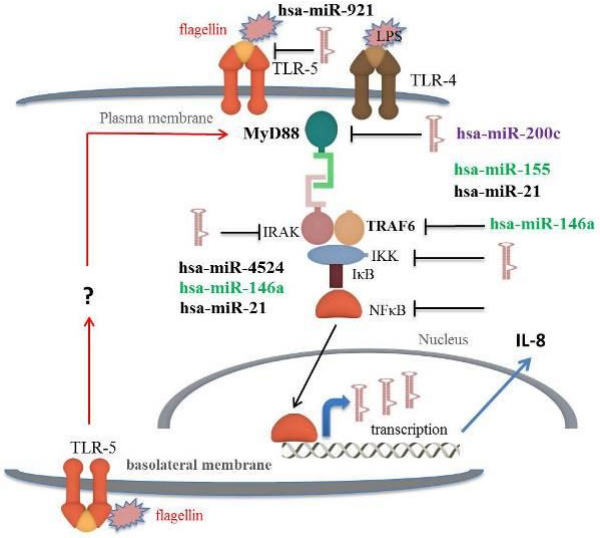
The gastrointestinal tract is home to an abundant, complex and balanced consortium of bacteria that orchestrate important immune and metabolic functions in the host. This intestinal microflora consists of commensal and symbiotic bacteria that are not only essential for the development of the immune system but also represent the first barrier against colonization by pathogenic bacteria. In order to protect against intruding pathogens systemic immunological host responses are activated by the pathogen-associated molecular pattern pathway via membrane-associated Toll-like receptors (TLR) resulting in the stimulation of nuclear factor kappa-B (NF-κB) signaling and the according induction of a variety of pro-inflammatory cytokines like IL-8.
NF-κB signaling is tightly regulated by a variety of cellular components to maintain the endotoxin tolerance of epithelial cells

to the continuous challenge by intestinal microbiota. Among others a crucial regulatory element are miRNAs.
Within our project we use human intestinal epithelial cells (T84) as well as monocytes (THP-1) as cell culture models to study miRNA responses, regulating more than 60% of all protein encoding genes of target cells in the presence of different bacteria.
MiR-155 as well as miR-146a are known to be negative regulators of the NF-κB pathway and the adaptor molecule MyD88, which is upregulated after LPS stimulation via TLR4. Beyond that we were able to show that after basolateral stimulation of T84 as well as THP-1 cells in particular IL-8 synthesis is induced, probably via TLR-5, as a flagellin antagonist. This might simulate a breach of barrier function during bacterial infection. Interestingly our data indicate that this cellular response seems to be more prominent after probiotic *E.coli* Nissle 1917 (EcN) than after enteropathogenic E.coli (EPEC) basolateral co-incubation. The innate immune system in the gut is challenged to distinguishing beneficial, probiotic EcN from pathogenic EPECs and to adjust the immune response properly.
Actually we are analyzing whether this differential immune response is reflected by the regulation of MyD88 by a set of miRNAs similar to the LPS challenge to TLR-4. This might reveal another differentiation mechanism depending on the probiotic or pathogenic character of the microbes.
We are correlating the miRNA expression profiles and their putative targets to the according bacterial factors, like flagellin or LPS, that trigger the differential miRNA responses. Employing a set of miRNAs, in particular probiotics-induced miRNAs, effecting different intermediates of the TLR pathway, may pave the way for mediating alterations of mammalian host signaling and might foster the development of new strategies for controlling host immune responses.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**Figure 1**




**219/PWP**

**Elucidating the mechanism responsible for Shiga toxin bacteriophage resistance of *E. coli* Nissle 1917**

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Enterohaemorrhagic *E. coli* strains (EHEC) are the causative agent for severe food borne diseases leading often to life threatening heamolytic uraemic syndrome (HUS). One of the most important virulent factors linked with pathogenicity of EHEC is Shiga toxin (Stx) production. The 2011 EHEC outbreak in Germany emphasizes the need for focusing on an effective prophylaxis. Furthermore, the use of antibiotics induced Stx production, which worsens the scenario and indicates the need for alternative strategy. Under stress conditions, EHEC produces Stx phages which infects other *E. coli* serotypes and convert them into Stx-producers. *E. coli* Nissle 1917, (EcN) is one of the most investigated probiotic bacteria licensed as "Mutaflor" for the treatment of various gastrointestinal disorders.
Earlier, we have shown antagonist activity of EcN against EHEC strains. And, we have also found that unlike *E. coli* K-12 serotypes like MG1655, DH5α and HB101, EcN cannot be infected by Stx-phages of EHEC. Hence, we believe EcN could be safely used to treat patients suffering from an EHEC infection. We hypothesize that sequence variations of LamB, the bacteriophage lambda cell surface receptor, could be responsible for the resistance (EcN) or sensitivity (*E. coli* K-12) to *stx*-phage infection. This hypothesis is based on the in-silico analysis of the *lamB* gene sequence of EcN which shows a significant difference at the 3' end in comparison to that of *E. coli* K-12 strain MG1655.
To investigate the role of the *lamB* gene, we created *lamB* deletion mutants of EcN and MG1655 by Lambda Red recombinase genome editing. Complementation of the deletion mutants with the original or the *lamB* gene of the other strain are in process. Analysis of all four complemented mutants for their sensitivity against *stx*-phages will show the importance of the LamB receptor in *stx*-phage resistance of EcN. Moreover, *stx* gene specific PCR, Phage plaque assay and Shiga toxin ELISA will be performed to elucidate the difference in infectivity of *stx*-phages for EcN and MG1655.
We believe that the data acquired in this study could enable us to understand the *stx*-phage resistance mechanism of EcN and present the reason for a further important safety feature of EcN as a potential therapeutic against EHEC infection

**References**

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[B] Rund et al, *IJMM*, 2013

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**220/PWP**

**Analysis of functional of TLR 5 in *Galleria mellonella***

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The principal components of the innate immune system are highly conserved between mammals and insects. Within the last decade the greater waxmoth *Galleria mellonella* has become a more and more used model for infection studies. *Galleria mellonella* as a model organism permits studies under humanized conditions by keeping the insects at 37°C to investigate the influence of temperature dependent virulence factors. Low costs, easy handling and a good statistical power are strong benefits of this model organism.
The recognition of bacterial compounds in *Galleria mellonella* results in the secretion of anti-microbial peptides (AMP). Except for toll like receptor 4 (TLR4) dependent immune responses not much is known about functional other TLR signaling in *G. mellonella*.
To investigate wether *G. mellonella* expresses functional TLR5 we treated *G. mellonella* with ultrapure flagellin or LPS and analyzed survival rates, AMP-growth inhibition assays as well as expression of antimicrobial peptides on RNA level from hemolymph of TLR ligand treated *G. mellonella*.
High dosis injection of ultrapure flagellin or LPS into the hemolymph of the host did not affect the survival within 4 days, whereas 60% of standard-purity LPS treated *G. mellonella* died after 4 days. In accordance the mRNA expression levels of anti-microbial peptide genes were significantly increased. Gloverin, Galiomycin or Gallerimycin were measured indicating the expression of a functional flagelling-recognizing TLR5 homologue in the hemolymph of *G. mellonella*.
The results show for the first time that besides TLR4 *G. mellonella* also possesses a functional receptor for the TLR5 ligand flagellin.

This opens new options to expand the work in the field of infection studies in *Galleria mellonella*.

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**221/PWP**

**A toxin-antitoxin module may regulate maintenance of the typical plasmids pMUT1 and pMUT2 in the probiotic *Escherichia coli* strain Nissle 1917**

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The non-pathogenic *Escherichia coli* strain Nissle 1917 (EcN) is used as a probiotic drug against intestinal disorders and diseases. One unique property of EcN is the presence of the two cryptic plasmids pMUT1 and pMUT2. Beside plasmid sequences little is known about their copy numbers, encoded proteins or their maintenance in EcN.
The determination of the plasmid copy numbers by quantitative real time PCR (qRT-PCR) in two independent RT-PCR dilution sets revealed an average of 20 and 7 copies for pMUT1 and pMUT2, respectively.
BlastN searches with the plasmid nucleotide sequences revealed pMUT1-homologous plasmids in *Citrobacter rodentium* (pCRP3), *Klebsiella pneumoniae* (pB1020) and in enterohemorrhagic *E. coli* (EHEC) O157:H7 (strain 86-24) (p9705). Sequences homologous to the entire pMut2 plasmid were not found in the NCBI database. 64 % of the plasmid including the *mobABCD* gene cluster shows 97 % identity to such a gene cluster in *Plesiomonas shigelloides*.
Computer-assisted annotation of open reading frames (ORF) predicted 5 and 7 ORF and the RNA elements RNAI and PK-repBA for pMUT1 and pMUT2, respectively. BlastP protein searches of the translated putative ORFs within the *E. coli* taxon discovered on pMUT1 beneath the known MobA protein a conserved global transcription regulator of the GntR family. On pMUT2 the toxin-antitoxin module (TA) RelB/RelE is encoded. Since plasmid-encoded TA modules help to maintain plasmids due to the "post-segregational killing" mechanism, the here found RelB/E pair might explain the ensured inheritance of the pMUT2 regulated via transcriptional control by pMUT1.
To date it is still unclear why and how EcN establishes the two cryptic plasmids pMUT1 and pMUT2. The postulated pMUT1-controlled expression of the TA module encoded on pMUT2 offers a promising three-component model to explain maintenance of both cryptic plasmids. Both plasmids together are exclusively found in EcN. Amplification of plasmid-specific sequences in a specific PCR assay enables to distinguish EcN from all bacteria including even closely related *E. coli* strains. This strain-specific PCR assay is reliably used within the molecular quality- and purity-control in the pharmaceutical production of EcN, active principle of the licensed drug MUTAFLOR®.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**222/PWP**

**Optimization of a cell-free *in vitro* expression system for synthesis of antimicrobially active microcin S in high quantities**

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**Introduction**: Due to steadily increasing numbers of antibiotic resistant bacteria, infections caused by these pathogens are a relevant problem. Therefore, detection of so far unknown antimicrobial substances is a promising tool to keep this development under control. To protect themselves against pathogens, some bacteria are able to secrete compounds with an antimicrobial effect. The probiotic *Escherichia coli* G3/10, which is a component of the drug Symbioflor 2, produces such an antimicrobial peptide, named microcin S (MccS). Among others, this class IIa microcinis suppressing the adherence of enteropathogenic *E. coli in vitro* [1]. To investigate its antimicrobial activity and its mode of action in more detail, quantitative amounts of MccS in high quality are needed.
Therefore, a bacteria based cell-free *in vitro* system was set up using a combination of different energy sources. After optimization of this system we are now able to synthesize high quantities of biological active microcin S.
**Methods**: For an effective *in vitro* expression, the microcin S gene (*mcsS*) was fused with a 6x His-tag to a T7 promoter system. To induce expression of MccS templates were then transferred into an *in vitro* system, composed of among others a crude cell extract of *E. coli* BL21 Rosetta 2(DE3), fructose-1,6-bisphosphate, ATP and NAD, all 20 amino acids and $Mg^{2+}/K^{+}$ buffer. Different combinations of the energy buffer composition were tested. Also the concentrations of all ingredients of the *in vitro* system were adjusted to reach high microcin S expression rates.
Performance of MccS synthesis was controlled by dot blot followed by immune detection. Antimicrobial activity against sensitive strains was determined via soft-agar diffusion tests and colorimetric detection of metabolism of tetrazolium salts.
**Results**: Highest quantities of 6x His-tagged MccS could be expressed *in vitro* by using high concentrations of Mg-glutamate. Fructose-1,6-bisphosphate was also found to be better suited as ATP regenerating substance than phosphoenolpyruvic acid. A combination of different sugars further could improve protein expression rate. Protein folding conditions were optimized by exclusion of any reducing substances from the reaction mixture. Biological activity of microcin S could be demonstrated by detection of inhibition zones in soft-agar containing a suitable indicator strain. Strength of antimicrobial activity depends on the structure of the microcin S expression construct.
**Conclusion**: We could show that *in vitro* protein synthesis with our expression system is a well suited tool to generate high yields of biological active MccS. Several improvements of the *in vitro* expression system have led to a significant increase in MccS protein yield, sufficient for further structural and functional investigations of this interesting antimicrobial peptide.

**Literature**

[1] Zschuettig, A. *et al.* (2012), PLoS One 7(3):e33351.

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**223/PWP**

**An abundance of antimicrobial substances governs microbial competition in the human nasal microbiota**

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The human nasal microbiota is highly variable and dynamic often enclosing major pathogens such as *Staphylococcus aureus*. The potential roles of bacteriocins or other mechanisms allowing certain bacterial clones to prevail in this nutrient-poor habitat have hardly been studied. Of 90 nasal staphylococcal strains, unexpectedly, the vast majority (82%) was found to produce antimicrobial substances in particular under habitat-specific stress conditions. Activity spectra were generally narrow but highly variable with activities against certain Gram-positive, Gram-negative, or both groups of bacteria. A representative bacteriocin was identified as a nukacin-related peptide whose inactivation strongly reduced the producer's capacity to limit growth of other nasal bacteria. Of note, the bacteriocin genes were found on mobile genetic elements exhibiting signs of extensive horizontal gene transfer and recombination events. Thus, continuously evolving bacteriocins appear to govern bacterial competition in the human nose and specific bacteriocins may become important agents for eradication of notorious endogenous pathogens.

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**224/PWP**

**Using a gnotobiotic mouse model to investigate the mechanisms of *Salmonella*-microbiota interaction in inflammation-induced pathogen blooms**

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*Salmonella enterica* serovar Typhimurium (*S.* Tm) infection induces acute gut inflammation, which is followed by dramatic changes in microbiota composition as well as by *Salmonella* overgrowth. These conditions are termed dysbiosis and pathogen "blooming", respectively.
Recently, first insights into the underlying mechanisms have been obtained. On the one hand, anaerobic electron acceptors and iron are selectively consumed by the pathogen. On the other hand, the microbiota might experience collateral damage caused by leukocytes, which infiltrate the gut lumen in response to *Salmonella*-induced inflammation. Yet, it is still unclear which of these two mechanisms is more important for the induction of pathogen "blooming": the altered nutritional environment or differential killing by the inflammatory immune response. Furthermore, it has remained elusive how the environment of an inflamed gut impacts on the different members of a normal microbiota.
To address this question in more detail, we employ a defined consortium of mouse adapted commensal bacteria, the Oligo Mouse Microbiota (Oligo-MM). The Oligo-MM consists of 12 strains representing 5 main Eubacterial phyla of the mammalian gut (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria*).
We are establishing *in vitro* culture assays to analyze the contribution of defined environmental changes on the Oligo-MM consortium. We studied the fate of each of the individual strain using a strain-specific real-time PCR assay.
Using this reductionist model we envision extending the current knowledge on gut inflammation-inflicted dysbiosis and, thereby, contribute to the development of new therapies to prevent pathogen "blooming" and collateral damage of the gut microbiota.

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**225/PWP**

**Analysis of the secretom of *Escherichia coli* strain Nissle 1917 (EcN)**

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In 1917 Alfred Nissle discovered and isolated the probiotic *E. coli* strain Nissle 1917, which shows antagonistic properties against some virulent enterobacteria by e.g. crosstalk with epithelial cells. EcN is a non-pathogenic *E. coli* strain that does not form cytotoxins, enterotoxins or pathogenic adhesion factors. Analysis of the whole genome of EcN led to the identification of so-called genomic islands (GEIs) on its chromosomes [1]. These GEIs are coding among other things for different Sat serine-proteases, whose specific functions are unknown by now. Other fitness factors which mediate antagonistic actions via secreted substances are for example the two microcins H47 and microcin M [2] or iron chelators of six different iron uptake systems (e.g. aerobactin, hemin-dependent iron acquisition system). The flagella of EcN mediates also antagonistic actions by inducing the human β-defensin 2 production in Caco-2 cells [3], and inhibits the invasion of the porcine gut epithelial cell line IPEC-J2 by aEPEC [4]. In addition it was shown that EcN even without physical contact with either the epithelial cells or the invasive bacteria inhibits invasion of human intestinal epithelial cells by *Salmonella enterica*, *Listeria monocytogenes* and other enteroinvasive pathogens [5]. Reduction of growth-rate of pathogenic enterobacteria and their shiga toxin production by EcN were demonstrated by different groups in the past [6,7]. At least for some of the antagonistic effects a secreted unknown substance seems to be responsible for the EcN effect. But currently it is not known if this is a protein or a low-molecular weight component.
The aim of this project is to identify the secreted EcN proteins after cultivation in M9 medium with different C-sources by SDS polyacrylamide gel electrophoresis followed by silver staining, HPLC and mass-spectrometry. For these analysis' samples are concentrated and dialyzed. Several proteins in the supernatants have been already detected, showing EcN to secret proteins under the applied culture conditions. These will be varied to find optimal conditions to maximize the amount of secreted proteins. Various molecular weight fractions of supernatants will be applied in invasion and shiga toxin assays to test for inhibitory activity. Subsequently protease treatment will be used to probe for the proteinaceous nature of the active component in the respective fraction. In addition a collection of EcN mutants will be employed with defined lack of secreted molecules.

**References**

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**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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## QUALITY MANAGEMENT IN DIAGNOSTIC MICROBIOLOGY (QSP)

**226/QSP**

**Qualitative and quantitative detection of BoNT from complex matrices: results of the first international proficiency test**

S. Worbs*[1], U. Fiebig[1], R. Zeleny[2], H. Schimmel[2], A. Rummel[3]
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**Introduction:** In order to develop innovative security solutions and to build up a network of EU CBRN laboratories, the Seventh Framework Programme for Research (FP7) was funding projects addressing the standardization of CBRN detection and identification. In this context, EQuATox successfully established a network of 35 expert laboratories from 20 countries worldwide working together on the quality assurance for the detection of biological toxins which are covered by the Chemical Weapons Convention and the Biological Weapons Convention (ricin, saxitoxin, staphylococcal enterotoxin B and botulinum neurotoxins).
**Results:** In the framework of EQuATox a first international proficiency test (PT) on the detection and quantification of botulinum neurotoxins (BoNT) was conducted. Sample materials included BoNT serotypes A, B and E spiked into buffer, milk, meat extract and serum. A variety of methods was applied by the participants combining different principles of detection, identification and quantification. Based on qualitative assays, 95% of all results reported were correct. Successful strategies for BoNT detection were based on a combination of complementary immunological, MS-based and functional methods or on suitable functional *in vivo* / *in vitro* approaches (mouse bioassay, hemidiaphrama assay, Endopep-MS assay). Quantification of BoNT/A, BoNT/B and BoNT/E was performed by 48% of

participating laboratories. It turned out that precise quantification of BoNT was difficult resulting in a substantial scatter of quantitative data. This was especially true for results obtained by the mouse bioassay which is currently seen as "gold standard" for BoNT detection.
**Conclusion:** The results clearly demonstrate the urgent need of certified BoNT reference materials and the development of methods replacing animal testing. In this context, the BoNT PT provided the valuable information that both the Endopep-MS assay and the hemidiaphrama assay delivered quantitative results superior to the mouse bioassay.

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## NATIONAL REFERENCE LABORATORIES AND CONSILIARY LABORATORIES (RKP)

**227/RKP**

**Enteropathogen prevalence in human enteric infections - unexpected results of a study of the diagnostic Network for Enteric Infections (NEI)**

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**Introduction**: During recent years, the number of notifiable enteric infections in Germany caused by viruses, bacteria, or protozoa has been increasing to about 3-500.000. However, the estimated number of unreported cases is still considerable, and the prevalence of most diarrhoea-inducing pathogens is largely unknown and needs to be established. Therefore, eleven German consulting and reference laboratories (CL HUS; CL Molecular Diagnosis of *Treponema*; CL *Whipple* Bacteria; CL Norovirus; CL Rotavirus; CL Gastrointestinal Infections; Bi-national CL *Listeria*; CL Adenovirus; NRC Tropical Infections; CL *Clostridium difficile*, NRC *Salmonella* and other Enteric Bacterial Pathogens) established the Network for Enteric Infections (NEI) to detect both notifiable and in reporting not yet considered potential diarrhoeal pathogens.
**Methods**: Stool samples were analysed by the eleven partners for the presence of about 25 different pathogens. From July 2012 till September 2013, the network in a population based study (550 individuals), with embedded case-control, prospectively analysed 84 diarrhoea stool samples and 128 closely matched control samples from three defined study regions in South-West Germany.
**Results**: Unexpectedly, in 62% of the diarrhoea samples at least one pathogen was found but surprisingly also in 56% of the control samples. In 44% of the diarrheal samples and in 39% of the healthy controls more than one pathogen was detected. Specifically, the following pathogens were identified most frequently: *Dientamoeba fragilis*, intestinal pathogenic *E. coli*, *Blastocystis hominis*, Norovirus, *Tropheryma whipplei*, and Adenovirus.
Conclusion: Our analysis shows that enteric pathogens are often the cause of acute diarrhea and which pathogens are commonly associated with disease in the respective study population. Additionally, the study provides indications that a) for some enteric pathogens molecular diagnostic methods, when applied exclusively, may produce misleading results and further that b) carriage of a pathogen without disease symptoms might be observed frequently.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**228/RKP**

**Serotype distribution and ampicillin-resistance of invasive *H. influenzae* isolates in Germany: results from five years of laboratory surveillance**

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The national reference laboratory for meningococci and *H. influenzae* (NRZMHi) has been serotyping invasive *H. influenzae* isolates since 2008. The typing data were merged with the national statutory notification data since 2009. Coverage of laboratory submissions could be increased from 60% in 2009 to 73% in 2013 by introducing active feedback to local health authorities.
From 2009 to 2013 the incidence for invasive *H. influenzae* infections have increased from 0.23/100,000 to 0.52/100,000. The serotyping results showed that the majority of isolates (80%) were unencapsulated, so-called non-typeable *H. influenzae* (NTHi). The most common capsule type found in Germany was *H. influenzae* serotype f (Hif; 13%). The second most common capsule type in 2012 and 2013 was Hie (4% and 3%, respectively). The previously most frequent Hib was only found in 4% of all isolates over the complete observation period. The augmented incidence rate in invasive *H. influenzae* disease was due to increased infections in the elderly caused by NTHi. The study showed that all unencapsulated invasive strains were NTHi, and none of them were strains with capsule locus mutation, which have been postulated in other studies.
Ampicillin resistance (MIC > 1µg/ml) was found in 14% of all tested isolates. The resistance rate remained at moderate levels over the observation period (2009: 11%, 2010: 9%, 2011: 18%, 2012: 14%, 2013: 17%). Beta-lactamase negative ampicillin resistant (BLNAR) isolates were found in low percentages, ranging from 1% (2010) to 6% (2011 and 2013).

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**229/RKP**

**Primary resistance of *Helicobacter pylori* to clarithromycin, levofloxacin and tetracycline in Germany in 2014/2015**

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**Objectives**: Knowledge of antimicrobial resistance of *Helicobacter pylori* is needed to prescribe adequate treatments. The aim of this ongoing prospective study is to keep the development of primary antimicrobial resistance of *H. pylori* to key antibiotics such as clarithromycin under surveillance and to continuously deliver updated data on resistance.
**Methods:** From October 2014 until April 2015, a total of 291 adult patients who had not received an eradication therapy in the past were enrolled. Clinical and epidemiological data were gathered; gastric tissue samples taken for the Helicobacter urease test were genotypically tested for mutations conferring resistance to clarithromycin, levofloxacin and tetracycline.
**Results:** Primary resistances were 8.6% for clarithromycin, 10.2% for levofloxacin and 0% for tetracycline. We did not observe any correlation between resistances, the underlying gastric disease and the patients' country of origin. There was no significant difference

in the resistance proportions among the different German federal states.
**Conclusions:** In our study population, the prevalence of clarithromycin and levofloxacin resistance is low; resistance to tetracycline does not exist. Patients without any prior eradication therapy can be treated empirically. Genotypic susceptibility testing is a reliable method that facilitates updating resistance data in regular intervals. These data may then be used to guide treatment recommendations.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**230/RKP**

**Report of the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2014**

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**Question**: Multidrug-resistance in *Enterobacteriacea*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drugs against gramnegative bacteria will be introduced into clinical practice within the next years. Among all resistance mechanisms the worldwide spread of carbapenemases is the most worrisome development. However, the correct identification of carbapenemases is challenging for the microbiological laboratory.
**Methods**: The National Reference Laboratory for Multidrug-Resistant Gramnegative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts allows the detection of still unknown β-lactamases.
**Results**: A total of 4791 isolates were investigated for carbapenemases in the National Reference Laboratory in 2014. Specimen sources were mostly rectal swabs (22.1%), urine (20.8%) and respiratory samples (19.3%). Carbapenemases were found in 1240 *Enterobacteriaceae* strains (46.3%), 312 *P. aeruginosa* (24.2%) and 525 *A. baumannii* (93.9%). The most frequent carbapenemases in *Enterobacteriaceae* were OXA-48 (31.2%), KPC-2 (26.5%), VIM-1 (15.5%), NDM-1 (15.1%) and KPC-3 (4.2%), OXA-181 (2.2%). OXA-232, OXA-162, OXA-244, NDM-5, GIM-1 and VIM-4 were found in less than 1.5% each. In *P. aeruginosa* VIM-2 was the most frequent carbapenemase (79.9%), followed by VIM-1 (5.0%). FIM-1, GES-5, IMP-7, IMP-13, IMP-28, NDM-1 and VIM-4 were found in less than 2.5% each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (74.1%) followed by OXA-72 (13.5%) and OXA-58 (7.9%). GIM-1, NDM-1 and NDM-9 were found in less than 1.5% each.
**Conclusions**: The most frequent carbapenemases in Germany are OXA-48 in *Enterobacteriaceae*, VIM-2 in *P. aeruginosa* and OXA-23 in *A. baumannii*. OXA-48 and NDM-1 are found with increasing frequency.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**231/RKP**

**Ascertainment of pneumococcal vaccination status among children <2 years of age with invasive pneumococcal disease in Germany**

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**Background and aims:** In 2006, a general recommendation for vaccination with pneumococcal conjugate vaccine (PCV) for children <2y was issued in Germany. Here, we report on the pneumococcal vaccination status of children <2y with invasive pneumococcal disease (IPD) in the years 2006-2014.
**Methods:** The German National Reference Center for Streptococci has been collecting pneumococcal isolates of IPD in children since 1997. Entries were reviewed for their vaccination status. Missing data were obtained by written request from the treating pediatricians.
**Results:** Among 842 entries, vaccination data could be obtained for 605 cases. Of these, 370 (61.2%) were vaccinated; PCV7: 27.3%, PCV10: 7.8%, PCV13: 49.7%, PCV7/13: 2.7%, PCV10/13: 0.8%, vaccine unknown: 11.4%.
Using the indirect cohort method a vaccine effectiveness (VE) of 80% could be calculated for PCV7 (at least one dose) for the period 2007-2010 for children <2y. For the six additional serotypes in PCV13 (PCV13-non-PCV7) a vaccine effectiveness (VE) of 81% could be calculated for the period 2010-2014.
In 2011-2014 a total of 62 children had IPD caused by a PCV13 serotype. Of these 37 were not vaccinated (<2mo: n=5, 2-4mo: n=16, 5-23mo: n=16). In 15 children vaccination was incomplete, mostly lacking the booster dose (n=10). In eight children IPD occurred after vaccination according to schedule, of which three had received all four doses including the booster dose. In two cases the vaccination status could not be obtained.
**Discussion:** The first results of this study show a high VE for PCV7 and PCV13 for IPD in children <2y. It is remarkable that among the remaining cases with vaccine type serotypes 60.3% of the children were not vaccinated. In many cases vaccination was late, the booster dose was not given, or otherwise incomplete schemes were applied. Eight cases of vaccination failure were detected which will be assessed for underlying disease.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**232/RKP**

**Comparison of different typing methods for discrimination of clinical *S. epidermidis* isolates**

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**Introduction:** During the last years reports about the emergence and outbreaks with multidrug-resistant (MDR) *S. epidermidis* in hospital-associated settings are increasing worldwide. Molecular typing of these strains is important to detect outbreaks and to follow the spread of successful clones.
**Objectives:** Several methods are available for typing of *S. epidermidis*, representing different technologies and varying with respect to hands-on-time, costs and discriminatory power. Here we compare the performance of *Sma*I macrorestriction analysis in PFGE, Multilocus Sequencetyping (MLST) and DiversiLab (DL) typing, a commercial rep-PCR based typing system, for discrimination of clinical *S. epidermidis* isolates in a suspected outbreak setting.
**Material & Methods:** Sequences types, PFGE-patterns and DL-fingerprints were obtained from a strain collection, which comprised 31 MDR *S. epidermidis* from two ICU´s (isolated over a one year period). In both settings epidemiologically related isolates were supposed. Sequence types were determined according to http://sepidermidis.mlst.net/; clusters were defined for PFGE-patterns (>90% similarity score, BioNumerics 7.1 Software) and DL-fingerprints (>95% similarity score, DL-typing Software).

Statistical tests were performed using the EpiCompare Software 1.0.
**Results:** MLST revealed five different sequences types (ST2, ST5, ST10, ST23, ST168); PFGE-typing grouped the isolates into eight clusters and into five DL-clusters. PFGE typing revealed the highest discriminatory index; DL-typing and MLST showed comparable discriminatory indices. Isolates exhibiting ST2 could be further resolved by PFGE and DL-typing forming four and three clusters, respectively. Epidemiologically related isolates (according to MLST and PFGE) were grouped into the same DL-cluster. However epidemiologically unrelated isolates, exhibiting different sequences types and generating distinct PFGE-clusters, could not be resolved by DL-typing and were partly grouped into similar DL-clusters.
**Conclusions:** PFGE revealed the highest discriminatory index for typing of *S. epidermidis*, but the method is laborious and time-consuming. DL provides a semi-automated and time-saving strain typing, although it is comparatively costly. Our results indicate that strains, which are different by DL-typing, are also unrelated when applying PFGE. But the discriminatory power of DL-typing for *S. epidermidis* is limited, because strains which form an identical DL-cluster may be unrelated when applying PFGE-analysis and/or MLST typing. Therefore, when DL-typing is initially used in outbreak investigations, application of a second typing method or considering also further strain characteristics (e.g., antibiotic profiling) for confirmation of the results is highly advisable.

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## ANTIMICROBIAL RESISTANCE IN DIFFERENT HOSTS (ZOP)

**233/ZOP**
**Influence of colistin resistance on pathogenicity and bacterial fitness in *Klebsiella pneumoniae***
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**Introduction**: Multidrug-resistant Gram-negative (MRGN) bacteria have disseminated worldwide during the last years. Thus, it is of definite interest to not only understand resistance mechanisms but the impact on cell physiology as well. One of the last-line antibiotics for treatment of infections with MRGNs is colistin (CT) which targets the lipopolysaccharide (LPS) component lipid A. Here, we characterized the colistin resistance mechanism in clinical *Klebsiella pneumoniae* isolates and the influence on CT resistance on bacterial fitness, pathogenicity and inflammation.
**Materials and Methods**: Antibiotic susceptibility was evaluated using antibiotic gradient test. Genes involved in lipid A synthesis were investigated by PCR analyses and whole genome DNA sequencing (WGS). Pathogenicity and bacterial fitness were determined using the LDH cell toxicity assay, the *Galleria* infection model and competitive growth kinetics respectively. The inflammatory response of differentiated THP-1 monocytes during infection was investigated by quantifying mRNA gene induction of IL-8 and TNF-α via qRT-PCR.
**Results**: CT-susceptible (MIC of 0.5 mg/L) and CT-resistant (MICs of 4-8 mg/L) isogenic *K. pneumoniae* isolates were recovered from a single infected patient who did not receive CT treatment before. Comparison of CT-susceptible (CT-S) and CT-resistant (CT-R) isolates by WGS revealed point mutations in the transcription factors *pmrA* (C515A) and *pmrB* (C284T), which encode for a regulatory two-component system involved in biosynthesis of the CT target lipid A. To prove that these mutations were responsible for the CT resistance, complementation assays were performed to restore the function of mutated genes and resulted in reduced colistin MIC values. Since formation of antibiotic resistance often correlates with a bacterial fitness cost, competitive growth kinetics using CT-S and CT-R isolates were generated but revealed no significant differences suggesting unimpaired bacterial fitness. Likewise, analysis of pathogenicity employing the *Galleria mellonella in vivo* infection model as well as the *in vitro* LDH release assay using A549 human lung epithelial cells showed no significant differences between the CT-S and CT-R isolates. In contrast, infection of monocytes with CT-R bacteria resulted in lower mRNA induction of the pro-inflammatory cytokine genes IL-8 and TNF-α.
**Conclusion**: We elucidated that single point mutations in *pmr*A and *pmr*B, which regulate synthesis of the CT target lipid A, lead to CT resistance in clinical isolates of *K. pneumoniae*. Interestingly, CT resistance did not impair bacterial fitness or pathogenicity, but resulted in lower induction of pro-inflammatory host genes which might confer an advantage during an immune response.

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**234/ZOP**
**ESBL-plasmids influence the chromosomally-encoded *csgD*-pathway in multi-resistant ST131 and ST648 *E. coli*, possibly contributing to their pandemic success in numerous habitats**
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ESBL-*E.coli* have become abundant all over the world, not only in a human clinical and community context but also in animals and the environment. Clonal lineages of ESBL-associated STs 131 and 648 are important pandemic multi-resistant bacteria. This study aimed to investigate the influence of ESBL-plasmid-encoded non-resistance genes on chromosomally-encoded features of ST131 and ST648 *E. coli.*
ESBL-carrying wild-type (WT) *E. coli* strains, their corresponding ESBL-plasmid-"cured" variants (PCV) as well as complementary ESBL-carrying transformants were comparatively analyzed in long-term colony, swimming motility and Omnilog® Phenotypic Microarray assays, whole-genome sequence and RNA sequence analysis. Differences were detected in several phenotypic tests including an enhanced curli and/or cellulose production and a reduced swimming capacity of some ESBL-carrying strains compared to their PCV. Omnilog® results pointed towards a similar metabolic behavior of the strains. RNA sequencing mostly confirmed the phenotypic results on a genomic level, revealing the chromosomally-encoded *csgD*-pathway as a key factor involved.
Phenotypic differences, the reversibility in transformants and RNA sequencing results clearly indicate and influence of ESBL-plasmids on chromosomally encoded features especially important for the subtle interactions between a sessile and planktonic way of life in multi-resistant *E. coli*, presumably contributing to their pandemic success.

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**235/ZOP**

**Whole-genome analysis of ESBL-producing *E. coli* of ST410 reveals interspecies transmissions of identical bacterial clones between avian wildlife, humans, companion animals and the environment**

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Extended-spectrum beta-lactamase (ESBL)-producing multi-resistant *E. coli* have been on the rise for years. Diseases including septicemia, pneumonia, and wound and urinary tract infections these bugs cause are not only severe but also increasingly difficult to treat due to limitations in antimicrobial therapies. While, their occurrence was initially restricted to solely a human and veterinary clinical context, recent findings have shown their prevalence in extra-clinical settings such as communities and the environment.
A total of 320 fecal samples were collected from wild birds during entry examinations in a small-animal clinic in Berlin, Germany, of which 7.5 % were ESBL-and/or AmpC-producing *E. coli*. Forty ESBL-producing human clinical (bacteremia) *E. coli* isolates were selected randomly from clinical isolates in a university medical center in Berlin, Germany. To determine the phylogenetic population structure of ESBL-producing isolates from wild birds and humans, multi-locus sequence typing (MLST) analysis was performed. The sequence type (ST) occurring in both sample groups was ST410. Three previously published ESBL-producing *E. coli* of ST410 from environment dog feces and one from a clinical dog isolate were also included in this study. All ST410 isolates were analyzed using pulsed-field gel electrophoresis (PFGE). Ten isolates from one clonal PFGE group with identical or almost identical macrorestriction patterns were chosen for generating whole-genome sequence data using the MiSeq platform (Illumina). Following raw data reprocessing through standard bioinformatics pipelines it was then possible to perform phylogenetic and single nucleotide polymorphism (SNP) analyses.
Within the ten ST410 strains from different hosts in the same region, almost genetically identical isolates were identified using Harvest and Chromopainter. As some of the stains differed by a few SNPs only, the study gives initial evidence for an ongoing interspecies transmissions of multi-resistant *E. coli* clones between avian wildlife, humans, companion animals and the environment underlining their zoonotic potential as well as the mandatory nature of the "One Health" approach to address the threat of multi-resistant ESBL-*E. coli* for human, animal and environmental health.

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**236/ZOP**

**Biochemical characterization of the metallo-β-lactamase KHM-2**

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**Objectives**: The production of carbapenemases by clinically relevant Gram-negative species is one of the most worrying developments in hospital-associated multidrug-resistance. In this context, the dissemination and diversification of Ambler class B metallo-β-lactamases (MBL) is especially important. In 2013, a carbapenem-resistant clinical *P. aeruginosa* isolate was referred to the National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria. This isolate harboured the novel $bla_{\text{KHM-2}}$ MBL gene, coding for a subclass B1 enzyme. Here we present the purification and biochemical characterization of KHM-2.
**Methods**: The KHM-2 encoding gene was cloned into the pBK-CMV vector and expressed in *E. coli* TOP10. The cells were lyzed by sonication and the lysate was cleared by centrifugation, followed by a desalting step. The enzyme was purified by a two-step Fast Protein Liquid Chromatography (FPLC). The first step was an ion exchange chromatography, followed by gel filtration. The purified enzyme was analyzed biochemically by *in vitro* hydrolysis assays by photometrically monitoring the absorbance changes with various β-lactam substrates. The kinetic parameters $K_m$ and $k_{cat}$ were determined by nonlinear regression using the Michaelis-Menten equitation. To serve as a reference, the KHM-1 enzyme was purified and characterized the same way.
**Results**: Biochemical analysis of KHM-2 showed that the enzyme was able to hydrolyze almost all tested substrates. Penicillin G and ampicillin were hydrolyzed with high turnover numbers but with relatively low affinity towards the enzyme. KHM-2 showed a very weak piperacillin hydrolysis. Most cephalosporins were hydrolyzed with high efficiencies. Regarding carbapenems, imipenem showed the highest hydrolysis rates, while meropenem and ertapenem were rather poor substrates. In comparison to KHM-1, KHM-2 showed higher rates for ceftazidime and imipenem, but lower rates for cefotaxime, meropenem and ertapenem. Both KHM-2 and KHM-1 were not able to hydrolyze aztreonam.
**Conclusion**: The biochemical characterization of KHM-2 and the comparison to KHM-1 further underline the diversification of subclass B1 metallo-β-lactamases and the resulting differences in catalytic behaviour between enzymes of the same group. The kinetic data for KHM-2 suggest that this enzyme is a potent carbapenemase that most likely can confer high carbapenem resistance levels in Gram-negative species of clinical importance.

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**237/ZOP**

**ESBL-carrying multi-resistance plasmids in clinical *Escherichia coli* isolates from food-producing animals**

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**Objective:** The aim of this study was to investigate the co-location of antimicrobial resistance genes on extended-spectrum β-lactamase (ESBL) gene-carrying plasmids in *Escherichia coli* isolates from diseased food-producing animals.
**Methods:** In total, 194 of 3670 *E. coli* isolates, collected from diseased animals in the German National Resistance Monitoring program GE*RM*-Vet during 2010-2013, were ESBL producers. Representative isolates and their plasmids were characterized by susceptibility testing to 28 antimicrobial agents, XbaI-macrorestriction analysis, multilocus sequencing typing (MLST), phylotyping, electrotransformation and conjugation experiments, replicon typing, S1 nuclease PFGE and PCR assays for the detection of resistance genes.
**Results:** In 140/1783 bovine, 46/819 porcine and 8/1068 avian *E. coli* isolates the presence of ESBL genes was confirmed by PCR and sequencing. Among the 50 representative ESBL-producing isolates 48 unrelated and two closely related XbaI-macrorestriction patterns as well as 23 MLST types were seen. The isolates belonged to phylogenetic groups A (n=27), B1 (n=9), B2 (n=1) or D (n=13). Transfer experiments revealed the presence of single ESBL genes on the plasmids [$bla_{\text{CTX-M-1}}$ (n=24), $bla_{\text{CTX-M-14}}$ (n=17), $bla_{\text{CTX-M-15}}$ (n=4), $bla_{\text{CTX-M-3}}$ (n=1) and $bla_{\text{SHV-12}}$ (n=4)], with 41/50 plasmids being conjugative. Multi-resistance (resistance to at least three classes of antimicrobial agents) was identified in 23 plasmids, 16 of them were conjugative and had sizes of 30-330 kb. These plasmids carried, most commonly, genes for resistance to sulphonamides (*sul1*, *sul2*, *sul3*), trimethoprim (*dfrA* genes), or tetracycline [*tet*(A), *tet*(B)]. The multi-resistance plasmids encoding CTX-M-1 belonged to the incompatibility groups IncI1

(n=2), IncF (n=1), FIA+FIB (n=2), IncHI2 (n=1), or IncX (n=1), all encoding CTX-M-14 to IncF, those encoding CTX-M-15 to IncF+FIA+FIB (n=2), IncI1 (n=1) or IncN (n=1), and those encoding SHV-12 to IncI1 (n=2) or IncF (n=1).
**Conclusions:** The presence of additional resistance genes on the ESBL-carrying plasmids suggests that co-selection of ESBL genes may occur even in the absence of β-lactam antibiotics and may lead to the presence and persistence of ESBL producers in animals and humans. Moreover, the identification of conjugative multi-resistance plasmids carrying ESBL genes in *E. coli* isolates from food-producing animals underlines the risks of resistance dissemination to humans as such isolates may enter the food chain.

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**238/ZOP**

***Staphylococcus stepanovicii* harboring *mec*C on a complete class E *mec* complex isolated from a wildlife rodent (*Myodes glareolus*)**

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**Introduction**: In recent years, the methicillin-resistance encoding gene *mec*A has been identified among coagulase-negative staphylococci (CNS) like *Staphylococcus fleurettii*, *Staphylococcus vitulinus* and further CNS. In 2011, a novel *mec*A homologue (*mec*C; EMBL FR821779) harbored by SCC*mec*XI was described for methicillin reistant *Staphylococcus aureus* (MRSA) from human and bovine origin, and later also from wildlife, companion animals as well as environmental sites including water.
**Objectives**: To gain deeper insights into the genomic region downstream of the chromosomal integration site (*att*BSCC) of the *mec*C-positive *S. stepanovicii*, we conducted whole genome sequencing (WGS).
**Materials & Methods**: The *Staphylococcus stepanovicii* strain IMT27065 (ODD4) was isolated in August 2011 from a fecal sample of a wild bank vole (*Myodes glareolus*) as part of a screening study focusing pathogens from wild rodents (Network "Rodent-Borne Pathogens"). Whole genome sequencing was carried out on a HiSeq (Illumina, USA). The reads were assembled using CLC Genomics Workbench 7.5 (CLC bio, Denmark) and open reading frames (ORFs) were predicted using Prodigal. Annotation of ORFs and prediction of (protein) coding sequences (CDS) was performed by The RAST Server. Putative CDS function and conserved domains were predicted with blastn and blastx using the NCBI database. For comparative genomic analyses Geneious 7.1.5 was employed.
**Results and Discussion**: Here we report on the entire nucleotide sequence of the region between the rRNA-methyltransferase (*orf*X)-like gene and the tRNA dihydrouridine synthase B (*orf*Y)-like gene in a *mec*C-positive strain (IMT28705, GenBank accession no. KR732654). Genome sequencing revealed that the isolate harbors a *mec*C gene which shares 99.2% nucleotide (and 98.5% amino acid) sequence identity with *mec*C from *S. aureus* strain LGA251. In addition, the *mec*C encoding region harbors the typical *bla*Z-*mec*C-*mec*R1-*mec*I structure (5,163 bp), corresponding with the class E mec complex. A similar structure (including *mec*B instead of *mec*C) was reported for *Macrococcus caseolyticus*, either as part of a transposon located on plasmids or within an SCC*mec* element. However, the region between the *orf*X and *orf*Y-like genes seems to lack transposases as well as *ccr* recombinase homologues. One the other hand, analysis of the 15bp direct repeats (DR) flanking *att*BSCC revealed similar DRs widely distributed downstream of *orf*X within the genus *Staphylococcus*, especially within SCC*mec* elements of MRSA, indicating the possibility of a broad genetic exchange.
**Outlook**: Our data highlights the necessity of research on putative transmission routes of resistance encoding factors from the environmental resistome in terms of wildlife reservoirs to opportunistic bacteria such as *S. aureus*.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**239/ZOP**

**Characterization of clinical *Acb*- (*Acinetobacter calcoaceticus-Acinetobacter baumannii*-) complex isolates of human and animal origin collected during a one year time-period**

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**Introduction**: The increasing number of infections due to multidrug resistant bacteria challenges human as well as veterinary medicine. Besides ESBL (extended-spectrum beta-lactamase) producing Enterobacteriaceae and MRSA (methicillin resistant *S. aureus*), multidrug resistant *A. baumannii* are more frequently isolated from veterinary clinical specimens. Moreover, the same clonal lineages are associated with *A. baumannii* infections in humans and animals, indicating a zoonotic transmission. Despite this, knowledge regarding the occurrence of *A. baumannii* in animal populations is scarce.
**Aims**: We therefore aimed to compare the distribution of the *Acb*-complex species among clinical isolates of human and animal origin. To assess the occurrence of antibiotic resistances of *A. baumannii* isolates, antibiotic susceptibility profiles have furthermore been determined.
Materials and methods: Clinical *Acb*- complex isolates from convenience samples of human and animal origin and associated metadata (host, specimen, month of isolation, geographical origin) have been collected during a one year time-period starting in February 2013. Since the *Acb*-complex species *A. baumannii*, *A. pittii*, *A. nosocomialis* and *A. calcoaceticus* are remarkably closely related, species identification has been performed based on restriction fragment length polymorphism (RFLP) of the 16S-23S intergenic spacer region. Susceptibility profiles have been generated by use of the VITEK 2 System (bioMérieux).
**Results**: In total, a collection of 657 *Acb*-complex isolates could be obtained [human isolates n=282 (*A. baumannii*: 58, *A. pittii*: 149, *A. nosocomialis*: 5, *A. calcoaceticus*: 23, pending: 47); animal isolates n=375 (*A. baumannii*: 164, *A. pittii*: 108, *A. nosocomialis*: 8, *A. calcoaceticus*: 60, pending: 35)]. 152 of the 222 *A. baumannii* isolates showed enhanced MICs (Minimum Inhibitory Concentrations) for less than three tested antimicrobial classes [human isolates n=51 (88%); animal isolates n=101 (61,6%)] and 70 *A. baumannii* isolates showed enhanced MICs for more than three antimicrobial classes [human isolates n=7 (12%); animal isolates n=63 (38,4%)].
**Conclusion**: While *A. pittii* was the most common pathogenic *Acb*-complex species associated with human clinical samples, *A. baumannii* has more frequently been isolated from animal specimens. Susceptibility profiles revealed a high prevalence of antimicrobial resistances among *A. baumannii* isolates of animal origin.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

# EPIDEMIOLOGY OF ZOONOTIC PATHOGENS (ZOP)

**240/ZOP**

**Nasal colonization of pig-exposed persons with *Enterobacteriaceae* and associated antimicrobial resistance**

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Extended-spectrum betalactamase (ESBL) producing *Enterobacteriaceae* (ESBL-E) have recently emerged among livestock affecting 44-56% of German pig holdings. Among humans, rectal ESBL-E colonization involves 6% of persons in the general population. For Methicillin-resistant Staphylococcus aureus (MRSA), which is also widely distributed in pigs, cattle and poultry, occupational contact with these animals, was identified as a major risk factor for nasal colonization. In Germany, about 80% of all pig farmers are colonized with MRSA in the nares.
In this study we hypothesized that contact with pigs, besides MRSA, might also facilitate colonization of the nares with ESBL-E.
Swabs from the anterior nares were obtained from pig-exposed persons (mostly farmers) in North-West-Germany between July and December 2014. Swabs were enriched in non-selective broth and plated on MacConkey and ESBL-selective agars. Species confirmation was done by matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS). Antimicrobial susceptibility testing was performed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.
Among 114 pig-exposed persons tested, *Enterobacteriaceae* were detected in the nares of 76 participants (66.7%). The predominant species were *Proteus mirabilis* (n=17, 14.9%), *Pantoea agglomerans* (n=13, 11.4%), *Morganella morganii* (n=9, 7.9%), *Citrobacter koseri* (n=9, 7.9%), *Klebsiella pneumonia*, *Escherichia coli* and *Proteus vulgaris* (each n=8, 7.0%). ESBL-E were not detected. Enterobacterial isolates were resistant against ciprofloxacin (3.4%), gentamicin (2.3%) and trimethoprim-sulfamethoxazole.
We found a very high rate of nasal carriage with enterobacteria among pig-exposed persons. However, antimicrobial resistance was overall lower compared to clinical isolates of the species detected. Although ESBL-E is highly prevalent among pigs, pig-exposed persons did not carry ESBL-E in their nares. This finding is important, because nasal colonization might cause endogenous infections or facilitate transmission of ESBL-E in the general population.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**241/ZOP**

**How Strongyloidiasis behaves in our community?**

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**Background:** Strongyloidiasis is considered a neglected tropical disease because is underdiagnosed due to its low parasitic load and uncertain clinical symptoms. Although not all the cities of Spain are endemic sites, cases of strongyloidiasis have been reported relatively often; in the Mediterranean coast, linked to the cultivation of rice. We aimed to make an epidemiological description of the cases of strongyloidiasis in our community.
**Methods:** We conducted a structured search using a database program to collect data of positive samples for *Strongyloides stercoralis* by serology or direct stool examination, between January 2004 and December 2014. Serology was performed by, using enzyme linked immunoabsorbant Assay technique (ELISA)**.**
**Results:** We have 20 positive stool samples of 14 patients for strongyloidiasis, four of them, also with positive serology. Six men and eight women.The age range was from 4 to 88 years. Be receiving corticosteroid therapy (5) and having HIV stage C3 (3), were the most commonly encountered risks factors while having malignancies treated with chemotherapy (2), alcoholism (2); were other risk factors, and being healthy (2). Ten patients were from South America; two were Spanish and two from Africa.50% (7) were asymptomatic with eosinophilia (count from 15% to 44%), 21.4% (3) urticaria, and 7.1% (1) diarrhea and abdominal pain. Two patients developed hyperinfection syndrome, in the context of AIDS and systemic lupus erythematosus, and were receiving corticoesteroids and cytotoxic agents. Both of them developed septic shock by gram-negative bacilli, associated with this syndrome.All of them received ivermectin, and progressed well, except from an old woman that had a reinfection 1 to 6 months after treatment, and also received albendazole.
**Conclusions:** In our study, the most common is to have an asymptomatic eosinophilia, followed by urticaria and hyperinfection syndrome. *Strongyloides* hyperinfection is more frequent in Human T-lymphotropic virus 1(HTLV-1), than in HIV, unless the patient also has immune reconstitution syndrome or receive corticosteroid therapy, like these patients: AIDS and Systemic Lupus erythematosus. Screening for *strongyloides* is necessary, before patients undergo any organ transplant, or should received any immunosuppressive therapy, especially in those coming from endemic areas. It is also required to follow-up after treatment by clinical and laboratory evaluations including stool examinations with culture of Baermann, agar plate, and serology.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**242/ZOP**

**Re-adaptation of Livestock-associated Methicillin-resistant *Staphylococcus aureus* to the human host: epidemiological screening for phi3-positive LA-MRSA CC398**

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**Question:** Livestock-associated Methicillin-resistant *Staphylococcus aureus* (LA-MRSA) of clonal complex CC398 are becoming clinically important in Germany especially in regions with high livestock density. To date, little is known about the re-adaptation process of this zoonotic *S. aureus* lineage to the human host. A previous host-adaptation-model could show ancestral MSSA CC398 spread from humans to livestock whereby acquiring antibiotic determinants like the SCC*mec* and *tetM,* but simultaneously losing bacteriophage phi3. Hypothesizing, that bacteriophage phi3 is involved in the on-going re-adaptation process of MRSA CC398 to the human host we analyzed the proportion of phi3-positive MRSA CC398 since their emergence until today.
**Methods:** A representative set of human MRSA isolates of clonal complex CC398 was screened by PCR for prevalence of phi3-associated genes (*chp, sak, scn* and *sea/sep*). Colonization- and infection-associated isolates were recovered from patients at the University Hospital of Muenster covering the time period from their first detection at our facility (2000) until recent times (2014). Within 2000 to 2006, all available isolates were tested, while in later years the first 15 isolates each quarter were tested.
**Results**: In total, 572 isolates were screened for the presence of the β-converting bacteriophage phi3 as well as a truncated *hlb*-gene. Based on BURP analysis, the study includes the most prevalent CC398 associated *spa* types t011 (51.4%), t034 (37.6%) and t108

(3.3%) as well as 21 other rarely occurring types. Among 92 isolates from 2000 to 2006, only one isolate (1.1%) was found carrying bacteriophage phi3. During 2007 to 2014, 480 isolates were selected and 17 isolates (3.5%) were positive for bacteriophage phi3. Most isolates carried bacteriophage phi3 with the immune evasion cluster (IEC) of type B (55.6%), followed by IEC E (22.2%), IEC A (11.1%) and IEC C (5.6%).
**Conclusions:** The increasing rate of human MRSA isolates of clonal complex CC398 carrying a β-converting, IEC-encoding bacteriophage phi3 could be a hind for an ongoing re-adaptation process of this zoonotic *S. aureus* lineage to the human host. The re-adaptation process promotes an increased colonization risk for the human host followed by a rising threat of human-to-human transmission.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**243/ZOP**

**Evidence for chlamydiae in free-living and captive great apes from Central Africa**

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**Background and Significance:** Disease, poaching, and habitat loss are the three major reasons for a dramatic decline of the great ape population in Central Africa.
Chlamydiae can infect a wide range of hosts and have a major impact on animal and human health worldwide. Members of the order *Chlamydiales* are obligate intracellular bacteria with a unique biphasic life cycle. Despite being the closest relatives of humans almost nothing is known about the prevalence of chlamydiae in great apes.
**Objectives:** The aim of this project was to learn more about the prevalence of chlamydiae in the critically endangered *G. gorilla gorilla* and the endangered *P. troglodytes troglodytes* from Central Africa, Gabon.
**Methods:** For that purpose, we screened ocular, vaginal, penis and rectal swabs obtained non-invasively during routine health checks of 12 captive great apes. Moreover, we investigated feces of wild-living great apes. All samples were collected according to the international guidelines applied at the primatology unit of the International Centre for Medical Research of Franceville (CIRMF). Extracted DNA samples were analyzed using a pan-*Chlamydiales* broad-range real-time PCR, a *Chlamydiaceae* specific real-time PCR, as well as a 16S rRNA PCR and sequencing.
**Results:** We found 7 samples to be positive for chlamydiae. These chlamydiae belonged to the non-*Chlamydiaceae* families and were detected in both chimpanzee and gorilla from captivity and wildlife population. Of interest, we found *Waddlia* in captive and wild-life apes. This emerging pathogen has been implicated in bovine and human miscarriage and is thought to be transmitted both zoonotically and through water which is contaminated with free-living *Waddlia*-infected amebae.
**Conclusions:** The chlamydial diversity and host range has been underestimated for a long time. With the first detection of chlamydiae in great apes from Gabon we identified chimpanzee and gorilla as new hosts for chlamydiae. Our results could help to gain deeper insight into the chlamydial evolution and pave the way for further studies on the prevalence and transmission of chlamydiae in primates and their potential for causing disease in African great apes threatened with extinction.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**244/ZOP**

**Serogenotyping and antimicrobial susceptibility testing of *Salmonella* spp. isolated from food samples in Lagos (Nigeria) using DNA microarrays**

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Food-borne salmonellosis is one of the most prevalent zoonotic diseases worldwide. It is important that serotype identification amongst culture-confirmed cases is carried out for epidemiological purposes.Particularly in developing countries,accurate serotype detection is cumbersome and sometimes inaccurate while inGermany, new rules for quality control increase costs of classical serotyping. This makes standardized DNA-microarray-based approaches, i.e., serogenotyping, an attractive option.
The aim of the study was to test a recently developed microarray technique by Alere Technologies GmbH (Jena, Germany) by serogenotyping local isolates of *Salmonella* spp. fromfood samples in Lagos, Nigeria, as well asto characteriseantimicrobial susceptibility patterns of these isolates phenotypically and genotypically. A total of 151 samples of meat,including beef, chicken, pork, and goat from different parts of these animals were purchased from various abattoirs and markets in sixadministrative unitsintheLagos state.
Out of 151 samples, 40 isolates were initially confirmed as *Salmonella* spp. using the API system, while 33 (82.5 %) of these 40 isolates were confirmed to be *Salmonella* by VITEK 2 and additionally with the microarray based genotyping system. These isolates were subsequently analysed by microarray-based serogenotyping as well as tested for antibiotic susceptibility using agar diffusion assays and the VITEK 2 system. Within this panel, nine different *Salmonella* serovars were found: *S.* Amoutive (n=8), *S.* Bargny (n=5), *S.* Drac (n=3), *S.* Ealing (n=5), *S.* Urbana (n=1), *S.* Hadar (n=1), *S.* Nyborg (n=3), *S.* Anatum (n=5) and *S.* Havana (n=2). Antibiotic susceptibility testing of 17 antibiotics with the VITEK 2 system showed that all the isolates were sensitive to imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin, moxifloxacin, tetracycline, tigecycline, fosfomycin, co-trimoxazole and piperacillin/tazobactam. One isolate of serovar *S.* Nyborg(0.03%) was resistant to ampicillin while another isolate belonging to *S.* Amoutive (0.03%) was resistant to ampicillin/sulbactam, cefuroxime, ceftazidime, cefotaxime and cefuroxime-axetil. Susceptibility testing of nalidixic acid was done by agar diffusion and revealed five isolates that were resistant (*S.* Amoutive, *S.* Drac, *S.* Bargny, *S.* Ealing and *S.* Havana, 15.0%).
This study indicates the presence of so far as rare described serovars in the panel of tested isolates from ready-to-eat food samples in Lagos, andaneed to frequently monitor antimicrobial resistance. The used system proved to be perfectly suited to replace serotyping.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

## HOST-MICROBE INTERACTION (ZOP)

**245/ZOP**

**Mechanisms regulating HPV8-mediated tumorigenesis**

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Beta human papillomaviruses (HPV) have been suspected to be carcinogenic in nonmelanoma skin cancers (NMSC), but the basis for potential viral contributions to these cancers is poorly understood. We previously developed a HPV8 transgenic mouse model, which recapitulates the HPV-induced SCC pathology. In nonviral preclinical models, VEGF-A has been identified as a critical regulator of NMSC. However, the role of VEGF-A in HPV-induced NMSC is not resolved, neither the question whether diverse cellular sources of VEGF-A may impact this process. In this study we dissected the contribution of epidermis- versus myeloid cell-derived VEGF-A in HPV8-mediated skin cancer using a combination of HPV8 transgenic mice and conditional gene targeting for VEGF-A. Here we show, that epidermis-specific deletion of VEGF-A results in complete abrogation of tumor initiation in HPV8 mice both spontaneous and under diverse tumor promoting conditions (UV light, skin wounding). In contrast, myeloid cell-derived VEGF-A is only critical in regeneration-induced tumorigenesis triggered by skin injury. Mechanistically, we show that blocking VEGFR2 inhibited injury-induced papilloma formation in HPV8 transgenic mice, indicating an important paracrine function of VEGF-A on tumor angiogenesis. Notably, papilloma-derived keratinocytes showed an upregulation of VEGFR1 and Nrp1 expression at the mRNA and protein level, suggesting the existence of an autocrine effect of VEGF-A on epidermal keratinocytes during tumorigenesis next to VEGF-A-mediated activities on angiogenesis. Taken together, here we provide novel mechanistic insights in distinct functions of epidermal- versus myeloid cell-derived VEGF-A in HPV8-mediated tumor development, which may have important implications for the prevention and treatment of HPV-mediated skin cancer.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**246/ZOP**

**Association of *Campylobacter jejuni ssp. jejuni* chemotaxis receptor genes with multilocus sequence types and source of isolation**

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**Introduction:** *Campylobacter jejuni* is the most prevalent bacterial pathogen causing acute enteritis worldwide. *C. jejuni's* flagellar locomotion is controlled by at least 11 different chemoreceptors. To date, relatively little is known about their distribution in a particular host and in subpopulations of different clonality.
**Objectives:** First, to assess the presence of chemoreceptor genes in each of the
*C. jejuni* genomes deposited in the NCBI database. Second, to investigate presence and distribution of the identified *C. jejuni* chemoreceptor genes and their variants in a collection of 292 MLST-typed isolates of human, bovine, chicken, and turkey origin.
**Materials and Methods:** Assessment of presence of chemoreceptor genes in each of the
*C. jejuni* genomes deposited in the NCBI database was done by BLAST analysis. Investigation of presence and distribution of the identified *C. jejuni* chemoreceptor genes and their variants in a collection of 292 MLST-typed isolates of human, bovine, chicken, and turkey origin was done by PCR.
**Results:** BLAST analysis of the published *C. jejuni* genomes led to the identification of two new variants of *tlp4* (*tlp4a* & *tlp4b*) and *tlp11* (*tlp11a* & *tlp11b*), a disrupted gene of *tlp5* and an already described splitted variant of *tlp7* while the remaining *tlp* genes are well conserved.
PCR analysis showed that *tlp1, tlp3, tlp6, tlp8, tlp9* are ubiquitous while *tlp2* and *tlp10* nearly ubiquitous.
*Tlp4a* was found to be present in only 33.56% of all tested isolates and significantly underrepresented in turkey isolates. Comparably, 29.5% of the isolates tested positive for *tlp4b*. *Tlp11a* was found to be present in 17.8% of the isolates while *tlp11b* was detected in 38.7%. Bovine isolates bear significantly higher levels of *tlp11a* as compared to *tlp11b*. Interestingly, *tlp4a, 4b, 11a* and *11b* share the same locus in the *C. jejuni* genome and are mutually exclusive to a certain degree but co-occur in a subset of isolates.
56.6% of all tested isolates were positive for intact *tlp5*, while the remaining 43.4% bore a disrupted *tlp5* gene.
Surprisingly, *tlp11a* demonstrates the same distribution as the splitted variant of *tlp7*. But t*lp7* splitted and unsplitted receptor variants taken together are ubiquitous.
**Conclusions:** Generally, majority of chemoreceptors genes are ubiquitous indicating the central importance of chemotaxis to *C. jejuni ssp. jejuni* while competing with the microbiota of its wide spectrum of hosts. The non-ubiquitous exceptions of *tlp4a/b* and *tlp11a/b*, *tlp5* and *tlp7* could be a result of the processes of adaptation to particular environments or hosts.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**247/ZOP**

**eIF2α tolerizes cells to *S. aureus* α-toxin by modulating its receptor**

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**Introduction:** Pore forming toxins (PFT) trigger multiple stress responses in target cells, including phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) (1-4). Previously, we have shown that this enables epithelial cells to endocytose *S. aureus* α-toxin pore complexes in an eIF2α-dependent manner (3). In contrast to human epithelial cells, murine fibroblasts (MEF) tolerate exposure to micro-molar concentrations of α-toxin.
**Aim:** The aim of the present study was to understand the basis of cellular tolerance to α-toxin observed with murine cells.
**Methods:** Using small molecular weight inhibitors of certain stress pathways and MEF variants that do not express GCN2, Ppp1r15b or phosphorylatable eIF2α (at serine 51), proteins implicated in defense of human epithelial cells againsta-toxin (3), we investigated the role of stress responses including regulated eIF2α-phosphorylation for MEFs tolerance to α-toxin.
**Results:** We found that balanced (de)phosphorylation of eIF2α is an essential requisite to protect MEF from *S. aureus* α-toxin. Amino acid deprivation-sensitive eIF2α kinase GCN2 proved to be responsible for basal eIF2α-phosphorylation in wild type MEF. Lack of GCN2 led to an increase in α-toxin-dependent eIF2α-phosphorylation, resulting in sustained attenuation of translation, and increased stress. In contrast, basal phosphorylation of eIF2α by GCN2 conferred tolerance to α-toxin in wild type MEF. Tolerance is selective and apparently due to modulation of ADAM10, a proposed α-toxin-receptor.
**Summary**: Nutrient stress in host tissue colonized or infected by bacteria may serve as an early trigger of protective measures against imminent cellular damage by toxins.

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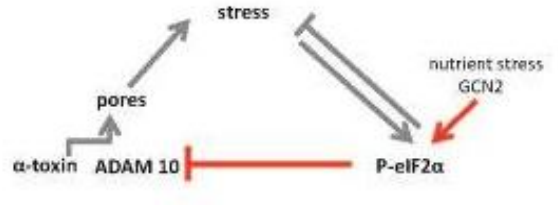
**Figure 1**

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**248/ZOP**

**Metabolic activity of IFN-γ induced persistent Chlamydia trachomatis infection and re-activation of persistent infection**

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**Introduction:** *Chlamydia trachomatis* is an obligate intracellular bacteria and intracellular growth strongly depends on host cell metabolic activities. In addition to acute infection, persistent *C. trachomatis* infection was supposed to be associated with the pathogenesis of chronic inflammation in the urogenital tract. The persistent state is reversible and persistent *C. trachomatis* can be re-activated by various triggers such as tryptophan treatment and low oxygen concentrations *in vitro*. Although the chlamydial infection varies host cell metabolism, little is known about host-pathogen metabolic characteristics in different state of chlamydial infections. We therefore elucidate metabolic activities of IFN-γ induced persistent *C. trachomatis* infection and re-activation of persistent infection by a novel metabolic analyzer.
**Materials and methods:** IFN-γ was used to form persistent *C. trachomatis* infection. Tryptophan was used for the re-activation ofpersistent infection. Cellular glycolytic and mitochondrial activities were measured by Seahorse XF24 analyzer in real time.
**Results and conclusions:** Productive *C. trachomatis* infection caused enhancement of glucose metabolism, glycolytic capacity and spare glycolytic capacity compared to non-infected control cells. On the other hand, these activities were dramatically reduced in IFN-γ induced persistent infection compared to productive infection. In the re-activation state, glycolytic activity was differentially regulated compared to the persistent state. Furthermore, productive *C. trachomatis* infection enhanced basal respiration, maximal respiration and proton leak compared to non-infected control cells in mitochondrial activity assay. In contract, IFN-γ induced persistent *C. trachomatis* infection and re-activation of persistent infection showed different metabolic characteristics in this assay. We conclude that productive, persistent and re-activation of *C. trachomatis* orchestrate cellular glycolytic and mitochondrial pathways in different manners for their intracellular survival.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**249/ZOP**

**Annexin A8 and its diverse function during influenza A virus infection**

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Influenza A virus (IAV) is a major public health concern, but so far a lot of host-pathogen interactions regulating IAV replication still remain poorly understood. Recent studies imply that proteins of the annexin family are involved in different stages of IAV infection {Musiol 2013}. Here, we examined the role of Annexin A8 for a successful IAV infection and its impact for a proper immune response. Previous reports demonstrate that Annexin A8 affects leukocyte recruitment to activated endothelial cells by supplying Weibel-Palade bodies with sufficient amounts of the P-selectin regulator CD63 {Poeter 2014}. In an attempt to determine the role of Annexin A8 during virus infection, an Annexin A8-depleted A549 lung carcinoma cell line was used and subsequently infected with various IAV strains. Interestingly, Annexin A8-depleted cells show lower virus titers in the supernatant compared to control cells, indicating a crucial role of Annexin A8 in IAV replication cycle. To gain further insights into the replication step that is affected by Annexin A8, we performed immunofluorescence assays. AnxA8-depleted cells possess lower amounts of viral nucleoproteins in the nucleus and reduced colocalization levels of the early endosomal marker Rab5 with viral envelope proteins during infection compared to control cells, suggesting a decrease of virus entry. To analyze the role of Annexin A8 *in vivo* we infected Annexin A8 knockout (AnxA8KO) and wild type (wt) mice with IAV. Strikingly, current results stress out that AnxA8KO mice are highly susceptible for virus infection in comparison to wt mice, which results in decreased survival rate after infection with IAV. We furthermore observed higher virus titers in lungs and tracheae in AnxA8KO mice. All these results among other findings indicate that AnxA8KO mice are unable to fight viral invaders by triggering a proper immune response.

Musiol, Agnes; Gran, Sandra; Ehrhardt, Christina; Ludwig, Stephan; Grewal, Thomas; Gerke, Volker; Rescher, Ursula (2013): Annexin A6-balanced late endosomal cholesterol controls influenza A replication and propagation. In: mBio 4 (6), S. e00608-13. DOI: 10.1128/mBio.00608-13.
Poeter, Michaela; Brandherm, Ines; Rossaint, Jan; Rosso, Gonzalo; Shahin, Victor; Skryabin, Boris V. et al. (2014): Annexin A8 controls leukocyte recruitment to activated endothelial cells via cell surface delivery of CD63. In: Nature communications 5, S. 3738. DOI: 10.1038/ncomms4738.

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**250/ZOP**

**The extracellular adherence protein (Eap) of *Staphylococcus aureus*: a proliferation and migration repressing factor as potential new cancer therapeutic agent.**

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The clinically important human pathogen *Staphyloccocus aureus* is equipped with a large arsenal of virulence factors allowing it to attach to various host cell structures, and to modulate the immune response of the host. One of these factors is the extracellular adherence protein Eap, a member of the "secretable expanded repertoire adhesive molecules" (SERAM) that possess adhesive and immune modulatory properties. We have previously shown that Eap impairs wound healing by interfering with host defense

and repair mechanisms, and that this secreted protein abates cell proliferation and migration of keratinocytes and endothelial cells.
Here we report that Eap also affects the proliferation and migration capacities of bladder cancer cells. By challenging cells of the invasive (T-24, J82) and non-invasive bladder (RT-112, 5637) cancer cell lines with Eap, a profound reduction in cell proliferation for three out of the four cell lines was observed. Additionally, fetal calf serum stimulated cells of the invasive bladder cancer cell line J82 exhibited markedly decreased migration capacities upon Eap challenge, when compared to unchallenged cells. Both findings suggest that Eap might serve as an interesting new therapeutic option to reduce growth and metastasis of bladder cancers.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**251/ZOP**

***Staphylococcus aureus* SCVs use intracellular persistence in human macrophages as a strategy evade the innate immune response**

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**Question:** *S. aureus* is able to invade and survive in non-professional phagocytes and can also survive engulfment by professional phagocytes. Here, bacteria can escape from the phagosomes and are released after the lysis of the host cells. *S. aureus* small colony variants (SCV) seem to be well adapted to the intracellular milieu and increasing evidence suggests that they can persist in non-professional phagocytes. We studied the potential of different *S. aureus* SCVs to persist within human macrophages and their ability to escape from these cells and the immune response and cytolytic effects that is triggered by different strains. Comparison of characteristics of clinical strains with site-directed mutants.
**Methods:** Human monocyte-derived macrophages were infected with different *S. aureus* SCV strains (clinical isolate 3878, menandione, hemin and thymidine dependent strains). Persistence and release from macrophages were monitored via protection assays and high resolution electron microscopy. Cytolytic effects of various strains were analyzed by FACS, apoptosis and necrosis were confirmed by independent assays. Inflammatory reaction was measured by IL-1 and TNF-α ELISAs.
**Results:** The isolate 3878 SCV is able to persist and survive in macrophages for more than 12 days and also displays low cytotoxic effects and a reduced pro-inflammatory effect when compared to wild type. Bacterial release started after 9-12 days. Defined site-directed mutants exhibit different patterns: Thymidine auxotrophs showed a decreased uptake by macrophages and were almost undetectable intracellular. Menadione auxotrophs were not able to persist for a longer duration of time. Hemin auxotrophs did not show a difference to its wildtype strain either. This could be attributed to high intracellular haemin content measured by hemin assays.
**Conclusion:** SCVs are able to persist intracellularly for several days without affecting the viability of macrophages which may have a potential role for dissemination of bacteria. Site-directed mutants cannot mimic the effects of clinical strains indicating that a complex phenotype is a pre-requisite for intracellular persistence.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**252/ZOP**

**Gp96 shedding induced by *Chlamydia trachomatis* prevents chlamydial re-infection**

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*Chlamydia trachomatis* is an obligate intracellular human pathogen with a biphasic developmental life cycle. The infectious elementary bodies (EBs) enter a host cell, transform into reticulate bodies (RBs) that use cellular metabolites to multiply. Re-infection of an infected cell during the replicative phase of chlamydial development may prevent formation of infectious EBs, interrupting the infectious cycle. Here, we report that Glucose Regulated Protein 96 (Gp96), a chaperone for cell surface receptors, binds to and facilitates adherence and entry of *C. trachomatis*. Gp96 expression was increased early in infection in a MAP kinase-dependent way, thereby increased chlamydial adherence and invasion. Gp96 co-precipitated with Protein Disulphide Isomerase (PDI), known to be involved in chlamydial host cell entry. During the replicative phase, Gp96 was depleted from infected cells and shed into the supernatant by activation of metalloproteinase TACE (ADAM17). Loss of Gp96 also reduced the activity of PDI on the cell surface. Reduced display of Gp96 on the surface of infected cells prevented chlamydial re-infection in a TACE-dependent manner, also demonstrated in primary cells derived from human fimbriae, the natural site of chlamydial infection. Our data suggest a role of infection-induced Gp96 shedding in the protection of the chlamydial replicative niche.

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**253/ZOP**

**Fighting Fire with Fire: A Patent for the Combined Application of Oncolytic Herpes Viruses and Antiangiogenic Agents in the Battle against Human Cancers**

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Specific elimination of tumor cells by replication-competent viral vectors is mediated through active viral replication, spread in tumor tissue and direct cytopathic effects. In addition, immune responses are induced against virally infected tumor cells while sparing normal cells. Recently, oncolytic vectors were constructed with mutations in neurovirulence genes or DNA synthesis genes. Many viral vectors including genetically modified viruses and angiogenic agents or their receptors have been chosen as potential drugs / treatment targets. The most frequently used attenuation of herpes simplex virus type 1 (HSV-1) was rendering the virus incapable of expressing an active gene product from both copies of the γ134.5 gene, also known as the neurovirulence factor. Some specific examples of γ134.5-deficient mutants are R3616, 1716 or G207. The virus construct G207 does not express both a functional γ134.5 gene product and an active ribonucleotide reductase (ICP6). This vector replicates in malignant cells via a lytic infection and resulting cell death, but is highly attenuated in non-dividing cells, thus, viral spread is limited to tumors only. G207 is non-neurotoxic. It has been shown not to cause disease in mice and non-human primates. Viral replication should only be restricted to malignant cells to prevent severe viral disease. These constructed vectors terminate cells by mechanisms different to standard anti-

cancer therapies; they offer another treatment modality which can be used in combination with chemotherapy, radiotherapy and gene therapies with additive or synergistic effects. Combination therapies are usually necessary to control tumorigenic diseases. Inhibiting angiogenesis represents another new field in current anti-cancer treatment development. Combining an oncolytic virus with antiangiogenesis is able to potentiate both treatment effects compared to each treatment modality alone in both primary and advanced disease. This combination might be beneficial for cancer patients in the future.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**254/ZOP**

**Host GTPase machinery implicated in the formation of *Legionella*-containing vacuoles**

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Undermining host vesicle trafficking machinery is pivotal for survival and pathogenesis of many intracellular pathogens. One model organism for studying host cell process modulation during infection is the Gram-negative accidental human pathogen *Legionella pneumophila*, which can cause a severe pneumonia termed Legionnaires` disease. *L. pneumophila* injects approximately 300 "effector" proteins into host cells through its Icm/Dot type IV secretion system (T4SS), in order to guarantee intracellular growth in a distinct pathogen compartment termed the "*Legionella*-containing vacuole" (LCV).
Our recent proteomics studies of purified LCVs from infected *Dictyostelium discoideum* amoebae or murine RAW 264.7 macrophages identified 13 small GTPases of the Rab family, implicated in the secretory or endosomal vesicle trafficking pathways [1]. Using fluorescence microscopy, 6 novel Rab proteins were confirmed to localize on LCVs harboring wild-type but not Δ*icmT* mutant *L. pneumophila*. Individual depletion of 20 GTPases by RNA interference indicated that endocytic GTPases (Rab5a, Rab14 and Rab21) restrict intracellular growth of *L. pneumophila*, whereas secretory GTPases (Rab8a, Rab10 and Rab32) implicated in Golgi-endosome trafficking promote bacterial replication. The down-stream effectors and functional roles of these GTPases during *L. pneumophila* infection and LCV formation are only incompletely understood.
The LCV proteomics analysis also suggested that large GTPases implicated in vesicle fusion and fission are candidate LCV components. Current experiments aim at the validation of the proteome data and an investigation of the functional roles of these host factors for *L. pneumophila* phagocyte infection and LCV formation. Thus, the phagocyte proteomes of purified LCVs are a valuable resource for further hypothesis-driven investigations of the complex process of pathogen vacuole formation.
[1] Hoffmann, C., *et al.* & Hilbi, H. (2014) Functional analysis of novel Rab GTPases identified in the proteome of purified *Legionella*-containing vacuoles from macrophages. *Cell Microbiol* 16: 1034-1052.

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**255/ZOP**

**A Recombinant *Yersinia* Outer Protein as a Potential Novel Cell-Penetrating, Anti-Inflammatory Therapeutic**

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*Yersinia* outer proteins (Yops) are plasmid-encoded, type 3 secretion system (T3SS)-dependent effector proteins of pathogenic *Yersinia* spp.. They mainly interfere with phagocytosis as well as central inflammatory signaling cascades. In the present study, one of these Yop effector proteins from *Yersinia enterocolitica* (pYV 8081) - purified as a recombinant protein from *Escherichia coli* - was investigated regarding its potential as a cell-penetrating, anti-inflammatory therapeutic.
A possible protein transduction domain (PTD) of ten amino acids was predicted within the N-terminus of this particular Yop. The recombinant Yop - as well as different fusion constructs thereof used later on - were found to be enzymatically active *in vitro* and in cell lysates. Using LDH-release and PI-uptake assays we showed that none of the constructs induced significant cytotoxicity neither in HeLa nor in monocytic THP-1 cells. An efficient and rapid uptake of the recombinant Yop alone without the need of an additional protein transduction domain was demonstrated using cell fractionation techniques and fluorescently labeled proteins in confocal microscopy and FACS-based quenched time lapse assays. We further investigated the mechanism underlying the observed uptake by applying inhibitors of distinct endocytosis pathways, which suggested macropinocytosis followed by endosomal trafficking as the major entry pathway. Based on these results, we tested different strategies to enhance cytoplasmic delivery of the recombinant Yop, e.g. by fusion to a PTD from another *Yersinia* effector - YopM -, which is already known to deliver cargo to the cytoplasm of eukaryotic cells. Finally, we assessed the desired immunomodulatory activity of the recombinant proteins within target cells by immunoblotting, quantitative real-time PCR and ELISA.
Taken together, our results indicate that next to rYopM, another recombinant Yop from *Y. enterocolitica* might as well be applicable as a self-delivering, anti-inflammatory therapeutic in the future. These kind of protein drugs ('biologics') provide a powerful tool to face the increasing need for more efficient therapeutics in auto-inflammatory diseases
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**256/ZOP**

**Autopenetrating bacterial effector proteins as biological therapeutics**

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In the last 20 years biologics including anti-TNFα antibodies have been discovered and are constantly further developed as very efficient treatment options for autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease or psoriasis. However, a bottleneck of any therapeutic drug including biologics is their bio-availability, which also involves their capacity to reach also intracellular targets. Cell-penetrating peptides (CPPs) can overcome this problem by passing cellular barriers and even delivering cargos into cells. Therefore, they are investigated as potential drug-delivering agents. Rueter *et al.* could demonstrate that recombinant Yersinia Outer Protein M (YopM) from *Yersinia enterocolitica* has the capacity to overcome cellular barriers autonomously and thus represents a bacterial CPP resp. cell-penetrating effector (CPE). Previously, virulence factors and effector proteins have been mostly targeted to counteract infection, however, as these factors have been largely optimized during co-evolution of bacteria with their respective hosts, they might be applicable also as tools to modulate and/or reduce detrimental immune responses. Hence, these factors might potentially be employed for therapeutic purposes. In this study the therapeutic capacity of bacterial effector proteins that are known to down-regulate pro-inflammatory cytokines or chemokines such as TNFα or IL-8 is investigated. Here, we demonstrate the functionality of a recombinant protease NleC, which has cell-penetrating abilities as demonstrated by immunofluorescence. Furthermore, we show that this CPE is functional as it specifically cleaves the p65 subunit of NF-κB in cell lysates as well as in stimulated whole cells and cleaves p65 in a dose- and time-dependent manner. We demonstrate that rNleC alone has a better efficacy in cleaving p65 than a fusion protein of NleC and a known CPP (Tat) emphasizing its endogenous cell-penetrating activity.

The cleaving event of p65 by rNleC is highly specific for stimulated cells restricting its activity only to those cells that are affected by auto-inflammatory processes. In summary, we found a self-delivering therapeutic agent with promising potential for treatment of auto-inflammatory diseases.

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**257/ZOP**

**Modulation of phagocyte migration by Legionella pneumophila effector proteins and the Lqs quorum sensing system**

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The opportunistic pathogen Legionella pneumophila causes a severe pneumonia termed Legionnaires´ disease. L. pneumophila uses a conserved mechanism to replicate within a specific "Legionella-containing vacuole" (LCV) in macrophages and amoebae. The bacterial Icm/Dot type IV secretion system (T4SS) governs the process of LCV formation and translocates over 300 different effector proteins into host cells. Moreover, L. pneumophila uses the Lqs quorum sensing system for cell-cell communication. This system produces the signaling molecule LAI-1 (3-hydroxypentadecane-4-one) through the autoinducer synthase LqsA, and phosphorylation signaling through the LqsS and LqsT sensor kinases converges on the response regulator LqsR (1).
Using under-agarose and scratch migration assays, we found that L. pneumophila inhibits in a T4SS- and dose-dependent manner the directed cell migration of infected Dictyostelium discoideum, macrophages or neutrophils. L. pneumophila lacking the T4SS substrate LegG1, an activator of the small eukaryotic GTPase Ran, hyper-inhibited the migration of amoebae or immune cells (2). Under these conditions, microtubule polymerization of the infected cells was significantly reduced. Using scratch assays and RNA interference, we observed that LegG1 promotes random cell migration in a Ran-dependent manner. Single cell tracking analysis of L. pneumophila-infected phagocytes indicated that velocity and directionality were decreased and microtubule polymerization was impaired.
Recent studies revealed that the migration of D. discoideum amoebae infected with L. pneumophila mutant strains lacking lqsA or lqsR is not impaired, similar to amoebae infected with a ΔicmT strain. Taken together, our findings indicate that L. pneumophila modulates phagocyte migration in an Icm/Dot- and Lqs-dependent manner.

**References**

1. Schell U, et al. & Hilbi, H. 2014. Phosphorylation signalling through the Legionella quorum sensing histidine kinases LqsS and LqsT converges on the response regulator LqsR. Mol Microbiol 92:1039-1055.
2. Simon S, et al. & Hilbi, H. 2014. Icm/Dot-dependent inhibition of phagocyte migration by Legionella is antagonized by a translocated Ran GTPase activator. Cell Microbiol 16: 977-992.

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**258/ZOP**

**Escape of *Staphylococcus aureus* from within Phagocytes - a multifactorial process**

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Although *Staphylococcus aureus* is not a classical intracellular pathogen, it can survive within phagocytes and many other cell types. However, the pathogen is also able to escape from cells by mechanisms that are only partially understood. We analyzed a series of isogenic *S. aureus* mutants for their capacity to destroy THP1 macrophages or HeLa cells from within each cell type. Mutation of both major virulence regulatory systems Agr and Sae resulted in a complete inability to escape from macrophages. Single Mutation of the *agrA* target gene *psmα1-4* and the sae target genes lukAB and pvl had an effect on the escape from macrophages which could be strengthened by simultaneous deletion of all three genes. However a complete inability to escape could only be induced by mutation of Agr and Sae which leads to the concept of a multifactorial process concerning the escape of *S. aureus* from within professional phagocytes. For the escape from HeLa cells *psmα1-4* was found to be the only factor, presumably due to the missing receptors for LukAB and PVL.

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**259/ZOP**

**Hyper-activation of ERK1/2 signalling results in IL-6 production during influenza A virus and *Staphylococcus aureus* co-infection**

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Bacterial co-infections are a major complication of influenza A virus (IAV) infections leading to severe illness and fatal outcomes. Recent findings suggest that beside the pathogen load, a dysregulated immune response as well as an enhanced inflammatory response of the host also contributes to increased morbidity and mortality. Mitogen-activated protein kinases (MAPKs) play an important role in both of these host responses and activate the transcription of pro-inflammatory cytokines and chemokines. Although several *in vivo* studies demonstrate elevated levels of cytokines and chemokines upon IAV and bacterial co-infections resulting in severe tissue damage, the underlying molecular signalling mechanisms still remain to be elucidated. However, this knowledge is crucial for development of new therapeutic approaches.
In the present study we focused on cellular signalling mechanisms in human lung epithelial cell lines (Calu-3, A549) resulting in a dysregulated inflammatory response upon co-infection with IAV and *Staphylococcus aureus* (*S. aureus*). Therefore we established an *in vitro* co-infection protocol including a serial pathogen incubation combined with an antibiotic wash.
Upon co-infection with IAV and *S. aureus* we observed an overexpression of cytokines and chemokines as described in *in vivo* models. Similar results were obtained, when *S. aureus* infection was restored by lipoteichoic acid (LTA) stimulation, but not with other bacterial components. Analyses of cellular signalling mechanisms regulating these inflammatory response genes revealed significantly increased activation of the MAPKs p38 and ERK1/2 in presence of both pathogens compared to IAV-infected cells. Blocking of p38 or ERK1/2 activity by the use of specific inhibitors showed that levels of the pro-inflammatory cytokine IL-6 are ERK1/2 dependent during co-infection.
Our data indicate a correlation of hyper-activation of MAPKs and overexpression of pro-inflammatory cytokines. We will provide deeper insights in the regulation of pathogenicity during IAV and *S. aureus* co-infections on a molecular level, which contributes to the lethal synergism of these pathogens.
Note: Parts of these data were presented at different meetings before.

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**260/ZOP**

**Regulation of cell death mechanisms after influenza A virus and *Staphylococcus aureus* super-infection**

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Bacterial super-infections are a major complication in influenza diseases resulting in significantly increased morbidity and mortality. Most of the fatal cases in the course of an influenza A virus (IAV) infection are a result of secondary pneumonia caused by different bacteria, among which *Staphylococcus aureus (S. aureus)* is one of the contributing pathogens**.** One potent, highly regulated cell defense mechanism in response to invading microorganisms is the programmed cell death (apoptosis) that eliminates individual cells without inducing an inflammatory response. In contrast necrosis is less controlled and results in the release of various cellular products causing inflammation. Thus, cell death mechanisms play an important role for the outcome of this disease. Although cell death mechanisms are very well analyzed during infections by either IAV or *S. aureus* alone, until today it is poorly understood how these processes are controlled in the presence of both pathogens. Both, IAV and *S. aureus* have evolved strategies to manipulate the host cell death machinery to increase their replication and survival.
Within the present study we focused on the regulation of apoptosis as well as the programmed necrosis (necroptosis) upon IAV and/or *S. aureus* infection.
In an *in vitro* coinfection model human lung epithelial cells (A549) were infected with different IAV and *S. aureus* strains. Induction of cell death was monitored by detection of various cellular factors on protein and mRNA level.
We were able to show that IAVs induce the expression of pro-apoptotic factors such as TRAIL or the cleavage of caspases and PARP. Although in the presence of *S. aureus* the activation of apoptosis-markers was reduced, cell-morphology was changed and cell-viability seemed to be decreased. Concomitantly, a marker of necroptosis, the mixed lineage kinase domain-like protein (MLKL), was strongly activated in presence of bacteria.
Our results indicate that *S. aureus* is able to inhibit the IAV-induced apoptotic cellular response. We hypothesize that the *S. aureus*-mediated switch between apoptosis to necroptosis supports intracellular bacterial survival and spread. Thus, we introduce a novel mechanism that might contribute to increased pathogenicity upon IAV and *S. aureus* coinfection.
Note: These data were presented in part at different meetings before**.**

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**261/ZOP**

**Impact of the Raf-MEK-ERK signaling cascade during influenza virus and *Staphylococcus aureus* coinfection *in vitro* and *in vivo***

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Influenza A virus (IAV) infections represent one of the major causes of severe respiratory diseases. Secondary bacterial pneumonia following IAV infection can increase pathogen load, resulting in higher morbidity, mortality and a general increase of disease severity. This is often due to a hyper-induction of cytokines and chemokines. Vaccination against the virus and use of different antivirals and antibiotics counteract single infections by both pathogens, but there is a high risk of failure and/or the emergence of resistant variants. Furthermore, these treatments are less efficient in coinfections. Thus, new pathogen-inhibiting strategies are required and targeting cellular factors might minimize the risk of resistance induction.
The aim of this study is the analysis of virus-supportive cellular Raf-MEK-ERK pathway as a potential target for anti-infective therapies. Inhibition of this pathway has been shown to result in retention of viral ribonucleoprotein complexes (vRNP) in the nucleus leading to reduced release of newly synthesized virus particles.
To investigate the influence of the Raf-MEK-ERK signaling pathway on pathogen load and cell-mediated responses during coinfection, human lung epithelial cells (A549) were infected with high and low pathogenic IAV strains and *Staphylococcus aureus* (*S. aureus*) strain 6850 in the presence or absence of specific MEK-inhibitors. Inhibition of pathogen-induced ERK activation lead to reduced viral titers. This was independent of the strain, demonstrating a general anti-viral potential in the case of IAV. Moreover, bacterial growth was reduced in the presence of the MEK-inhibitor U0126. In addition to that, inhibition of pathogen-induced ERK activation resulted in reduced cytokine and chemokine levels. Furthermore, Balb/c mice were infected with both pathogens in the presence or absence of U0126. Blockade of ERK activation caused significantly reduced lung bacterial titers.
Taken together, these data indicate that the activation of ERK plays an important role in pathogenisis during coinfection *in vitro* and *in vivo*. Therefore targeting cellular factors as a new therapeutic approach against coinfections seems to be promising and will be further investigated.
Note: Parts of these data were presented elsewhere (GfV Meeting, Bochum).

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**262/ZOP**

**Differential IL-8 release of brain microvascular and peripheral human endothelial cells after meningococcal infection**

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**Background**: *Neisseria meningitidis* (*Nm*, meningococcus), a gram-negative Diplococcus, is a major cause of epidemic meningitis and septicemia worldwide. To establish systemic infection, *N. meningitidis* attaches to and invades into host endothelial cells, thus triggering an inflammatory response with subsequent release of cytokines. Previously published data showed an LPS-independent increase of the murine IL-8 analogue KC in lungs of mice challenged with *Nm* (Zarantonelli *et al*., 2006).
**Methods**: We infected human brain microvascular endothelial cells (HBMEC) and peripheral endothelial cells (EA.hy926) with colonizing and invasive *Nm* isolates and determined adherence to and invasion into both cell lines using gentamicin protection assay. Supernatants of infected cells were collected and used to determine IL-8 release applying BD Opt-EIA ELISA Kit.
**Results**: We observed a differential release of IL-8 from brain endothelial cells compared to peripheral endothelial cells. Besides, we were able to show that infection with invasive or colonizing *Nm* strains causes a differential Interleukin-8 release.
**Conclusions**: Our data indicate that brain endothelial cells respond with an excessive IL-8 release compared to peripheral endothelial cells after meningococcal infection. These findings may provide a better comprehension of meningitis pathology.
Zarantonelli, Maria Leticia; Huerre, Michel; Taha, Muhamed-Kheir; Alonso, Jean-Michel (2006): Differential role of lipooligosaccharide of *Neisseria meningitidis* in virulence and inflammatory response during respiratory infection in mice. Infection and Immunity 74(10), S. 5506-5512. DOI: 10.1128/IAI.00655-06

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**263/ZOP**

**_Neisseria meningitidis_ infection result in a S-phase arrest of the cell cycle of immortalised and primary human brain endothelial cells.**

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**Introduction:** Studies have shown that pathogens can alter the host cell cycle. Published transcriptomic data from our group showed that *N. meningitidis* can alter host cell cycle gene expression.
**Objectives:** To investigate the effect of Nm MC58 (Sg B) infection on the cell cycle of human brain microvascular endothelial cells (HBMECs), to identify bacterial molecules acting as cyclomodulins and to elucidate the mechanism(s) involved in cell cycle alteration.
**Materials & Methods:** The immortalised cell line HBMEC/ciβ and primary HBMECs were used to investigate cell cycle alterations by propidium iodide and 5-ethynyl-2'-deoxyuridine (EdU) labeling. DNA content measurement was used to investigate the effects of mutants defective of adhesins and invasins, live, heat-killed and Nm supernatant and *E. coli* recombinantly expressing the Nm opacity proteins on the host cell cycle. Transcriptomic screening of host cell cycle genes was conducted and immunoblotting and immunofluorescence was used to examine the effect of Nm infection on host cell cycle proteins.
**Results:** Nm MC58 arrested HBMEC/ciβ (2h & 24h p.i.) and pHBMEC (3h p.i.) at S-phase, which was reversed in the presence of the $H_2O_2$ scavenger sodium pyruvate. Increased levels of γH2A.X was identified in infected cells. Live bacteria and bacterial supernatants also induced a S-phase arrest and not heat-killed organisms. Infection with *E. coli* recombinantly expressing the opacity proteins resulted in a S-phase arrest, indicating that the Opc and Opa proteins act as cyclomodulins. Transcriptomic analyses revealed changes in the quantities of many mRNAs encoding cell-cycle molecules, including the inhibitors $p21^{Waf1/Cip1}$ and cyclin G2. Immunofluorescence revealed higher fluorescent signals of nuclear p21 and cyclin G2 in infected cells, while genetic ablation and pharmacological inhibition of p21 and cyclin G2 abrogated the S-phase arrests. Nm infection, infection with *E. coli* recombinantly expressing the opacity protein and treatment with bacterial supernatant all resulted in significantly increased levels of 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative stress.
**Conclusion:** Our results indicate that *N. meningitidis* infection induces a reversible p21-dependent cell cycle arrest in immortalised and a cyclin G2-dependent arrest in primary HBMECs and requires the expression of bacterial Opc and Opa proteins.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**264/ZOP**

**Identification of a new meningoccocal factor activating the ASM/ceramide system**

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**Introduction**: *Neisseria meningitidis* (*Nm*), an obligate human pathogen, is a causative agent of septicemia and meningitis worldwide. The interaction with brain endothelial cells is central to the pathogenicity of meningococcal meningitis. Recent studies demonstrated that distinct ceramide enriched membrane microdomains are important in this process. Ceramide can be generated via the salvage pathway through the action of sphingomyelinases, or the *de novo* synthetic pathway through the action of ceramide synthases.
**Aim/Hypothesis**: The aim of the study was to understand the role of the acid sphingomyelinase (ASM) and sphingolipid-enriched membrane microdomains during the process of meningococcal adhesion to and invasion into brain endothelial cells.
**Material and methods**: We employed human brain microvascular endothelial cells as an *in vitro* model to analyse whether *Nm* stimulates surface ceramide display on brain endothelial cells. The role of ASM and ceramide-enriched microdomains was analyzed using clickable ceramide-analogs, flow cytometry and confocal immunofluorescence microscopy. In order to identify meningococcal factors responsible for activating the ASM/ceramide system isogenic meningococcal mutants were constructed.
**Results**: *Nm* causes transient activation of ASM followed by ceramide release in brain endothelial cells. In response to *Nm* infection, ASM and ceramide are displayed at the outer leaflet of the cell membrane. Interestingly, we observed that a defined set of pathogenic isolates of the ST-11/ST-8 clonal complex were restricted in their ability to induce ASM and ceramide release, which was parallelled by less invasiveness[(1)]. We now extended our study to isolates belonging to serogroup C ST-11 cc outbreak strains from France and comparatively analysed adhesion and invasion properties of these isolates and their capacity to induce ceramides on endothelial cells. We further addressed the potential contribution of meningococcal factors, including PorB, NarE and VapD-like proteins, to activation of the ASM/ceramide system. Using clickable ceramide-analogs and a *mCherry*-expressing strain we initiated studies on the membrane organization of cortical plaques.
**Conclusion**: Our results unravel a differential activation of the ASM/ceramide system by the species *Nm* determining its invasiveness into brain endothelial cells.

**Reference**

1. Simonis A, Hebling S, Gulbins E, Schneider-Schaulies S, Schubert-Unkmeir A. Differential activation of acid sphingomyelinase and ceramide release determines invasiveness of Neisseria meningitidis into brain endothelial cells. PLoS Pathog. 2014 Jun;10(6):e1004160. PubMed PMID: 24945304. Pubmed Central PMCID: PMC4055770. Epub 2014/06/20. eng.

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**265/ZOP**

**Transcriptome analysis of _Streptococcus gallolyticus_ subsp. _gallolyticus_ with regard to collagen binding ability as virulence factor**

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**Introduction:** *Streptococcus gallolyticus* subsp. *gallolyticus* (SGG) is recognized as an emerging pathogen in about 20% of streptococcal-caused infective endocarditis cases. It was postulated that collagen-binding ability is the key virulence feature of SGG in humans. For a better understanding of this host-SGG interaction, changes in the transcriptome of SGG in reaction to the binding to collagen matrix was analyzed.
**Methods:** Binding of SGG to human immobilized collagen type I was verified by crystal violet staining after two hours of incubation. For transcriptome analysis, RNA was extracted from two SGG strains in BHI me<dium bound or not bound (control) to human collagen. Two biological replicates which showed RNA integrity numbers above eight were used for further experiments. The RNA was processed, one-color labeled and hybridized to microarrays (8x15K design) with 5-6 specific oligonucleotides per gene. Analysis was done by Direct Array (Oaklabs, Hennigsdorf, Germany) and $\log_2$ values were only appropriate when higher than +1 or smaller than -1 with a *p*-value smaller than 0.05.
**Results:** The binding-ability of SGG to collagen is strain-dependent. Therefore two different strains were chosen for

transcriptome analysis. Strain A shows a weak binding-ability whereas strain B binds strong to collagen type I. When strain A is bound to collagen two regions in the genome were upregulated. One region contains genes which are related to the streptococcal phage P9, most coding for head morphology and tail structure proteins. The other region is a TnGBS-related integrative and conjugative element, which includes genes of the virulence-associated type IV secretion system. Strain B regulates 48 targets down and 30 up, when bound to collagen. Downregulated are especially genes which products are related to carbohydrate metabolisms like glycolysis. Upregulated are genes of diverse transport-proteins of different compounds, fatty acid synthesis as well as genes of e.g. a peptidase and a lipase.

**Conclusion:** The expression of phage and transposon proteins in strain A indicates that the cells start to build a biofilm and provide conjugation. Strain B regulates it metabolism down, when bound to collagen, and instead of that expresses more transporting molecules to take up e.g. nitrogen. Additionally there could be a hint of virulence because it expresses peptidase, sortase A and lipase.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**266/ZOP**

**The Role of miRNAs in the Cord factor mediated inhibition of IFN gamma induced gene expression in murine macrophages**

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The mycobacterial cord factor, trehalose-dimycolate (TDM), is the most abundant glycolipid in the cell wall of virulent mycobacteria. It is recognized by the C-type lectin receptor Mincle, which activates an intracellular signaling cascade involving FcRγ, Syk and Card9. This leads to the induction of inflammatory cytokines and promotes the immune response of the host organism, e.g. by $T_H1$ and $T_H17$ activation. However, besides its function as a PAMP, TDM per se is sufficient to block phagosomal maturation and suppresses inflammatory responses in macrophages.

Extensive transcriptome microarray analysis revealed both antagonistic and synergistic effects of TDM on IFN-γ induced gene expression in macrophages, suggesting that TDM mediates at least partially inhibitory effects of mycobacteria on IFN-γ signaling. To provide a better insight into how TDM reprograms host macrophages, we will investigate in this project whether microRNAs (miRNAs) are involved in the cord factor mediated negative regulation of IFN-γ-induced responses.To identify changes of host miRNA levels by the cord factor, miRNA profiling of macrophages after TDM stimulation will be performed using Agilent microarray technologies. Bioinformatic analysis will predict target genes of the identified miRNAs and the role of potential candidate genes in TDM-mediated interference of IFN-γ-induced inflammatory responses in macrophages will be analyzed in more detail.

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**267/ZOP**

**Comparison of the activity of the effector AnkG from different *Coxiella burnetii* strains**

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The obligate intracellular bacterium *Coxiella burnetii* is the causative agent of the zoonotic disease Q-fever. This disease is often a mild flu-like illness, but can develop into an atypical pneumonia or hepatitis. Furthermore, the infection can lead to chronic infection which is typically characterized by bacterial endocarditis and is potentially fatal. *C. burnetii* pathogenesis depends on a functional type IV secretion system (T4SS), used to translocate bacterial proteins into the host cell in order to manipulate host cell pathways. To date over 130 effector proteins have been identified, however their functions mainly remains elusive. We have demonstrated that the T4SS effector AnkG inhibits pathogen-induced host cell apoptosis. It is believed that this activity is essential for the establishment of a persistent infection. However, the mode of action of AnkG is still not fully understood.

Here, we compared the sequences of *ankG* encoded by 41 different *C. burnetii* strains and classified the strains according to the *ankG* sequence into three different groups. The first group contains the reference strain Nine Mile and twenty two additional strains expressing a 338 amino acid protein. The second group includes fourteen *C. burnetii* strains. The *ankG* sequence of this group contains in comparison to the first group two base pair deletion directly after amino acid 82. This causes a frameshift and a premature stop at amino acid 92. Thus, AnkG from the second group is identical in the first 83 amino acids but harbors 9 different amino acids at the C-terminus compared to the first group. The four isolates of the third group carry an amino acid exchange at position 11 (Isoleucine to Leucine) and one base pair insertion directly after amino acid 28. This base pair insertion causes to a frameshift and this leads to premature stop at amino acid 51. Thus, AnkG from this group is identical in the first 28 amino acids, but harbour 23 different amino acids at the C-terminus compared to AnkG from Nine Mile. As we have demonstrated previously that the first 69 amino acids of AnkG Nine Mile are necessary and sufficient for anti-apoptotic activity, we analysed whether AnkG from second and third group would influence host cell survival. Our results demonstrates, that in contrast to the anti-apoptotic activity of AnkG from the first group, the truncated AnkG of the second group did not interfere with cell death induction, while the truncated AnkG from the third group displayed pro-apoptotic activity. However, whether these truncated AnkGs are still substrates of the T4SS is questionable, as the translocation signal is located at the C-terminus of the protein, and is currently under investigation.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**268/ZOP**

**The role of the effector AnkF for the trafficking of the *Coxiella burnetii*-containing parasitophorous vacuole**

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*Coxiella burnetii*, the causative agent of the human zoonotic disease Q fever, is a Gram-negative obligate intracellular pathogen. Upon inhalation and uptake by human alveolar macrophages the pathogen is able to reside and replicate in an acidic phagolysosomal compartment. Bacterial protein synthesis is necessary and thus may directly influence the biogenesis of the *C. burnetii*-occupied parasitophorous vacuole (PV). In agreement with this assumption, the type IV secretion system (T4SS) was shown to be essential for establishing a replicative PV. The T4SS is a multi-protein complex known to translocate bacterial effector proteins into the host cell to manipulate host cell pathways. Here we are focusing on the effector protein AnkF and its role in the establishment of the replicative PV.

*L. pneumophila* expressing and translocating the *C. burnetii* T4SS effector AnkF into the host cell is as infective as the respective control bacterial strain, but is defective in establishing a replicative, calnexin-positive *L. pneumophila*-containing vacuole. To address how AnkF might interfere with intracellular trafficking, we performed a yeast two-hybrid screen using a HeLa cell library to identify potential host cell interacting proteins. The type III intermediate filament (IF) protein Vimentin was identified as a potential binding partner. Several reports indicate that the machinery required for vesicle transport is modulated by IFs. We aimed to confirm the interaction of AnkF with Vimentin by

fluorescence microscopy. Thus, GFP or GFP-AnkF were co-expressed with HA-tagged Vimentin in CHO cells and analyzed by confocal microscopy. We observed co-localization of GFP-AnkF and HA-Vimentin. Interestingly, the expression of GFP-AnkF led to perturbation (agglutination) and altered localization of Vimentin, suggesting that the expression of GFP-AnkF might interfere with the function of Vimentin and thereby with vesicular transport. We further investigated the localization of this IF protein in *C. burnetii* infected cells. At 24 h post-infection ~50% of the PVs were decorated with Vimentin. At 72 h post-infection this rate was increased to ~90%. Next, we analyzed whether the expression of AnkF or the infection with *C. burnetii* leads to alterations in Vimentin mRNA or protein level. Our data demonstrate that neither AnkF expression nor *C. burnetii* infection lead to changes in Vimentin mRNA or protein level.
Taken together, our data indicate that the *C. burnetii* effector AnkF alters vesicular trafficking, possibly by binding to Vimentin. AnkF does not alter Vimentin on transcriptional or translational level, but changes its intracellular localization. This might lead to the observed decoration of the PV with Vimentin. We are currently investigating where AnkF is localized during *C. burnetii* infection and whether and how Vimentin influences bacterial replication as well as PV-biogenesis.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**269/ZOP**
**Functional characterization of the IgM degrading enzyme $IdeS_{suis}$ of *Streptococcus suis***

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*Streptococcus suis* is an important porcine pathogen responsible for high economic losses in swine production due to meningitis, arthritis, serositis and other pathologies. Moreover, it is also an emerging zoonotic agent.
Recently, we identified a highly specific IgM protease designated $IdeS_{suis}$ in *S.suis*. Since IgM is an important activator of the classical complement cascade, the working hypothesis is investigated that cleavage of IgM by $Ide_{Ssuis}$ is a complement evasion mechanism playing a crucial role in host pathogen interactions during early adaptive immune responses.
The aim of the current study is to functionally characterize the IgM protease $IdeS_{suis}$ and its interaction with the immune system. First of all, a recombinant protein with a point mutation in the cysteine of the putative catalytic centre of $IdeS_{suis}$ was generated and tested with regard to its IgM cleaving activity. The inability of rIdeSsuis containing the point mutation to cleave porcine IgM classifies it as a cysteine protease like other members of the IdeS-family. As a next step, abrogation of complement activation by $rIdeS_{suis}$ and the point mutated variants was tested in a haemolysis assay. The point mutated $rIdeS_{suis}$ did not prevent haemolysis induced by porcine IgM directed against erythrocytes in contrast to wt $rIdeS_{suis}$. This result indicates that IgM cleavage activity of $rIdeS_{suis}$ is crucial for complement evasion. For further functional analysis, a *S. suis* mutant expressing only point mutated $IdeS_{suis}$ is investigated. This mutant is compared to the wt and the complete mutant in blood survival assays. To find out exactly which functions of the complement system are inhibited by $IdeS_{suis}$ activity different read out parameters for complement activation such as C5b-C9 and wCD11R3 are measured. Furthermore, complement inhibitors are used in these assays to inhibit specific functions of the complement system.
In summary, this project is designed to elucidate the role of IgM cleavage by *S.suis* in complement evasion and host-pathogen interaction in general.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**270/ZOP**
**Deregulation of cell survival and death signals in macrophages by *Yersinia***

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Death of host cell is a common outcome in the interaction of pathogenic microbes with the host immune response that may have substantial consequences for the development of inflammation and disease. Enteropathogenic *Yersinia enterocolitica* triggers apoptosis in macrophages by injecting the YopP effector protein through type III secretion into host cells. YopP exerts an acetyltransferase activity that deactivates several TLR-induced signaling pathways which impedes the development of a coordinated immune response and finally affects cell survival, leading to macrophage apoptosis. The mechanisms involved in *Yersinia*-induced apoptosis are, however, less clear. Our studies focus on the exploration of the signaling pathways that regulate death and survival of *Yersinia*-infected macrophages. Our data show that the RIP-1 kinase is centrally implicated in *Yersinia*-induced apoptosis downstream from TLR signaling. RIP-1 is central regulator of the cellular immune response that may signal cell survival and inflammation, as well as different modes of cell death. We provide a model how immunomodulation by Yersinia may provoke apoptosis in infected macrophages.

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**271/ZOP**
**Host interaction and adaptation of *Escherichia coli* in the urinary tract**

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**Introduction**: The main cause of urinary tract infection (UTI) are uropathogenic *E. coli* (UPEC). Besides causing symptomatic urinary tract infection, *E. coli* can also asymptomatically colonize the human bladder. During asymptomatic bacteriuria (ABU), bacterial carriage in the bladder is high without provoking overt host immune responses or symptoms.
Uropathogenic *E. coli* and commensal strains can be discriminated based on the presence and absence of additional DNA elements contributing to specific virulence traits. Unlike UPEC, which cause symptomatic infections, the ABU isolate 83972 lacks functional virulence-associated genes. For several ABU strains the weak host response can be explained by poor cell contact, which is essential to trigger the innate host defense. This may constitute a key mechanism of their persistence. The analysis of the molecular mechanisms of bacterial adaptation upon *in vivo* growth is essential for the understanding of ABU. Our aim is to elucidate determinants responsible for turning a pathogen into a commensal.
**Material and methods**: To study bacterial persistence and *in vivo* adaptation of *E. coli* in the human urinary tract, we pheno- and genotypically investigated strain ABU 83972 with its clinical re-isolate SN25. The *E. coli* SN25 genome was sequenced using Illumina sequencing technology. Raw sequence reads of the draft genome were quality trimmed and mapped to the annotated reference genome of *E. coli* 83972. Strain SN25 was investigated regarding single nucleotide polymorphisms (SNPs). To determine a SNP in either a coding or noncoding region, a coverage of 78 sequences and a frequency of 85% divergent nucleotides at a given position was applied. Additionally, the transcriptome of the clinical

re-isolate SN25 and the parental strain 83972 upon *in vitro* growth was compared by RNA sequencing.
**Results**: With the set of chosen parameter, 48 SNPs were detected. Four of these variants are located within a noncoding, the remaining 44 within a coding region, respectively. Of these, 32 variants represent synonymous SNPs, whereas 12 non-synonymous SNPs result in amino acid changes of the corresponding protein. Individual genes containing non-synonymous SNPs were either deleted in wildtype strain 83972 or reconstituted in re-isolate SN25 and their impact on bacterial phenotypes was analyzed. The transcriptome comparison revealed 153 differentially regulated genes.
**Discussion**: The RNA-seq data provide insights into differential gene expression in the *E. coli* strain 83972 relative to its re-isolate SN25. In combination with the draft genome sequence, candidate genes were selected for further in-depth analysis regarding their allocation to regulatory networks and their contribution to altered phenotypes of re-isolate SN25 as a result of *in vivo* adaptation and interaction with the human host.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**272/ZOP**
**Comparative analysis of cytokine and chemokine production of epithelial and primary immune cells after stimulation with asymptomatic and symptomatic urinary tract isolates**
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**Introduction**: Urinary tract infection (UTI) is a worldwide occurring disease with an estimate of more than 10 million cases per year in Western Europe. The most prevalent causative agent of UTI is *Escherichia coli*. Besides symptomatic UTI, *E. coli* also causes asymptomatic bacteriuria (ABU). ABU strains cause only mild or no symptoms during carriage.
During ABU, *E. coli* grows in high numbers ($10^5$ to $10^8$ bacteria per milliliter urine), but the patients don't suffer from symptoms or only experience mild symptoms. A recent study showed that the *E. coli* ABU strain 83972, but not symptomatic uropathogenic *E. coli*, is able to actively suppress the eukaryotic RNA-Polymerase II. Thus, *E. coli* strain 83972 actively suppresses cellular processes of the host and consequently protects itself from an overwhelming immune response. Therefore, *E. coli* strain 83972 is currently tested as an alternative therapy to treat patients with recurrent urinary tract infections.
**Aims**: Based on the finding that *E. coli* ABU strain 83972 is able to actively suppress cellular processes by inhibiting the RNA polymerase II activity, we comparatively determined the cyto- and chemokine levels in supernatants of eukaryotic cells after infection with strain 83972. Decreased cyto- and chemokine levels caused by strain 83972 would explain the asymptomatic carriage and beneficial effects experienced by the patients.
**Materials and Methods**: We tested *E. coli* 83972, a reisolate of ABU strain 83972 from a deliberately inoculated patient as well as the symptomatic uropathogenic *E. coli* isolate CFT073. Several cell lines were infected during this study: bladder epithelial cells T24 and 5637, kidney cells A-498 and the monocyte cell line THP-1 as well as THP-1 differentiated to macrophages. Cells were infected at an MOI of 10 for 4 hours and bacterial interaction with host cells was assessed. After infection supernatants were collected and the cyto- and chemokine levels were determined by quantitative ELISA. The quantified cyto- and chemokines included IL-6, IL-8, IL-10, GROα/CXCL1 and TNFα.
**Results**: Secretion of cyto- and chemokines by epithelial and immune cells after infection with asymptomatic and symptomatic *E. coli* urinary tract isolates occurred in a cell- and strain-specific manner. In contrast to published results, IL-6 expression could not be detected for T24 or 5637 bladder epithelial cells upon bacterial infection. Although *E. coli* 83972 showed a weaker interaction with host cells than symptomatic UPEC strain CFT073, IL-8 expression by bladder epithelial cells was higher upon infection with ABU isolate.
**Summary**: Our results show that asymptomatic and symptomatic isolates of urinary tract infections trigger different cyto- and chemokine release depending on the infected cell type.

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**273/ZOP**
**The role of the novel putative protease JEP in *Staphylococcus aureus* host interaction**
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**Question:** The increasing numbers of methicillin-resistant *Staphylococcus aureus* (MRSA) strengthens the need for new effective antibiotics and a protective vaccine. In general, human-adapted *Staphylococcus (S.) aureus* strains are used to study *S. aureus* pathogenicity in mice. However, it is known that *S. aureus* acts highly host-specific. The recently characterized mouse-adapted *S. aureus* strain JSNZ may be a promising tool to develop more appropriate infection models. Interestingly, JSNZ secretes huge amounts of a putative serine protease, called JSNZ extracellular protease (JEP). Like other bacterial extracellular proteases, JEP could be important for colonization and infection. It is known that bacterial extracellular proteases are able to degrade host tissue, facilitate bacterial spread and manipulate host immune responses. The aim of this study was to elucidate the role of JEP in *S. aureus* host interaction.
**Methods:** The strain JSNZ was sequenced and the location of *jep* was determined with the software Geneious 1.6.1. The prevalence of *jep* in human and murine *S. aureus* isolates was analyzed by multiplex-PCR. Anti-JEP antibodies were determined by ELISA. Recombinant JEP and a JEP mutant (Ser194Ala) were applied to study the effect of the protease on the adaptive immune response. Read-outs included proliferation assays and cytokine measurements. Currently, we are investigating whether JEP is required for survival in different growth media.
**Results:** The *jep* gene showed up to 48 % sequence homology to *S. aureus* serine proteases-like proteins (SplA-F). Our genome analysis showed that *jep* is located on a Sa1int phage within the virulence module, indicating that JEP is a virulence factor. The *jep* gene was only present in murine strains, but not in human *S. aureus* isolates. Surprisingly, we could not detect any antibodies against JEP in the sera of naturally colonized and infected laboratory mice. There was no measurable effect on proliferation and cytokine secretion by immune cells.
**Conclusions:** The data suggest that JEP might manipulate the host immune response. The underlying mechanisms remain to be elucidated. Further studies using peptide libraries and substrate screenings will provide insights into the specificity of the JEP enzyme and its role in *S. aureus* colonization and infection.

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**274/ZOP**

**Molecular characterization of the SasC-mediated biofilm accumulation in *Staphylococcus aureus***

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In device-related infections with staphylococci, the bacteria are embedded in a three-dimensional matrix known as biofilm. Biofilm formation is a two-step process that requires primary adhesion to surfaces followed by accumulation of bacteria into multilayered cell clusters. Recently, we identified the *S. aureus* surface protein C (SasC), which mediates intercellular adhesion and biofilm formation. We found that the functional domain is located within the N-terminal region that is expressed by subclone 1 [1]. It contains three short repeats (SR) with 40 aa each, two longer repeats (LR) with 140 aa each, and a FIVAR domain (found in various architectures), which has a putative sugar binding function. To further narrow down the domain/s mediating intercellular adhesion and biofilm accumulation, we constructed further subclones expressing different domains of the N-terminal region. The characterization of subclones 3, 5 and 6 in aggregation and biofilm assays suggested that at least two different mechanisms are involved in SasC-mediated intercellular adhesion and biofilm formation. Furthermore, to characterize putative homotypic and/or heterotypic interactions of particular domains, we expressed and purified the different repeat domains as His-tagged fusion proteins. Subsequently, far-western blot analysis and $BS^3$-crosslinking experiments were performed to identify putative interactions. Data from far-western blot analysis indicated that interaction of the N-terminal domain with the three SR (referred to as insert 1) may be influenced by the presence of zinc, while others, when both of the LRs (referred to as insert 4) are involved, are not. The effect of zinc could also be observed with SasG-mediated cell aggregation [2], although both proteins do not share significant sequence similarities. $BS^3$-crosslinking experiments demonstrated that the N-terminal domain forms multimers in the presence of the crosslinking molecule $BS^3$, independently of the presence of zinc. In a different, still ongoing approach, DNA fragments encoding distinct N-terminal domains were analyzed for mediating interaction using a bacterial-two-hybrid system (BACTH). This method is based on the interaction of two subunits of the enzyme adenylate cyclase, which are fused to the N-terminal domains. In conclusion, the *S. aureus* surface protein SasC mediates cell aggregation and biofilm formation and probably involves at least two different mechanisms specified by different domains within the N-terminal portion of the protein.

**References**

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**275/ZOP**

**Regulation of type VI secretion systems in uropathogenic *E. coli***

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**Introduction**: Type VI secretion systems (T6SS) are complex machineries allowing Gram-negative bacteria to deliver different types of effectors into competing bacteria or eukaryotic host cells. Accordingly, one could assume that this kind of secretion system can contribute directly or indirectly to pathogenesis. Indeed, the genomes of uropathogenic *Escherichia coli* (UPEC) often harbor one or more gene clusters coding for this type of secretion system. As the T6SSs seem to be tightly regulated and therefore mostly inactive under standard laboratory growth conditions, the aim of this study was to find regulators enhancing the expression of T6SS. This is meant to be a first step to get a better understanding under which conditions these secretion systems may be active and contribute to the fitness and/or pathogenicity of *E. coli*.
**Material & Methods**: An *in silico* screen for putative transcription factor binding sites was combined with the construction of plasmid-based and chromosomal promoter-reporter gene fusions to core genes of the secretion systems in different genetic backgrounds. The data of the transcriptional regulation are complemented with the detection of the marker protein Hcp.
**Results**: The screen revealed the nucleoid-associated protein H-NS acting as a potential transcriptional repressor of genes coding for core elements of T6SSs. Preliminary data revealed further regulators influencing gene expression of T6SS core genes e.g. RpoS.
**Conclusion**: The T6SSs in UPEC are encoded on genomic islands. As H-NS is often involved in regulation of genes acquired by horizontal gene transfer, it is not surprising to identify H-NS being a potential repressor. Beside this, there are clearly further factors resp. regulators influencing each other to direct gene expression of T6SS in UPEC.

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**276/ZOP**

**Investigation of *Staphylococcus aureus* and *Streptococcus pneumoniae* induced signal transduction during phagocytosis in human THP-1 cells**

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*Staphylococcus aureus* and *Streptococcus pneumoniae* are major human pathogens responsible for a wide range of diseases, including life-threatening infections like pneumonia and sepsis. The innate immune system is the first line of defense when pathogenic bacteria enter different compartments of the human body. Thereby, macrophages recognize surface exposed pathogen-associated molecular patterns of invading bacteria which leads to their phagocytosis and eradication as well as to the presentation of antigens to cells of the adaptive immune system. The phagocytosis and intracellular killing of the laboratory strain
*S. aureus* SA113, the methicillin resistant community associated *S. aureus* USA 300 and the low encapsulated *S. pneumoniae* strain 35A was investigated using the human monocyte cell line THP-1. After PMA-induced differentiation to macrophages, time-dependent phagocytosis of bacteria was examined using double immune fluorescence microscopy and antibiotic protection assays. The participation of various signal pathways involved in cytoskeleton rearrangement was analyzed by the use of different pharmacological inhibitors. Furthermore, the phosphorylation status of selected signal kinases (PI3K, AKT, MAPK, ERK and JNK) was evaluated by SDS-PAGE and Western blotting using whole cell lysates from different time points of bacterial infection.
Taken together, we present a comparative study of phagocytosis of different *S. aureus* strains and *S. pneumoniae* by the human monocyte cell line THP-1 with a focus on the time-dependent phosphorylation of selected signal kinases.

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**277/ZOP**

**PIP5K1γ90 drives local production of phosphatidylinositol-4,5-bisphosphate to direct integrin-mediated uptake of *Staphylococcus aureus***

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**Questions:** *Staphylococcus aureus*, a Gram-positive coccoid bacterium, is becoming a major healthcare challenge on a global scale due to accumulation and widespread occurrence of antibiotic resistance. It can invade non-phagocytic cells in an integrin-dependent manner by exploiting protein constituents of focal adhesions. As localization and activation of several focal adhesion proteins can be regulated by phosphatidylinositol-4,5-bisphosphate (PI-4,5-$P_2$), we investigated the role of PI-4,5-$P_2$ and PI-4,5-$P_2$ producing enzymes during cellular invasion of *S.aureus*.
**Methods and Results:** Under microscopy, it was observed that integrin-mediated uptake initiated by staphylococcal fibronectin-binding protein (FnBP) was accompanied by enrichment of PI-4,5-$P_2$ in the vicinity of cell-associated bacteria. Targeting of an active phosphoinositide 5-phosphatase, which catalyzes the dephosphorylation of PI-4,5-$P_2$, to the plasma membrane significantly reduced bacterial invasion. Knockdown of individual phosphatidylinositol -4-phosphate 5-kinase via siRNA revealed that phosphatidylinositol-4-phosphate-5-kinase γ (PIP5K1γ) played a major role in bacterial internalization. The 90 kDa isoform of PIP5K1γ is known to associate with talin and FAK at integrin-rich focal adhesion sites and this enzyme was strongly recruited to sites of bacterial attachment. Selective genetic deletion of this isoform reduced bacterial invasion, which could be rescued by re-expression of active, but not by re-expression of inactive PIP5K1γ90. In PIP5K1γ90-deficient cells, overall PI-4,5-$P_2$ levels in the plasma membrane were unaltered, but local accumulation of PI-4,5-$P_2$ at bacterial attachment sites and FAK tyrosine phosphorylation were obviously reduced.
**Conclusions:** These results highlight the importance of local synthesis of phosphatidylinositol 4, 5-bisphosphate by a focal adhesion-associated lipid kinase to promote integrin-mediated internalization of *S. aureus* by non-phagocytic cells.

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## ZOONOSES (ZOP)

**278/ZOP**

**Human invasive sarcocystosis: insights in an emerging parasitic human disease from an outbreak in the tropics**

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*Sarcocystis nesbitti*, a putatively reptile-associated intracellular parasite has recently caused two concomitant outbreaks of a febrile eosinophilic myositis syndrome in Malaysia affecting > 300 patients, half of them international travelers. First documented in Malaysia accidentally in autopsy cases 50 years ago, *Sarcocystis* parasites had caused a small symptomatic outbreak in the 1990s in a jungle village, but had not come to further attention until recently. One of the outbreaks in Malaysia is still ongoing, and the source of infection is still unclear. The biphasic clinical picture and serum parameter changes have been characterized in returning travelers, as well as early and late-phase cytokine changes in infected individuals. Molecular diagnostic tests (PCRs) have been developed. Serological assays are, however, still needed. These food-/water-borne infections demonstrate the potential of zoonotic protozoal parasites to cause large unexpected outbreaks that might be associated with reptile host population changes.

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**279/ZOP**

**Development and Performance Evaluation of a Phase Specific Enzyme Linked Immunosorbent Assay and Lineblot for Serological Diagnosis of Coxiella / Q-fever Infection in Humans and Animals**

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The pathogen Coxiella burnetii is a gramnegative bacterium and belongs to the family Rickettsiaceae. It is the causative agent of Q-Fever, an acute rickettsial disease.
It can be considered the most infectious disease in the world, as a human being can be infected by a single bacterium. It can be found worldwide, including tropical countries, with the exception of New Zealand. In Europe it appears as hepatitis rather than pneumonia as in the United States.
The most common manifestation is flu-like symptoms. The fever lasts approximately 7 to 14 days. The disease can progress to an atypical pneumonia, which can result in a life threatening acute respiratory distress syndrome (ARDS). Occasionally, Q-fever causes hepatitis, which may be asymptomatic or becomes symptomatic with malaise, fever, liver enlargement and pain in the right upper quadrant of the abdomen.
The chronic form of Q-fever is very similar to inflammation of the inner lining of the heart, which can occur months or decades following the infection. It is fatal if left untreated, however with the correct treatment the mortality rate falls under 10%.
Acute infection: IgM and IgG antibodies to phase 2 antigen present.
Chronic Infection: IgG and IgA antibodies to phase 1 antigen present
The aim of this work was to develop a serological assay to detect IgG and IgM antibodies against Coxiella in serum, plasma and milk. Native antigen preparations were used to coat 96 well microtiterplates and to print lineblots. For the detection anti-human IgG and IgM antibodies or a protein A/G conjugate, able to detect IgG and IgM simultaneously, are used. Samples used for the evaluation originated from humans, cows, sheep and goat.
For the human as well as for the veterinary assay values for sensitivity and specificity of > 90% could be achieved for both, Phase 1 and Phase 2. Cut off values have to be adjusted for each species. Veterinary data still needs more validation.

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**280/ZOP**

**Serological survey of *Bartonella* spp., *Borrelia burgdorferi*, *Brucella* spp., *Coxiella burnetii*, *Francisella tularensis*, *Leptospira* spp., *Echinococcus*, Hanta-, Tick-borne encephalitis (TBE)- and Xenotropic murine leukemia virus-related virus (XMRV) infection in employees of two forestry enterprises in North Rhine-Westphalia (NRW), Germany, 2011-2013**

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In Germany, knowledge of the prevalence of zoonoses at the regional level in different risk populations is remains incomplete.
Within the network of consiliary laboratories for zoonoses we initiated a survey of 722 employees of forestry enterprises in NRW from 2011 to 2013 in order to collect basic data on various zoonoses. Exposures associated with seropositivity were identified to gain insight into possible risk factors for infection with each pathogen.
We collected blood samples and interviewed the participants during on-site appointments. The serum samples were tested for IgG antibodies via ELISA screening test, Western blot, IFT, EIA, NT. The data were analysed by logistic regression using SPSS.
41.2% of participants were found to be seropositive for anti *Bartonella*-IgG, 30.6% for anti *Borrelia burgdorferi*-IgG, 14.2% for anti *Leptospira*-IgG, 6.5% for anti *Coxiella burnetii*-IgG, 6.0% for anti Hantavirus-IgG, 4.0% for anti *Francisella tularensis*-IgG, 3.4% for anti TBE-virus-IgG in non-vaccinated participants, and 1.7% for anti *Echinococcus*-IgG. No participant was found to be seropositive for anti *Brucella*-IgG or anti XMRV-IgG. Participants seropositive for *B. burgdorferi* were 3.96 times more likely to work professionally in the forest (univariable analysis: OR 3.96; 95% CI 2.60-6.04; p<0.001); and participants seropositive for Hantavirus 3.72 times more likely (univariable analysis: OR 3.72; 95% CI 1.44-9.57; p=0.007). Participants seropositive for *Bartonella* were 2.07 times more likely to live in a rural area, or in a small or medium town (univariable analysis: OR 2.07; 95% CI 1.08-3.98; p=0.029). The relatively high seroprevalence for *Leptospira* found in this study could be linked to living conditions rather than work exposure. No typical risk for exposure to *C. burnetii* and *Echinococcus* was identified in the study.
The survey collected for the first time regional data on ten zoonoses in NRW. We found a high percentage of participants seropositive for B. *henselae* and *F. tularensis*. Forestry workers appear to have more contact to *B. burgdorferi*-infected ticks and have a regionally diverse risk for acquiring Hantavirus-infection. NRW does not appear to be a typical endemic area for *C. burnetii* and *Echinococcus.* The epidemiology of zoonoses is of great importance for public health. Knowledge of regional risk factors facilitates the development of efficient prevention strategies and the implementation of the prevention measures in a sustainable manner.

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**281/ZOP**

**Leptospirosis outbreak in strawberry harvesters in Germany**

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**Question**: In industrialized countries such as Germany, travelling abroad, recreational activities linked to freshwater exposures and residential exposures such as gardening and owning pets are major risk factors for human leptospirosis. Due to the modernisation of farming practices, outbreaks associated with agricultural exposure risks seemed to have minor importance. In 2007, the first major leptospirosis outbreak linked to field work was reported in Germany since the 1960s. Here we report a further outbreak among predominantly Polish strawberry pickers in Germany in 2014.
**Methods**: An outbreak investigation was performed by local, federal and national public health and veterinary institutions, which included the trapping of 64 mice, voles and shrews of one strawberry field to identify the potential outbreak source. Blood and serum samples were tested by IgG and IgM ELISA and MAT, rodent kidney samples by real time PCR, *secY* sequencing and multi locus sequence typing (MLST).
**Results**: Between June and August 2014, 45 leptospirosis cases among strawberry pickers working on two different farms were officially registered in the German federal state Lower Saxony. Leading symptoms were sudden onset of high fever (>38.5°C), generalized body/muscle pain and an increase of renal or liver enzymes. 47% of the patients were hospitalised. For fifteen cases laboratory evidence for leptospirosis was reported, all other patients had similar working exposure and characteristic symptoms, which was regarded as sufficient evidence for notification as leptospirosis. Only the patient which had displayed the earliest onset of symptoms was tested positive in the MAT with an antibody titer of 1:200 against *L. kirschneri* serovar Grippotyphosa. Two patient's urine or EDTA blood could be analyzed by molecular methods and *L. kirschneri* was identified.
In 67% of the rodent kidneys *Leptospira*-specific DNA was detected, the infecting species was identified as *L. kirschneri* sequence type 110, which corresponds to the serogroup Grippotyphosa.
**Conclusions**: Due to the ever increasing number of seasonal workers in German agriculture, as well as more frequent warm and wet summers and increased rodent numbers due to mild winters, further agriculturally associated outbreaks can be expected in future. Therefore, prevention measures including personal protective equipment and rodent control as well as future information campaigns are currently subjects under discussion.

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**282/ZOP**

**TCC capacity for monitoring complement inhibition in patients with atypical hemolytic uremic syndrome treated with eculizumab**

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**Question**: Atypical hemolytic uremic syndrome (aHUS) is a devastating disease characterized by thrombus formation in the microvasculature and associated with complement dysregulation. The recommended treatment is eculizumab, an expensive humanised monoclonal antibody, which binds C5 and prevents the assembly of the terminal complement complex (TCC, C5b-9). The study objective was to find a reliable marker for the time point of the next dosage, i.e. when complement control was still sufficient

not only under normal, but also under possibly occurring pathophysiological conditions, such as an infection.
**Methods**: We report on 7 patients with aHUS, in whom serial measurements of SC5b-9 (the soluble form of C5b-9), and the TCC capacity (SC5b9 after *ex-vivo* activation) were performed over 3.8 years of therapy.
**Results**: SC5b-9 levels were elevated prior to eculizumab treatment compared to healthy individuals, but did not show any significant difference during maintenance intervals. Therefore we generated an assay to determine the TCC capacity and established reference cut-off levels. Eculizumab treatment resulted in a profound decrease of TCC capacity.
**Implications**: Thus, measuring the TCC capacity may represent a novel and simple assay to determine the level of complement inhibition in patients treated with eculizumab, i.e. the residual capacity of inhibition at pathophysiological stages, and thus the time point of next dosage. This determination is likely equally useful for eculizumab treatment in *Escherichia coli*-induced hemolytic uremic syndrome (eHUS).

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**283/ZOP**
**_Bacillus cereus_ biovar _anthracis_ - an emerging pathogen affecting African rain forest areas**
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*Bacillus anthracis* (*Ba*) is known worldwide as a monomorphic species causing anthrax in a large variety of mammalian species, especially ruminants. In Africa, the disease is mainly active in arid savannah regions. Therefore, it was surprising to find, in 2001, chimpanzees in the Taï National Park in Côte d'Ivoire that had apparently died of an anthrax-like disease. Since the first observations, bacteria that exhibit microbiological and molecular features pointing both to *B. anthracis* and to less virulent members of the *B. cereus* group have been isolated from different animal species in rain forest regions of Africa. Based on their untypical features, the bacteria were designated as *B. cereus* biovar *anthracis* (*Bcbva*).
The unusual bacilli were characterized by standard bacteriological methods and shown to be non-haemolytic like *Ba*, but motile and resistant to the diagnostic gamma phage like other *B. cereus*. On molecular level, the bacteria possess the two virulence plasmids encoding the toxins and capsule of *Ba* in a non-*B. anthracis* chromosomal background. Whole genome sequencing revealed unique genomic regions that can be used for specific detection. Gene regulation of toxin and capsule genes was assessed by reverse transcriptase PCR and shown to be similar in *Bcbva* and classic *Ba*, where virulence gene expression is induced by growth under in vivo mimicking conditions in a $CO_2$-enriched atmosphere and controlled by the regulator AtxA.
Animal experiments using mice and guinea pigs to determine $LD_{50}$ and mean time to death revealed comparable virulence as in *Ba*. However, deletion of the capsule plasmid which results in strong attenuation in *Ba* had only a slight effect on virulence of *Bcbva*. This effect can be ascribed to the synthesis of a second capsule type composed of hyaluronic acid which is encoded by a gene cluster on the toxin plasmid. Synthesis of this polysaccharide capsule was displayed by electron microscopy and specific staining of bacterial extracts separated by polyacrylamide gel electrophoresis. Like the typical polyglutamic acid capsule of *Ba*, production of the hyaluronic acid capsule in *Bcbva* is controlled by AtxA. A mutation in the corresponding gene cluster prevents hyaluronic acid capsule synthesis in *Ba*.
In a German-African cooperation project which is funded by DFG, we currently analyze the epidemiology of *Bcbva* in Côte d'Ivoire by studying the distribution of the bacteria and by performing seroprevalence studies in humans and animals all over the country. Although human cases caused by *Bcbva* were not yet described, exposition of the population is likely due to hunting and consumption of bush meat. Based on the untypical characteristics of *Bcbva*, it can probably not be diagnosed as anthrax-causing pathogen based on the simple methods that are still widely used in African laboratories. Therefore, an important goal of the project is to strengthen the diagnostic capacities in Côte d'Ivoire.

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**284/ZOP**
**Biomarkers demonstrate varying disease activity in different stages of alveolar echinococcosis**
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**Question**: Alveolar echinococcosis (AE; infection with the larval stage of the fox-tapeworm *Echinococcus multilocularis*), is a parasitic zoonosis mainly affecting the liver. The parasitic tissue grows infiltratively and has a tendency to metastasize. The disease is staged according to the WHO PNM-system, based on the radiological extend of the lesion(s). It is assumed that PET-positivity reflects immune cell activity at the parasite-host interface, and thus disease activity. The aim of this study was to identify biomarkers which might reflect clinical activity of AE.
**Methods**: We analyzed sera of 160 patients (aged 18-94 years; mean age 57 years; m:f ratio 0.72:1) in different clinical stages of AE for levels of biomarkers possibly reflecting disease status. Patients were grouped according to their clinical PNM stage (I-IV) and positron emission tomography (PET) result status (positive [n=101; mean age 59 years; m:f ratio 0.74:1] versus negative [n=59; mean age 54 years; m:f ratio 0.69:1]). Biomarkers analyzed were soluble interleukin 2 receptor (sIL-2R), cytokeratin fragments (Ck18F-M30 and Ck18F-M65), total IgE, and parasite-specific IgE.
**Results**: There were significantly higher mean levels of total IgE and parasite-specific IgE in the patient cohort with positive PET status than in the cohort with negative PET status ($p<0.001$), largely exceeding the respective reference range. This result was seen in all clinical stages of AE. Both total IgE and parasite-specific IgE concentrations showed a bimodal distribution with highest levels in stage II and IV of PET-positive AE.
Higher mean levels of sIL-2R were detected in the patient cohort with positive PET status than in the cohort with negative PET status. However, in the clinical stage sub-analysis, this effect was only seen in stage II and IIIb. In both patient groups with positive and negative PET status, sIL-2R levels were highest in the earliest (I/II) and latest (IV) disease stages, but still within the reference range.
Also, higher mean levels of Ck18F-M30 and -M65 were measured in the patient cohort with positive PET status than in the cohort with negative PET status. M30 levels steadily increased, however moderately, with the clinical stage in the complete study cohort; this effect was mainly due to the PET-positive cohort, and not seen in the PET-negative group. M65 concentrations showed a bimodal distribution in both PET cohorts, with peaks in stage II and IV.
**Conclusions**: Highest levels of the cellular immunological response marker sIL-2R and the parasite-responsive antibody class IgE and specific IgE are seen in stages II and IV, pointing towards a stronger immunological activation in these disease phases when compared to other stages of AE. A similar result is seen with the apoptosis and necrosis marker Ck18F-M65, demonstrating an

excess necrosis in these stages in both PET cohorts when compared to levels with the apoptosis marker Ck18F-M30. Ck18F-M30 however, mirrors the clinical stage of AE. Total IgE and parasite-specific IgE levels reflect PET status, and thus disease activity, in all clinical stages of AE.

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**285/ZOP**

**Occurrence of Extended-spectrum betalactamase producing *Enterobacteriaceae* and Methicillin-resistant *Staphylococcus aureus* on pig farms and among pig farmers in North Rhine-Westphalia**

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**Background:** Extended-spectrum betalactamase (ESBL) producing *Enterobacteriaceae* (ESBL-E) and Methicillin-resistant *Staphylococcus aureus* (MRSA) have recently emerged in pig holdings.
In this study, we assessed the simultaneous occurrence of ESBL-E and MRSA in dust and faecal samples from farms in North Rhine-Westphalia. In addition, ESBL-E rectal carriage as well as nasal MRSA colonization was investigated among the farmers.
**Methods:** Between May and September 2014, five dust samples and five faeces samples, respectively, were collected at 51 pig holdings in North Rhine-Westphalia.
From the farmers working on the farms from which environmental samples were taken, a nasal swab and a stool sample was obtained in addition. Dust samples were enriched in broth supplemented with 6.5% NaCl, then in phenol red broth containing ceftixozime and then plated on MRSA ID agar (bioMérieux). Swabs from the anterior nares were enriched in 6.5% NaCl broth and plated on MRSA ID agar. Environmental faeces and farmers' stool samples were enriched in non-selective broth and then streaked on ESBL chromogenic agar (bioMérieux). Species confirmation was done by MALDI-ToF MS and susceptibility testing by VITEK2 automated systems using EUCAST clinical breakpoints.
**Results:** Preliminary results show that MRSA was detected in dust and environmental faeces samples on 49 farms (96%) and ESBL-E on 31 (61%) farms ($MRSA^+/ESBL\text{-}E^+$, n=30, 59%; $MRSA^+/ESBL^-$, n=19, 37%; $MRSA^-/ESBL\text{-}E^+$, n=1, 2%; $MRSA^-/ESBL\text{-}E^-$, n=1, 2%). All ESBL-E isolates were *Escherichia coli*. All isolates (n=105) were susceptible to meropenem, 18% resistant to gentamicin, 33% to ciprofloxacin and 67% to SXT. Nasal MRSA carriage was detected in 70/73 (96%) and rectal ESBL-*E. coli* carriage in 5/73 (7%) of the farmers, respectively.
**Conclusions:** Rectal ESBL-*E. coli* colonization of pig farmers was comparable to studies assessing respective carriage in the general community (6%) in Germany and was less than indicated by a study among Dutch poultry farmers (33%). However, our findings that all farmers carried MRSA in the nares confirm previous findings and underline the importance of preventive measures to forestall endogenous infections in this group of persons.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**286/ZOP**

**Sequential continuous flow PCR for detection of *Bacillus anthracis, Brucella melitensis, Burkholderia mallei, Coxiella burnetii, Francisella tularensis* and *Yersinia pestis* using a microfluidic chip system**

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**Question**: A microfluidic chip system for continuous flow PCR was tested for the detection of the six biological agents. These species can cause severe zoonotic diseases. In this study, the bacteria were tested for all six targets by sequential on-chip PCR assays within less than two hours.
**Methods**: A microfluidic chip system (microfluidic ChipShop GmbH, Jena, Germany) was used for the detection of *Bacillus anthracis*, *Brucella melitensis*, *Burkholderia mallei*, *Coxiella burnetii*, *Francisella tularensis* and *Yersinia pestis*. The chip has a meander shaped microchannel that is guiding the liquid above different heating zones required for thermocycling in PCR assays. 25 µl of PCR mixture were prepared each with single PCR assays and a maximum of 1 ng DNA extracted from the different bacteria species. Another PCR mixture was prepared including a PCR assays specific for detection of *bacteriophage lambda* DNA as internal amplification control. All seven PCR mixtures including the different primer sets and one DNA extract were sequentially pumped through the microchannel using a neMESYS syringe pump (cetoni GmbH, Korbußen, Germany). The aqueous PCR mixture plugs where thereby separated by 25 µl of mineral oil. Amplicons were analysed via agarose gel electrophoresis.
**Results**: As expected, only the corresponding DNA yielded in the specific PCR product. The internal amplification control could be detected in all experiments, too. One major advantage of this sequential approach is the increased reaction speed compared to a series of single continuous flow PCR assays. A single PCR lasted 57 min while the sequential PCR assay for all targets could be performed within less than two hours.
**Conclusion**: DNA from all selected pathogens could be detected successfully by sequential continuous flow PCR with high velocity.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**287/ZOP**

**Detection of thermophilic *Campylobacter* in air samples from broiler chicken and pig farms after DNA purification with a microfluidic chip system**

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**Question**: A microfluidic chip system for DNA purification was evaluated with air samples collected in broiler chicken and pig farms. The samples were tested for the presence of thermophilic *Campylobacter* species including *C. jejuni, C. coli* and *C. lari*.
**Methods**: Air samples were collected in broiler chicken and pig farms using a portable air sampling apparatus (Coriolis µ, Bertin Technologies, Montigny-le-Bretonneux, France). Sampling was done at two different distances from the ground (30-60 cm and 150 cm). Collected particles were suspended in 1x phosphate-buffered saline containing 0.01% Tween-20 and 10 mM ascorbic acid. Following a combined thermal, enzymatical and chemical lysis DNA was purified using a microfluidic chip system (ChipGenie P, microfluidic ChipShop GmbH, Jena, Germany). The purification process was based on reversible attachment of DNA to particles with a silica shell and paramagnetic core. For each analysis 2 ml sample solution were used and finally 100 µl eluate were obtained after the DNA purification process. Detection was carried out by quantitative real-time PCR using *Campylobacter* species specific assays detecting *mapA*, *ceuE* and *gyr* genes.

**Results**: Three different thermophilic *Campylobacter* species were detected in both of the investigated farms. Particularly high concentrations corresponding to 100 genome equivalents (GE) per 10 litre air and more were detected for *C. coli* and *C. lari* in a height of 30 cm for the broiler chicken farm and 150 cm for the pig farm. *C. jejuni* was found in few samples of both farms with concentrations of 30 GE per 10 litre air and less. Detection of *Campylobacter* from samples directly added to the PCR mixture without DNA preparation did not yield any positive result, probably due to inhibition.
**Conclusion**: The microfluidic chip system is suitable for preparation of DNA from air samples to enable detection of thermophilic *Campylobacter* species by subsequent PCR. In addition to the widely spread *C. jejuni* and *C. coli* also *C. lari* was detected. An effective removal of potential inhibitors from samples as prerequisite for PCR is presumed.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**288/ZOP**

**Acute Q-fever infection after contact to fawn cadaver in Thuringia, a case report**

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A 48 years old man presented in July 2013 with fever and fatigue. Clinical examination revealed a reduced general condition, fever and sinus tachycardia. Basic laboratory testing showed thrombocytosis and marked elevation of C-reactive-protein. Chest X-ray demonstrated the presence of an infiltrate in the right upper lobe, subsequently confirmed by computed tomography. There was no clinical improvement after initial antibiotic treatment with ceftriaxone. No pathogen was isolated from sputum or blood culture. Due to the non-specific clinical picture further serologic investigation was performed (Brucella spp., Coxiella burnetii, Francisella tularensis, Borrelia spp., Leptospira spp., Listeria spp., Chlamydia spp., Mycoplasma und CMV). Based on positive serology results for Coxiella burnetii (IgG-phase 2 ELISA: 41.1, IgG-phase 1 ELISA: negative, IgA ELISA: negative, IgM ELISA: negative, IgG-phase 2 IFT: 1:128, IgM-phase 2: 1:64, IgG-phase 1 IFT: negative) the diagnosis of acute Q-fever with Q-fever-pneumonia was considered. Specific antibiotic treatment was initiated with ciprofloxacin 500 mg oral bid for 14 days. The patient responded well to treatment and fever subsided within 24 hours. Further serology tests performed after 2 and 8 months ruled out chronic coxiella infection.
Retrospective anamnesis to clarify the origin of the infection revealed that 14 days before onset of acute disease the patient had buried two fawn cadavers. Analyses of soil samples taken from the burial site in September 2013 by real-time PCR revealed the presence of C. burnetii-DNA. The cadavers were not available for investigation due to decomposition and game damage. This is the first case report of an acute Q-fever infection in which the source of infection can be linked to deer.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**289/ZOP**

**Characterisation of Shiga toxin glycosphingolipid receptors by a combination of thin-layer chromatography, overlay immunodetection and desorption electrospray ionisation mass spectrometry (DESI MS)**

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**Introduction**: Glycosphingolipids (GSLs) are constituents of vertebrate cell membranes playing important roles in cell recognition and modulation of membrane-associated proteins like receptors, transducers and transporters [1]. Furthermore, GSLs act as receptors of pathogens such as uropathogenic *Escherichia coli* (UPEC) [2] or virulence factors like Shiga toxins (Stxs) released by enterohemorrhagic *E. coli* (EHEC) [3]. As a consequence of their structural heterogeneity, analysis of GSLs demands for the combination of analytical methods such as thin-layer chromatography (TLC) and mass spectrometry (MS).
**Aims**: In this project we are developing a hyphenation of the ambient desorption ionisation technique DESI with a high resolution Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer to enable accurate and precise MS analysis of immunodetected GSLs from TLC plates.
**Materials and Methods**: Neutral GSLs from human erythrocytes were applied bandwise onto normal phase silica TLC plates, separated, and detected by antibody-overlay assays. To that end plates were incubated with either a specific anti-GSL antibody or an Stx, its respective toxin-specific antibody followed by incubation with an alkaline phosphatase-labelled secondary antibody and staining with 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Immunodetected GSLs were desorbed and ionised directly from the plate by use of DESI. Mass analysis was achieved with a 7 T FT-ICR mass spectrometer. A $CO_2$ laser was used for infrared multiphoton dissociation (IRMPD) MS/MS experiments.
**Results**: TLC-separated Stx GSL receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) were detected by overlay assays with GSL-specific antibodies as well as Stx1a, Stx2a and Stx2e subtypes, combined with secondary antibodies and subsequent staining with BCIP. Stained bands were probed by DESI FT-ICR MS and immunopositive GSLs could be desorbed and ionised directly from the TLC plate without interferences from the antibodies, Stxs or the staining reagent. Scanning the plate in the direction of chromatographic development furnished 2D spectra which showed the distribution of the different Gb3Cer and Gb4Cer lipoforms on the plate. IRMPD tandem MS experiments yielded fragmentation patterns allowing for structural elucidation of Stx receptors both with respect to glycan sequence as well as ceramide moiety.
**Conclusion**: Here we demonstrate the potential of the combination of planar chromatographic separation, overlay assay immunodetection and DESI MS directly on TLC plates for the characterisation of GSL receptors of bacterial virulence factors exemplarily shown for various anti-GSL antibodies and Stx subtypes.

**References**

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**290/ZOP**

**Distribution and characterization of *astA* encoding the heat-stable enterotoxin of enteroaggregative *Escherichia coli* (EAST1) in enterohemorrhagic *E. coli***

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**Background**: The *astA* gene was first identified in enteroaggregative *Escherichia coli* (EAEC) as a locus encoding the EAEC heat-stable enterotoxin (EAST1). In addition to EAEC, it also occurs in several other *E. coli* pathotypes. However, little is known about the distribution of *astA* among enterohemorrhagic *E. coli* (EHEC). Here we investigated the frequency, serotype distribution, allelic variations and transcription of *astA* in a large collection of EHEC patients´ isolates. Moreover, we analyzed the phylogeny of the *astA*-harboring strains
**Methods**: Presence of *astA* was screened with PCR, alleles were determined by sequence analysis of the *astA* amplicons, phylogeny using multilocus sequence typing (MLST), and transcription using quantitative reverse transcription PCR.
**Results**: The *astA* gene was idenitified in 8.3% of 252 EHEC strains. It was associated with particular serotypes and MLST types. Sequence analysis revealed four different *astA* alleles, three of which were identified in EHEC for the first time. The gene was transcribed in all strains but the transcriptional levels significantly differed among strains of different serotypes and MLST types.
**Conclusions**: These data indicate that the *astA*-encoded EAST1 toxin might play yet unknown role in the pathogenesis of EHEC-mediated diseases.

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**291/ZOP**

**In quest of plant-derived STEC: Contamination or habitat - a genotypic approach**

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**Introduction:** Enterohemorrhagic *E. coli* (EHEC), the human pathogenic subgroup of Shiga toxin-producing *E. coli* (STEC) are zoonotic pathogens which can cause large food- and waterborne outbreaks. Though it is generally accepted that ruminants are the main reservoir of these bacteria, the search for the origins of many large outbreaks, including the recent O104:H4 outbreak in central Europe, also points toward vegetable sources.
**Objectives:** In this study, we want to answer the question, if and how plant derived STEC genetically differ from clinical, animal, or environmental isolates.
**Materials and Methods:** To achieve our goal we set up a strain collection of 170 isolates from different origin (33 plant, 86 human, 38 environment, and 13 from animal sources). All strains were whole genome sequenced and these data were used to determine *in silico* multilocus sequence typing (MLST) data and the so-called MLST+, a gene-by-gene allelic approach. Additionally, multilocus variable-number tandem repeat analysis (MLVA) based on 10 regions was performed.
**Results:** To our knowledge, the largest European collection of plant-derived STEC could be established. Altogether, we could identify based on the MLST data 65 different sequence types (ST) in the strain collection, with a very high heterogeneity in the plant isolates (19 STs). The resulting minimum spanning trees based on the established typing methods (MLST and MLVA) showed that the plant isolates do not cluster together. Interestingly, also the gene-by-gene approach resulted not in a clustering of plant associated strains. In a closer look we even did not find alleles in which the plant isolates differ from isolates of the other origins.
**Conclusions:** Taken together these data suggest that there are no plant-specific STEC. The strains may survive in/on plants, but there are no specific characteristics on a genetic level. However, we have to take into account that the amount of samples in this collection is limited and that there may be populations that could be found performing excessive field sampling.

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**292/ZOP**

**Glycosphingolipid-doped model membranes as valuable tools for sensitive real time bioaffinity analysis of Shiga toxin receptors using a surface acoustic wave sensor**

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**Introduction:** Shiga toxins (Stxs) of Stx-producing *Escherichia coli* (STEC) are responsible for damage of the renal and cerebral microvascular endothelium that can lead to severe sequelae such as the life-threatening hemolytic uremic syndrome [1]. Stxs bind to oligosaccharides of globo-series neutral glycosphingolipids (GSLs), which decorate the plasma membrane of target endothelial cells [2]. GSLs are integral constituents of cholesterol-rich microdomains in the outer leaflet of the plasma membrane called *lipid rafts*. These clusters may play a pivotal role in receptor-mediated binding and internalization of Stxs [3]. So far the initial interaction of the various Stx-subtypes with clustered GSL receptors is poorly understood.
**Objective:** Our aim was to develop a lipid bilayer model membrane approach based on label-free real time interaction analysis of Stx receptors using a surface acoustic wave (SAW) sensor.
**Materials and Methods:** Liposomes were prepared with varying content of phospholipids, cholesterol and Stx receptors Gb3Cer or Gb4Cer. Lipid mixtures were used for the production of multilamellar vesicles, which were sized to small unilamellar vesicles (SUVs) by extrusion. Bilayer formation on a 11-mercaptoundecanoic acid modified gold biosensor surface was accomplished by the adsorption of linker lipid-containing SUVs. Spreading and burst of adsorbed SUVs to stable lipid bilayers were mediated *via* divalent cations allowing for binding of membrane lipids onto the modified sensor surface. The formation of stable artificial membranes with lipid domains was probed by atomic force microscopy (AFM) under fluid conditions. Real time interaction measurements with affinity purified GSL specific antibodies and Stx1a-, Stx2a-, and Stx2e-subtypes were recorded with the SAW biosensor (sam5, NanoTemper Technologies).
**Results:** Lipid bilayer formation on the modified biosensor was strongly influenced by linker lipid-containing SUVs and by the amount of divalent cations used as a mediator for spreading and burst of SUVs. Properties of GSL-containing microdomains within the lipid bilayers were portrayed by AFM. Real time interaction analysis indicated binding of globo-series GSLs with receptor-specific antibodies and various Stx1a-, Stx2a-, and Stx2e-subtypes. Moreover, analysis of data from kinetic measurements revealed novel insights in the course of association and dissociation processes of this type of carbohydrate (GSL) - protein (Stx) interaction. Collectively, our study provides substantial methodological improvements in analyzing the molecular mechanism of Stx-interaction with surface-exposed lipid-bound oligosaccharides.
**Conclusion:** The biosensor technique will support the development of anti-adhesion drugs to alleviate the course of STEC-infections.

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**293/ZOP**

**Cholesterol depletion alters membrane microdomain composition and Shiga toxin susceptibility of Vero and MDCK cells**

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**Introduction:** Enterohemorrhagic *Escherichia coli* (EHEC) produce Shiga toxins (Stxs), the major EHEC-derived virulence factors. Stxs cause extraintestinal complications such as the hemolytic uremic syndrome [1]. Vero-B4 cells, which originate from monkey kidney epithelial cells, represent the gold standard for evaluation of Stx-mediated cellular cytotoxicity. MDCKII cells, a kidney cell line of dog origin, are widely used for studying epithelial barrier function [2]. The *lipid raft*-association of Stx receptor glycosphingolipids (GSLs) globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) is believed to be one requirement for efficient Stx binding and subsequent internalization, whereby cholesterol stabilizes clustering of GSLs in *rafts*.
**Objectives:** Here, we aimed in determining the effect of cholesterol depletion of the plasma membrane on the stability of *lipid rafts* and the Stx-mediated cytotoxicity toward Vero and MDCKII cells.
**Materials and Methods:** Vero-B4 and MDCK II cells were cultivated under serum free conditions and Stx-mediated cytotoxicity was measured with the crystal violet cell cytotoxicity assay. Cholesterol depletion of both cell lines was performed by cellular exposure to cyclodextrin. The association of cholesterol and Stx-receptor GSLs with microdomains was determined by analyzing detergent-resistant membranes (DRMs), which were prepared by sucrose density gradient centrifugation [3]. Subsequently, Stx-receptors were identified by thin-layer chromatography immunodetection with specific antibodies, and their structures were determined by electrospray ionization (ESI) mass spectrometry [4].
**Results:** Vero-B4 and MDCKII cells were found to express similar amounts of globo-series GSLs with binding capacities toward various Stx-subtypes. Sucrose density gradient centrifugation indicated canonical DRM distribution of GSLs and the *lipid raft* marker sphingomyelin. However, different content and distribution of cholesterol in DRM preparations of MDCKII and Vero-B4 cells suggest different susceptibilities toward Stxs and cyclodextrin treatment (cholesterol depletion) of the two investigated cell lines. Furthermore, recent experiments gave first evidence for cyclodextrin to enhance or reduce the cytotoxic effects of Stxs, suggesting a cholesterol-dependent influence on the susceptibility of Vero-B4 and MDCKII cells toward Stx.
**Conclusion:** This investigation supports the hypothesis that the association of Stx receptors with *raft* microdomains and their interplay with *raft*-associated lipids such as cholesterol might have functional impact for Stx-GSL interaction and Stx-mediated cell damage of target cells.

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**294/ZOP**

**Real-time PCR assays for the detection and subtyping of Shiga toxin genes of enterohemorrhagic *Escherichia coli***

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**Background**: Enterohemorrhagic *Escherichia coli* (EHEC) produce different types of Shiga toxins (Stx) which are their key virulence factors. The two major Stx types, Stx1 and Stx2, are encoded by the $stx_1$ and $stx_2$ genes, respectively, which are further subdivided into several subtypes ($stx_{1a}$, $stx_{1c}$, $stx_{1d}$, $stx_{2a}$, $stx_{2b}$, $stx_{2c}$, $stx_{2d}$, $stx_{2e}$, $stx_{2f}$ and $stx_{2g}$) with conventional PCR (Scheutz *et al*. 2012). The aim of the present study was to develop a real-time PCR protocols for a rapid detection of all known $stx_1$ and $stx_2$ subtypes.
**Methods**: 10 EHEC reference strains harboring various $stx_1$ and/or $stx_2$ subtypes, 42 strains of the hemolytic uremic syndrome (HUS)-associated EHEC (HUSEC) collection, and 50 EHEC human isolates whose *stx* subtypes had been determined by conventional PCR were tested with 11 real-time PCRs.
**Results**: Using the 10 EHEC reference strains and 42 HUSEC strains, the real-time PCR results demonstrated 100% concordance with conventional PCR subtyping. Among the 50 human isolates, two $stx_1$ subtypes ($stx_{1a}$ and $stx_{1c}$) and four $stx_2$ subtypes ($stx_{2a}$, $stx_{2b}$, $stx_{2c}$ and $stx_{2d}$) were identified. Forty strains were positive for a single *stx* gene (16 for $stx_{1a}$, 24 for $stx_{2a}$) and 10 for combinations of two different *stx* subtypes using the real-time PCR. Also these results were in 100% agreement with conventional PCR results.
**Conclusion**: We conclude that the real-time PCR protocols developed here represent a rapid and reliable method for *stx* subtyping in diagnostic laboratories.

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**295/ZOP**

**Shiga toxin receptors of human intestinal epithelial cells**

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**Introduction**: Shiga toxins (Stxs) released by pathogenic enterohemorrhagic *Escherichia coli* (EHEC) are the major virulence factors involved in life-threatening complications like the hemolytic uremic syndrome (HUS) [1]. Stxs belong to the class of $AB_5$ toxins and consist of an enzymatically active A-subunit and five identical B-subunits responsible for binding to the high and less effective glycosphingolipid (GSL) receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer), respectively. Stxs severely damage microvascular endothelial cells [2,3], which is considered to be the leading event in the development of HUS. However, the involvement of Stxs in human intestinal epithelial cell injury still remains an open question.
**Aims**: The aim of this study was to investigate 1) the GSL composition of human intestinal epithelial cells with emphasis on Stx receptors Gb3Cer and Gb4Cer and 2) their putative association with *lipid rafts*, which is believed to play an essential role in Stx binding and subsequent cellular internalization.
**Materials and Methods**: GSLs were extracted from *in vitro* propagated human intestinal epithelial cell lines. Co-extracted phospholipids and triglycerides were removed by alkaline saponification; neutral GSLs were isolated by anion exchange chromatography. GSLs were detected by thin-layer chromatography (TLC) overlay assays with anti-Gb3Cer and anti-Gb4Cer antibodies. *Lipid raft* association of Stx receptors was examined using detergent-resistant membranes (DRMs), which were prepared by sucrose density gradient centrifugation, followed by TLC overlay analysis of DRM-associated GSLs.
**Results**: With this work we show that human intestinal epithelial cells do express GSLs of the globo-series such as Gb3Cer and Gb4Cer, which represent potential receptors for Stx1a and Stx2a subtypes. The GSL structures were determined by TLC

immunostaining combined with electrospray ionization mass spectrometry. Furthermore, we provide some preliminary results on the association of Stx receptors with DRMs, suggesting a putative involvement of *lipid rafts* in Stx-mediated cytotoxicity of human intestinal epithelial cells.
**Conclusions**: The data support the hypothesis that a membrane organization of Stx receptors in microdomains might be involved in Stx-mediated cytotoxicity of the intestinal epithelium.

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**296/ZOP**
**Thin-layer chromatography MALDI mass spectrometry imaging of Shiga toxin glycosphingolipid receptors**
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**Introduction:** Shiga toxins (Stxs) of enterohemorrhagic *Escherichia coli* (EHEC) play pivotal roles in a wide range of clinical outcomes from diarrhea to hemolytic uremic syndrome (HUS). The injury of endothelial cells of kidney and brain by Stxs is a key event in the development of HUS [1]. Stxs bind to glycosphingolipid (GSL) receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) exposed on the cell surface of endothelial cells. However, the transport of Stx through the bloodstream and its delivery to target cells is still controversially debated [2], whereby leukocytes might act as cargo vehicles.
**Aims:** The aim of this study was to develop a sensitive strategy for full structural characterization and comprehensive visualization of Stx GSL receptors of monocytic cells directly on the thin-layer chromatography (TLC) plate employing infrared matrix-assisted laser desorption/ionization mass spectrometry imaging (IR-MALDI-MSI).
**Materials and Methods:** Lipids were extracted from *in vitro* propagated monocytic THP-1 cells [3] and co-extracted phospholipids were removed using phospholipase C (PLC) as recently published [4]. GSLs were separated by TLC and Stx receptors were detected by overlay immunostaining. A companion chromatogram was soaked with glycerol MALDI matrix and subjected to MALDI-MSI. A Synapt G2-S mass spectrometer (Waters) equipped with an optical parametric oscillator laser ($\lambda$ = 2.94 µm; 20 Hz, focal spot size ~ 50 µm) was used to acquire the MS imaging data with a pitch size of 100 µm x 100 µm.
**Results:** Top-down scanning of chromatograms revealed structural information of TLC-separated GSLs, ranging from monohexosylceramide (GlcCer) to Stx receptor trihexosylceramide Gb3Cer and tetrahexosylceramide Gb4Cer and even minor polyglycosylceramides. Only PLC-pretreated crude lipid extracts are required avoiding laborious GSL purification procedures. This novel MALDI TLC imaging approach operates on sub-microgram scale and allows for full structural characterization of the entire repertoire of heterogeneous GSLs with regard to variability in their individual oligosaccharide and ceramide moieties, e.g., of Stx receptors carrying sphingosine (d18:1) and variable fatty acyl residues with C16 to C24 chain lengths. The identity of selected GSLs was corroborated by subjecting selected molecular ions to tandem MS, performed also directly from the TLC plates.
**Conclusions:** Our approach thus enables highly sensitive imaging of Stx GSL receptors and unravelling of their structural diversity in lipid extracts of small-sized cell samples and provides a supplementary tool for MALDI-MS imaging of tissue sections [5].

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**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

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**297/ZOP**
***Salmonella* Dublin exhibits serovar-specific increased SPI-1/4 activity and virulence**
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**Introduction**: During the infection process *Salmonella enterica* has to overcome the intestinal barrier formed by polarized epithelial cells. For that most *Salmonella* serovars rely on the function of two co-regulated secretion systems encoded by *Salmonella* Pathogenicity Island 1 (SPI-1) and SPI-4. The type one secretion system (T1SS) of SPI-4 secretes the giant non-fimbrial adhesin SiiE, which mediates intimate contact of *Salmonella* to microvilli on the apical membrane of the host cell. The SPI-1-encoded type three secretion system (T3SS) manipulates the actin skeleton and triggers the internalization into epithelial cells. It was shown that SPI-4 deletion mutants are highly attenuated in calf but not in chicken infection models. Therefore, SPI-4 might represent a host-specific colonization factor[(1)].
**Objectives:** We asked whether there is a correlation between SPI-1/4 activity with the *Salmonella* serovar and/ or with the host organism.
**Materials and Methods:** The activity of both secretion systems was determined for a collection of *Salmonella enterica* strains with the focus on isolates adapted to and isolated from cattle (sv. Dublin) and chicken (sv. Gallinarum). The SiiE secretion levels were determined via a SiiE-specific ELISA and transcriptional activities of SPI-4 and SPI-1 were analyzed with luciferase-based reporter constructs. Quantitative RT-PCR was used to determine the mRNA levels of key SPI-1/4 regulating transcription factors. Additionally, the secretion of the two SPI-1 effector proteins SopA and SopB was investigated using Western Blot. The capability to invade polarized (MDCK) and non-polarized (Hela) epithelial cells was assessed for selected serovars with altered expression and secretion levels.
**Results:** Compared to serovar Typhimurium, we were able to detect elevated levels of SiiE secretion for serovars Dublin and Gallinarium. Using different luciferase reporter plasmids in sv. Dublin we could reveal that the increased SPI-1/4 transcription depends on the bacterial host. Two *S.* Dublin strains displayed an enhanced and altered SPI-1 effector secretion. Further characterization of selected *S.* Dublin strains showed increased invasion rates into MDCK and HeLa cells. In contrast, the serovar Gallinarum strains were attenuated in the two infection models.
**Discussion:** SPI-1/4 expression and activity is a serovar-specific characteristic which does not correlate with the site of isolation. We demonstrated that cattle-adapted *S.* Dublin displayed increased activities of SPI-1/4 that correlated with elevated epithelial cell invasion rates. Together with the previous *in vivo* results this adaptation of *S.* Dublin points towards a paramount role of both secretion systems for cattle infections. Further characterization of the underlying regulatory circuits might reveal important mechanisms of *Salmonella* host adaptation.

**Reference**
1: Morgan *et al.* Mol. Micro. 54 (2004)

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**298/ZOP**

**_N_-acyl modified amino sugars alter the expression of Shiga toxin glycosphingolipid receptors of epithelial cells**

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**Introduction:** Shiga toxins (Stxs) of enterohemorrhagic *Escherichia coli* (EHEC), which cause the postdiarrheal hemolytic uremic syndrome, bind to oligosaccharides of globo-series glycosphingolipids (GSLs) exposed from the outer half of the plasma membrane of various types of endothelial cells [1, 2]. However, recent investigations suggest that besides endothelial cells intestine and/or kidney epithelial cells are involved in Stx-mediated diseases. Principally, the capability of up- and downregulation of Stx GSL receptor content in endothelial or epithelial cells offers a convenient tool for the analysis of receptor-mediated cellular damage of target cells caused by the various Stx subtypes.
**Aims:** This study was aimed at unravelling altered expression of the Stx GSL receptors globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer) upon exposure of epithelial cells to *N*-acyl modified amino sugars.
**Materials and Methods**: Various *N*-acyl-D-glucosamine derivatives were synthesized and structurally characterized by NMR. Epithelial cells were grown in serum free medium supplemented with Glc*N*-derivatives using nontoxic doses. Lipids were extracted and neutral GSLs were isolated by anion exchange chromatography. GSLs were separated by thin-layer chromatography (TLC) and Stx receptors Gb3Cer and Gb4Cer were identified by TLC overlay immunodetection and quantified by densitometry [3].
**Results:** Highly purified *N*-acyl glucosamine derivatives were shown being nontoxic in the employed epithelial cell culture systems. After incubation in cell culture medium supplemented with Glc*N*-derivatives, TLC overlay analysis of isolated cellular GSLs with anti-Gb3Cer and anti-Gb4Cer antibodies revealed altered expression of Stx receptor GSLs, which depended on the type of epithelia cells (intestine *versus* kidney) and the applied derivative. Increase as well as decrease of GSL content (and thus of Stx receptors), ranging from highly enhanced to considerably reduced expression (in comparison to control cultures without Glc*N*-derivatives), were obtained and quantified by scanning densitometry of immunopositive GSL bands detected in TLC overlay assays.
**Conclusions:** Here we show that *N*-acyl glucosamines are capable of triggering up- or downregulation of Stx GSL receptor expression of *in vitro* propagated epithelial cells.

**References**

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[3] Kouzel *et al.* (2013) *J Lipid Res* 54, 692-710

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**299/ZOP**

**Genotypic characterization of bovine Shiga toxin-producing *Escherichia coli* (STEC) strains with persistent or sporadic colonization types**

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**Question:** Certain STEC strains not yet identified as human pathogenic EHEC strains can persist in cattle at herd or even at single animal level over several months and may serve as gene reservoir during the genesis of highly virulent zoonotic pathogens. Reduction of human risk for acquiring EHEC infections thus particularly requires strategies to control STEC strains persisting in cattle. We therefore aimed at identifying gene patterns associated with the STEC colonization types in the bovine host.
**Methods:** We monitored 4 cattle farms over 28 months and isolated 85 persistent (pSTEC, shedding $\geq$ 4 months) and 72 sporadically colonizing STEC (sSTEC, shedding $\leq$ 2 months) strains. Additionally, 16 STEC from sampling a cohort of calves were included. Genoserotype and MLST were determined for all 173 strains by whole genome sequencing and the strains were probed with the „*E. coli* Genotyping“ DNA microarray (Alere Technologies GmbH, Germany).
**Results:** While all pSTEC belonged to only 4 genoserotypes (O26:H11, O156:H25, O165:H25, O182:H25), 28 genoserotypes were present in sSTEC with O157:H7 being the most prevalent. The microarray analysis identified 135 unique gene patterns based on the occurrence or absence of 104 virulence-associated genes (VAGs) or VAG-variants. The VAG-patterns clustered with the genoserotypes and MLST types of the strains. Comparing 122 strains with known colonization type and only one representative of each VAG-pattern, pSTEC possessed significantly more often the genes *astA, stx1, eae, lpfA, efa-1/lifA, espB, espJ, nleA, nleB,* and *nleC* ($Chi^2$, $p \leq 0.01$), sSTEC more often *cdtB, stx2,* and *toxB* ($Chi^2$, $p < 0.01$).
**Conclusions:** Microarray analysis including only known VAGs did not allow identification of gene profiles characteristic for a persistent colonization type in bovine STEC. Analysis of the sequencing data is currently underway to differentiate the genomic backgrounds more clearly.

This study is funded by the German Research Foundation (DFG).

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**300/ZOP**

**Switching the O-antigen gene cluster in *Escherichia coli* - an opportunity for Shiga toxin-producing *E. coli* (STEC) to persist longer in cattle?**

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**Question**: Cell-surface polysaccharides are targets for host immune responses limiting the ability of bacteria to survive and persist in their hosts. The O-antigen polysaccharide constitutes the outermost part of the lipopolysaccharide layer. In *E. coli*, the O-antigen biosynthesis gene cluster is flanked by the *wca* gene and the *his* operon. In this study, we compared the whole genome

sequencing data of two STEC and one EPEC strain isolated from cattle with special reference to their O-antigen gene clusters.
**Methods**: The entire genomes of two STEC strains with identical MLST ST 300, but different serogroups (O182:H25 and O156:H25) and an outgroup O156:H8 EPEC strain were de novo sequenced by the PacBio RS II system, assembled and completely annotated.
**Results**: The identical to MLST ST 300 of O182:H25 and O156:H25 was mirrored in the high homology of core genome and virulence-associated genes. The genes of the *stx*1a encoded bacteriophage, the genes of the complete LEE locus including ζ-intimin as well as the flagellar and chemotaxis genes were >99.99% identical in their nucleotide sequences. Instead, fundamental differences were discovered in the O-antigen gene cluster sequences in the region between the *wca* gene and the *his* operon. In contrast, the O-antigen gene clusters of the O156:H8 and O156:H25 strains were sequence-homologous, but significant differences were detected in the remaining genome including the virulence gene patterns (e.g., *stx*-negative, LEE locus with θ-intimin).
**Conclusions**: The results implicate that specific STEC strains replace their O-antigen gene cluster to change their phenotype and to evade the host immune response, possibly to improve their persistence.

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**301/ZOP**

**Decreased STEC shedding by cattle following passive and active vaccination based on recombinant *Escherichia coli* Shiga toxoids**

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**Question:** The principal virulence factor of Shiga toxin (Stx)-producing *Escherichia coli* (STEC), the eponymous Stx, modulates cellular immune responses in cattle, the primary STEC reservoir. We examined whether immunization with genetically inactivated recombinant Shiga toxoids ($rStx1_{MUT}$/$rStx2_{MUT}$) influences STEC shedding and transmission in a calf cohort.
**Methods:** Twenty-four calves were passively (colostrum from immunized cows) and actively (intra-muscularly at 5th and 8th week of life) vaccinated. Further 24 calves served as unvaccinated controls (fed with low anti-Stx colostrum, placebo injected).
During the observation period of one year calves were monitored for humoral immune response by Vero cell assay and cellular immune responses after re-stimulation *in vitro* with whole cell lysates (WCL) of farm-specific *E. coli* strains (stx-positive and negative) as well as toxoid preparations. STEC-shedding was assessed by stx1/stx2-specific multiplex PCR of fecal samples (n=191).
**Results:** Colostral anti-Stx1 and anti-Stx2 were effectively transferred to the calves but antibody titers differed significantly between vaccinated and control group until the16th week of life. In the 25th and 54th week of life, differences vanished and Stx2-neutralising antibodies were no longer detectable. Using CD25 expression by CD4 and CD8 T memory cells as flow cytometry based read-out, T cells from vaccinated animals responded more pronounced to WCL and $rStx2_{MUT}$ re-stimulation *in vitro* than those of control calves. Less fecal samples from vaccinated animals were *stx1* and/or *stx2* positive at 3rd, 16th, 25th, and 54th week of life than samples from control animals (34 % versus 49 % of samples taken over the entire observation period, Fisher's exact, p=0.04).
**Conclusions:** Results presented here imply that vaccination of cattle at calves' age with Shiga toxoids leads to a more pronounced T cell mediated immune response to STEC strains present in the cohort and a sustained reduction in STEC shedding by the animals in cattle herds.

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**302/ZOP**

**Experimental evaluation of faecal *Escherichia coli* as biological indicator of contacts between domestic pigs and Eurasian wild boar**

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**Question:** Domestic pigs and Eurasian wild boar (*Sus scrofa*) share several important viral and bacterial pathogens. Therefore, direct and indirect contacts between domestic pigs and wild boar present a risk of pathogen spill-over and can lead to long-term perpetuation of infection. Biological indicators could be a powerful tool to understand and characterize contacts between wild boar and domestic pigs. Here, faecal *E. coli* were explored as potential biological indicator under experimental conditions.
**Methods:** Within an animal trial domestic pigs (group 2, n=8) were brought into contact with faecal material of wild boar (group 1, n=8). Before and three to five weeks after transmission of faeces fecal samples of both groups were collected, coliforme bacteria isolated, and the bacteria tested by CHEF-PFGE for clonal relatedness. The study was meant as test and calibration phase for potential field studies.
**Results:** Eighty-eight individual *E. coli* clones were detected by *Xba*I restriction and PFGE analysis. Selecting only one isolates representing a distinct clone from an individual faecal sample, 123 *E. coli* isolates were further analysed. Overall, 17 different clones were found in several animals of a group (1 or 2) or both samples from one animal. Additionally, five clones were detected in group 1 as well as in contact group 2.
**Conclusions:** The data gained in our pilot study suggest that faecal *E. coli* can be used as biological indicator of contact between wild boar and domestic pig. Based on these promising results, future field studies will especially target the practicability of *E. coli* microbiome molecular typing as surrogate of contacts at the wildlife-livestock interface.
This study is funded by the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 311931 (ASFORCE).

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**303/ZOP**

**Assessing the removal of *E.coli* during drinking water production using a qPCR based method**

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Assessment of hygienic water quality is based on the cultivation of microorganisms. Techniques used in drinking water production, like artificial groundwater recharge, may convert fecal microbial indicators as *E. coli* to a viable but non culturable (VBNC) state which will not be detected by cultivation-based methods so that

microbial pollution in water supplies may be underestimated. Molecular methods can serve to complement cultivation-based methods regarding detection of non-culturable cells.
In this study the efficiency of filtration techniques used for artificial groundwater recharge and drinking water production was examined. The removal of bacterial fecal indicator *E. coli* depending on common input concentrations and varying filter velocities was analyzed. The investigations were carried out simulating the technical process of slow sand filtration in a pilot plant. *E. coli* was detected by Colilert® Quanti-Tray® and SYBR green-based quantitative *real time* PCR (qPCR).
The results show a total removal of *E. coli* during slow sand filtration under common operating conditions and also simulating a worst case scenario with high bacterial input by surface runoff. The maximum removal efficiency for *E. coli* was 2.6 logs (evidence by Colilert®) and 3.6 logs (detection by qPCR) respectively.
At elevated filter velocities a clear effect of the actual operating conditions was observed. High filter velocities caused a breakthrough of *E. coli* detected by Colilert® as well as by qPCR. After normalization of the operating conditions a complete removal was observed again.
The molecular method and the culture-based method Colilert® showed similar effects. The correlation coefficient between the two methods was 0.95. By qPCR, however, higher results on average of 2 logs were found. Therefore, an integrated method for exclusion of dead cells and free DNA is necessary to applicate molecular methods in the hygiene assessment of water samples.
The results confirm that slow sand filtration can effectively restrain also non-culturable microorganisms even in worst-case scenarios considering the generally acknowledged rules of technology.

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**304/ZOP**

**Isolation and functional characterization of the novel *C. botulinum* neurotoxin A8 subtype**

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**Introduction:** Botulism is a severe neurological disease caused by the complex family of botulinum neurotoxins (BoNT). Based on the serotypes known today, a classification of serotype variants termed subtypes has been proposed according to sequence diversity and immunological properties. However, the relevance of BoNT subtypes is currently not well understood. Here we describe the isolation and functional characterization of a novel *Clostridium botulinum* strain from a food-borne botulism outbreak near Chemnitz, Germany.
**Methods:** The novel strain was subjected to whole genome sequencing and Sanger sequencing. Functional characterization included analysis of binding to mammalian surface receptors, detection of endopeptidase activity by mass spectrometry and testing of the biological activity in a mouse phrenic nerve hemidiaphragm assay.
**Results:** Comparison of the novel toxin's gene sequence with published sequences identified it to be a novel subtype within the BoNT/A serotype designated BoNT/A8. The neurotoxin gene is located within an ha-orfX+ cluster and showed highest homology to BoNT/A1, A2, A5 and A6. Unexpectedly, we found an arginine insertion located in the HC domain of the heavy chain, which is unique compared to all other BoNT/A subtypes known so far. Functional characterization revealed that the binding characteristics to its main neuronal protein receptor SV2C seemed unaffected, whereas binding to membrane-incorporated gangliosides was reduced in comparison to BoNT/A1. Moreover, we found significantly lower enzymatic activity of the natural, full-length neurotoxin and the recombinant light chain of BoNT/A8 compared to BoNT/A1 in different endopeptidase assays. Both reduced ganglioside binding and enzymatic activity may contribute to the considerably lower biological activity of BoNT/A8 compared to BoNT/A1. Despite its reduced activity the novel BoNT/A8 subtype caused severe botulism in a 63-year-old male.
**Conclusion:** To our knowledge, this is the first description and a comprehensive characterization of a novel BoNT/A subtype which combines genetic information on the neurotoxin gene cluster with an in-depth functional analysis using different technical approaches. Our results show that subtyping of BoNT is highly relevant and that understanding of the detailed toxin function might pave the way for the development of novel therapeutics and tailor-made antitoxins.

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**305/ZOP**

**Biofilm formation and autoaggregation studies of HUSEC autotransporters**

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**Introduction**: Enterohemorrhagic *Escherichia coli* (EHEC) naturally occur in the intestinal tract of ruminants which are colonized asymptomatically. Through their feces, EHEC are shed into the environment and can persist on vegetables, in water or on meat products. Humans get mainly infected by ingestion of fecal contaminated food which is not adequately cooked. During EHEC infections several virulence factors are expressed that can lead to severe outcomes such as hemorrhagic colitis (HC) and the life-threatening hemolytic-uremic syndrome (HUS). The mechanisms of human colonization by EHEC are still not fully understood.
Biofilm formation is believed to contribute to the persistence of EHEC in the environment or during infections. Due to enclosure in a self-produced matrix bacteria show enhanced resistance against desiccation, host immune defenses and antibiotics.
**Objectives**: In this study, we investigated the capability of clinical isolates of HUS-associated enterohemorrhagic *E. coli* (HUSEC)[1] strains to form bacterial aggregates and biofilms *in vitro*. Moreover, we examined the expression of the autotransporter Calcium-binding antigen43 homologue (Cah) and EHEC autotransporter A (EhaA) which are believed to mediate aggregation and contribute to biofilm formation. By comparing different *ehaA*/*cah* alleles among HUSEC strains, we aim to functionally characterize these autotransporters with regard to their roles in biofilm formation and EHEC pathogenesis.
**Materials and Methods**: Biofilm formation was studied in a classical microtiter plate assay using crystal violet for biofilm staining. Autoaggregation was checked in distinct time intervals from minimal liquid cultures by OD measurement. These two phenotypes were compared between HUSEC wildtypes and *E. coli* K-12 that heterology express the autotransporter alleles. In addition, the expression of autotransporters could be shown via Western Blot using antibodies raised against EhaA or Antigen43.
**Results**: Only a small subset of HUSEC formed biofilms *in vitro*. 81 % of HUSEC encode for the *ehaA* gene and 31 % for the *cah* gene but not all *ehaA* or *cah* positive EHEC strains do actually express these proteins. The difference in expression could be due to DNA sequence variations of autotransporter alleles among HUSEC.
**Conclusion**: Further studies will help to clarify the roles of different alleles of EHEC autotransporters in biofilm formation and EHEC virulence.

**Reference**

[1]Mellmann et al., *Emerg Infect Dis* 2008, **14** (8), 1287-1290

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**306/ZOP**

**Serological and molecular epidemiology of crimean-congo hemorrhagic fever in ghaemshahr county in the mazandaran province of Iran**

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**Introduction***:* Crimean-Congo Hemorrhagic Fever (CCHF) is a tick-borne viral hemorrhagic fever disease. During the transmission cycle of the disease, ticks play both vector and reservoir roles for the CCHF virus (CCHFV). CCHF is an endemic disease in different provinces within Iran.
**Materials and Methods**: This study describes CCHFV in Ghaemshahr county of Mazandaran Province, a province in the northern part of Iran.By using an ELISA method, IgG antibodies against CCHFV were detected in 4 (4.8%) of 84 sheep sera samples collected from 4 villages.
**Results**: Forty sera were obtained from human in contact with the examined sheep, none of which had IgG antibodies against CCHFV. Molecular analysis on the ticks by RT-PCR detected the CCHFV genome in 1.7% of hard tick samples.Sequence analysis demonstrated that the CCHFV genomes isolated from ticks were 100% identical to those isolated from corresponding livestock.
**Conclusion:** This study confirms the circulation patterns of the virus in this region, so people in close contact with livestock and health care workers should be alerted.

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**307/ZOP**

**Crimean-Congo Hemorrhagic Fever: A Molecular Survey in Damqam district, Semnan Province, Iran**

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**Introduction:** Crimean Congo Hemorrhagic Fever (CCHF) is an arboviral zoonotic disease that is asymptomatic in infected animals, but a serious threat to humans. Numerous genera of ticks serve as both vector and reservoir of CCHF virus. CCHF is an endemic disease in different provinces of Iran.
**Material and Methods:** We tried to catch ticks persistinglivestock (Camel, Sheep, Cow and Goat) in Damqan district in spring 2013.
**Results:** We discovered presence of Rhipicephalusand *Hyalomma* genera of ticks in studied district with 5 species. The most frequent tick was*Rh.sanguineus*. We also found *Hy. marginatum, Hy.dromedarii,Hy.anatolicum*and*Hy. schulzei*. Reverse Transcription-polymerase Chain Reaction (RT-PCR) showed CCHFV in 17 hard tick samples including all identified species.
**Conclusion:** This study confirms the circulation of the virus in the afore-mentioned region and so, humans of this region who are in close contact with livestock including health-care workers should be alarmed. Additional information will be presented in near future.

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**308/ZOP**

**Isolation and characterization of lytic bacteriophages specific for enterohemorrhagic *Escherichia coli* of serotype O104:H4**

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**Introduction**: Enterohemorrhagic *Escherichia coli* (EHEC), a highly pathogenic subgroup of intestinal pathogenic *E. coli* (IPEC), can cause hemolytic uremic syndrome (HUS) as severe post-infective complication. In 2011 the so far biggest foodborne European HUS epidemic occurred in Northern Germany. It was caused by an unusual enteroaggregative *E. coli* (EAEC) of serotype O104:H4 that produces Shiga toxin (Stx) 2. Since antibiotic treatment of EHEC infections is not recommended due to an undesirable release of Stx, an interruption of the infection chain via contaminated food is a conceivable approach. In this context, lytic bacteriophages have been shown to eliminate or strongly reduce contaminating EHEC on various foods such as fresh-cut fruits, vegetables, and lettuce.
**Aim**: Objective of this study is the isolation of *E. coli* O104:H4-specific lytic phages out of water samples from different origin as well as the selection and characterization of candidate phages that could be used for the eradication of this highly pathogenic strain from the food chain.
**Methods**: Lytic bacteriophages were isolated from sterile filtered water samples by selective cultural enrichment with a stx-negative derivative of the outbreak strain from 2011 and *E. coli* MG1655 (K-12) as positive control following a protocol of Merabishvili *et al.* (PLoS One, 2012;7(12):e52709). Candidate lytic phages were isolated, purified and characterized by sequencing. Furthermore, their specificity was evaluated with representative *E. coli* reference strains of different serotypes. Future experiments will elucidate if the phages eliminate or reduce contaminating *E. coli* O104:H4 in water and various foods.
**Results**: In this ongoing project we analyzed 95 water samples of different origin so far (waste water, n=2; water from swimming pools/ponds, n=3; well water, n=6; groundwater, n=13; surface water, n=38; drinking water, n=33). Lytic phages were isolated from wastewater and surface water exclusively (2/2 and 13/38 samples, respectively). As expected, the presence of *E. coli* in water samples correlated with the presence of corresponding lytic phages. Preliminary results indicate, that the specificity of phage isolates varies as shown by their ability to infect the aforementioned indicator strains: a single phage isolate was lytic for *E. coli* O104:H4 exclusively, whereas 10 isolates infected only MG1655 and four phage isolates infected both indicator strains.
**Conclusion**: Lytic Bacteriophages that are able to infect *E. coli* O104:H4 can be isolated from water samples that are contaminated with *E. coli*. Current experiments will help to characterize the isolates in more detail on a genomic and phenotypic level.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

**309/ZOP**

**Know your neighbourhood: Antibiotic resistance profiling of staphylococcal species from livestock environments neighbouring LA-MRSA**

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In this study we focused on the antibiotic resistance profiles of livestock-associated coagulase-negative staphylococci (LA-CoNS) to assess their risk potential for horizontal gene transfer of known or novel antibiotic resistance genes into LA-MRSA. Among 400 LA-CoNS isolates from dust and manure samples in diverse animal husbandries, we identified 20 different staphylococcal species, with *S. sciuri* being the most abundant species (45%), followed by *S. simulans* (14%), *S. chromogenes* (10%), *S. pasteuri* (7%) and *S. haemolyticus* (6%).
High resistance rates were detected for tetracycline (70%), oxacillin and penicillin (65%), fusidic acid (52%), clindamycin (55%) and erythromycin (40%), while for other antibiotics, e.g. rifampicin, vancomycin, gentamicin and quinupristin-dalfopristin, LA-CoNS showed high susceptibility.
Moreover, intrinsic resistance to fusidic acid and a lower susceptibility to moxifloxacin were found among *S. sciuri*, which dominated the population in the livestock environment. We also tested the MIC levels of daptomycin, a last resort antibiotic in the treatment of MRSA infections, and detected an alarmingly high tolerance to daptomycin (average MIC levels of 4 µg/ml) among LA-CoNS and *S. sciuri*. In particular, two *S. sciuri* isolates were demonstrated to exhibit high-level daptomycin resistance. Future work aims at the identification of the molecular mechanism behind the daptomycin resistance phenotype to weigh the danger of horizontal gene transfer to MRSA/VRSA.

**Presentation:** Tuesday, September 29, 2015 from 15:00 – 17:00 in the industrial exhibition.

## MICROBIOLOGICAL DIAGNOSTICS (DVV)

**310/DVV**

**Rickettsioses - serology as challenging diagnostic tool**

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Rickettsioses are caused by obligate intracellular Gram negative bacteria. Rickettsiae (R) are divided into two main groups within the genus Rickettsia by serologic properties: spotted fever group rickettsiae (SFG) and typhus group (TG) rickettsiae. They are among the most neglected vector borne diseases. Due to variable and non-specific clinical signs ranging from subclinical to fatal, they are not easily distinguished from other febrile conditions without specific tests and therefore mostly not included in the differential diagnosis. Direct proof of the causative agent is often difficult due to the lack of sensitive methods, suitable specimens and the limited time frame in which a direct detection is possible. Therefore, even to date, diagnosis is mainly based on case history, clinical findings and detection of antibodies e.g. by immunofluorescence assays. However, such serological diagnosis of rickettsioses is often hampered by significant cross-reactivity within the respective serogroups of rickettsia.
In order to gain more detailed information about the serologic response in human rickettsioses we compared diagnostic results of a commercially available conventional IFA test (Fuller Labs Inc.) with an inhouse microimmunofluorescence (MIF) test containing antigen spots of *R. felis*, *R. helvetica*, *R. monacensis*, *R.slovaca* and *R. raoultii*, known to be prevalent in Germany. Immunological responses in several cases of autochthonous and imported rickettsioses were analyzed to determine differences in their reactivity pattern in the MIF assay.
The results of the suspected murine typhus cases differed significantly from each other. In one case a clear and exclusive serologic response against TG-rickettsiae was observed. Another case, however, revealed cross-reacting IgG antibodies against *R. felis* antigen, as well as broadly cross-reacting IgM antibodies against *R. conorii* and all SFG-rickettsiae included in the MIF test. In a case of rickettsiosis acquired after a tick bite in Germany, MIF testing indicated *R. monacensis* as possible cause by fourfold titer difference in IgM antibodies.
Our results indicate that accurate interpretation of serological results remains demanding, even when combinations of different rickettsial antigens are used for testing. Further studies using confirmed samples ranging from the acute phase of a rickettsial infection to samples obtained in convalescent phase are needed for the improvement of interpretation of serological test results in rickettsioses.

**Presentation:** Monday, September 28, 2015 from 8:30 – 8:45 in room Roter Saal 1.

**311/DVV**

**Validation of a novel *Bacillus anthracis* PCR Kit within the National Laboratory Network for Diagnostic of BT-relevant Agents (NaLaDiBA)**

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**Introduction**: NaLaDiBA is a BBK funded network of national laboratories working on the detection of highly pathogenic agents in extraordinary biological risk situations, for now using real-time PCR only. Since *Bacillus anthracis* is one of the major biological agents with the potential to be used in bio-terroristic events, it is especially important to detect it in environmental samples as fast and as sensitively as possible. The virulence of *B. anthracis* isolates is determined by the presence of two virulence plasmids, namely pXO1 (encoding anthrax toxin) and pXO2 (encoding the capsule). There are reports on *B. thuringiensis* and *B. cereus* also bearing anthrax virulence plasmids and therefore causing anthrax-like disease. Thus a reliable diagnostic tool addressing chromosomal and plasmid-encoded targets is preferable to using three single assays. Therefore the NaLaDiBA consortium developed a *B. anthracis* PCR kit in collaboration with altona Diagnostics GmbH. The result is the RealStar® Anthrax PCR Kit 1.0 detecting the chromosome of *B. anthracis* (target *dhp61*), the protective antigen gene *pag* on pXO1 and the capsular biosynthesis gene *capC* on pXO2. The reaction is monitored by the amplification of an internal control (IC) and its sensitivity was shown by Probit analysis using plasmids.
**Aim**: The aim of this study was to validate the specificity and the usability of this novel *B. anthracis* PCR kit by means of testing the huge collection of different *B. anthracis*, other *Bacillus spp.* and further, non-related bacterial isolates within the NaLaDiBA consortium.

**Methods**: The coordinators of NaLaDiBA invited the participating NaLaDiBA laboratories to list their available bacterial isolates. Afterwards they were equipped with a sufficient number of the RealStar® Anthrax PCR Kits and tested their isolates with the novel kit. PCR results and information about the usability were analyzed.
**Results**: Almost 100 *B. anthracis* and almost 100 related and non-related bacterial isolates were analyzed with the novel *B. anthracis* PCR kit. Preliminary results show high specificity and easy handling compared with using three single assays for the same PCR targets.
**Discussion**: The RealStar® Anthrax PCR Kit 1.0 provides fast and reliable detection of samples suspected to harbor virulent or non-virulent *B. anthracis* or *Bacillus spp.* and is also easy to handle.

**Presentation:** Monday, September 28, 2015 from 8:45 – 9:00 in room Roter Saal 1.

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**312/DVV**

**Direct blood culturing on solid medium for rapid diagnostics of bloodstream infections**

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**Introduction**: Bloodstream infection represents an acute condition, which requires immediate antimicrobial therapy. At this early time-point, microbiological findings are not available and antimicrobials are chosen empirically. However, it has been shown that rapidly available results of identification and antimicrobial susceptibility testing enable earlier adaptation of antimicrobial therapy and improve patient outcome.
**Objectives**: We aimed to investigate whether direct blood culturing on solid medium could provide more timely results compared to the currently widely applied liquid-based automated blood culture systems.
**Materials and methods**: Eleven clinical and reference strains of common pathogens including bacteria and yeasts were each mixed in different concentrations in a Falcon tube with 10 ml human blood taken from healthy volunteers. Written informed consent was obtained from volunteers prior to blood donation. Seeded blood was proceeded using Isolator 10 (Wampole, USA) tubes. After lysis of blood cells and centrifugation, supernatant was removed and sediment was distributed onto solid medium. After the growth became visible, biomass was subjected to matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry for species identification. Additionally, inoculated blood was added to BACTEC aerobic bottles (for yeasts also specific mycosis bottle) and monitored by BACTEC automated blood cultures system until the positive signal.
**Results**: In all cases (i.e. for all bacterial and fungal pathogens in all concentrations used), species identification from directly incubated agar plates was achieved earlier than BACTEC system produced positive signal for growth detection. The mean time difference between BACTEC growth detection and species identification from shortly incubated solid medium was 4.6 hours.
**Conclusion**: Direct cultivation on solid medium provides identification result earlier and more detailed than liquid-based automated system detected growth, which enables only Gram staining at this point. This finding suggests that the usefulness of direct blood cultivation on solid medium should be re-evaluated in the era of rapid microbiology.

**Presentation:** Monday, September 28, 2015 from 9:00 – 9:15 in room Roter Saal 1.

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**313/DVV**

**Comparison of the new PCR/ESI-MS platform IRIDICA with quantitative culture for detection of bacterial pathogens in bronchoalveolar lavage fluids of patients with suspected pneumonia**

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**Objectives**: To compare culture-based standard methods to detect bacteria in bronchoalveolar lavage fluids (BAL) of patients with suspected pneumonia to a new PCR and electro-spray ionisation mass spectrometry (PCR/ESI-MS) platform (IRIDICA).
**Methods**: 115 BAL samples of patients with suspected pneumonia from the Hannover Medical School (MHH), Hannover, Germany, were collected and submitted for quantitative culture. Specimens were processed according to standard operating procedures, culture plates were photographed at high resolution, and remaining material was frozen. Subsequently, archived material was processed using recommended protocols for DNA extraction, amplification and detection using the IRIDICA *Bacterial antibiotic susceptibility and Candida (BAC) assay*® (Abbott Molecular, Des Plaines, Il, USA). Comparisons between culture and IRIDICA diagnostics were performed for potential respiratory pathogens that were detected by culture in concentrations of $10^3$ CFU/ml or higher.
**Results**: Results were available for the PCR/ESI-MS assay approximately 6 hours after the start of processing; positive culture-based results were available after 18 to 72 hours. In 31 BAL specimens that yielded significant results by culture, 42 respiratory pathogenic bacteria were detected at a concentration of $10^3$ CFU/ml or higher. Of these 42 possible pulmonary pathogens, 30 were also detected by PCR/ESI-MS, while 12 potential pathogens were not detected. In a subset of 22 BAL where culture detected a single significant pathogen, detection by PCR/ESI-MS was concordant in 17 cases (missed species: *H. parainfluenzae* (n=2), *Achromobacter xylosoxidans* (1), *S. aureus* (1), *P. aeruginosa* (1)). In the 8 BAL specimens with growth of two different pathogens, PCR/ESI-MS detected at least one of these in all specimens, and both in 3 specimens (missed species: *Citrobacter* sp. (1), *K. oxytoca* (1), *S. marcescens* (2) *Achromobacter xylosoxidans* (1)). One BAL yielded 4 possible pathogens, of which 2 were detected in PCR/ESI-MS.
27 of the tested BAL specimens did not yield any significant bacterial pathogen, but tested positive by the PCR/ESI-MS approach. Potential pathogens exclusively detected by IRIDICA in these BAL included *S. pneumoniae*, *S. pseudopneumoniae*, *H. influenzae*, *H. parainfluenzae* and *S. agalactiae*.
57 BAL samples were negative in both culture and PCR/ESI-MS.
**Conclusion**: The IRIDICA PCR/ESI-MS is a new molecular diagnostic test procedure performed directly on clinical specimens. Here, we show that IRIDICA detected 71% of potential pathogens isolated by culture at a significant concentration (77% in specimen containing a single pathogen). IRIDICA reported potential pathogens in 27 specimens where culture had not yielded significant results. These specimens will be further evaluated by 16S rDNA sequence based analysis.

**Presentation:** Monday, September 28, 2015 from 9:15 – 9:30 in room Roter Saal 1.

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**314/DVV**

**Inferring *Staphylococcus aureus* Virulence and Resistance Traits from Whole Genome Sequences - Comparison of the Alere Indentibac© Microarray and the Respective *In Silico* Typing Scheme**

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**Question:** *Staphylococcus aureus* is a major bacterial pathogen causing a variety of diseases ranging from wound infections to severe bacteremia or intoxications. One extensive molecular typing method for *S. aureus* virulence and resistance is the Identibac© *S. aureus* Genotyping microarray (Alere Technologies GmbH, Jena, Germany). We adapted the respective typing scheme for whole genome sequencing (WGS) data.
**Methods:** 157 diverse human community-associated *S. aureus* isolates from Germany (n = 66), Gabon (n = 36), Tanzania (n = 36) and Mozambique (n = 19) were previously typed with the Alere Identibac© microarray according to the manufacturer's instructions. WGS was performed using the Illumina MiSeq system, subsequent sequence analysis was conducted using SeqSphere+ v2.0 (Ridom GmbH, Muenster, Germany). Predefined query sequences based on the microarray probes were searched in the de novo assembled contigs within a sequence similarity of ≥ 95 % and an overlap of ≥ 99 %. For genes that exhibited too much diversity and would result in false negative WGS results using the applied thresholds (mainly surface proteins involved in host immune evasion), only their presence based on conserved parts of the coding sequences was determined. Genes that were only detected partially were regarded as absent for further analyses.
**Results:** In total, 192 unique resistance (n = 64), virulence (n = 104), regulatory (n = 15) and species identification (n = 9) targets were analyzed by microarray and WGS. The presence of 96.1 % of all targets was equally identified using microarray and WGS analysis (39.7 % present, 56.4 % absent). 3.2 % of all targets were positive in the microarray but negative in WGS. The remaining targets (0.8 %) were detected by WGS only, presumably due to mutations in those sequence parts supposed to bind to the microarray probe. 1.2 % of all targets were incomplete due to premature contig termination. Multilocus sequence typing (MLST) clonal complexes inferred from combinations of microarray targets were confirmed by WGS-adapted MLST. Moreover, SCC*mec* types concluded by the microarray were also confirmed by WGS *in silico* typing. On sequence level, 0.6 % of all targets were found to comprise a frameshift mutation, resulting in potentially altered proteins.
**Conclusion:** *S. aureus* virulence and resistance traits can be reliably inferred *in silico* from WGS. In addition, WGS enables the detection of different allelic variants and pseudo genes.

**Presentation:** Monday, September 28, 2015 from 9:30 – 9:45 in room Roter Saal 1.

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**315/DVV**

**Improvement of DNA isolation from pathogenic fungi by semiautomated platforms**

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Manual extraction of fungal DNA from clinical samples (e.g. blood, respiratory secretions) is time-consuming and laborious. Additionally, the quality and quantity of the extracted DNA is often poor and is greatly dependent on the extraction methods chosen. In search of a semiautomated nucleic acid extraction platform with low to medium sample throughput, we compared the performances of EZ1 (Qiagen), Maxwell-16 (Promega), MagNa Pure Compact (Roche Diagnostics) and Arrow (NorDiag) with two commercial manual procedures regarding their ability to isolate DNA from *Pneumocystis jirovecii* and *Aspergillus fumigatus* from serial dilutions of respiratory patients specimens. Prior to DNA extraction no sample pretreatment of any kind was performed (e.g. sputasol, centrifugation). Detection limit was determined using two commercial real-time PCR assays (*P. jirovecii* Real-TM, Sacace and MycXtra *Aspergillus*, myconostica). Additionally, the total yield of extracted DNA for each platform and dilution was measured using a commercial quantification assay (Quant-iT dsDNA Broad Range assay, Invitrogen). In our hands the Maxwell-16 and MagNA Pure Compact platform showed the best performance for the extraction of fungal DNA from clinical samples with regards to detection limit. Both systems extract DNA from up to 16 samples simultaneously in approximately 45 minutes with minimal hands on time and it is feasible to perform multiple runs per workday. Compared to manual preparation procedures preparation time can be reduced by 30 to 50% without significant increase in costs. Especially the fact that the Maxwell-16 platform uses as little as 150µl of sample volume to extract fungal DNA makes the platform recommendable whenever higher sample volumes are difficult to obtain. Additionally, the quality and quantity of fungal DNA both platforms provide was superior to manual extraction methods, making pretreatment of the samples needless.

**Presentation:** Monday, September 28, 2015 from 9:45 – 10:00 in room Roter Saal 1.

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## EUKARYOTIC PATHOGENS (EKV)

**316/EKV**

**Cell wall integrity signaling in pathogenic fungi.**

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Cell wall stress induces a conserved signaling pathway in fungi. This pathway, the cell wall integrity (CWI) signaling pathway, was subject of extensive studies in the model yeast *Saccharomyces cerevisiae*. The CWI pathway consists of several components such as cell wall stress sensors, Rho GTPases, guanine nucleotide exchange factors and multiple protein kinases. Several lines of evidence suggest a role of CWI signaling in the pathogenicity of fungi. Though, the CWI signaling pathways of pathogenic fungi were only marginally characterized. In this lecture I will briefly review our current understanding of the respective pathway in the major fungal pathogens. A special focus will be set on the link to virulence, resistance against killing by immune cells and antifungal drug susceptibility.
It consists of several components, starting with several stress sensors at the cell surface. Upon cell wall stress these sensors activate downstream Rho GTPases which in turn activate the protein kinase C. The protein kinase C subsequently activates a MAP kinase module. The final MAP kinase is assumed to control transcription factors that regulate the expression of genes involved in cell wall biogenesis. Several lines of evidence suggest a role of CWI signaling in the pathogenicity of fungi. Though, the CWI signaling pathways of pathogenic fungi were only marginally characterized. In this lecture I will review our current knowledge of the pathways of the major fungal pathogens. I will mainly focus the links to named the cell wall integrity (CWI) pathway.
Environmental stress often results
The cell wall integrity signaling pathway of fungi is essential for survival in the presence of was well characterized in the model fungus baker's yeast.

**Presentation:** Wednesday, September 30, 2015 from 8:30 – 9:00 in room Roter Saal 2.

**317/EKV**

**The putative 2,4-dienoyl-CoA reductase of *Leishmania* represents a novel virulence factor**

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*Leishmania* spp., are medically important protozoan parasites that are transmitted as flagellated extracellular promastigotes during blood sucking by sandfly vectors to vertebrate hosts. The parasites are phagocytosed by host phagocytes in which they transform into non-flagellated amastigotes and reside within a membrane-bound compartment known as the parasitophorous vacuole. The comparison of proteomes of amastigotes, purified from their intracellular habitat, to extracellular promastigotes showed that enzymes involved in ß-oxidation of unsaturated fatty acids, such as the putative 2,4-dienoyl-CoA reductase (DECR), are particularly abundant in intracellular amastigotes. These findings were supported by proteome derived predictions for amastigote metabolism. In order to test the relevance of ß-oxidation in amastigotes, we have started to generate *decr*-deficient parasites. *In vitro* and *in vivo* infection experiments demonstrated that *decr*-deficient *L. major* lost virulence. This outcome suggests that DECR activity is essential for intracellular survival and replication. Furthermore, we could partially restore the virulence of *L. major* deficient in DECR by genetic complementation. Phylogenetic analysis of DECR revealed that this protein is present in a broad range of kinetoplastids, including genera *Leishmania*, *Trypanosoma* and *Angomonas*. However, DECR is absent or not entirely detected in extracellular kinetoplastids indicating that this enzyme could be essential for intracellular parasitism. Moreover, *L. major* DECR possess a significant degree of homology with prokaryotic DECRs and no homology with other eukaryotic DECRs, suggesting an early event of prokaryote-to-eukaryote lateral gene transfer during kinetoplastids evolution. In conclusion, proteomics analyses permitted the identification of a novel virulence factor in *Leishmania*, which represents a possible target for the development of anti-parasitic drugs.

**Presentation:** Wednesday, September 30, 2015 from 9:00 – 9:15 in room Roter Saal 2.

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**318/EKV**

**A novel *Candida glabrata* phenotype induced by high $CuSO_4$ concentrations**

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Phenotypic switching in microorganisms is a reversible morphological change based on epigenetic changes. Pathogenic yeasts can use this process to quickly adapt to changes in their environment. In contrast to other morphological transitions which affect whole populations, phenotypic switching occurs often only in a subset of a given population. Well-characterized phenotypic switches are e.g. the mucoid-smooth switch in *Cryptococcus neoformans*, which controls virulence properties, and the white-opaque switch in *Candida albicans*, which controls mating. Until today, two switching systems have been described in the important pathogenic yeast *Candida glabrata*. One can be distinguished by graduated colony colouration on copper containing agar [1] and controls *C. glabrata* virulence properties [2]. The second systems leads to a change from yeast to pseudohyphal growth, corresponding to smooth and irregular wrinkled colonies, respectively [3].

We observed a novel phenotypic switch of *C. glabrata* after exposure to sublethal copper concentrations. The cell morphology roughly resembled the elongated phenotype of *C. albicans* opaque cells. When propagated at standard growth conditions, a subpopulation continuously maintained the elongated phenotype, while a significant proportion of the population switched back to the typical *C. glabrata* yeast growth form. We furthermore found that the phenotype seems largely unconnected to the known switching systems of *C. glabrata*, as the elongated phenotype exhibited all different colony colouration phenotypes associated with the core switching system on copper containing agar. In general, the growth rate of the elongated phenotype is reduced, raising the possibility that this phenotype presents an adaptation to long-term stress exposure.

To obtain more insights into the function of this novel *C. glabrata* phenotype, we started functional analyses, including comparative transcriptional analysis and stress tests under different *in vitro* conditions. With the characterization of this switching system we hope to shed more light on epigenetic regulation and its relation to stress resistance in *C. glabrata*.

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**Presentation:** Wednesday, September 30, 2015 from 9:15 – 9:30 in room Roter Saal 2.

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**319/EKV**

**Differentiation of *Exophiala dermatitidis* strains from various sources by analysing the characteristics of hyphal formation in an *in vivo Caenorhabditis elegans* infection model**

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**Introduction**: The dimorph black-yeast-like fungus *Exophiala dermatitidis* is reported as a cause of fatal phaeohyphomycoses, including infections of the central nerve system of immunocompetent Asian-born patients (PA). In Europe the fungus could be isolated out of the lung of 5-10 % of patients with cystic fibroses (CF).The infection pathway of systemic infections in Asian patients, as well as the role of *E. dermatitidis* as a pathogen in the lung of CF-patients are still unknown.

**Objectives**: In this work we analysed differences between *E. dermatitidis*-strains based on their origin of isolation. We will present our results of differences in hyphal formation and their role in virulence in an *in vivo C. elegans* infection model.

**Methods**: A total of 22 *E. dermatitidis*-strains, separated in groups characterised by location of isolation (CF-patients, PA-patients, environmental isolates), were analysed in morphology. Analyses of micromorphology were performed by plating yeast-like cells on RICE-Agar and incubate them at 35 °C under $O_2$ deficient conditions. Hyphal cells were measured in length after 24 h and 48 h and were evaluated statistically. Synchronised *C. elegans* nematodes were infected via feeding on lawns of yeast-like cells over a period of 24 h and 48 h. The infected worms were moved to a BHI-Buffer-mix and were observed over a period of three days. The characteristic features of *E. dermatitidis* infection in the intestine of *C. elegans* were retained via imaging; statistical evaluation was done using the Kaplan Meier Schaetzer statistical test.

**Results**: The isolates from the PA-strains showed a rapid growth of hyphal structures within the first 24 h with a length in hyphal-cells over 10 µm, whereas the isolates from CF-patients formed hyphal structures with a similar growth of hyphal-cells after 48 h. The strains from environment never showed hyphal-cell growth over a length of 10 µm.

Three characteristic features of *E. dermatitidis* infection in *C. elegans* could be detected: proliferation of yeast-like cells in the intestine of *C. elegans* without any visible hyphal structures; non-invasive hyphal formation growing out of anus, mouth or vulva; invasive hyphal formation with hyphal spikes from inside of the worm through the cuticular to outside. Strains isolated from CF-patients showed more often than the PA-strains a high proliferation of yeast-like cells in the intestine of infected *C. elegans* after an infection period of 24 h. In contrast the isolates from the PA-strain-group showed the highest potential in hyphal formation after an infection period of 48 h.
**Conclusions**: The isolates from Asian patients could be separated from isolates out of CF patents and those of the environment, by means of growth velocity of hyphal structures. Hyphal formation could be shown as a virulence factor in a *C. elegans* infection model and could be mainly assigned to the strain group of Asian patients isolates.

**Presentation:** Wednesday, September 30, 2015 from 9:30 – 9:45 in room Roter Saal 2.

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**320/EKV**
**A novel assay to study protein-protein interactions in filamentous fungi using the C-terminal domain of the *Aspergillus fumigatus* Lah protein that anchors Woronin bodies to the septal pore.**
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Infections by the human pathogenic mold *Aspergillus fumigatus* are a major cause of mortality in immuno-compromised patients.
Hyphae of filamentous fungi are subdivided by septa into so-called compartments. Septal pores connect these compartments thereby allowing communication, e.g. the exchange of molecules. Woronin bodies (WB) are fungal specific organelles that, after wounding, seal the pores and thereby save the fungus still intact compartments.
WB are tethered to the septal pore by Lah, a protein of 5538 amino acids. Its C-terminal 1000 amino acids (LahC) have been shown to contain all information that is required for the targeting and the interaction with a so far unknown receptor at the rim of the septal pore.
Sequence analysis of the particularly conserved C-terminal 500 amino acids indicates several structural elements, e.g. a coil-coiled domain. Using a series of truncated LahC-GFP fusion constructs we have identified a minimal functional LahC domain. Using this domain we have established a novel assay to analyze protein-protein interactions in filamentous fungi and successfully applied it for the interaction between the proteins PalA and PacC.

**Presentation:** Wednesday, September 30, 2015 from 9:45 – 10:00 in room Roter Saal 2.

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**321/EKV**
**Virulence determinants of environmentally acquired pathogenic fungi help to escape from soil amoeba predation**
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Invasive fungal infections in immunocompromised patients are an increasing health and financial burden and present a particular danger as these organisms are not susceptible to classical antibiotics and options for early diagnosis are often poor. While some fungal pathogens like *Candida albicans* are well adapted to a commensal life style within the human host and infection usually results from endogenous sources. Other yeast pathogens and all filamentous fungi are usually acquired from environmental sources. Little is known on how such free-living organisms could have gained the ability to overcome the innate immune system and invade host tissues. *Aspergillus fumigatus* is a classical example of such an environmentally acquired pathogen. In nature, the fungus lives as a ubiquitous saprophyte which has prompted the hypothesis that its virulence potential could be acquired through interactions with predatory microorganisms. Amoeba are professional phagocytes that are abundant in nature and prey on a wide range of microorganisms. Of all amoeba, *Dictyostelium discoideum* has become a leading non-mammalian model for host-pathogen interactions and presents a powerful tool for investigating the evolutionary origin of virulence. It belongs to the myxomycetes which also includes mycophagous species. During *in vitro* confrontations, phagocytic interactions between both organisms showed similarities to *A. fumigatus* encounters with macrophages of the human immune system. While white, naked conidia were rapidly ingested by *Dictyostelium*, uptake of those covered with the green pigment DHN-melanin was drastically reduced. We have further investigated a complex chemical interplay between the two organisms which resulted in delayed fungal germination and induced amoeba aggregation and lysis, respectively. Using LC-MS analysis we identified gliotoxin, a non-ribosomal peptide with immunosuppressive properties, as the major amoebacidal metabolite of *A. fumigatus*. As a further step, we have extended our model to the environmentally abundant amoeba *Protostelium mycophaga*, which is actively feeding on a variety of yeasts species. We will present first results on the food spectrum of this amoeba as well as a common mechanism which helped the fungi to escape predation.

**Presentation:** Wednesday, September 30, 2015 from 13:00 – 13:15 in room Roter Saal 2.

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**322/EKV**
**Fungal genes associated with *Candida albicans* translocation through intestinal epithelial barriers**
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*Candida albicans* can be found as a commensal on mucosal surfaces of most healthy humans. However, under certain predisposing conditions, this fungus can become pathogenic, leading to superficial or even life-threatening systemic infections. Several studies suggest that the major source of *C. albicans* cells during systemic infections is the commensal population of the intestinal tract. However, the molecular mechanisms of *C. albicans* translocation from the gut - as the main reservoir - into the bloodstream, from where the fungus can infect almost all organs, remain to be characterized. The aim of this project is therefore to identify fungal factors and processes associated with damage of and translocation through intestinal epithelial tissue *in vitro* in order to describe potential pathogenicity factors required for intestinal tissue invasion and dissemination *in vivo*. We have screened *C. albicans* mutant libraries for a reduced ability to damage intestinal epithelial cells. We found 170 out of 1929 mutants to cause less damage than the corresponding *C. albicans* wild type. The identified genes have predicted functions in filamentation, biofilm formation, vacuolar or cell wall organization, stress response and transcriptional processes. Importantly, we also found a number of unknown function genes associated with damage of intestinal epithelial cells. Nine of these *C. albicans* mutants are currently phenotypically characterized to elucidate the corresponding gene's role during interaction with intestinal epithelial cells. Moreover, these mutants are analyzed for their invasive potential and effects on the epithelial barrier function in an *in vitro* translocation model to investigate the relation of epithelial damage and *C. albicans* translocation. Next,

transcriptional profiling data of *C. albicans* during *in vitro* infection of intestinal epithelial cells will be used to verify our screening results and to extend the set of genes and molecular pathways associated with intestinal tissue invasion and fungal translocation.

**Presentation:** Wednesday, September 30, 2015 from 13:45 – 14:00 in room Roter Saal 2.

## GASTROINTESTINAL INFECTIONS (GIV)

**323/GIV**

**A transferable plasticity region in *Campylobacter coli* allows isolates of an otherwise non-glycolytic food-borne pathogen to catabolize glucose**

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K. Schmidt-Hohagen[7], D. Schomburg[7], W. Eisenreich[2]
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Thermophilic *Campylobacter* asymptomatically persist in agricultural and domestic animals but are currently the most common cause for severe bacterial gastroenteritis in humans. Contrary to other enteropathogenic bacteria, it is commonly accepted that *Campylobacter* are unable to catabolize carbohydrates except fucose as carbon- and energy sources. However, in our present study we unambiguously found that several *Campylobacter coli* strains are able to grow with glucose as growth substrate. Isotopologue profiling experiments with $^{13}$C-labeled glucose suggested that glucose is metabolized via the pentose phosphate and Entner-Doudoroff pathways in these *C. coli* strains and that glucose utilization not only fuels the energy metabolism but also enables *de novo* synthesis of amino acids and cell surface carbohydrates. In agreement with our isotopologue profiling data, whole genome sequencing of these glucose-catabolizing *C. coli* isolates identified a genomic island located within a ribosomal RNA gene cluster that encodes for all ED pathway enzymes and a glucose permease. Strikingly, we observed *in vitro* that a non-glycolytic *C. coli* strain was able to grow on glucose as sole energy source after natural transformation with chromosomal DNA of a glycolytic *C. coli* by acquisition of the ED pathway encoding plasticity region. Interestingly, growth analysis in line with genome comparisons indicated that glucose catabolism in *C. coli* was preferentially associated with isolates from human and porcine origin. In summary, our study revealed for the first time the ability of a *Campylobacter* species to catabolize glucose and provide new insights into how genetic macrodiversity through horizontal gene transfer shapes the metabolic capacity of these clinically relevant food-borne pathogens.

**Presentation:** Monday, September 28, 2015 from 17:45 – 18:00 in room Congress Saal.

**324/GIV**

***Different gastric microbiota compositions in two populations with high and low gastric cancer risk in Colombia***

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Among the inhabitants of the Colombian state of Nariño, stomach cancer rates in the Andean region around Túquerres are strikingly higher than in the coastal region around Tumaco. This is in contrast to the very similar levels of *H. pylori* infection, but is associated with differences in human and bacterial ancestries. In order to investigate whether bacteria other than *H. pylori* contribute to the differences in susceptibility between the inhabitants of the two regions, we analysed the composition of the gastric microbiota of individuals from both regions (n=20 each). In spite of very high within-population variability, we found significant differences in stomach microbiota between the two populations. We identified operative taxonomic units (OTUs) and phylogenetic clades with significant abundance differences between the two towns. This included two OTUs significantly more abundant in Túquerres, which were identified as *Leptotrichia wadei* and as a member of the genus *Veillonella*, respectively, and 16 OTUs significantly more abundant in Tumaco. Tumaco-specific OTUs included an OTU identified as a member of the genus *Staphylococcus* which was found in 35% of the Tumaco samples. Additionally, we identified OTUs correlated with patient characteristics such as diagnosis of intestinal metaplasia of the stomach epithelium. We also tested for correlation of the microbiota composition with the population, ancestry and *cag*PAI status of the infecting *H. plyori* strains.
Follow-up studies to test candidate bacterial strains for their accelerating or protective effect on the development of *H. pylori*-induced preneoplastic lesions in animal models are under way.

**Presentation:** Monday, September 28, 2015 from 18:00 – 18:15 in room Congress Saal.

**325/GIV**

**Nucleoside uptake in *Vibrio cholerae* and its role in the transition fitness from host to environment**

F. Zingl*[1], D. Vorkapic[1], T. Gumpenberger[1], K. Pressler[1]
S. Lackner[1], A. Seper[1], J. Reidl[1], S. Schild[1]
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The water-born pathogen *Vibrio cholerae* transits between the aquatic reservoir and the human host and consequently adapts to different environmental conditions and nutrient sources along its life cycle. As it became evident recently, *V. cholerae* utilizes extracellular DNA (eDNA) in vivo as well as during persisting in bacterial biofilms. eDNA could be a versatile nutrient source along the different stages of the life cycle. By the use of two extracellular nucleases Dns and Xds, *V. cholerae* can degrade eDNA to the nucleoside level. Transporting nucleosides across cell membranes is conserved in branches of life as this is a major source of de novo synthesis of nucleic acids and secondary metabolites in many cell types. *Escherichia coli* encodes two predominant nucleoside uptake systems, which are part of the CytR (cytidine repressor) regulon: NupG belongs to the Nucleoside:H+ Symporter family, whereas NupC has been characterized as concentrative nucleoside transporter (CNT). The three transporters of *V. cholerae* VCA0179, VC1953 and VC2352 are closely related to NupC. VC2352 represents a bacterial member of the CNT family, which uses a sodium gradient for effective transport like the three representatives nucleoside transporters in humans hCNT1, hCNT2 and hCNT3. Thus, VC2352 could be used as a model to identify candidates for pharmacological nucleoside- and nucleobase-

derived chemotherapy. In this study, we investigated the nucleoside uptake via identification and characterization of the three nucleoside transport systems in *V. cholerae* using growth analysis and uptake assays. Based on our results VC2352 seems to be the dominant nucleoside transporter. Nevertheless, all three transporters are functional and can contribute to the utilization of nucleosides as a sole source of carbon or nitrogen. We found that the transcriptional activity of these three distal genes is promoted or antagonized by Crp or CytR, respectively. The three transporters seem to play an important role in biofilm formation as transporter mutants exhibit higher biomass within static biofilm conditions. According to our results, nucleoside uptake via the herein described transporters enhances fitness upon transition from the host into low carbon environments along the lifecycle of *V. cholerae*.

**Presentation:** Monday, September 28, 2015 from 18:15 – 18:30 in room Congress Saal.

## GENERAL AND HOSPITAL HYGIENE (HYV)

**326/HYV**

**New aspect in norovirus outbreak management: How much isolation is necessary?**

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**Question**: Norovirus infections lead to symptoms of acute gastroenteritis in people of all ages and settings. Due to high virus infectivity, easy transmissibility and its environmental resistance, several norovirus outbreaks can be registered within healthcare settings. Besides other aspects, outbreak management includes patient isolation and cohorting into groups on the basis of symptomatic, exposed asymptomatic and unexposed asymptomatic status. Here we investigate to what extent exposed asymptomatic patients (=contact patients) suffer from symptoms of acute gastroenteritis after exposure to symptomatic patients.
**Methods**: Norovirus outbreaks (as defined in the German act on protection and prevention of infectious diseases in man, Infektionsschutzgesetz [IfSG]) were observed between November 2014 and May 2015 at the University Hospital Muenster, Germany. Stool samples were tested using norovirus RT-PCR in patients with typical gastroenteritis symptoms (vomiting ± emesis ± diarrhoea). Contact patients were defined as those patients, who were exposed to symptomatic patients (independent from length of contact time) and did not develop symptoms within the minimum incubation time of 6 hours. Occurrence of symptoms of these contact patients were noted within 96 hours (mean double incubation time).
**Results**: In total seven norovirus outbreaks were observed between November 2014 and May 2015. 59 patients suffered from symptoms of acute gastroenteritis, 39 of them were tested positively for norovirus. After exposure to symptomatic patients, 11 of 14 contact patients did not develop any typical gastroenteritis symptoms within the average double incubation time of 96 hours. Two stool samples of contact patients with only one out of three typical symptoms were tested negatively. Here underlying diseases were accounted for presented symptoms.
**Conclusions**: The majority of contact patients remained asymptomatic. In the context of norovirus outbreaks it remains to be considered if exposed asymptomatic patients and unexposed asymptomatic patients can be cohorted, while symptomatic patients should be isolated.

**Presentation:** Monday, September 28, 2015 from 8:30 – 8:45 in room Weißer Saal 2.

**327/HYV**

**Silver containing surfaces lack antibacterial activity under dry conditions**

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**Objectives**: There is a need to prevent cross-contamination on inanimate surfaces in healthcare associated environments. A number of surfaces on the market promise to fulfill this need. Most commercial products are based on silver as the antimicrobial compound. In this study we investigated the antibacterial activity of different commercial surfaces as well as pure silver and different copper alloys using a newly developed touch transfer assay modeling fingerprint transmission.
**Methods:** Sterile uncoated ceramic tiles (4.8 x 4.8 cm) were inoculated with *Enterococcus faecium* ATCC 6057 and dried under ambient conditions. Disinfected fingers or gloved hands covered with sterile cotton gloves and tempered by touching blood agar were used to take up bacteria by gently pressing a finger on the precontaminated smooth tile for 10 s. Subsequently, bacteria were transferred by pressing for 10 s on different disinfected surfaces and a sterile ceramic tile as a control. JIS Z 2801 assays were performed to confirm antibacterial activity of surfaces under wet conditions. Bacterial recovery from surfaces was done using sterile wetted foam swabs. Quantitative culture was performed on blood agar at 37 °C for 24 to 48 hours.
**Results:** Following a descriptive transmission protocol a stable transmission rate was observed using disinfected skin as well as tempered sterile gloves. Only minor differences between the transferred numbers of bacteria were observed for 12 independent investigators, each of whom conducted 10 experiments, indicating a highly reproducibility of the new method**.** Surfaces with confirmed antimicrobial activity in the JIS Z 2801 assay were investigated using the new touch transfer model. For these experiments a microbial bioburden of about 1000 CFU of the reference strain was used to be transferred to the respective surfaces by touch transfer. Unexpectedly we observed no significant reduction of bacterial contamination by silver containing surfaces and pure silver. In contrast, all copper containing alloys displayed at least a tenfold reduction of the bacterial load.
**Conclusions:** We successfully established a new, highly reproducible method modeling cross contamination caused by touch transfer. Using the new method we were able to demonstrate that several silver containing surfaces with confirmed antimicrobial activity in the JIS Z 2801 assay lacked effectiveness under dry conditions. This data indicate that the JIS Z 2801 assay is not suitable to evaluate the impact of antibacterial surfaces in the setting of contamination by contact to the human skin. In contrast to silver containing materials copper alloys displayed an antibacterial activity even under dry conditions.

**Presentation:** Monday, September 28, 2015 from 9:30 – 9:45 in room Weißer Saal 2.

**328/HYV**

**Monitoring of *Legionella* in drinking water plumbing systems by cultivation and quantitative real-time PCR**

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**Introduction**: It is necessary to evaluate the hygienic-microbiological situation of a drinking water plumping systems (DWPS) by having access to little information. This in turn has important implications for the design and implementation of monitoring and remediation measures. Using cultural detection methods may underestimate the health risk by disregarding potential infectious bacteria in the VBNC state.

**Objectives**: The aims of the study were to gain a deeper insight into *Legionella* contaminated DWPS and to investigate the potential of quantitative real-time PCR (qPCR) to evaluate health risks caused by *L. pneumophila* in DWPS.
**Methods**: Nine contaminated buildings were monitored for water quality at the outlets (objective b) according to German drinking water ordinance (GDWO) and ISO 19458 (2006) over a period of six month. Additionally, in one building variations of *Legionella* in the course of a day were monitored by sampling every two hours.
*Legionella* spp. was analysed by culture methods following GDWO and DIN EN ISO 11731-2 (2008). After sample filtration and DNA extraction using Chelex Resin 100 and proteinase K (Walsh et al. 1991) qPCR was done with primer and probe sequences for amplification of the *L. pneumophila mip* gene (Shannon et al. 2007, modified for higher heat stability). Detection limit was 20 GU/µL DNA extract and DNA recovery rate was 50%, resulting in a calculated detection limit of 200 GU/L primary water sample volume.
**Results**: Cultivation results show high spatial and temporal variability (up to 4 log) of *Legionella* concentrations over half a year (n = 777) as well as within one day (n = 32, e.g. 10 am: 11,900 CFU/100ml, 8 pm: 18 CFU/100ml). Continuous exceedance of the GDWO technical threshold level at an outlet was rarely detected. Three buildings showed no permanent detectable contamination.Gene concentrations varied from 3 to 6 log within a building, and up to 3 log within one tap. Less than 20% of sampling points in a building were always qPCR positive. Gene detection but failed colony growth indicates VBNC states. Negative qPCR results but colony growth could not always be explained by matrix effects, as internal standards showed.
**Conclusions**: The observed long and short term variation of *L. pneumophila* should result in a re-evaluation of guidelines concerning sampling and monitoring strategies. Due to false-negative qPCR results, qPCR seems currently not suitable to be exclusively used to determine drinking water quality, but can support culture methods in restore contaminated DWPS.
Shannon KE, Lee D-Y, Trevors JT, Beaudette LA, 2007. Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment. Sci. Total Environ. 382, 121-129
Walsh PS, Metzger DA, Higuchi R, 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10, 506-513

**Presentation:** Monday, September 28, 2015 from 9:45 – 10:00 in room Weißer Saal 2.

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**329/HYV**
**Infection control in hospitals - monitoring the progress of the implementation of recommendations in North Rhine-Westphalia (NRW)**
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**Introduction**: Understaffing is an often mentioned challenge in hospital hygiene.
AIM: In 2009 the Commission for Hospital Hygiene and Infectious Disease Prevention (KRINKO) released a recommendation on the organisational and staffing conditions for infection prevention in hospitals .The „Verordnung ueber die Hygiene und Infektionspraevention in medizinischen Einrichtungen“ - HygMedVO regulates hospital hygiene in a legally binding way in NRW since 1989.
**Material and Methods**: In 2011 the Ministry of Health, Equalities, Care and Ageing (MGEPA) and the Hospital Federation North Rhine-Westphalia (KGNW) conducted a survey in all NRW hospitals inter alia on numbers and educational level of infection control staff and participation in the national nosocomial infections surveillance system (KISS). In 2013MGEPA repeated a part of this surveywithin the thirdfederal state-wide survey on MRSA management in hospitals in NRW.
**Result**: While there was a lack of 32 infection control nurses (Hygienefachkraefte) in 2011, in 2013 111 infection control nurses more than mandated were recorded. Even though there may be regional differences in offer and demand in general the needs of infection control nurses in NRW hospitals can be met. Furthermore in 2011 61 % of the hospitals took part in at least one module of KISS; increasing to 77% in 2013. In 2013 the most used KISS module among hospitals in NRW was HAND KISS (62%). Additionally almost 50% of hospitals took part in MRSA KISS or in ITS KISS. Furthermore in 2013 94% of hospitals report to have a microbiological counselling via telephone, but only 54% of hospitals employ a medical microbiologist or hygienist in the hospital.
**Summary**: Between 2011 and 2013 the realisation of the KRINKO recommendations on organisational and staffing conditions for infection prevention has improved. Even if it is not possible to employ medical microbiologists or hygienists in each of almost 400 hospitals in NRW, a quick support of specialists is necessary for effective infection control measures especially in case of continuing nosocomial transmissions (outbreaks). Cooperation in a network of professionals, including infection control doctors (Krankenhaushygieniker), infection control nurses, medical pharmacists, antibiotic stewardship experts, public medical officers, medical microbiologists or hygienists, specialists in environmental medicine, national reference centres and consultant laboratories can be constructive for outbreak management.

**Presentation:** Monday, September 28, 2015 from 17:00 – 17:13 in room Weißer Saal 2.

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**330/HYV**
**Disinfection of gloves: feasible, but pay attention to the combination!**
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**Background**: Compliance with hand hygiene is complicated by indications for hand disinfection in rapid succession during the care of one patient. In these situations disinfection of medical gloves could facilitate workflow and optimize compliance rates.
**Methods**: Therefore we analyzed the efficacy of disinfecting medical gloves comparing five different hand disinfection solutions (Sterilium, Sensiva, Descoderm, Desderman pure, Promanum pure) and three different gloves (Nitril Blue Eco-Plus, Vasco, Latex Med Comfort). The investigation was performed according to DIN EN 1500:2013. For all combinations 10 analysis were performed (N=150) including right and left hand examination, disinfection efficacy after the first and the fifth contamination with *E.coli* K12 NCTC 10538 (DIN EN 1500:2013), recovery rates after the first contamination, reduction efficacy, fingertip culture, and check for tightness.
**Results**: The disinfection efficacy for all combinations was better with gloves than without gloves, respectively. For eight combinations the disinfection efficacy was always >5.0 log10. Sensiva performed best with all gloves followed by Descoderm with only one peculiarity in the fingertip analysis by using the Latex glove. Detailed data on the reduction efficacy and the tightness for all combinations are shown in the figure.
From the glove perspective the Nitril Blue Eco-Plus was disinfectable best with all five disinfecting solutions, whereas Vasco Braun reached disinfectant efficacy of 5.0 log10 with two disinfection solutions and the Latex Comfort reached disinfectant efficacy of 5.0 log10 only with Sensiva, respectively.
In the check for tightness only the Vasco Braun showed no leaks in all samples followed by the Nitril Blue Eco with one, and the Latex Comfort with seven leaks after investigation.
Statistical analysis will we provided.
**Conclusion**: The disinfection efficacy for the different combinations was greater than for the ungloved hands. However, there seem to be relevant differences within both, different gloves and different disinfectants. Thus before giving a recommendation efficacy and the compatibility should be determined.

**Presentation:** Monday, September 28, 2015 from 17:13 – 17:26 in room Weißer Saal 2.

**Figure 1**

**Table: Results of microbiological** (disinfection efficacy of 5.0 log10 and sterile fingertip cultures in N1 =10 samples) **and mechanical** (tightness after investigation in N2= 20 gloves) **analysis**

| Products (brands) | Endpoint | Sterilium | Sensiva | Descoderm | Desderman pure | Promanum pure |
|---|---|---|---|---|---|---|
| Vasco Braun | reduction efficacy 5.0log10 (N) | 9/10 | 10/10 | 10/10 | 9/10 | 10/10 |
| | lowest reduction efficacy | 4.44 log10 | >5.0 log10 | >5.0 log10 | 4.34 log10 | >5.0 log10 |
| | leaky gloves (N) | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 |
| Nitril Blue Eco-Plus | reduction efficacy 5.0log10 (N) | 10/10 | 10/10 | 10/10 | 9/10 | 10/10 |
| | lowest reduction efficacy | >5.0 log10 | >5.0 log10 | >5.0 log10 | 4.84 log10 | >5.0 log10 |
| | leaky gloves (N) | 1/20 | 1/20 | 0/20 | 0/20 | 0/20 |
| Latex Med Comfort | reduction efficacy 5.0log10 (N) | 8/10 | 10/10 | 9/10 | 9/10 | 8/10 |
| | lowest reduction efficacy | 0 Complete cover over (fingertip) | >5.0 log10 | >5.0 log10 2 CFU/ml (fingertip) | >5.0 log10 11 CFU/ml (fingertip) | 3.43 log10 |
| | leaky gloves (N) | 3/20 | 1/20 | 1/20 | 2/20 | 0/20 |

One sample consisted of right and left hand investigation and disinfection testing after the first and the fifth (re-) contamination

## 331/HYV

**Do WiFi-based hand hygiene dispenser systems increase compliance with hand hygiene?**

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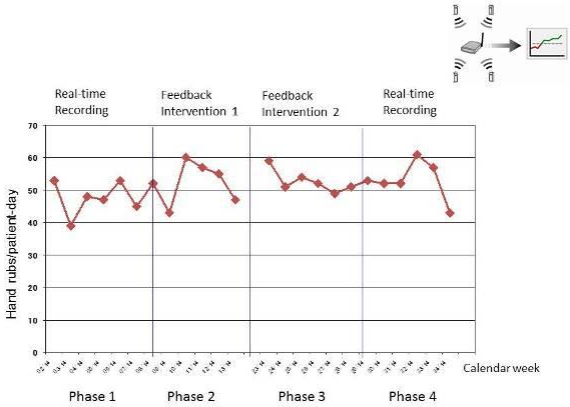
**Introduction**: Despite being the cornerstone in infection control compliance with hand hygiene remains disappointing. New dispenser technologies may help increasing compliance rates.
**Methods**: The impact of WiFi-based hand hygiene dispensers with the possibility to visualize real-time hand hygiene compliance was investigated at a cardio-surgical intensive care unit. The study was divided into four parts:

- Exchange of 50 % of standard dispensers the WiFi compatible ones; information to the staff about the possibility to see the own data and to perform analysis by their own
- Weekly data analysis by the infection control staff followed by presentation and discussion of the results in interdisciplinary team rounds
- Exchange of the remaining dispensers by WiFi compatible ones and continuing the intervention of phase 2
- No intervention; maintenance

**Results**: The introduction of WiFi based dispensers in phase 1 does not affect the number of hand rubs per patient day compared to baseline rates. In contrary, during phase 2 the number of daily hand rubs per patient day increased by about 13 % from about 47 HR/PD to about 53 HR/PD, respectively. Daily hand hygiene activities remained constant during phase 4. The volume for each hand rub continuously increased during the study from about 1,6ml to about 2,8ml, respectively. Statistical analysis will be provided.
**Conclusion**: The exchange from standard to WiFi-based dispensers without any intervention did not increase compliance. The possibility to visualize the own data was not used by the ward-specific staff. Weekly feedback by the infection control team seems to increase hand hygiene activities moderately and the volume of disinfectant per individual hand rub considerably. The effect seems to be sustained. Thus new devices may be an additional tool aiming at increasing compliance rates.

**Presentation:** Monday, September 28, 2015 from 17:26 – 17:39 in room Weißer Saal 2.

**Figure 1**




## 332/HYV

**A curriculum shows improvement of skill sets of infection control nurses (ICN) to facilitate in-house training and sustainable collaboration with infection control link nurses (ICLN). The HYGPFLEG-Project**

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[2]*Klinken der Stadt Cologne gGmbH, Institut fuer Hygiene, Cologne, Germany*

**Background:** Purpose of the HygPfleg-Project was to develop and pilot-test a multi-modular curriculum for ICN. ICN were trained to link didactic and psychological skills with infection control, enabling them to provide in-house training for ICLN using a train-the-trainer-approach. Therefore a questionnaire was developed to assess improvements of didactic skills of ICN during a 4 day curriculum.
**Methods**: A pre-; post-; post-post-test design was used to measure the ICNs' level of didactic competence. Initial instrument development was based on an extensive literature review. Face validity was provided by 5 independent experts. Participants (N = 126) were surveyed using a questionnaire to assess their level of didactic skills with 7 hospital related real world scenarios (case vignettes), each with several options of action. Each option was rated on a 5-point Likert scale from 'strongly preferred' to 'strongly not preferred'. Participants were asked to complete the questionnaire at three time points (day 1 = T0 etc., 3, and 4). A summary score was calculated; score range 0 - 92 with higher scores indicating higher skill sets. A univariate ANOVA with repeated measures was used and the demographics of the participants were analyzed.
**Results**: Demographic data show that 82.4% of participants were older than 41 Years; 85.6% of them were female. 53.2% of them had worked less than 5 years as ICN, 16,2% worked 5-10 years, and 30.6% over 10 years. Almost all of them (94.4%) had worked in a hospital for more than 10 years. Only complete data sets (n = 73) were included in an ANOVA. Descriptive statistics showed at time point one $M(T0)$ = 68.63 ($SD$ = 7.05), at two $M(T1)$ = 71.14 ($SD$ = 5.50) and three $M(T2)$ = 70.75 ($SD$ = 5.40). After the correction of degrees of freedom, the results showed significant differences between the three time points with moderate test strength, $F$ (1.87, 134.52) = 7.06, $p$ = .002, $\eta_p^2$ = .089. Post Hoc analyses showed significant differences between 'T0' and 'T1' ($p$ = .005), 'T0' and 'T2' ($p$ = .025), but no significant differences between 'T1' and 'T2' ($p$ = 1.00). All Post Hoc analyses were Bonferroni adjusted.
**Conclusion**: Although participants were all highly qualified ICN with profound working experience, the results demonstrate a significant improvement of didactic skills over time. It can be concluded that the HygPfleg-Project can be used to successfully improve didactic skills of ICN. This may support the improvement of the quality of educational programs for ICLN and may contribute to sustainable collaboration between ICN and ICLN.

**Presentation:** Monday, September 28, 2015 from 17:39 – 17:52 in room Weißer Saal 2.

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**333/HYV**

**Evaluation of an Infection Control Newsletter (NL) as one HygPfleg-Project-Strategy to ensure sustainable collaboration with infection control link nurses (ICLN)**

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**Background**: The HygPfleg-Project developed and pilot-tested a curriculum for infection control nurses (ICN). ICN were trained to link didactic and psychological skills with infection control, enabling them to provide in-house training for ICLN. Several strategies to ensure long-term collaboration with ICLN were included in the curriculum, one being the implementation of an infection control newsletter (NL).
**Aim**: In mid 2013, HygPfleg-trained ICN of the City of Cologne Hospitals' institute of hospital hygiene implemented a NL. Almost two years later its reception and impact was evaluated.
**Methods**: A 10-item questionnaire was developed and sent to all 3 recipient groups (n=170; 90 ICLN, 25 infection control physicians (ICP), 55 nurse managers (NM)).
Recipients were asked to classify items in 3 categories: "I agree", "I disagree", "I don't know". For each item overall-rates and rates per recipient group were calculated.
**Results**: Overall response rate was 30% (51/170). (ICLN 30%, ICP 12%, NM 38%).
45 (88%) found topics important and up to date (48, 94%). 44 (86%) stated the NL was easy to read, 21 (41%) found it entertaining. 40 (78%) positioned the NL on ward visible to all staff, 27 (53%) discussed topics with colleagues. 37 (73%) considered changes on ward after reading the NL.
32 (63%) reported an increase of personal competence through reading the NL.
13 (25%) reported changes on the ward after reading the NL, for instance improved personal hygiene or hand hygiene, and enforced isolation precautions. 40 (78%) found the publication interval adequate (every 2-3 months and on occasions, e.g. seasonal influenza).
Differences between the evaluation of ICLN and NM were not significant.
**Conclusions**: Overall, feedback can be considered positive. Dissemination is high (78% positioned NL visible to all colleagues on the ward), discussions and hence involvement in infection control are triggered (73% considered changes, 53% discussed contents of NL with colleagues). NL can contribute to a successful collaboration between ICN and ICLN especially as an important and effective tool to quickly disseminate information. A NL alone however is not sufficient to achieve the implementation of infection control measures (only 25% stated actual changes after reading the NL). Public positioning of NL on the ward might be a sucessful supplementary strategy to initiate behaviour change, since information is provided repeatedly and in a non-threatening way to all professionals on the ward.

**Presentation:** Monday, September 28, 2015 from 17:52 – 18:05 in room Weißer Saal 2.

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**334/HYV**

**Reduction of nosocomial infections in intensive care units. An interventional prospective multicenter study**

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**Questions**: Hospital acquired infections on intensive care units accounts for a substantial morbidity and mortality. Aim of this multicenter study was the reduction of nosocomial infections such as catheter related blood stream infections (CRBSI), ventilator associated pneumonia (VAP) and urinary tract infections (UTI).
**Methods**: This prospective observational multicenter study with a preintervention - postintervention design includes 24 intensive care units from primary to tertiary care centers. The study comprimises three phases: preintervention (for baseline), intervention and postintervention over a 4,5year period from 2009 until 2013. The interventional period using a multidirectional approach including continuous staff education with for example promoting hand hygiene compliance, feedback of infection rates and implementing evidence based guidelines. Infections rates were surveyed according to the protocol of the German National Reference Center for the Surveillance of Nosocomial Infections (NRZ). On the basis of these pre- and postinterventional surveillance data, device rates (number of devices per 100 patient days) and device associated infection rates (number of infections per 1000 device days) were statistically evaluated.
**Results**: The device associated infection rates such as CRBSI decreased significantly from 1,7 to 1,3 in the postinterventional period (IRR 0.72, 95% CI: 0,55 to 0,95, p=0.021). For VAP there was significant reduction from 6.1 to 5.2 (IRR 0.81, 95%CI: 0,68 to 0,95, p=0.016) and for UTI a significant reduction from 2.0 to 1.1 (IRR 0.54, 95% CI: 0,42 to 0,68, p<0.001). This went along with a significant reduction of the device rates in the postinterventional period: central venous catheter IRR 0,92, p<0,001; urinary tract catheter IRR 0,95, p<0,001 and endotrachal tube IRR 0.95, p<0.001.
**Conclusions:** An evidence- based multimodal intervention progamm resulted in an overall reduction of device associated infection rates along with a significant reduction of device rates. The increased general awareness of CRBSI, VAP and UTI have had a beneficial effort which seems to be more effective with educational interventions involving more than one active element that are administered over time.

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**Presentation:** Monday, September 28, 2015 from 18:05 – 18:18 in room Weißer Saal 2.

**335/HYV**

**Emergence and Control of Linezolid-resistant Staphylococcus epidermidis (LRSE) in an intensive care unit**

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**Objectives**: Linezolid (LZD), an oxazolidinone antibiotic, is approved for the treatment of infections caused by gram-positive bacteria including *Staphylococcus epidermidis*. We report the emergence of colonisation and infection with Linezolid-resistant *Staphylococcus epidermidis* (LRSE) in an intensive care unit (ICU) at a teaching hospital.
**Methods**: Routine surveillance of nosocomial infections revealed colonisation but one infection with LRSE affecting 14 patients during a 15 month period in an ICU. Isolates were typed using rep-PCR. Infection control measures included active surveillance cultures of all patients and environmental screening. The consumption of LZD in 2014 was reviewed and then restricted. Patients colonised or infected with LRSE were treated under contact precautions starting in August 2014. Infections were classified according to CDC definitions.
**Results**: In July 2014 a cluster of 3 patients with LRSE was suspected. In August two and in September three additional patients were detected, giving a total of 14 patients colonised or infected with LRSE. One patient developed a bloodstream infection (BSI). 13 patients had received LZD prior to colonisation with the resistant strain. Transmission of LRSE in two clusters was confirmed by rep-PCR with five and seven isolates, respectively.
An increase in LZD usage was noted between February and July 2014 in the ICU due to 10 patients with MRSA infections. From October to December 2014 no further patients acquired a LRSE. The usage of LZD had been reduced significantly.
**Conclusion**: The study revealed the emergence of LRSE in an ICU. After a BSI with LRSE in September infection control measures were reinforced, including the restriction of LZD. Treatment of many MRSA infections with LZD seems to facilitate the spread of LRSE. Reduction of LZD usage and strengthening of contact precautions were effective infection control measures.
To retain LZD as a therapeutic antibiotic any usage of this reserve agent should be well considered.

**Presentation:** Wednesday, September 30, 2015 from 8:30 – 8:45 in room Weißer Saal 2.

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**336/HYV**

**Whole Genome Sequencing of *Klebsiella pneumoniae* Isolates from a Neonatal Intensive-Care Unit of a University Medical Center**

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**Background:** Preterm infants are at great risk for developing complications caused by bacterial infections. Therefore, German infection control guidelines recommend weekly screenings for potential pathogens on neonatal intensive-care units (NICU). This group of bacteria also includes *Klebsiella (K.) pneumoniae*. However, the genetic diversity and the clinical relevance of *K. pneumoniae* isolates are incompletely understood.
**Aim**: The purpose of this retrospective study was to assess the genetic diversity of *K. pneumoniae* isolates collected on a NICU over a one year time period and to compare the results of whole genome sequencing (WGS) with conventional typing methods such as Rep-PCR.
**Methods**: *K. pneumoniae* isolates from microbiological screenings of newborns were collected over a one year time frame (October 2013 to November 2014). Commercial Rep-PCR was performed on 120 isolates (at least one isolate per child). Strains showing >95% similarity in PCR patterns were grouped in one cluster. Stable colonization with one strain was presumed, and patients were assigned to the clusters according to their colonizing isolate. Subsequently, whole genome sequencing (WGS) was performed on 43 samples (at least one isolate of each Rep-PCR Cluster) on an Illumina Miseq System. Multi-locus sequence typing (MLST) and extended MLST was performed and resistance and virulence genes were analyzed.
**Results**: *K. pneumoniae* strains could be grouped into 36 clusters according to Rep-PCR patterns, each of which consisted of 1 to 9 isolates from different patients. WGS and MLST analysis revealed that some of the Rep-PCR clusters could be grouped together. Four new sequence types (ST) were found during the MLST analysis and submitted to the Institute Pasteur *K. pneumoniae* database. Two of the new ST are related genotypes, one is completely diverse and one relates to the most common sequence types (like 23 and 258). One strain of one patient developed an ESBL-resistance phenotype after eight weeks being at the ward and after antibiotic treatment.
**Conclusions**: Rep-PCR analysis and WGS of *K. pneumoniae* isolates on two NICUs over one year showed a high diversity of genotypes found on our NICU. Rep-PCR patterns resulted in more clusters than found using WGS probably due to inter-experimental variation. Further analysis of the WGS and epidemiological data is needed to reveal the clinical relevance of the isolates, their resistant patterns and the relationship between prevalence of colonizing factors.

**Presentation:** Wednesday, September 30, 2015 from 9:30 – 9:45 in room Weißer Saal 2.

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**337/HYV**

**Bacterial Colonization of Newborns in a NICU Setting: Results from Three Years of Microbiological Screening**

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**Background**: German infection control guidelines recommend weekly microbiological screening for all newborns treated on a neonatal intensive care unit (NICU). The purpose is to detect colonization with potential pathogens, which could lead to subsequent infections, at an early time point and to realize and prevent transmission events. Little is known about the pharyngeal and anal colonization during the first weeks of life in an NICU setting.
**Aim**: The aim of this retrospective study was (1) to analyze the kinetics and stability of colonization with potential pathogens in newborns, (2) to determine the effect of early antibiotic therapy on the colonization, and (3) to calculate the prevalence of potential pathogens on a NICU.
**Methods**: We employed the results from weekly microbiological screening swabs from our institution over a time frame of three years (4/2012 - 3/2015). Longitudinal colonization kinetics were calculated from inborn children, who did not receive antibiotic therapy. Antibiotic usage data were used to compare children with and without antibiotic therapy during their first week of life. Bed occupancy data were used to calculate the prevalence of potential pathogens on the NICU. Results from consecutive screening materials were analyzed to obtain information about the stability of colonization by different pathogens.

**Results**: 9490 screening swabs (4203 anal swabs and 5287 throat swabs) from 1551 patients were included in the analysis.
Pharyngeal colonization was dominated by coagulase-negative staphylococci (CoNS) during the first two weeks of life, followed by enterococci and viridans streptococci after this time point. Colonization with *Staphylococcus (S.) aureus* and members of the Enterobacteriaceae family gradually increased over the first two months of life.
Enterococci and CoNS were present in half of the anal swabs taken during the first week of life and Enterococci quickly became prevalent in almost all swabs over the following weeks. Enterobacteriaceae became detectable in ca. 80% of the children during the first eight weeks of life with *Escherichia (E.) coli* being the most often isolated organism.
Antibiotic therapy during the first week of life was associated with decreased colonization with *S. aureus* and Enterobacteriaceae on weeks 2 and 3 of life.
Colonization with Enterococci, *S. aureus*, and Enterobacteriaceae showed a high degree of stability, whereas colonization with *Acinetobacter (A.) baumannii* and *Pseudomonas (P.) aeruginosa* was a more transient phenomenon.
Prevalence of potential pathogens on the NICU was highly versatile over the three year period.
**Conclusions**: Microbiological screening of newborns reveals typical colonization patterns in a NICU setting. Potential pathogens such as *A. baumannii* and *P. aeruginosa* are often transient colonizers, which could serve as an argument for high frequency (weekly) screening. Overall prevalence of pathogens on a NICU is highly variable over time, which hints at frequent transmission events.

**Presentation:** Wednesday, September 30, 2015 from 9:15 – 9:30 in room Weißer Saal 2.

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**338/HYV**

**Can Screening predict the causative pathogen of infection in very low birth weight infants?**

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**Aim**: The Robert Koch Institute Commission on Prevention of Hospital Infections (KRINKO) recommends screening of all very low birth weight (VLBW) infants (birth weight <1500g) for colonisation with MRSA, multi-resistant gram-negative rods (MRGN) and *Serratia* spp., *Enterobacter* spp. and *Pseudomonas aeruginosa.* Aims of the screening are: 1. to adapt initial antibiotic therapy to the colonising (resistant) bacteria and 2. to monitor transmission on neonatal intensive care units (NICU). We evaluated the results of this screening in our neonatal intensive care with regard to these aims.
**Methods**: Based on the surveillance data obtained in 2014 using the NeoKISS system, we identified VLBW infants with infections according to NeoKISS criteria and correlated the pathogens isolated from clinical samples with the pathogens isolated by KRINKO screening. Additionally, for all VLBW infants with positive screening results, we analysed if there was evidence of pathogen transmission.
**Results**: In 2014 72 infants with a birth weight <1500g were treated in the NICU. According to NeoKISS criteria nine of these infants developed clinical signs of infection: one baby had pneumonia and necrotizing enterocolitis (no pathogen detection from clinical samples) and eight babies had catheter related blood stream infections. In six of these cases pathogens were isolated from blood cultures and all were gram-positive cocci (*S. epidermitidis* (2), *S. capitis* (3), *S. haemolyticus* (1), in two cases enterococci were detected in addition to staphylococci). Eight of the nine infants with signs of infection had previously been screened for colonisation. Three neonates were rectally colonized: One was colonized with *P. aeruginosa*, one with two different *E. cloacae* strains (wild type and 2MRGN) and one with *E. cloacae* and *E. coli* (2MRGN). No baby had an infection due to the pathogen detected by screening. 0f 63 neonates documented in NeoKISS without infection, seven were colonized with pathogens detected by screening (one 3MRGN *E. coli*, one 3MRGN *K. pneumoniae*, one MRSA, 3 *P. aeruginosa*, 3 *E. cloacae*). One baby was colonized with *P. aeruginosa* and 3MRGN *K. pneumoniae.* None of them had infection due to these pathogens. Median time to first detection of colonisation for all neonates with colonisation was 40 days. There was no evidence for transmission between the infants during the study period (same strain, overlapping time of hospitalization).
**Conclusion**: Screening of VLBW infants according to KRKINO on NICU
- did not predict the causative pathogen in cases of infection
- did not reveal transmission during the study period

**Presentation:** Wednesday, September 30, 2015 from 9:45 – 10:00 in room Weißer Saal 2.

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**339/HYV**

**Hospital epidemiology and molecular characterization of vancomycin-resistant *Enterococcus faecium* bacteraemia strains from a university hospital**

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Vancomycin-resistant *Enterococcus faecium* (VRE*fm*) has emerged as an important nosocomial pathogen worldwide because of its rapid spread, limited treatment options and high-level morbidity and mortality especially in intensive care and neutropenic patients. The aim of this study was to characterize the molecular epidemiology and virulence factors of VRE*fm* from bacteremia cases from 2006 to 2015. A total of 23 VRE *E. faecium* strains were available for further analysis to determine the genetic relatedness using rep-PCR (DiversiLab), PFGE, MLST, and MLVA; the presence of both vancomycin resistance genes vanA and vanB, and virulence factors such as enterococcal surface protein (esp), hyaluronidase (hyl), and collagen adhesion (acm).
MLST was completed on all 23 isolates and 10 sequences types were identified, all exept one belonging to the clonal complex 17 (CC17). MLVA, PFGE and rep-PCR analyses are still in progress. The vanA gene was present in 5/23 and the vanB gene in 18/23 isolates. The distribution of the esp, hyl, and acm genes showed 19 esp positive, and 16 hyl positive and 23 acm positive isolates. Only two strains were negative for both virulence genes esp and hly.
In sum our analysis of the molecular epidemiology and genetic relatedness by different assays revealed that our VRE*fm* bacteremia strains represent at least 10 different MLST types and therefore are not associated with the dissemination of particular epidemic clones. The suitability of the different molecular typing methods for the analysis of VRE*fm* hospital epidemiology will be critically discussed.

**Presentation:** Wednesday, September 30, 2015 from 8:45 – 9:00 in room Weißer Saal 2.

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**340/HYV**

**Molecular typing of local VRE excludes spread of endemic VRE strains**

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Enterococci reside in the human colon and are typically transmissed via stool contamination and on inanimate hospital surfaces. Although they do not express a high amount of virulence factors their environmental resistance, biofilm forming potential and the acquisition of antibiotic resistances have increased their significance as nosocomial pathogens detected in blood stream infections and infections caused by medical devices. Furthermore, acquisition of resistance to vancomycin leaves only limited and expensive options for treatment; increased spread of vancomycin-resistant enterococci (VRE) among hospitalized patients aggravates this problem. It has previously been proposed that the nation-wide increase in VRE in the hospitals might be due to endemic spread of VRE in the population. Here, we present the results of a local study where we analyzed the epidemiology of VRE during a study period of one year. *rep*-PCR analysis with Diversilab was used to determine strain similarity and was found to be a useful, rapid and easy-to-handle tool. Finally, comparison of different departments revealed that transmissions can occur within a department. However, no relevant strain homology was observed in different departments. Lastly, analysis of our data excludes the presence of endemic strains within the local population.

**Presentation:** Wednesday, September 30, 2015 from 9:00 – 9:15 in room Weißer Saal 2.

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**341/HYV**

**Multiplex PCR testing during a gastroenteritis outbreak attributed to Norovirus provided important additional information which influenced infection control measures.**

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**Background**: In February 2015 an outbreak of gastroenteritis was detected in a 38-bed (double rooms only) internal nephrology/gastroenterology ward of a 752-bed maximum care hospital. In total 18 patients and 4 staff members were affected. Stool samples of all patients were analysed using multiplex PCR.
**Aim**: Aim of the study was to determine whether broad diagnostics during an outbreak attributed to *Norovirus* infection can produce important additional information that might influence infection control measures.
**Methods**: Case definition for suspected *Norovirus* infection was sudden onset of diarrhoea and/or vomiting.
After testing a stool sample of the first symptomatic patient positive for *Norovirus*, samples of patients who subsequently developed symptoms matching the case definition were analysed by multiplex PCR testing. The multiplex PCR essay included *Clostridium difficile* toxin A and B (CDT A/B). Stool samples of symptomatic staff members were not obtained.
Infection control measures included single-room or cohort isolation of patients, splitting of the ward in segments for symptomatic and non-symptomatic patients, assigning of staff to segments, personal protection equipment for staff and enforced cleaning and disinfection.
**Results**: Of all 18 patients who matched the case definition, 5 (28%) were tested positive for *Norovirus*, including the index patient. 3 (17%) patients were tested positive for CDT A/B and 2 (11%) patients were tested positive for *Norovirus* and CDT A/B. One (6%) patient was diagnosed with EPEC (enteropathogenic *Escherichia coli*). 7 (39%) patients were tested negative for all pathogens included in the multiplex PCR essay.
**Conclusions**: The RKI suggests that after testing 5 patients positive for *Norovirus* during an outbreak, all further cases of gastroenteritis with similar symptoms and of the same cohort need not be tested. Here 12 patients matched the RKI-criteria for no further testing. Of these 12 patients, 2 were tested positive for *Norovirus*, one was tested positive for CDT A/B, one was tested positive for CDT A/B and Norovirus and one was tested positive for EPEC. In 7 cases no pathogen was detected. Especially the detection of CDT A/B clearly influenced infection control measured since cohort isolation of *Norovirus* and *Clostridium difficile* (C. diff.) can lead to *C. diff.* transmission. Also, an early diagnosis of *C. diff.* infections is important to start the required anti-infective therapy.

**Presentation:** Wednesday, September 30, 2015 from 13:30 – 13:45 in room Weißer Saal 2.

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**342/HYV**

**Control of Multidrug Resistant *Pseudomonas aeruginosa* by Environmental Disinfection and Surveillance**

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**Background**: Severely immunocompromised patients are at great risk of developing nosocomial infections with *Pseudomonas (P.) aeruginosa*, which is often preceded by colonization with the pathogen. This is complicated by the increasing occurrence of multidrug-resistant (MDR) isolates. Water-associated devices such as sinks, water drains, and toilets play an important role as environmental sources for this pathogen. Since 2010, patients on a hematologic ward in our institution are infrequently found to be colonized or infected by Carbapenem-resistant *P. aeruginosa* isolates, which express Metallo-Betalactamase (MBL) genes. This has led to the installment of extensive infection control measures including patient screening and isolation as well as subsequent disinfection of rooms after occupancy by a MBL-*P. aeruginosa* positive patient.
**Aim**: The aim of this descriptive analysis was to assess the environmental contamination with *P. aeruginosa* during different cleaning and disinfection regimens as well as its correlation with colonization of patients with MBL-*P. aeruginosa*.
**Methods**: After noticing a series of new colonizations and infections with MBL-*P. aeruginosa* in August 2014, a new environmental surveillance was installed, which included weekly microbiological screening of sinks, toilets, and shower drains of all patient rooms. Detection of MBL-*P. aeruginosa* in these specimens led to immediate cleaning and disinfection of the bathroom and re-sampling. Two rounds of thorough environmental cleaning and disinfection were performed in November 2014 and February 2015 on all rooms by an external company. Standard toilets were replaced by rimless toilets basins in May 2015.
Microbiological screening results from rectal swabs taken on admission and at least weekly thereafter were used to determine colonization rates in patients. Environmental specimens were analyzed for the presence of *P. aeruginosa*.
**Results**: Thorough environmental cleaning and disinfection of sink siphons, shower drains and toilets lead to a decrease of the occurrence of *P. aeruginosa*.
Sampling three locations (drain pipe, flush water pipe, basin) during replacement of all 20 toilets revealed the presence of *P. aeruginosa* in 29/60 (48,3%) of the sampled locations. 10/29 (34,5%) of these isolates harboured MBL-genes.
The combined efforts correlated with a decrease of MBL-*P. aeruginosa* colonizations in patients without any observed infections.
**Conclusions**: Aquatic reservoirs play an important role as sources for nosocomial infections with MBL-*P. aeruginosa*. Our results show that these pathogens can be recovered from the wastewater tubing, from where retrograde contamination can likely occur.

Therefore, efforts should be undertaken to identify these contaminations and to quickly remove them in order to minimize patient exposure. Patient screening demonstrated in our case, that thorough environmental cleaning and disinfection correlates with reduced colonization rates in patients.

**Presentation:** Wednesday, September 30, 2015 from 14:15 – 14:30 in room Weißer Saal 2.

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**343/HYV**

**Influenza 2015: Management of hospitalized patients in a season with high influenza activity**

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Because of a high infectivity with a basic reproductive rate between 2 and 6, rapid identification of influenza cases in a hospital setting is important for clinical and hygiene management. In the present work, we therefore investigated the clinical characteristics of influenza from the season 2015. In addition, a strict hygiene management with a bundle was followed and its effect on the rate of nosokomial influenza cases was observed.
**Methods:** in this observational study, patients with respiratory symptoms were enrolled which were hospitalized during January and March 2015. Each patient was tested by a rapid PCR for influenza A / B and clinically examindend by a standard protocol for characteristics according to the case definition for influenza: fever / cough / headache / muscle aches / pneumonia / ARDS. Patients with a positive influenza test were managed in line with the national hygiene guidelines including the following bundle: strict isolation of patients or cohorting / hand hygiene / using gloves / use of surgical masks in the context of a direct contact to patients However, because of an increasing rate of influenza patients during the observational period, hygiene management was enforced: hospital staff was provided to use surgical masks from the beginning until the end of each working day. Effectiveness of measures was evaluated by monitoring the rate of nosokomial influenza infections (CDC criteria).
**Results:** A total of 235 patients (mean age 77 years) with a positive influenza A / B PCR were enrolled (Distribution of influenza A strains: pandemic H1N1: 8 % / seasonal H1N1 / H3N2: 82 % / influenza B: 10 %). With regard to clinical characteristics of influenza A, only the frequency of cough was increased when compared to a PCR negative group with respiratory symptoms (48 % vs. 23 %, $p<0.01$). Body temperature, frequency of headache, malaise, pneumonia did not differ between both groups. During the observational period, 47 patients acquired influenza by nosokomial transmission. However, the ratio of nosokomial infections / community acquired influenza turned from 0.48 to 0.19 after introducing the use of surgical masks for the whole staff from the beginning until the end of each working day.
**Discussion:** During the influenza season 2015, discrimination of influenza from other respiratory infections in elderly hospitalized patients was only possible by a combination of clinical signs and laboratory testing. Furthermore, the general use of surgical masks by hospital staffs seem to be most effective for prevention of nosokomial infections in a season with a high influenza burden.

**Presentation:** Wednesday, September 30, 2015 from 13:45 – 14:00 in room Weißer Saal 2.

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**344/HYV**

**First Results of the German consulting center for infection control outbreak registry**

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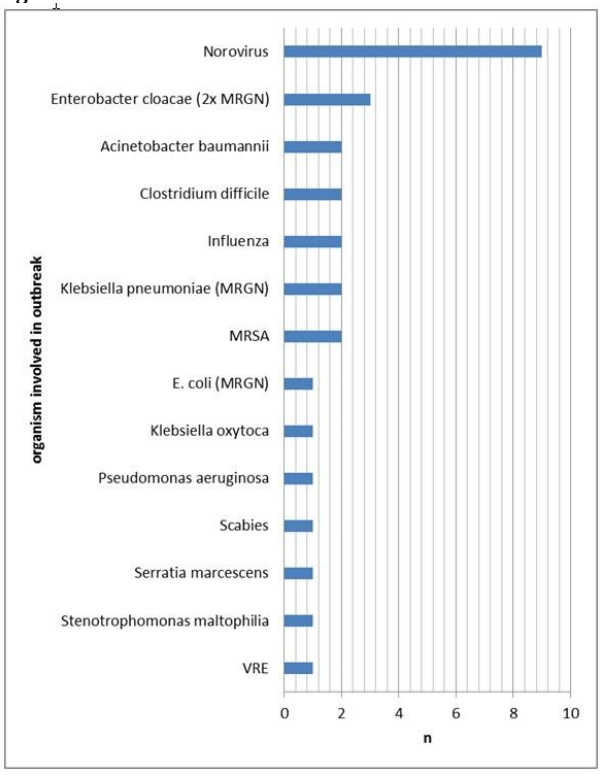
**Question**: Outbreaks of infectious diseases and/or colonization pose an increasing burden on hospitals and the health system in general and can be a threat to patient safety.
Methods: At the end of 2013 we implemented a quality assurance registry of outbreak investigations (> more than 5 patients or health care workers involved and/or organism of special infection control interest) performed by the Deutsches Beratungszentrum fuer Hygiene (German Consulting Center for Infection Control and prevention) in Freiburg. Now we analyzed the registered outbreaks until January 2015.
**Results**:Figure 1 shows an overview of the organisms involved in the 29 registered outbreaks. Norovirus was the leading causative organism and gram negative bacteria dominated the group of bacterial outbreaks. Outbreaks lasted between 6 and 185 days. 24% of outbreaks were related to colonization only. Within 29 outbreaks we had 187 infected patients, 50 colonized patients und 92 infected health care workers (64 x norovirus, 20 x influenza, 8 x scabies). No deaths were recorded. Several risk factors and improvement potentials for future outbreaks could be identified.
**Conclusion**:A structured and goal directed outbreak management especially in the initial phase of an outbreak is essential for an efficient and fast termination of an outbreak. Lack of staff compliance with vaccination, misuse of personal protective equipment and lapses in absence from work for the required time can play an important role for prolonged outbreak situations esp. with viral outbreaks and scabies.

**Presentation:** Wednesday, September 30, 2015 from 13:00 – 13:15 in room Weißer Saal 2.

**Figure 1**

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**345/HYV**

**Detection of Outbreaks in Microbiological Data – a Comparison**

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**Introduction**: Clusters of pathogens with an epidemiological link are called outbreaks. Early detection of outbreaks is very important for infection control professionals. So they can initiate infection control measures on time and limit the range of the outbreak. There are different methods to detect outbreaks in microbiological data. The aim of this investigation is to compare different methods for outbreak detection and to find a practical method for the clinical practice.

**Method**: The microbiological data from a tertiary hospital were used for the analysis. We investigated the frequency of six different pathogens over a period of 3 years. There were used three different algorithms to detect clusters of pathogens. Detected clusters were validated based on three known outbreaks.

Method 1: Prediction intervals were calculated from historical data. Different prediction intervals were used depending on the frequency of a pathogen within the analyzed data. A normal distribution prediction interval for common pathogens, a poisson distribution prediction interval for rare pathogens and a score prediction interval for very rare pathogens. (1)

Method 2: Secondly the method of cumulated sums (CUSUMs) was used – a method from statistical process control. Different methods for different frequencies of pathogens were used, one for common and one for uncommon pathogens. (2)

Method 3: The last implemented method is the Farrington algorithm. Thereby a regression model is adapted. It is adjusted for over dispersion, season and a timely linear trend. (3)

**Results**: All algorithms detected all three known outbreaks.

Outbreak of vancomycin resistant Enterococcus was detected by the method of predictione interval at the same time like conventional methods. The method of CUSUMs detected the outbreak earlier.

Outbreak with multiresistant Acinobacter baumanii started at 14 October 2014 and was detected with all methods at the same time point.

3rd known outbreak, an outbreak with carbapenem-resistentant Klebsiellie pneumoniae, started at 02 October 2013. All three methods detected the outbreak in April. At this time, the outbreak was not known in hospital.

**Discussion**: The algorithms detected all known outbreaks at the same time or before they were detected in the department. Prospectively the selection of the algorithms should be done automatically. The selection should reduce the number of false alarms, but still detect all outbreaks.

**References**

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**Presentation:** Wednesday, September 30, 2015 from 13:15 – 13:30 in room Weißer Saal 2.

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**346/HYV**

**Whole Genome Sequencing Enables Exact Delineation of Cluster-Related *Acinetobacter baumannii* Isolates in a Nosocomial Setting**

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**Background:** Multidrug resistant (MDR) *Acinetobacter baumannii* are increasingly detected in clinical samples and challenge infection control particularly in intensive care units (ICU). To investigate whether these pathogens are transmitted nosocomially, in the past pulsed field gel electrophoresis was frequently used as a typing method. Today, whole genome sequencing (WGS) emerged as a novel tool for bacterial typing. Here, we investigate the ability of WGS to elucidate the epidemiology of a cluster of MDR *A. baumannii*.

**Methods**: During routine surveillance, we noticed an increase of *A. baumannii* cases at a single ICU at the University Hospital Muenster. Within four months, eleven MDR and two susceptible *A. baumannii* were detected among patients and, initiated by the infection control team, environmental swabs were taken. All *A. baumannii* were subjected to WGS using the MiSeq platform (Illumina Inc., San Diego, USA). After sequencing, quality-trimming and de novo assembly all coding regions were extracted and compared in a gene-by-gene approach (MLST$^+$) using the SeqSphere$^+$ software version 2.0 beta (Ridom GmbH, Muenster).

**Results**: In total, 16 *A. baumannii* isolates (eleven from patients [one infection and ten cases of colonization] and five environmental samples) were sequenced. WGS and subsequent analysis using a gene-by-gene analysis of 3319 genes ("MLST$^+$") present in all strains included resulted in one cluster comprising eleven isolates (eight human and three environmental isolates) with an identical allelic profile; two additional isolates differed in only one gene. One patient's MDR *A. baumannii* and two further environmental isolates were clearly separated from the cluster (>1600 genes differed). One additional *A. baumannii* isolate that was detected after the end of the study period was genetically unrelated to the cluster fostered by the easy portability and storage of data.

**Conclusion**: In summary, WGS in combination with MLST$^+$ yielded clear-cut differentiation of cluster-related and unrelated isolates. The typing results enabled the implementation of targeted control measures to prevent additional infections. One future challenge is the establishment of a universal nomenclature to facilitate the inter-laboratory comparability of WGS-based typing data.

**Presentation:** Wednesday, September 30, 2015 from 14:00 – 14:15 in room Weißer Saal 2.

# INFECTION IMMUNOLOGY (IIV)

**089/IIV**

**Are specific antibodies protective in *S. aureus* infection?**

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U. Voelker[2], M.- C. Roghmann[5], B. C. Kahl[6], E. D. Renner[4]
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*Staphylococcus aureus* is one of the leading human pathogens causing skin and soft tissue infection, as well as blood stream infection and consequently sepsis. The increase of antibiotic resistant *S. aureus* strains and the lack of new antimicrobial agents illustrate that alternative therapeutic strategies are urgently required. One approach is the development of an anti-*S. aureus* vaccine. Currently all clinical vaccine trials have failed and the question remains if specific antibodies are at all able to protect against *S. aureus* infection. To address this question we quantified *S. aureus* specific IgG in human sera.

Sera from different patient cohorts and from healthy individuals were analyzed for IgG binding to extracellular *S. aureus* proteins of an USA300Δ*spa* mutant by Simple Western assays and IgG binding to *S. aureus* cells by ELISA. Antigen specific IgG was determined by Flexmap technology using 64 recombinant *S. aureus* proteins.

*S. aureus* bacteremia patients with high amounts of *S. aureus* specific IgG at the beginning of disease showed a lower risk for developing sepsis than patients with low amounts. Information of IgG binding to eight recombinant *S. aureus* proteins enabled stratification of sepsis and non-sepsis patients with a principal component analysis. Hyper-IgE-syndrome (HIES) patients who carry heterozygous STAT3 mutations are highly susceptible to *S. aureus* infections and had low amounts of specific IgG. IgG substitution was beneficial to the clearance of their *S. aureus* infection signs in spite of the persisting Th17 defect. In contrast, cystic fibrosis (CF) patients showed a robust specific IgG response. Although CF patients have high loads of *S. aureus* in their airways, they are usually protected from *S. aureus* sepsis.

The debate about the protective potential of specific antibodies in *S. aureus* infection is ongoing. Cellular immunity is considered to be most important for protection against *S. aureus* infection. Our data, however, show that high levels of *S. aureus* specific serum IgG are associated with clinical protection. Furthermore, specific antibodies can mediate protection even in patients with low Th17 cell counts. These data encourage further vaccine developments based on the induction of specific antibodies.

**Presentation:** Monday, September 28, 2015 from 9:30 – 9:45 in room Congress Saal.

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**347/IIV**

**The human adaptive immune response to *Staphylococcus aureus* - the high cost of control**

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S. Michalik[6], F. Schmidt[7], U. Voelker[6]
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**Question:** *Staphylococcus aureus* is a dangerous pathogen both in hospitals and in the community. Due to the crisis of antibiotic resistance, there is an urgent need of new strategies to combat *S. aureus*-infections, such as vaccination. Since vaccine trials have failed, it must be questioned whether the human adaptive immune system is at all able to control *S. aureus*.

Each of us is frequently or even continuously in contact with *S. aureus*. This means that in most cases equilibrium can be maintained between microorganism and host for many years before, in a minority of individuals, *S. aureus* turns into a pathogen. Dissecting the natural adaptive immune response to *S. aureus*, therefore, promises insight into its protective potential.

**Methods:** Because of the extraordinary complexity and diversity of the species *S. aureus* we have opted for a personalized immune proteomics approach to characterize the antibody response to *S. aureus*. Studying *S. aureus*-specific human T-cells is challenging because bacterial products interfere with standard T cell assays. We have generated recombinant *S. aureus* proteins to probe the human T cell memory of *S. aureus*, taking the antibody binding patterns as a lead for antigen selection. Finally, a prospective clinical study was conducted to study the specific antibody response to *S. aureus* blood stream invasion. A bead-based multiplex assay (FlexMAP®) with 64 recombinant *S. aureus* proteins was developed for this purpose.

**Results:** First, we observed *S. aureus* strain-specific IgG responses in healthy adults as well as bacteremia patients showing that the antibody response to *S. aureus* is antigen-driven, most probably requiring T cell help. Second, we found a robust T cell memory response in healthy adults with high frequencies of T cells being activated by single *S. aureus* antigens. The whole *S. aureus*-specific T cell pool was estimated to comprise 3.6% of peripheral blood T cells with around 30-fold differences between individuals (range 0.2-5.7%). When exposed to *S. aureus* antigens, the T cells released predominantly but not solely Th1/Th17 cytokines. Finally, a prospective study in patients with *S. aureus* infection complicated by blood stream invasion revealed that robust immune memory of *S. aureus* - reflected by strong serum IgG antibody binding to *S. aureus* antigens - was associated with clinical protection from sepsis.

**Conclusions:** In summary, our findings show that the immune control of *S. aureus* comes at a high cost to the immune system. They lend support to the notion of a vaccine to protect against the most serious complications of *S. aureus* infection.

**Presentation:** Wednesday, September 30, 2015 from 8:30 – 8:45 in room Congress Saal.

**348/IIV**

**Hypoxia inducible factor α (HIF-1α) controls granulysin expression in human T cells**

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**Question:** Granulysin produced by cytotoxic lymphocytes is a major component of the adaptive immune response to restrict growth of intracellular bacteria. The biologically active 9 kDa form of granulysin is a saposin-like lipid-binding protein that binds to the bacterial cell wall and allows the entry of granzymes into the bacterial cytosol. Granzymes provoke bacterial killing by reactive oxygen species. Since we have recently shown a hypoxia mediated up-regulation of granulysin in human T cells we asked whether this finding is mechanistically linked to HIF-1α. HIF-1α is a transcription factor which is known to be a key environmental and metabolic sensor that controls T cell biology.
**Methods:** Cell culture. Preparation of antigen presenting cells and short term T-cell lines. IFNγ enzyme-linked immunosorbent assay. Quantitative LightCycler PCR. Flow cytometry. Nuclear and cytoplasmic protein extraction. Immunoblot. Chetomin mediated inhibition of HIF-1α expression. L-Mimosine mediated stabilization of HIF-1α.
**Results:** To investigate the regulation of granulysin expression in human T cells we generated *Mycobacterium tuberculosis* (*M.Tb*)-specific short term T cell lines. We found an *M.Tb*-extract dependent up-regulation of HIF-1α and granulysin that was enhanced under microaerophilic oxygen conditions. However Bovine Serum Albumine- and *Streptococcus pyogenes*-specific T cell lines derived from *M.Tb*-naïve donors lacked HIF-1α up-regulation in response to *M.Tb*-extract, and did not modulate granulysin expression. Microbial antigens such as *Listeria monocytogenes*-, *Candida albicans*-, *Helicobacter pylori*-, and *Streptococcus pyogenes*-extract failed to trigger IFNγ release, as well as granulysin expression. Chetomin, a pharmacological inhibitor of HIF-1α, resulted in significantly reduced HIF-1α and granulysin protein levels. In contrast L-Mimosine, a prolylhydroxylase inhibitor that prevents HIF-1α degradation at physiological oxygen conditions, stabilized the expression of HIF-1α and granulysin.
**Conclusion:** These results provide evidence that HIF-1α links antigen specific T-cell activation and the expression of antimicrobial effector molecules.

**Presentation:** Wednesday, September 30, 2015 from 8:45 – 9:00 in room Congress Saal.

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**349/IIV**

**Policing the cytosol: Inflammasome activation in primary human macrophages is dependent on flagellin**

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Rapid detection of intracellular pathogens is crucial for the innate immune system to establish a robust response towards potentially harmful bacteria. Murine Naip (NLR family, apoptosis inhibitory protein) 1, Naip2 and Naip5/6 are host sensors that detect the cytosolic presence of bacterial type III secretion system (T3SS) needle, T3SS rod protein and flagellin, respectively. In mice, this triggers formation of the NLRC4 (NLR family CARD domain-containing protein) inflammasome. NLRC4 acts as a platform for activation of Caspase-1, which promotes two major events: release of the pro-inflammatory cytokines IL-1β and IL-18, and the induction of a pro-inflammatory form of cell death termed pyroptosis. Previous studies using human-derived macrophage-like cell lines indicate that human macrophages sense the cytosolic needle protein, but not bacterial flagellin. Here, we show that primary human macrophages readily sense cytosolic flagellin. Infection of primary human macrophages with *Salmonella* or the delivery of flagellin via the anthrax Protective Antigen pore elicits robust cell death and IL-1β secretion that is dependent on flagellin. We show that flagellin-detection requires a full-length isoform of the human Naip sensor. This full-length Naip isoform is robustly expressed in primary macrophages from healthy human donors, but drastically reduced in monocytic tumor cells, THP-1 and U937 cells, rendering them insensitive to cytosolic flagellin. However, ectopic expression of full-length Naip rescues the ability of U937 cells to sense flagellin. Resembling their murine counterparts, the human Naip-NLRC4 sensor complex targets leucine residues in the C-terminal region of flagellin. Strikingly, the depletion of the human Naip isoform dramatically reduced inflammasome activation in response to intracellular flagellin delivered via the anthrax Protective Antigen pore or by *Salmonella* infection. In conclusion, human Naip functions to activate the inflammasome in response to flagellin, similar to murine Naip5/6. These results provide compelling evidence that primary macrophages are equipped with enhanced defense mechanisms.

**Presentation:** Wednesday, September 30, 2015 from 9:00 – 9:15 in room Congress Saal.

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**350/IIV**

**The role of the transcription factor IRF8 in *S. aureus* infection**

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**Question:** *Staphylococcus aureus* (*S. aureus*) is a gram-positive bacterium which is part of the human resident flora but is also responsible for a variety of hospital- and community-acquired infections ranging from superficial skin infections to severe tissue infections and life-threatening conditions such as sepsis. Monocytes/macrophages play a crucial role in the host defense against *S. aureus* by detection of staphylococcal elements and production of proinflammatory and immunomodulating cytokines. In our studies we observed that a specific anti-inflammatory $CD163^+$ macrophage population is missing in the bone marrow and spleen of Interferon regulatory factor 8 $(IRF8)^{-/-}$ mice. Since the $CD163^+$ macrophages exert enhanced phagocytic activity in comparison to $CD163^-$ cells we examined whether the lack of this population in $IRF8^{-/-}$ mice influences the course of *S. aureus* infection.
**Methods:** For the *in vivo* experiments C57BL/6 wild type (wt) and $IRF8^{-/-}$ mice were inoculated subcutaneously with $2x10^7$ CFU of *S. aureus* SH1000 into the left hind footpad. The right footpad served as control. Footpad swelling was measured daily and mice were sacrificed on day 4 and 8 to analyze the bacterial load in the foot and selected organs. The infiltration of $CD163^+$ cells in the infected tissue was monitored by fluorescence microscopy.
For the *in vitro* experiments we infected freshly isolated spleen cells from wt and $IRF8^{-/-}$ mice for 2h with *S. aureus* SH1000 and subsequently analyzed the killing of the bacteria in the cells.
**Results:** Infection of wt mice with *S. aureus* induced a strong footpad swelling of the infected foot as compared to the control foot. Significant swelling was observed already on day 1 after infection and increased with a time. After 4 days post infection footpad swelling began to decline. The immunofluorescence staining revealed strong infiltration of $CD163^+$ macrophages already during the early stages of infection. In contrast, $IRF8^{-/-}$ mice showed a significantly higher footpad swelling after *S. aureus* infection as compared to wt mice. Moreover, no decrease in footpad swelling from day 4 to day 8 after infection was observed in $IRF8^{-/-}$ mice. Monitoring of bacterial load revealed significant reduction of bacteria amounts in the popliteal and inguinal lymph nodes of wt mice on day 8 after infection but a high bacterial load was still detected in the lymph nodes of the $IRF8^{-/-}$ mice.
Monitoring of the killing of bacteria during the *in vitro* infection of spleen cells isolated from wt and $IRF8^{-/-}$ mice revealed a lower killing rate of *S. aureus* in the $IRF8^{-/-}$ spleen cells as compared to wt cells.
**Conclusions:** The $IRF8^{-/-}$ mice lacking a specific $CD163^+$ macrophages population in the bone marrow and spleen represent a useful model to study the role of $CD163^+$ cells in *S. aureus*

infection. We could show that lack of IRF8-/- enhances the susceptibility to infection with *S. aureus*. Further studies are needed to decipher the exact role of $CD163^+$ macrophages in *S. aureus* infection outcome.

**Presentation:** Wednesday, September 30, 2015 from 9:15 – 9:30 in room Congress Saal.

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**351/IIV**

**The detrimental role of C5aR in meningococcal sepsis**

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**Introduction**: The complement system is a critical innate determinant protecting the host against infectious agents. It key functions are 1) opsonization of microorganisms to enhance their phagocytic uptake, 2) lysis via the membrane attack complex and 3) initiation of inflammation to attract immune cells to sites of infection. It is well established that the lytic pathway of the complement system is essential for the control of disseminated *N. meningitidis* infection, which usually present as sepsis or as meningitis. Yet, besides the assembly of the membrane attack complex, the inflammatory split fragment C5a is released during activation of the complement cascade in invasive meningococcemia. C5a activates its corresponding G-protein coupled receptor, C5aR, on multiple target cells, foremost including granulocytes and macrophages, which are then recruited to sites of infection and triggered to clear invading microorganisms. However, unbridled or sustained complement activation yields unphysiologically high C5a concentrations, which exacerbate inflammatory conditions and lead to paralysis of cellular effectors. Given the importance of complement activation during meningococcal sepsis, we hypothesized that besides the beneficial effects of complement lysis due to the membrane attack complex, there might also be a concomitant detrimental effect mediated by the C5a/C5aR-axis. Hence, we speculated that C5aR-activation may impact on the pathophysiology of the disease.
**Methods**: As in vivo model for meningococcal sepsis, the mouse intraperitoneal infection model was used to compare WT and C5aR-/- genotypes. To induce lethal sepsis, 1E+05 CFU of N. meningitidis strain MC58 were administered intraperitoneally and the mice were supplemented with iron dextran to allow for bacterial growth. Clinical scoring was applied and survival rates, bacteremia and plasma cytokines were measured.
**Results**: Upon intraperitoneal challenge, complement anaphylatoxins C3a and C5a were detected in plasma of the mice. There was a striking correlation between N. meningitidis CFU counts in blood and the plasma concentration of C5a, which makes a contribution of C5a to pathophysiology plausible. Indeed, when subjected to infection, $C5aR^{-/-}$ mice displayed ameliorated symptoms, significantly higher survival rates and lower levels of bacteremia as well as cytokines in comparison to WT mice.
**Conclusions**: In this work, we describe the detrimental effect of the C5a/C5aR-axis during meningococcal sepsis. While assembly of the membrane attack complex is necessary to kill invasive meningococci, the production of C5a appears to be a downside to the strong complement activation during meningococcal sepsis that accounts for disease pathophysiology. Therefore, C5aR may be an interesting target for immune modulation in meningococcal disease to ameliorate symptoms and enhance survival.

**Presentation:** Wednesday, September 30, 2015 from 9:30 – 9:45 in room Congress Saal.

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**352/IIV**

**The mycobacterial cord factor TDM: dual role in cross-regulation of IFNγ responses**

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Tuberculosis still causes more than a million deaths per year. An important mycobacterial danger signal is trehalose-6'6-dimycolate (TDM), also known as the mycobacterial cord factor. Its recognition via C-type lectin Mincle leads to efficient activation of macrophages, including G-CSF and NO production. However, immune-evasive effects such as delay of phagosomal maturation have also been observed. We aim to get a broader view on the effects of the mycobacterial cord factor as a PAMP as well as a bacterial effector molecule. To this end, we performed genome-wide transcriptional profiling from bone-marrow-derived macrophages. Firstly, we compared the natural cord factor TDM versus the synthetic analogue and experimental adjuvant TDB. Secondly, we studied cross-regulation of both TDM and TDB with the pro-inflammatory cytokine interferon-gamma (IFNγ), a T cell cytokine essential for protective immunity against TB. Analysis of TDB and TDM-stimulated macrophages revealed great overlap between both stimuli, with TDM inducing a broader range of responses. With respect to IFNγ responses, different properties of TDB/TDM could be observed. On the one hand, selected pro-inflammatory responses showed enhancement upon co-treatment with IFNγ plus cord factor, confirming earlier results regarding the PAMP function. On the other hand, some pro-inflammatory processes were dampened in co-treated macrophages. This restrictive effect on IFNγ responses can be observed for regulation of MHCII as well as the 65 kDa GTPase Gbp1. Functional studies on both targets will help to identify regulatory pathways especially for dampening effects of the cord factor. With this broader view of cord factor-mediated regulation in murine macrophages, we define a transcriptional reprogramming indicating both pro-inflammatory and immune-evasive effects.

**Presentation:** Wednesday, September 30, 2015 from 9:45 – 10:00 in room Congress Saal.

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**353/IIV**

**The *Candida albicans* factor H binding molecule Hgt1p - *in vivo* evidence that it functions as virulence factor**

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**Question:** The complement system is tightly controlled by several regulators. In particular Factor H (FH) is preferentially acquired by pathogens conveying resistance to complement attack.
The aim of the study was to determine whether the FH binding molecule „high affinity glucose transporter 1" (CaHgt1p) of *Candida albicans*, a potentially life-threatening yeast, is a significant virulence factor *in vivo*.
**Methods**: The gene coding for this molecule was initially identified by probing an expression library and homozygous deletion mutants of the respective gene have been constructed previously. An *in vivo* study employing the *Galleria mellonella* model has now been used to investigate whether this complement evasion molecule is a virulence factor, i.e., whether Galleria inoculated with the knock-out mutant (i.e. lacking CaHgt1) are surviving longer than those inoculated with the wild type.
**Results:** Especially at 30°C, but also at 37°C, *Galleria* larvae inoculated with 10^5 homozygous *hgt1Δ/Δ* deletion mutant yeast cells per larva significantly (pHGT1 was reintegrated, or inoculated with the wild type strain.

**Conclusions:** The multifunctional complement evasion molecule CaHgt1p is not only a complement inhibitor, but also a virulence factor, corroborating *in vitro* data.

**Presentation:** Wednesday, September 30, 2015 from 13:15 – 13:30 in room Roter Saal 2.

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**354/IIV**

**Investigating the role of fungal morphology in the pathogenicity of *Candida albicans* infections**

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*Candida albicans* can cause superficial as well as life-threatening systemic infections. The morphological plasticity of the fungus - the ability to grow in the yeast or hyphal form - is believed to play an important role for virulence. However, the relative contribution of the different morphologies to pathogenicity are not well characterized. Hyphal formation is known to be essential for tissue invasion *in vitro* and has therefore been linked to pathogenicity in systemic infection. Yet, we found that a *C. albicans eed1*Δ mutant, defective in hyphal maintenance, still causes high mortality rates in a systemic infection model, challenging the assumption that hyphal formation *per se* is indispensable for pathogenicity.
To investigate the role of morphology for infection, we constructed conditional knock-out mutants, in which gene expression and morphology can be controlled *in vivo* by doxycycline. *EED1*, a regulatory factor essential for maintenance of hyphal growth, and *NRG1*, a negative regulator of the yeast-to-hypha transition, were chosen as target genes.
In an invasion based intraperitoneal infection model, mice challenged with the yeast forms showed mild symptoms, whereas hyphal formation led to significantly more severe disease. Furthermore, hyphal formation was associated with higher organ damage and an increase of inflammatory cytokines in the peritoneal fluid. In contrast, fungal morphology had no influence on fungal burden and the number of immune cells migrating to the site of infection. These results support the hypothesis that hyphal formation is essential for tissue invasion *in vivo* and that invasion-based damage of epithelial cells is necessary for cytokine induction.
In contrast, in a systemic infection model t-*EED1* yeasts caused mortality rates comparable to the wild type. Interestingly, fungal burden of these yeasts increased significantly in brain and kidneys within 24 hours post infection. In contrast, the yeast form of tet-*NRG1* was strongly attenuated in virulence, suggesting that morphology *per se* is not essential for *C. albicans* virulence. Further analyses are planned to unravel the underlying pathogenicity mechanisms of t-*EED1* yeast cells during systemic infections and to find out which differences between t-*EED1* and t-*NRG1* yeast cells contribute to the different virulence phenotypes.

**Presentation:** Wednesday, September 30, 2015 from 13:30 – 13:45 in room Roter Saal 2.

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**355/IIV**

**Complement Receptor 1 mediated control of *Leishmania* infection in inflammatory human macrophages.**

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Complement Receptors (CRs) are expressed on the surface of human monocyte derived macrophage (hMDM). Particularly, Complement Receptor 1 (CR1) can mediate *Leishmania* promastigote phagocytosis in hMDM. We hypothesized that CR1-mediated phagocytosis and subsequent activation enables control of *Leishmania* parasites in inflammatory human macrophages.
Investigating the expression of CR1 on both inflammatory macrophages (hMDM I) and anti-inflammatory macrophages (hMDM II), it was found that CR1 is significantly higher expressed on hMDM I when compared to hMDM II cells. To elucidate on how the receptor is used by *Leishmania* parasites to infect the macrophages, we modulated CR1 on hMDM I prior to infection. Blocking of CR1 on hMDM I resulted in a significant lower uptake of *L. major* parasites, when compared to the corresponding isotype control. Furthermore, we modulated CR1 using a siRNA knockdown (KD) approach. In line, we could show CR1 KD cells to internalize significantly less parasites as compared to the non-target control.
Since CR1 is highly expressed specifically on inflammatory cells, we evaluated the role of this receptor in the intracellular survival and stage transformation of *Leishmania* parasites. *L. major* infection was analyzed over time after the modulation of CR1 with blocking antibody. We found that even though blocking CR1 reduced uptake, intracellular development increased significantly up to 50 %.
In all, our data demonstrate CR1 to be a marker for hMDM I and suggest that CR1 mediated phagocytosis can activate the pathway, able to control *Leishmania major* development in inflammatory human macrophages.

**Presentation:** Wednesday, September 30, 2015 from 14:00 – 14:15 in room Roter Saal 2.

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**356/IIV**

**Cutaneous $Na^+$ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense**

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I. Siegert[2], A. Maronna[4], D. Wendelborn[2,3], P. Linz[3], K. Binger[5]
M. Gebhardt[5], M. Heinig[5,6], P. Neubert[3], F. Fischer[2], S. Teufel[7,8]
J.- P. David[7,8], C. Neufert[9], N. Rakova[5], C. Kueper[10], F.- X. Beck[10]
W. Neuhofer[10], D. Mueller[5], G. Schuler[4], M. Uder[11], C. Bogdan[2]
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**Introduction:** Large amounts of $Na^+$ are stored in the skin. Skin $Na^+$ storage can be induced experimentally by dietary salt. Experimental studies suggest that $Na^+$ storage creates a microenvironment of hyperosmolality in the skin which is also a characteristic feature of inflamed tissue and of lymphatic organs. The biological advantage of increased skin $Na^+$ concentrations is unknown.
**Methods:** $Na^+$, $K^+$ and water measurements were performed after dry ashing of the skin. $^{23}Na$ MRI and $^{23}Na$ spectroscopy were used

to quantifiy Na in infected human skin. The effect of high salt on LPS-induced macrophage(MΦ)-activation and on *Leishmania*-infected MΦ was analyzed. After two weeks on low salt diet (<0.1% NaCl, tap water) or high salt diet (4% NaCl, 0.9% saline in the drinking water), we infected hind footpads of FVB mice and/ or $LysM^{WT}$ $Nfat5^{fl/fl}$ (control) and $LysM^{Cre}$ $Nfat5^{fl/fl}$ mice (FVB background) with stationary-phase *L. major* promastigotes and monitored the course of infection.

**Results:** We found that $Na^+$ accumulated at the site of bacterial skin infections in humans and in mice. We used the protozoan parasite *Leishmania major* as a model of skin-prone macrophage infection to test the hypothesis that skin-$Na^+$ storage facilitates antimicrobial host defense. Activation of macrophages in the presence of high NaCl concentrations modified epigenetic markers and enhanced p38 mitogen-activated protein kinase (p38/MAPK)-dependent nuclear factor of activated T cells 5 (NFAT5) activation. This high-salt response resulted in elevated type-2 nitric oxide synthase (*Nos2*)-dependent NO production and improved *Leishmania major* control. Finally, we found that increasing $Na^+$ content in the skin by a high-salt diet boosted activation of macrophages in an *Nfat5*-dependent manner and promoted cutaneous antimicrobial defense.

**Discussion:** We show in humans and in mice that skin-$Na^+$ accumulation occurs during cutaneous bacterial infections and endogenously boosts antimicrobial capacity in MΦ. Our findings support the idea that salt metabolism is a physiological component in cutaneous immunological barrier formation to ward off infections. Salt deposition might serve as an ancient mechanism to aid in immune-mediated pathogen removal.

**Presentation:** Wednesday, September 30, 2015 from 14:15 – 14:30 in room Roter Saal 2.

## CLINICAL MICROBIOLOGY AND INFECTIOLOGY (KMV)

**357/KMV**

**Risk factors for lung function decline in cystic fibrosis patients with persistent *Staphylococcus aureus* recovered from the airways**

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**Questions:** *Staphylococcus aureus* is often cultured from the airways of cystic fibrosis (CF) patients. Our aim was to determine risk factors for worse lung function in patients with persistent *S. aureus*.

**Methods:** Inclusion criteria for the non-interventional prospective longitudinal multi-center study were CF, age above 6 years, persistent *S. aureus* from respiratory specimens. Exclusion criteria were persistence of *Pseudomonas aeruginosa* or *Burkholderia cepacia* complex. Co-infection with other CF pathogens (*Stenotrophomonas maltophilia*, *Aspergillus fumigatus*, *non-TB mykobacteria*, *Achromobacter xylosoxidans*, MRSA), lung function, antibiotic therapy, IL-6- and IgG-levels against *S. aureus* antigens were assessed. Results were analyzed using standard statistics and generalized linear mixed models.

**Results:** A mean of seven visits was analyzed for 195 patients from 17 centers. Nasal *S. aureus* carriers (n=122) were more likely male (p=0.00075), less likely co-infected by *Stenotrophomonas maltophilia* (p=0.0003) and experienced better lung function (p=0.042). Patients with *S. aureus* small colony variants (SCVs, n=84) were older (p=0.0066), had worse lung function (p=0.0011), were more likely treated with trimethoprim/sulfamethoxazole (p=0.0078). Patients co-infected with *S. maltophilia* (n=44) and *A. fumigatus* (n=60) had worse lung function (p=0.0103; p=0.0048). CF patients mounted higher IgG-levels against staphylococcal antigens compared to healthy nasal carriers. IL-6 levels positively correlated with *S. aureus* density in sputa (p=0.0016), with patients with SCVs (p=0.0209), exacerbations (p=0.00411) and co-infections with *S. maltophilia* (p=0.0195) and *A. fumigatus* (p=0.0496).

**Conclusions:** In CF patients with chronic *S. aureus*, non-nasal *S. aureus* carriage, female gender, *S. aureus* SCVs and co-infection with *S. maltophilia* or *A. fumigatus* are independent risk factors for worse lung function in CF.

Clinical trial registered with www.clinicaltrials.gov (NCT00669760).

**Presentation:** Wednesday, September 30, 2015 from 13:00 – 13:15 in room Weißer Saal 1.

**358/KMV**

**Prevalence and characterization of unusual *Staphylococcus aureus* strains with a mucoid phenotype recovered from the airways of cystic fibrosis patients**

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**Objectives:** Chronic respiratory airway infections are a major concern for cystic fibrosis (CF) patients. *Staphylococcus aureus* is frequently isolated from the respiratory tract, beginning in early childhood and often persisting for many years. While mucoid bacterial colony morphology has been described for *Pseudomonas aeruginosa*, the most prominent pathogen in CF, no data are available for *S. aureus*. Recently, we identified mucoid isolates from airway specimens of *S. aureus*. To determine the prevalence and the underlying mechanism of mucoid *S. aureus* phenotypes, we analyzed *S. aureus* isolates collected during two independent studies including 371 CF patients [one prospective multicenter study (n=195, 2 years) and one longitudinal prospective study of two CF centers in Muenster, Germany (n=176, 21 years)].

**Methods:** Mucoid isolates were identified phenotypically by macroscopic analysis of growth behavior on Columbia blood agar. The *intercellular adhesion* (*ica*) promoter region of all *S. aureus* isolates as well as the entire *ica* operon of selected mucoid strains were sequenced. Biofilm formation was measured by a microtiter plate assay. Mucoid and normal isolates of one patient were characterized in terms of capsule expression and phagocytosis by neutrophils.

**Results:** In eight patients, mucoid *S. aureus* isolates were found in the respiratory tract (prevalence of 2.2%). In contrast to normal *S. aureus* isolates, mucoid isolates were strong biofilm producers. A 5 bp-deletion in the *ica* promoter region was associated with the mucoid phenotype in all patients. Subsequent non-mucoid isolates of a patient infected for nine years, which also carried the 5 bp-deletion, harbored a compensatory mutation in *icaC* or *icaA*. Transformation of these non-mucoid strains with a plasmid vector,

expressing either intact *icaC* or intact *icaA*, restored the mucoid phenotype in each respective strain. In contrast to the normal phenotype, mucoid strains were protected against phagocytosis by neutrophils. Mucoid isolates outcompeted normal isolates over time.
**Conclusions:** Albeit mucoid *S. aureus* strains occur rarely during chronic airway infection in CF, biofilm hyper-production seems to be an effective strategy for protection against phagocytosis by neutrophils. Outcompeting of normal isolates over time in one patient indicates a survival advantage of mucoid isolates in the airways of CF patients.

**Presentation:** Wednesday, September 30, 2015 from 13:15 – 13:30 in room Weißer Saal 1.

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**359/KMV**
**Nasal *Staphylococcus aureus* carriage is not restricted to the anterior nares**
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**Question:** Nasal carriage of *Staphylococcus aureus* has been identified as source and risk factor for subsequent invasive infections and the anterior nares are perceived as the species' natural habitat. Thus, methods for detection as well as eradication approaches for *S. aureus* including methicillin-resistant (MRSA) strains focus on the anterior nasal sites, neglecting the posterior parts of the nasal cavity. We present a surgical approach to investigate *S. aureus* presence in precisely defined anatomical parts of the human nose by extensive cultivation enrolling individuals with and without local inflammation signs.
**Methods:** Nasal swab and tissue samples were collected from individuals with (n = 18) and without (n = 16) chronic (rhino-) sinusitis/polyposis during surgery. Samples were taken from four defined nasal sites: the anterior and posterior vestibule and the inferior and middle meatus. Samples were cultivated on liquid and solid media and identification was based on MALDI-TOF mass spectrometry. Methicillin resistance and *spa* types were determined on molecular basis.
**Results:** Amongst the 34 patients, 21 (61.8%) were characterized as *S. aureus* carriers. Overall, 21 different *spa* types were identified with three patients colonized by several *spa* types. Two individuals (5.9% of all patients) carried MRSA. Amongst *S. aureus* carriers, 9/21 (42.9%) revealed colonization throughout all nasal swab and tissue samples. Another five individuals were tested positive in all of the swab samples. All but one patients showed *S. aureus* colonization of the posterior vestibule. In one patient, *S. aureus* was only detected in the meatus tissue. 15/21 (71.4%), 16/21 (76.2%) and 17/21 (81.0%) were found to be colonized in the anterior vestibule, the inferior and the middle meatus, respectively. In four patients (19.0% of carriers), *S. aureus* was exclusively isolated from one nasal sampling site. Stratification into individuals with and without chronic inflammatory nasal diseases did not result in significant differences of *S. aureus* colonization patterns.
**Conclusions:** Since the posterior vestibule was found to be colonized in almost all individuals who were categorized as *S. aureus* carriers, this region is redefined as the principle *S. aureus* habitat and should not be neglected in nasal MRSA detection and eradication strategies.

**Presentation:** Wednesday, September 30, 2015 from 13:30 – 13:45 in room Weißer Saal 1.

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**360/KMV**
***S. aureus* bacteremia in patients with rheumatoid arthritis - Data from the INSTINCT cohort**
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**Background**: Patients with rheumatoid arthritis (RA) are believed to be at increased risk of severe infections. Infections mainly associated with RA are septic arthritis and pulmonary infections, however, data partially date back from the 70s and only few systematic or prospective evaluations have been published. *S. aureus* is one of the most common causes of invasive bloodstream infections and osteoarticular infections (OAI). Triggered by the clinical observation of severe and disseminated manifestations of *S. aureus* bacteremia (SAB) in RA patients we decided to characterize the epidemiology, clinical course and outcome of RA patients with SAB in our cohort.
**Methods**: Data were analyzed from a prospective cohort study (INSTINCT, INvasive *STaphylococcus aureus* INfection CohorT) from two study sites in Germany (Cologne and Freiburg) between 2006 and 2014 including detailed clinical, epidemiological, diagnostic and treatment data. Patients were followed up for one year. Patients with RA were identified through their medical records and/or patient interview. RA and non-RA patients were compared by Student's t-test or Fisher's exact test as appropriate. Survival was analysed using Kaplan-Meier curves and log-rank test as well as a multivariable Cox model.
**Results**: 1069 patients with SAB where included in the analysis. Among these, we identified 31 patients with RA. Patients with RA and SAB were more often female, infection was less common nosocomial and required more frequently surgical intervention . The main portal of entry were skin and soft tissue, however, in a high proportion of patients no traceable portal of entry could be detected (for a detailed comparison of clinical and epidemiological data see Table 1). Compared to non-RA patients, RA patients experienced significantly more often OAI (including septic arthritis, [vertebral] osteomyelitis, prosthetic joint infections) and showed a higher rate of hematogenous dissemination (38.7 vs. 15.8%) as well as multiple OAI (22.6 vs. 5.5%). Patients with RA had a substantially higher mortality than non-RA patients (Figure 1). Multivariable analysis including immunosuppressive therapy confirmed RA to be an independent risk factor for mortality and OAI.
**Conclusions**: Patients with RA and SAB are highly vulnerable to develop osteoarticular infections with *S. aureus* like septic arthritis and osteomyelitis. Hematogenous dissemination with distant metastatic foci is common. These often involve other joints, may include prosthetic joint infections and involve not only RA-affected regions. Therefore, a high degree of clinical suspicion and a thorough examination including appropriate imaging are needed to detect all foci timely and to involve other disciplines like orthopedic or hand surgery for focus eradication/debridement. Hypotheses for this increased susceptibility include RA-associated erosive joint alterations, immune dysfunction as well as immunosuppressive agents. Further studies should elucidate these underlying mechanisms.

**Presentation:** Wednesday, September 30, 2015 from 13:45 – 14:00 in room Weißer Saal 1.

**Figure 1**

| | All n=1069 | RA n=31 | Non-RA n=1038 | P value |
|---|---|---|---|---|
| Age, median (IQR) | 66.0 (53.0; 74.0) | 72.0 (64.0; 76.5) | 66.0 (53.0; 74.0) | 0.013# |
| Female | 368 (34.4%) | 19 (61.3%) | 349 (33.6%) | 0.003 |
| **Mode of acquisition** | | | | 0.002 |
| Comm. acqu. not healthcare-ass. Comm. acqu. | 188 (17.6%) | 12 (38.7%) | 176 (17.0%) | |
| healthcare-ass. | 317 (29.7%) | 11 (35.5%) | 306 (29.5%) | |
| Nosocomial | 564 (52.8%) | 8 (25.8%) | 556 (53.6%) | |
| **Portal of entry** | | | | <0.001 |
| Intravascular catheter/device-related | 394 (36.9%) | 2 (6.5%) | 392 (37.8%) | |
| SSTI | 213 (19.9%) | 13 (41.9%) | 200 (19.3%) | |
| Respiratory tract | 49 (4.6%) | 2 (6.5%) | 47 (4.5%) | |
| Other | 101 (9.4%) | 2 (6.5%) | 99 (9.5%) | |
| Unknown | 312 (29.2%) | 12 (38.7%) | 300 (28.9%) | |
| **Main focus** | | | | <0.001 |
| Non-deep-seated foci | 453 (42.4%) | 6 (19.4%) | 447 (43.1%) | |
| Osteoarticular infection | 167 (15.6%) | 15 (48.4%) | 152 (14.6%) | |
| Endocarditis and complications | 103 (9.6%) | 2 (6.5%) | 101 (9.7%) | |
| Pneumonia and/or pleural empyema | 57 (5.3%) | 2 (6.5%) | 55 (5.3%) | |
| Deep-seated foci (wo osteoart. inf.) | 91 (8.5%) | 3 (9.7%) | 88 (8.5%) | |
| Unknown | 198 (18.5%) | 3 (9.7%) | 195 (18.8%) | |
| **Underlying conditions** | | | | |
| Other autoimmune dis. or conn. tissue dis. | 67 (6.3%) | 0 | 67 (6.5%) | 0.254 |
| Malignancy | 323 (30.2%) | 0 | 323 (31.1%) | <0.001 |
| Organ or bone marrow transplant | 60 (5.6%) | 0 | 60 (5.8%) | 0.412 |
| Corticosteroid use | 99 (9.3%) | 11 (35.5%) | 88 (8.5%) | <0.001 |
| Immunosuppressive therapy | 106 (9.9%) | 13 (41.9%) | 93 (9.0%) | <0.001 |
| Antineoplastic chemotherapy | 136 (12.7%) | 0 | 136 (13.1%) | 0.025 |
| **Orthopedic implant** | 120 (11.2%) | 10 (32.3%) | 110 (10.6%) | 0.001 |
| Orthopedic implant infection | 20 (1.9%) | 3 (9.7%) | 17 (1.6%) | 0.018 |
| Previous inj. therapy intraarticular | 14 (1.3%) | 2 (6.5%) | 12 (1.2%) | 0.060 |
| Previous inj. therapy intramuscular | 44 (4.1%) | 1 (3.2%) | 43 (4.1%) | 1.000 |
| **Disseminated infection** | | | | |
| Presence of sec. foci of hematogenous diss. | 176 (16.5%) | 12 (38.7%) | 164 (15.8%) | 0.002 |
| Sec. hematogenous osteoart. manifest. | 64 (6.0%) | 7 (22.6%) | 57 (5.5%) | 0.002 |
| Surgical therapy/intervention | 310 (29.0%) | 19 (61.3%) | 291 (28.0%) | <0.001 |

Table 1: Patient characteristics and clinical data of patients with *S. aureus* bacteremia. #t-test, all others Fisher's exact test

**Figure 2**

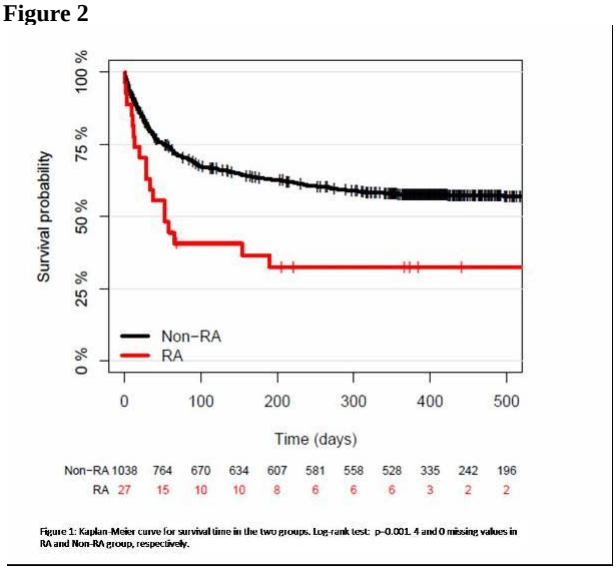


Figure 1: Kaplan-Meier curve for survival time in the two groups. Log-rank test: p<0.001. 4 and 0 missing values in RA and Non-RA group, respectively.

## 361/KMV

## CASE REPORT: Generalized Vaccinia after Oncolytic Virotherapy

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A 36-year-old male patient suffering from glioblastoma underwent surgery, but the tumor recidivated despite chemotherapy. Therefore the patient agreed to receive oncolytic therapy with different viruses, starting in September 2013, including an individual treatment attempt with Vaccinia virus administered intraarterially. Nine days post administration the patient was hospitalized showing multiple pustular lesions with central ulceration predominantly located in the face.
Directly after hospitalization we received crust, swab, whole blood and serum samples for diagnostics. We were able to detect high amounts of Orthopoxvirus (OPV)-DNA by real-time PCR in the crust and swab samples and low amounts in the blood sample, indicating a generalized vaccinia with concurrent viremia. Sanger sequencing of the HA ORF as well as whole genome sequencing from cell culture propagated virus (Life Ion PGM) revealed a Vaccinia virus infection which was attributed to the therapeutic Vaccinia virus Dryvax administration. Interestingly, the wild-type strain and not a genetically modified variant was used in this treatment attempt. An OPV-specific immune response in the patient was indicated by an increase in anti-OPV IgM and IgG antibody titer over the course of infection.
As a consequence of the laboratory results, the patient received Cidofovir and Vaccinia immune globulin and slowly recovered under isolation. Twenty-five days post oncolytic Vaccinia virus treatment the lesions were mostly healed and virus from swabs could no longer be propagated in cell culture. He left the hospital with advice for behavior to prevent further spread of the virus.
Unfortunately, the tumor continued to grow despite treatment and thus was extirpated 4 month after administration of VACV. Nucleic acid from VACV and another oncolytic virus administered was detected in the tumor tissue by metagenomics (Illumina MiSeq), proving the successful delivery of the virus.
The treatment of tumors with viruses seems to be a promising approach and complications as presented here are reported only rarely. Nevertheless, administration of life virus always involves the risk of replication related complications. Therefore, treatment parameters including virus variant, amount of virus and route of administration have to be considered carefully.

**Presentation:** Wednesday, September 30, 2015 from 14:00 – 14:15 in room Weißer Saal 1.

## 362/KMV

## Infection of a neonate by the psychrophilic bacterium *Psychrobacter sanguinis*

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*Psychrobacter* species are psychrophilic, fastidious gram-negative coccobacilli which belong to the family of *Moraxellaceae* and commonly live in deep sea and cold environments like Antarctica. Being primarily isolated from environmental specimens until recently, there are now several reports about the isolation of *Psychrobacter* species from hospitalized patients. However, clinically relevant characteristics of *Psychrobacter* (*P.*) species like antibiotic susceptibility or virulence are completely unknown. Here we describe the isolation of *P. sanguinis* from a newborn and investigation of growth kinetics, antibiotic susceptibility and pathogenicity *in vivo*.
A female baby was born in the restroom of the central station in Frankfurt and admitted to the Neonatology ward of the University Hospital with presentation of hypothermia (33°C) and clinical signs of infection. The newborn was treated empirically with ampicillin and cefotaxime. To identify the infection focus several swabs were taken and a gram-negative coccobacilli was recovered repeatedly from the umbilicus which was identified as *P. sanguinis* by 16S rRNA gene sequencing. Before, biochemical identification approaches using Vitek2 and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) failed to identify this bacterium. The isolate grew slowly on blood agar at different temperatures, ranging from 4° to 37°C. Therefore, we used different media to analyze growth kinetics over 36 h. *P. sanguinis* grew in brain heart infusion (BHI) medium supplemented with 5% FCS and in the *Bartonella* medium BALI whereas no growth was observed in BHI without FCS, LB or 2xYT media.
Antibiotic susceptibility testing via antibiotic gradient tests applying 55 antibiotics and clinical breakpoints for *Moraxella catarrhalis* and PK/PD data revealed an overall susceptible phenotype showing only resistance to ampicillin. *In vivo* virulence of the clinical isolate was assessed in the *Galleria mellonella* infection model (larva of the Greater wax moth). After injection of $10^6$ *P. sanguinis* 50% of larvae died after 24 h, whereas $10^7$ CFU of an ATCC reference strain was needed for the same killing. Mean lethal dose (LD50) of the *P. sanguinis* isolate was lower compared to the ATCC strain and several *Acinetobacter* non-*baumannii* strains (which are loosely related to *P.*) but higher than for *A.*

*baumannii* ATCC 17978 and 19606 indicating a moderate virulence of the *P. sanguinis* isolate.
Taken together, *P. sanguinis* presumably has been mis- and underdiagnosed in the past given the fact that cultivation and identification using biochemistry and MALDI TOF-MS is difficult. The hypothermia of the newborn has most likely selected for *P. sanguinis*. We hypothesize that *P.* species are opportunistic pathogens with virulence properties comparable to *Acinetobacter* non-*baumannii* species.

**Presentation:** Wednesday, September 30, 2015 from 14:15 – 14:30 in room Weißer Saal 1.

## FOOD MICROBIOLOGY AND HYGIENE (LMV)

**363/LMV**

**Prevalence of MRSA and VRE on retail raw meat**

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**Introduction:** Several reports described the contamination of retail meat products with multi-resistant bacteria. However, prevalence differs between different regions and between different meat products. Moreover, the degree of contamination of single samples has rarely been addressed. In this study, we aimed to elucidate the prevalence and bacterial load of Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococcus* spp. (VRE) on different raw meat products in (Northern-)Germany.
**Methods:** Microbes where detached from the whole retail meat surface of 347 samples (chicken, turkey and pork) by ultrasonic treatment. MRSA was quantified using a modified protocol of the LightCycler® MRSA Advanced Test. Direct culture and enrichment of MRSA and VRE was performed according to standard laboratory procedures.
**Results:** We could detect MRSA in approximately 12% of all samples. In 75% of these samples hospital acquired (ha-)MRSA and in 25% livestock associated (la-)MRSA was detected. Overall, a median of $2.2*10^3$ and $3.5*10^2$ MRSA GE copies per Gramm retail meat could be determined for la-MRSA and ha-MRSA, respectively. Approximately 14% of the samples carried VRE belonging to *E. faecalis* and *E. faecium* containing either *vanA* and/or *vanB* resistance genes. MRSA and VRE were mostly found on chicken and turkey meat. No MRSA could be detected on pork meat samples.
**Conclusion:** A significant percentage of poultry meat products were contaminated with MRSA and /or VRE. The molecular method applied for the detection of MRSA might to serve as a screening method for the quantitative detection of la- and ha-MRSA on meat products.

**Presentation:** Tuesday, September 29, 2015 from 9:15 – 9:30 in room Weißer Saal 1.

**364/LMV**

**Comparison of culture-dependent and -independent methods to detect the *Pseudomonas* community structure on oak leaf lettuce**

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The genus *Pseudomonas* plays an important role in the lettuce leaf microbiota. While some species are phytopathogens, cause human infections or are associated with food spoilage, some species have beneficial bioremediation and biocontrol activities. The aim of this study was to investigate the occurrence and diversity of *Pseudomonas* spp. on oak leaf lettuce by comparing culture-dependent and -independent methods.
Lettuce samples were treated with a Pulsifier™ device to remove bacteria from the leaf surface. Serial dilutions were prepared from the bacterial solutions and plated on Standard-I agar, *Pseudomonas* selective agar with cetrimide fucidin cephalosporin supplement, and Cetrimide agar. One hundred presumptive *Pseudomonas* isolates were confirmed by PCR reaction with *Pseudomonas*-specific primers. The species variation among the isolates was investigated by amplification of the 16S rRNA- and the *rpoB*-(RNA polymerase beta subunit) gene. The amplicons were analyzed by Restriction Fragment Length Polymorphism with *Alu*I and *Rsa*I. Based on the restriction patterns, selected isolates were sequenced and the sequence data was compared with the sequences of *Pseudomonas* reference strains deposited in the GenBank of the National Center for Biotechnology Information.
The biomass of the remaining bacterial solution was harvested and after DNA isolation the *Pseudomonas*-specific PCR was performed. The PCR product was ligated into the pGEM®-T Easy vector and transformed into *Escherichia coli* JM109. Transformant colonies were selected by blue-white screening. Plasmid-DNA of 100 clones was isolated and the insert of each clone was amplified using M13-f/M13-r primers. Restriction analysis, sequencing with T7 and SP6 primers, and analysis of the sequence data was carried out as described above.
All detected *Pseudomonas* species belong to the *P. fluorescens* lineage. In the culture-dependent analysis, 73% of the isolates belong to the *P. fluorescens* subgroup. In the culture-independent analysis 46% of the sequences matched the *P. fluorescens* subgroup and *P. extremaustralis*. The results of this study suggest that pseudomonads found by plating methods indeed represent the relevant part of the *Pseudomonas* community on oak leaf lettuce.

**Presentation:** Tuesday, September 29, 2015 from 9:30 – 9:45 in room Weißer Saal 1.

**365/LMV**

**Cereulide biosythesis in emetic *Bacillus cereus*: An unusual mega enzyme complex forming an unusal depsipeptide toxin**

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The incidence of foodborne intoxications caused by bacterial toxins has been steadily increasing in Europe throughout the last years. Especially the toxin cereulide, produced by a specific class of *Bacillus cereus* is increasingly recognized as a serious threat that occasionally is implicated in severe clinical manifestations including acute liver failures. Cereulide is an ionophoric dodecadepsipeptide, composed of alternating α-amino and α-hydroxy acids $(D\text{-}O\text{-}Leu\text{-}D\text{-}Ala\text{-}L\text{-}O\text{-}Val\text{-}L\text{-}Val)_3$, that is produced non-ribosomally by an enzyme complex with an unusual modular structure, named cereulide synthetase (Ces NRPS). [1,2]. The *ces* gene locus is encoded on a mega virulence plasmid related to the *Bacillus anthracis* toxin plasmid pXO1. A screening approach of emetic strains revealed a huge diversity of chemical toxin variants with highly variable toxigenic potential [3]. All cereulide variants are produced by a single NRPS synthetase in the same strain at the same time. All emetic strains analyzed so fare produce the same set of variants but in significantly different concentrations. NRPS product assembly usually displays a strict correlation between the enzymatic domain sequence of the synthase and the position of the amino acid building blocks in the peptide product. However, UPLC-TOF MS metabolite analysis and *in silica* gene cluster analysis revealed dipeptides rather than single amino or hydroxy acids as the basic modules in depsipeptide assembly, thus violating the canonical NRPS biosynthetic logic and proposing a novel mechanisms biosynthesis of ester bond containing NRPS products [4|. Transcriptomics, proteomics and metabolomics were employed for gaining insights into the complex and tightly controlled regulatory network that link toxin synthesis to the bacterial metabolism and nutritional status of the cell. Results from ongoing studies will be presented and the embedment of biosynthesis of this unusual toxin in *B. cereus* life cycle will be discussed.

**References**

[1] Ehling-Schulz et al., BMC Microbiol 2006
[2] Magarvey et al., JACS 2006

[3] Marxen et al., ABC 2015
[4] Marxen et al., Sci Rep 2015

**Presentation:** Tuesday, September 29, 2015 from 9:45 – 10:00 in room Weißer Saal 1.

## MICROBIAL PATHOGENICITY (MPV)

**123/MPV**

**Role of a major immunodominant protein GlpQ in *Staphylococcus aureus* pathogenicity.**
**Why does *S. aureus* secrete a glycerophosphodiesterase?**

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*Staphylococcus aureus* is a commensal bacterium and also a major human pathogen. The emergence of Methicillin-Resistant *S. aureus* (MRSA) strains limits the use of existing antibiotics to cure infections and new anti-microbial approaches are needed. In a recent study a group of 11 conserved proteins, that provoked a high immune response in bacteremia patients were identified and thus could constitute new antimicrobial targets. Interestingly, one of the most immunodominant proteins, GlpQ, is a secreted glycerophosphodiesterase predicted enzyme but its putative role in infection has been underestimated. Importantly, in contrast with many virulent factors, *glpQ* is not redundant as it is widely present in most *S. aureus* genomes. Why does *S. aureus* secrete a glycerophosphodiesterase? Our hypothesis is that GlpQ might degrade phospholipid-derived substrates present in the host cell membrane for nutrient mobilization and survival inside the host. Conversely, by degrading the host derived-phospholipids GlpQ can contribute to pathogenesis.
Using an *in vitro* activity assay and a recombinant MBP-GlpQ protein, we were able to identify glycerophosphocholine (GPC) and glycerophosphoinositol (GPI) as specific substrates for GlpQ. GPC derives from phosphatidylcholine, the major phospholipid present in the host cell membrane, after the acyl chains have been cleaved. GPI derives from phosphatidylinositol, an important signaling lipid present in host cell membranes. In an *in vivo* approach, we used filtrated *S. aureus* culture supernatants (wild type (WT) or *glpQ* mutant of different *S. aureus* strains) using GPC or GPI as a substrate. Mutant strains showed reduced glycerophosphodiesterase activity.
To reveal if GPC can be metabolized by *S. aureus*, we used a chemically defined medium, SNM3X, using GPC as a sole carbon source. We observed that *S. aureus* WT could growth well but GlpQ mutant strains showed a decreased growth in the presence of GPC. Therefore, GPC can be used as a carbon source for *S. aureus* and GlpQ is important to metabolize this compound.
Interestingly, other glycerophosphodiesters derived from the phospholipids phosphatidylethanolamine and phosphatidylserine showed also specificity for GlpQ activity.
Together, our findings suggest that GlpQ is able to degrade deacylated phospholipids present in the host cell, for nutrient mobilization and survival inside the host. Whether GlpQ activity towards host glycerophosphodiesters contribute directly to pathogenicity is under investigation.

**Presentation:** Monday, September 28, 2015 from 9:45 – 10:00 in room Congress Saal.

**366/MPV**

**$Ca^{2+}$ binding sites within BIg domains of the giant *Salmonella* adhesin SiiE are essential for secretion**

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The giant non-fimbrial adhesin SiiE of *Salmonella enterica* mediates the first contact to the apical site of host cells. This 595 kDa protein containing 53 repetitive BIg domains is the only known substrate of the SPI4-encoded type 1 secretion system (T1SS). SiiE is transiently retained within the secretion system and later released in the supernatant. In the majority of BIg domains, five conserved aspartate or glutamate residues are present. Recently, the crystal structure of BIg50-52 was solved, revealing two $Ca^{2+}$ binding sites per BIg domain formed by the conserved D residues. Bound $Ca^{2+}$ ions are important for the rigidity of the protein. There are two distinct types of $Ca^{2+}$ binding sites within SiiE. To test whether these sites play a role in SiiE structure and function, we created several plasmid and chromosomal encoded SiiE mutants with aspartate to serine exchanges of the conserved residues. Besides SiiE secretion, also other characteristics like SiiE retention and SiiE dependent invasion were attenuated according to the number of exchanged D residues. The severity of each of these phenotypes increased with an increased number of exchanges of D residues. We established a model with $Ca^{2+}$ ions acting as a pulling force for SiiE secretion, with type I $Ca^{2+}$ binding sites being more important than type II $Ca^{2+}$ binding sites.

**Presentation:** Monday, September 28, 2015 from 17:00 – 17:15 in room Congress Saal.

**367/MPV**

**Envelope stress and post-transcriptional regulation by sRNAs in *Yersinia enterocolitica***

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The Gram-negative *Yersinia enterocolitica* is a food-borne pathogen and the third most common cause of gastrointestinal infections in Germany. *Y. enterocolitica* contains a wide range of virulence factors that are associated with the bacterial envelope, e.g. adhesins, secretion systems and siderophore receptors. In Enterobacteriaceae, envelope homeostasis is ensured by several envelope stress response (ESR) pathways, including the alternate sigma factor RpoE-dependent ESR. RpoE activation leads to production of periplasmic chaperones and proteases that remodel the envelope-associated proteins. Moreover, in *Escherichia coli* and in *Salmonella enterica*, RpoE induces the expression of two conserved small non-coding RNAs (sRNA), MicA and RybB, which together with the RNA chaperone Hfq, destabilize transcripts encoding outer membrane proteins (OMPs) [1]. The impact of post-transcriptional mechanisms in the ESR has so far not been examined in pathogenic Yersiniae. However, our previous mutational analysis demonstrated that the RNA chaperone Hfq modulates the production of several OMPs in *Y. enterocolitica* [2].
In this study we examined whether Hfq is involved in the RpoE-dependent ESR and assessed the role of MicA and RybB in the production of OMPs in *Y. enterocolitica*.
Using gene fusions with *gfp*, we showed that a *Y. enterocolitica* strain mutated in *hfq* exhibits increased expression of *rpoE* as well as the RpoE-dependent chaperones *fkpA* and *degP*, suggesting that Hfq downregulates the RpoE-dependent ESR. The overexpression of the sRNA RybB from an inducible promoter inhibited the production of several OMPs, including OmpA and OmpC. Moreover, this RybB-mediated repression of OMPs required a functional Hfq protein.
Taken together, our results indicate that the RNA chaperone Hfq and the conserved sRNA RybB are involved in remodelling the bacterial surface of *Y. enterocolitica*. Ongoing studies are investigating whether RybB and MicA also control the production of surface virulence factors of *Y. enterocolitica*.
1. Vogel J, Papenfort K (2006) Small non-coding RNAs and the bacterial outer membrane. Curr Opin Microbiol 9: 605-611.
2. Kakoschke T, Kakoschke S, Magistro G, Schubert S, Borath M, Heesemann J and Rossier O (2014) The RNA chaperone Hfq impacts growth, metabolism and production of virulence factors in *Yersinia enterocolitica*. PLoS One 9: e86113.

**Presentation:** Monday, September 28, 2015 from 17:15 – 17:30 in room Congress Saal.

**368/MPV**

**The bacterial flagellum of *Salmonella*: protein export mechanisms of a macromolecular machine**

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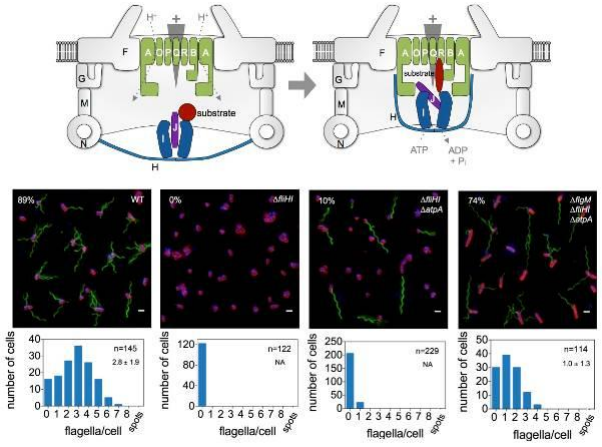
**Background:** Gastrointestinal infections by *Enterobacteriaceae* pose a serious health risk in developing and developed countries. One causative agent of gastrointestinal diseases is the gram-negative, food-borne pathogen *Salmonella enterica* serovar Typhimurium. In order to succeed in invading its host, *Salmonella* employs a wide variety of virulence factors, such as flagella and needle-like injectisome systems for directed movement and secretion of effector proteins. A type-III protein secretion (T3S) apparatus is utilized to secrete building blocks of the flagellum, structural subunits of the injectisome complex, as well as virulence effectors into host cells.
**Question:** The T3S apparatus utilizes both the energy of the proton motive force (PMF) and ATP hydrolysis to energize substrate unfolding and translocation. However, the role of the T3S ATPase and other export apparatus components remained unclear.
**Methods:** We used bacterial genetics, fluorescent microscopy and reporter secretion assays to probe the function of the T3S apparatus *in vivo*.
**Results:** We report formation of flagella in the absence of T3S ATPase activity by mutations that increased the PMF and flagellar substrate levels. We additionally show that increased PMF bypassed the requirement of the Spi1 virulence-associated T3S ATPase for secretion. We thus demonstrate that the actual export process is energized by the means of the proton motive force and does not require ATP hydrolysis.
Finally, we performed a genetic screen to dissect the minimally essential components of the flagellar T3S apparatus. We show that most integral-membrane components are essential and all cytoplasmic components are dispensable for export, emphasizing the importance of the core inner-membrane export apparatus components for the function of type-III secretion systems.
**Conclusions:** In summary, our data support a role for T3S ATPases in enhancing secretion efficiency under limited secretion substrate concentrations and reveal the essential core components of the T3S apparatus.

**Presentation:** Monday, September 28, 2015 from 17:30 – 17:45 in room Congress Saal.

**Figure 1**




**369/MPV**

**The pentose phosphate pathway regulator RpiRc acts as an attenuator of virulence in *Staphylococcus aureus***

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Central metabolism is intimately connected with virulence determinant production in pathogenic bacteria. In *Staphylococcus aureus* this linkage is mediated by at least six regulatory elements, CcpA, CcpE, CodY, Rex, and the RpiR homologs RpiRb and RpiRc. We have previously shown that RpiRc promotes the transcription of pentose phosphate pathway genes such as *rpiA* (encoding ribose phosphate isomerase A) and *zwf* (encoding glucose-6-phosphate 1-dehydrogenase), and to decrease the production of *RNAIII*, one of the master regulators controlling exoprotein synthesis in this clinically important human pathogen.
Here we report that RpiRc also affects infectivity of *S. aureus*. Our transcriptional analyses demonstrated that inactivation of *rpiRc* in *S. aureus* strain SA564 significantly increased the transcription of the α-toxin encoding gene *hla*, one of the major virulence factors of *S. aureus*. Challenging mice with SA564 and its *rpiRc* mutant, respectively, revealed symptoms of increased pathogenicity for the *rpiRc* mutant in a murine abscess model. Complementation of the mutant with the *rpiRc* wild-type allele restored this phenotype, demonstrating RpiRc as a negative regulator of virulence in *S. aureus*.

**Presentation:** Tuesday, September 29, 2015 from 8:30 – 8:45 in room Congress Saal.

**370/MPV**

**The Lipid-Modifying Multiple Peptide Resistance Factor Is an Oligomer Consisting of Distinct Interacting Synthase and Flippase Subunits**

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Phospholipids are synthesized at the inner leaflet of the bacterial cytoplasmic membrane but have to be translocated to the outer leaflet to maintain membrane lipid bilayer composition and structure. Even though phospholipid flippases have been proposed to exist in bacteria, only one such protein, MprF, has been described. MprF is a large integral membrane protein found in several prokaryotic phyla, whose C terminus modifies phosphatidylglycerol (PG), the most common bacterial phospholipid, with lysine or alanine to modulate the membrane surface charge and, as a consequence, confer resistance to cationic antimicrobial agents such as daptomycin. In addition, MprF is a flippase for the resulting lipids, Lys-PG or Ala-PG.
Here we demonstrate that the flippase activity resides in the N-terminal 6 to 8 transmembrane segments of the *Staphylococcus aureus* MprF and that several conserved, charged amino acids and a proline residue are crucial for flippase function. MprF protects S. aureus against the membrane-active antibiotic daptomycin only when both domains are present, but the two parts do not need to be covalently linked and can function in trans. The Lys-PG synthase and flippase domains were each found to homo-oligomerize and also to interact with each other, which illustrates how the two functional domains may act together. Moreover, full-length MprF proteins formed oligomers, indicating that MprF functions as a dimer or larger oligomer. Together our data reveal how bacterial phospholipid flippases may function in the context of lipid biosynthetic processes.

**Presentation:** Tuesday, September 29, 2015 from 8:45 – 9:00 in room Congress Saal.

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**371/MPV**

**Insights into *Staphylococcus epidermidis* primary attachment - Evidence for a new mode of fibronectin binding**

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**Objective**: *S. epidermidis* is the leading cause of biofilm-related implant infections. Biofilm formation essentially relies on tight binding of *S. epidermidis* to surface deposited extracellular matrix components, e.g. fibronectin(FN). The aim of this study was to decipher the molecular basis of extracellular matrix binding protein Embp-mediated *S. epidermidis* adherence to surface immobilized FN.
**Methods**: To this end, the involvement of defined Embp-modules in this process was tested by heterologous in trans expression of the native export motif fused to repetitive found in various architectures (FIVAR) or FIVAR-GA repeats, followed by anticipated cell wall anchor domains. Expression of FIVAR repeats alone or in combination with FIVAR-GA repeats resulted in increased binding of *Staphylococcus carnosus* to surface immobilized FN. By the use of biochemical methods the minimal structural unit of the FIVAR region and GA-module sufficient for FN binding were identified.
**Results**: Strikingly, *S. epidermidis* is incapable of recruiting soluble FN via Embp to its surface, suggesting the involvement of cryptic FN domains that are only accessible during resolution of the globular conformation of the FN molecule during fibrillogenesis. Since immobilization of a recombinant FN Type III subdomain (rFN12-14) strongly augmented bacterial binding, these domains are obviously sufficient for *S. epidermidis* - FN interactions. Protein-interaction-mapping suggests binding in FN12 apart from so far known binding mechanisms.
**Conclusion**: In conclusion, we here provide molecular evidence demonstrating the crucial role of defined Embp modules in staphylococcal adherence to FN and we present first insights into a yet unknown FN binding mechanism in staphylococci.

**Presentation:** Tuesday, September 29, 2015 from 9:00 – 9:15 in room Congress Saal.

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**372/MPV**

**Mechanistic studies of MrpJ paralogs in *Proteus mirabilis***

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The enteric Gram-negative bacterium *Proteus mirabilis* is associated with a significant number of catheter-associated urinary tract infections. *P. mirabilis* exhibits robust swimming and swarming motility, yet it produces a variety of adherence proteins. Both adherence and motility, mediated by fimbriae and flagella, respectively, contribute to UTI. Hence, strict regulation of these antagonistic processes is essential for successful disease progression.
**Objectives:** The transcriptional regulator MrpJ, which is encoded by the *mrp* (mannose-resistant *Proteus*-like) fimbrial operon, has been shown to repress both swimming and swarming motility. Recent work in our laboratory elucidated MrpJ as a global regulator of virulence-associated genes. The *P. mirabilis* genome encodes an additional 14 *mrpJ* paralogs, the majority of which also negatively affect motility. Our study aims to elucidate the differences in target genes or mechanism of action between these transcriptional regulators, necessitating their coexistence in this important uropathogen.
**Materials and Methods:** In order to study these regulatory proteins, we tested expression of potential target genes by qRT-PCR. Mechanistic studies focused on promoter deletion analyses of transcriptional LacZ reporters, as well as the production of chimeric fusion proteins in order to assess domain functionality.
**Results:** Transcriptional analysis of strains overexpressing individual paralogs revealed differences in regulation of target genes. Notably, several MrpJ paralogs autoregulate their own operons. We identified an MrpJ-responsive region in the *mrp* promoter, resulting in positive autoregulation of the fimbrial operon. We narrowed the MrpJ binding site to a 100 bp region located about 150-250 nucleotides upstream of the transcriptional start. Current studies focus on AtfJ, an MrpJ paralog associated with ambient temperature fimbriae (ATF). As with MrpJ, we observed positive autoregulation, albeit at a much greater magnitude. Interestingly, the *atfJ*-responsive element is placed at a greater distance (-487 to -655 nt) from the start of transcription of the *atf* operon. Chimeric fusion proteins between AtfJ and two other MrpJ-type proteins demonstrated that the C-terminal domain of AtfJ is necessary but not sufficient for transcriptional activation of the *atf* operon. Interestingly, several chimeric proteins eliciting negligible LacZ responses retained the ability to repress motility, suggesting that these two protein functions are not invariably linked.
**Conclusion:** Our findings support the hypothesis that *mrpJ* paralogs fulfill non-identical functions. In addition to the possible use of different mechanisms of regulation, these results strengthen the importance of MrpJ-type regulators in *P. mirabilis* biology.

**Presentation:** Tuesday, September 29, 2015 from 9:15 – 9:30 in room Congress Saal.

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**373/MPV**

**The advantage of being diverse: High abundance of mobile genetic elements contributes to genome plasticity and adaptation in a hypervariable *Staphylococcus epidermidis* strain**

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*Staphylococcus epidermidis* is a nosocomial pathogen which is known for its extraordinary phenotypic and genetic flexibility, resulting in heterogeneous gene expression patterns of metabolic, virulence and resistance-associated genes. The molecular background of this diversity in *S. epidermidis* populations and its possible role in the infection process is still poorly understood. Here we report the genome sequence of a hypervariable clinical *S. epidermidis* strain which was recovered from an immunocompromised patient during an eventually fatal infection. The strain, which belongs to the biofilm-forming clonal lineage ST2, constantly generates variants differing in biofilm matrix production and antibiotic susceptibilities. In addition, biofilm-negative variants occur in high frequencies ($10^{-4}$ to $10^{-5}$) that lack the PIA-biofilm-matrix-mediating *ica* locus along with the oxacillin resistance-mediating *mecA* gene.
Genome sequencing of the variants revealed a remarkably high number of mobile genetic elements (MGEs) which comprised 64 insertion sequence (IS) elements (grouping into six different IS families), three prophages, two plasmids, two transposons and a novel mosaic SCC*mec* island. Notably, the multiple IS copies, which featured as repetitive sequences, significantly contributed to genome flexibility by serving as crossover points for homologous recombination events triggering DNA inversions and deletions in the variants.
When assessing the overall genome structure of the strain, a deviation of the termination site *terC* from its normal 180° position opposite to the replication origin *oriT* was noticed. This type of a so called 'imbalanced genome structure' was described before for coagulase-negative staphylococci, but not in the more pathogenic species *Staphylococcus aureus*. Interestingly, analysis of the biofilm-negative variants, which arise regularly from biofilm-forming parent populations, revealed the loss of a 250-kb DNA fragment located near the origin of replication. Spontaneous

deletion of the fragment moves the *terC* site into a nearly 180° position, generating a balanced genome structure similar to that of *S. aureus*. The fragment harbours genes that might be regarded as dispensable for the bacterial cell such as biofilm- and resistance-associated genes, but comprises also a number of metabolic traits. The possible biological impact of this phenomenon for the population dynamics and adaptation power of *S. epidermidis* is discussed.

**Presentation:** Tuesday, September 29, 2015 from 9:30 – 9:45 in room Congress Saal.

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**374/MPV**

**Novel insights into Factor H acquisition by *Staphylococcus aureus***

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Immunocompromised people have a high risk of nosocomial infections caused by *Staphylococcus aureus* (*S. aureus*). Moreover the spread of antibiotic resistant *S. aureus* strains illustrates the increasing relevance of this facultative pathogenic bacterium in infection. One basis to develop therapeutics against pathogenic microbes is the identification and characterization of immune evasion proteins. *S. aureus* expresses a variety of proteins that bind human complement regulators and uses these attached host proteins for complement evasion.
The human complement regulator Factor H binds to *S. aureus* as evaluated in a whole cell elution assay. Using a protein microarray two novel staphylococcal Factor H binding proteins were identified: Complement regulator acquiring surface protein 7 (CRASP7) is a moonlighting protein that binds to Factor H and besides is involved in purine biosynthesis. The second novel identified Factor H binder is CRASP8, a member of a family of superantigen-like proteins.
CRASP7 and CRASP8 were recombinantly expressed. The purified recombinant staphylococcal proteins bind the complement regulator Factor H in ELISA studies. Factor H, when bound to both staphylococcal proteins maintains regulatory activity by acting as a cofactor for the protease Factor I which cleaves complement component C3b into inactive C3b (iC3b). With this cleavage complement amplification at the level of the C3 convertase is blocked. CRASP7, but not CRASP8 additionally binds Complement Factor H related protein 1 (CFHR1) which is an inhibitor of the Terminal Complement Complex. In addition both staphylococcal proteins also bind the human plasma proteins C4, C7, C4BP and Vitronectin.
The findings show that staphylococcal CRASP7 and CRASP8, when binding Factor H and additional complement regulators and components, help the microbe to overcome the human complement attack. We are currently analyzing how CRASP7 and CRASP8 influence complement activity and at which level of the cascade inhibition occurs in detail.

**Presentation:** Tuesday, September 29, 2015 from 9:45 – 10:00 in room Congress Saal.

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**375/MPV**

**DXD Motif-Dependent and -Independent Effects of the *Chlamydia trachomatis* Cytotoxin CT166**

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The Gram-negative, intracellular bacterium *Chlamydia trachomatis* causes acute and chronic urogenital tract infection, potentially leading to infertility and ectopic pregnancy. The only partially characterized cytotoxin CT166 of serovar D exhibits a DXD motif, which is important for the enzymatic activity of many bacterial and mammalian type A glycosyltransferases. That led to the hypothesis that CT166 possess glycosyltransferase activity. CT166-expressing HeLa cells exhibit actin reorganization including cell rounding, which was attributed by us to the inhibition of the Rho-GTPases Rac/Cdc42 (Thalmann, J. *et al.*, *PLoS One* **2010**, *5*, e9887).
The aim of this study was to further characterize the cytotoxin CT166 of *Chlamydia trachomatis* serovar D clarifying its importance for infection. With view to the presence of a DXD motif, its role for enzymatic activity was addressed giving clue to the kind of protein and type of mechanism of CT166. Signal pathways and cellular features that are affected by CT166 were depicted in more detail.
For *in vitro* characterization of the cytotoxin, infection experiments with CT166 expressing *Chlamydia trachomatis* serovar D/UW3 and non-expressing serovar $L_2$/434 were performed. Furthermore, using ectopic overexpression of CT166 and mutated CT166 (point-mutated DXD motif) in TRex$^{TM}$-HeLa, effects on signal pathways, cell cycle progression, cell division and migration were addressed.
Exploiting the glycosylation-sensitive Ras(27H5) antibody, we here show that CT166 induces an epitope change in Ras, resulting in inhibited ERK and PI3K signaling, delayed cell cycle progression and multinucleated cell formation. Consistent with the hypothesis that these effects strictly depend on the DXD motif, CT166 with the mutated DXD motif causes neither Ras-ERK inhibition nor delayed cell cycle progression and multinucleation. In contrast, CT166 with the mutated DXD motif is still capable of inhibiting cell migration, suggesting that CT166 with the mutated DXD motif cannot be regarded as inactive in any case.
Taken together, CT166 affects various fundamental cellular processes, strongly suggesting its importance for the intracellular survival of chlamydia.

**Presentation:** Tuesday, September 29, 2015 from 17:45 – 18:00 in room Congress Saal.

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**376/MPV**

**Inhibition of host cell protein translation during infection with *C. trachomatis***

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*Chlamydia trachomatis* is an obligate intracellular pathogen. It is the most common sexually transmitted bacterial pathogen and infects the epithelium of the urethra of men and endocervix of women, causing inflammation. After uptake, *Chlamydia trachomatis* is found in a membranous vacuole in the cytoplasm, the so called inclusion, where it is protected from host defences and phagolysosomal degradation. Numerous changes in the host cell functions such as apoptosis, cell division, organelle structure and immune signaling are known to be effected by *Chlamydia*. It is believed that this is achived by secretion of effector proteins through the type III-secretion system into the cytosol. Instances are however rare where an identified effector protein has been clearly linked to an observed effect. One prominent, secreted protein is the chlamydial protease CPAF whose physiological substrates and biological role have yet to be unraveled.

To further investigate the effect of infection on host proteins we established a proteomic map of degradation and synthesis of host cell proteins during chlamydial infection using SILAC-labelling. A considerable reduction of host translation associated proteins was seen in these experiments. By labeling newly synthesized proteins using Click-IT-Chemistry we analyzed host cell protein synthesis during chlamydial infection. HeLa cells infected with *C. trachomatis* showed a substantial reduction of *de novo*-protein synthesis from about 24 h p. i. This was comparable to the treatment of cells with 150 ng/ml cycloheximide for 2 h. Interestingly, we could detect a large increase in the 80S ribosome population and a minor reduction in the polysomal fractions by ribosomal profiling. Ectopic expression of CPAF in human cells could reproduce the increase of the 80S ribosome fraction.
*C. trachomatis* reduces protein synthesis of the infected cell, very likely through targeting of ribosomal protein translation. This may be relevant to counter a host response to infection and may explain numerous cell-biological effects in chlamydial infection.

**Presentation:** Tuesday, September 29, 2015 from 18:00 – 18:15 in room Congress Saal.

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**377/MPV**

***Pasteurella multocida* Toxin mediated bone resorption is mediated through differentiation of macrophages and dendritic cells into osteoclasts**

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*Pasteurella multocida* are gram-negative bacteria, which cause respiratory diseases in a number of animals depending. Toxigenic serotype A and D strains produce the *Pasteurella multocida* toxin (PMT), a classical AB toxin containing a deamidase activity as its catalytic function. Intracellularly the toxin constitutively activates heterotrimeric G proteins and induces downstream signalling cascades involved in cytoskeletal rearrangement, proliferation, differentiation or survival of cells. Pathologically, PMT causes porcine atrophic rhinitis characterized by an increased number of osteoclasts and bone resorption at the nasal turbinate bones. Physiologically, the formation of osteoclasts from monocytes or macrophage progenitor cells is initiated by the cytokines RANKL and MCSF. During differentiation, cells fuse into big, multi-nucleated cells. A sealing zone, characterized by accumulation of F-actin, is formed, which allows the cells to attach and resorb bone on the apical side of the osteoclast.
To characterise PMT-induced osteoclasts, we differentiated bone marrow-derived macrophages (BMDM) in the presence of MCSF/RANKL or PMT. The formation of osteoclasts was quantified by the presence of multiple nuclei and the expression of the tartrate resistant phosphatase (TRAP). When we compared TRAP-stained RANKL and PMT-generated osteoclasts, we found significant changes in the morphology as the ring-like structure of classical osteoclasts was missing in PMT-treated cells. This was corroborated in stainings of the actin cytoskeleton, where the Rac1-mediated formation of the actin ring was missing. Instead we found the GTPase RhoA to be highly activated, leading to the formation of stress fibers and the loss of the actin ring. Recently it was claimed that activation of G protein signalling by PMT is sufficient to generate osteoclasts. However, PMT also triggers the release of pro-inflammatory, osteoclastic cytokines. Using IL-6- and TNF-a-specific inhibitors we found that these cytokines play a central role in PMT-mediated osteoclast formation. In contrast to classical osteoclastogenesis that depends on the cytokine RANKL, the RANKL inhibitor and decoy receptor osteoprotegerin (OPG) did not inhibit PMT-mediated osteoclast formation. Interestingly, we found that plasmacytoid dendritic cells (pDC) but not GM-CSF/IL-4-differentiated BMDC were able to form TRAP-positive osteoclasts in the presence of PMT. However in contrast to BMDM, the transdifferentiation of pDCs depended on the RANK-RANKL interaction, and their differentiation was only observed in the presence of RANKL-expressing B-cells.
In summary, PMT is able to circumvent the activation of the immune system by changing DC and macrophage activity by re-directing their differentiation into the formation of osteoclasts and thus reducing their ability to act as the primary immune defence.

**Presentation:** Tuesday, September 29, 2015 from 18:15 – 18:30 in room Congress Saal.

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**378/MPV**

**Identification of Cyp40 as a novel drug target for the development of new therapeutic strategies against bacterial ADP-ribosylating toxins**

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Some severe diseases e.g. diphtheria are caused by bacterial AB-type toxins. These toxins consist of two functional distinct domains: the binding/translocation (B-) domain facilitates receptor-mediated endocytosis and membrane translocation of the enzyme (A-) domain from acidified endosomes into the host cell cytosol. In recent years, we demonstrated that the host cell factors Hsp90 and the peptidyl prolyl *cis/trans* isomerases (PPIases) cyclophilin (Cyp) A and FK506-binding proteins (FKBP) 51 facilitate the membrane translocation of ADP-ribosylating *Clostridium (C.) botulinum* C2 toxin, *C. perfringens* iota toxin and *C. difficile* CDT toxin [1,2]. Interestingly, recombinant fusion toxins harboring an ADP-ribosyltransferase domain - but not toxins with different enzyme activities - also require these host cell factors for membrane translocation [3]. These findings led to the hypothesis that the demand of Hsp90/PPIase might be a common characteristic for ADP-ribosylating toxins. Moreover, it is known that Hsp90, FKBP51 and FKBP52 act in a multi-chaperone complex during the activation of steroid hormone receptor complexes and their translocation to the nucleus in the cell, suggesting that a similar complex might facilitate the membrane translocation of ADP-ribosylating toxins [4]. Interestingly, we identified Cyp40, which also interacts with the Hsp90 multi-chaperone complex, as a novel interaction partner for ADP-ribosylating toxins demonstrated by dotblot analysis, isothermal titration calorimetry and pull down experiments [5]. Furthermore, we showed that the non-immunosuppressive Cyclosporin A-derivative VK112 inhibits the intoxication of mammalian cells with clostridial ADP-ribosylating toxins as well as their membrane translocation indicated by less rounded i.e. intoxicated cells in the presence of VK112 (see Fig. 1 & 2) [5]. These findings provide a potential starting point for the development of novel pharmacological strategies in order to inhibit intoxication and by that prevent clinical symptoms caused by ADP-ribosylating toxins.

**References**

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**Presentation:** Wednesday, September 30, 2015 from 13:00 – 13:15 in room Congress Saal.

**Figure 1**

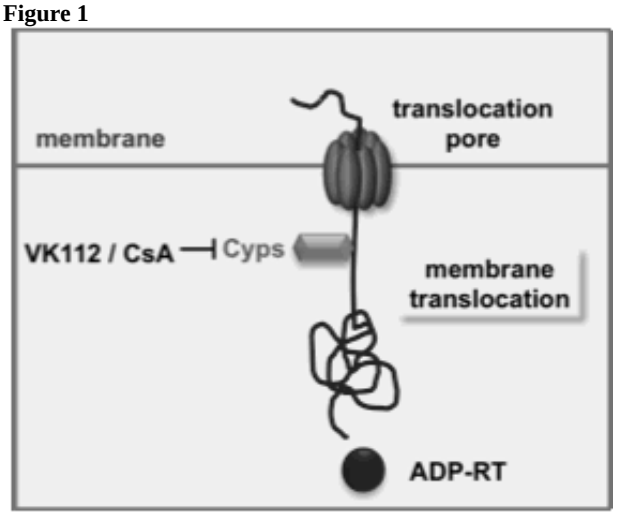
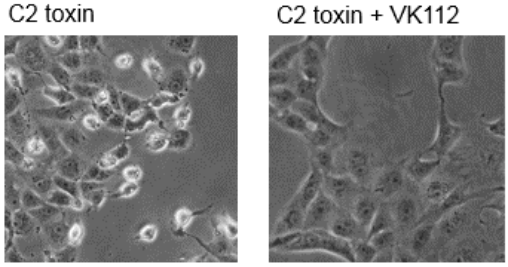



**Figure 2**




**379/MPV**

**A novel mechanism for bacterial outer membrane vesicle biogenesis and its role in microbial pathogenesis**

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Outer membrane vesicles (OMVs) are spherical, bilayered, membranous structures that are naturally released from the outer membrane of Gram-negative bacteria. Although OMVs have important biological roles in pathogenesis and intercellular interactions, the mechanism of OMV formation is far from being fully understood. Here we show that deletion or repression of a pathway involved in retrograde trafficking of phospholipids from the outer to the inner membrane increase OMV production in Gram negative pathogens using the distantly related species *Haemophilus influenzae* and *Vibrio cholerae* as model organisms. Disruption of the retrograde lipid trafficking system results in distinct changes in th lipidome of the OMVs and hypervesiculation without compromised outer membrane integrity. Interestingly, iron limitation, which could also serve as a signal in the host, leads to downregulation of the system and consequently increased OMV production. Indeed, we provide evidence that hypervesiculation in the initial phase of the infection might be advantageous for the colonization fitness of the pathogens. In summary, we propose a new model for OMV biogenesis based on the accumulation of distinct phospholipid-species in the outer membrane, which can be regulated by the cell via expression of the retrograde lipid trafficking system. Since this system is highly conserved among Gram negative bacteria this could be a first general model for OMV biogenesis.

**Presentation:** Wednesday, September 30, 2015 from 13:15 – 13:30 in room Congress Saal.

**380/MPV**

**Structural analysis of the choline-binding protein CbpL important for virulence of *Streptococcus pneumoniae***

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**Introduction:** Four families of surface proteins decorate the cell surface of the human pathogen *Streptococcus pneumoniae*. Besides lipoproteins and LPXTG proteins, also present in other Gram-positive bacteria, the pneumococcus presents the non-classical surface proteins and the choline-binding protein family. Choline binding proteins (CBP) show a modular organization including at least the choline-binding domain (CBD) and a domain exerting a biological function. The CBD interacts with choline molecules from teichoic and lipoteichoic acids, attaching the whole protein to the peptidoglycan layer. In this work we have characterized the role of the unique choline-binding protein CbpL in pneumococcal virulence and we show the three-dimensional structure of CbpL subdomains.
**Methods:** The CbpL containing the CBD and the excalibur domain as a functional domain were expressed as His-tagged fusion proteins and for X-ray crystallography the His-tags was cleaved off by the TEV protease. The impact of CbpL on phagocytosis and virulence in an acute mouse infection model was assessed by generating a CbpL-deficient mutant.
**Results**: The CbpL has a modular organization and the CBD in CbpL is located between two functional domains in the protein core. One of the functional domains of CbpL is the N-terminally located excalibur domain. The CBD of CbpL displays eight choline-binding sites. Four of them follow the canonical sequence while the other four are different. The alternate configuration of canonical and non-canonical sites is a unique property of CbpL among CBP family and the specific structural features of this module will be provided at a resolution of 1.5 Å. Similar the structural features of the excalibur domain will be introduced, which is most likely essential for the interaction with the host. The *cbpL*-mutant in *S. pneumoniae* D39 was attenuated in the acute pneumonia mouse model and phagocytosis of CbpL-deficient pneumococci was increased.
**Conclusion:** The CbpL choline-binding domain is formed by 9 repeats of two β-strands each, giving a total of 9 choline-binding sites. The functional excalibur domain is suggested to be involved in pneumococci-host interactions and essential for virulence.

**Presentation:** Wednesday, September 30, 2015 from 13:30 – 13:45 in room Congress Saal.

**381/MPV**

**The *Acinetobacter baumannii* trimeric autotransporter adhesin Ata and its linkage to adhesion, invasion and pathogenicity**

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*Acinetobacter baumannii* is an emerging nosocomial pathogen causing difficult to treat, severe infections worldwide. However, very little is known about virulence factors of *A. baumannii*. We hypothesised, that the recently identified trimeric *Acinetobacter baumannii* autotransporter adhesin (Ata) is an important virulence factor in this pathogen and therefore investigated Ata-mediated adhesion to different targets, invasion into host cells and virulence *in vitro* and *in vivo*.

*A. baumannii* ATCC 19606 and ATCC 17978 (WT) and respective isogenic *ata* deletion mutants (Δ*ata*) were generated and analysed in infection assays. In the *Galleria* infection model, 10% of WT *A. baumannii* ($10^5$ CFUs) infected caterpillars survived 24 h post infection, whereas 50% of infected larvae with Δ*ata* ($10^5$ CFUs) were still alive after 5 days. To monitor Ata-mediated virulence on human cells, LDH release of infected HUVECs were determined after 24 h. Here, 38% of WT and 22% of Δ*ata* infected HUVECs showed cell death respectively.
Since adhesion is a crucial and early step during infection, binding to extracellular matrix proteins und human cells was investigated. Bacterial adhesion was quantified by fluorescence microscopy and densimetric image analysis, determining relative fluorescence units of CD31-Alexa647 conjugated primary human endothelial cells (HUVEC) and CFSE stained bacteria, as well as by amplifying bacteria specific genes to calculate genomic equivalents using qRT-PCR. Deletion of *ata* led to a statistical significant decreased adhesion (up to seven-fold) to collagen I + II and laminin as well as HUVECs under static conditions. To analyse adhesion under blood stream infection conditions, HUVECs were infected with *A. baumannii* in laminar flow chambers under shear stress. Compared to static experiments, differences of WT and D*ata* adhesion were similar, confirming the previous results, but total binding rates decreased five-fold. When employing an *ex vivo* dynamic infection model using human umbilical cord veins, bacterial binding rates were six-fold higher in dynamic infected umbilical cord veins compared to the flow chamber model. Invasion into HUVECs was analysed *in vitro* by determination of intracellular bacteria after gentamycin treatment. Interestingly and in contrast to the adhesion assays, invasion into HUVECs was not significantly different between WT and Δ*ata*.
Our results indicate that Ata mediates adhesion to ECMs and human host cells *in vitro* and in the *ex vivo* umbilical cord vein infection model under dynamic flow conditions. Accordingly, virulence of *A. baumannii in vitro* and *in vivo* was dependent on Ata. Thus, Ata critically regulates virulence in *A. baumannii* and might represent an attractive target for new therapy approaches in the future.

**Presentation:** Wednesday, September 30, 2015 from 13:45 – 14:00 in room Congress Saal.

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**382/MPV**

**LecA - a multifunctional pathogenicity factor of *Pseudomonas aeruginosa* with a bivalent role in bacterial host cell invasion**

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*Pseudomonas aeruginosa* (*P.a.*) is a Gram-negative human pathogen which colonizes and infects diverse tissues. The invasion of host cells by *P.a.* significantly contributes to its pathogenicity. The invasion process, in particular the role of the interaction of *P.a.*-lectins with host glycosphingolipids (GSLs), is not fully understood. By using giant unilamellar vesicles as biomimetic membrane models and cell-based approaches we recently showed that binding of LecA, a lectin expressed by *P.a.*, to the host GSL Globotriaosylceramide (Gb3) triggers plasma membrane bending and engulfment of *P.a.* independently of actin, by a mechanism which is termed the *lipid zipper (1)*. However, our data also show that the complete invasion process requires the action of Arp2/3 in conjunction with LecA. This suggests a role for actin (polymerization) in bacterial invasion beyond the lipid zipper. Abelson tyrosine kinase (Abl) is an important host cell factor that regulates the actin cytoskeleton for efficient cell entry of *P.a. (2)*. As yet it is unknown which factor(s) of *P.a.* activate Abl kinase. Here we show that LecA engages GSLs to activate Abl kinase for efficient entry into lung epithelial cells. Inhibition of host GSL synthesis in general or specific depletion of Gb3, prevents Abl activation by LecA. On the other hand, wild-type Chinese hamster ovary cells that do not express Gb3 could be sensitized for LecA-dependent Abl activation by heterologous expression of Gb3, which was not found in the wt-cells. In conclusion, our data suggest that LecA broadly influences the invasion process. Therefore, compounds, which interfere with this specific bacterial factor might also help to treat *P.a.* infections. In this regard, we tested a divalent ligand for LecA that was identified from a galactoside-conjugate array and which shows a very high LecA-affinity ($K_D$ = 82 nM) *(3)*. This ligand exerts a strong, inhibitory effect (up to 90%) on the invasiveness of *P.a.* even when applied at rather low concentrations (0.05-5 μM). Therefore, this ligand could be a potential candidate for further drug development to face *P.a.* infection. In summary, our data provide evidence for LecA as a multifunctional pathogenicity factor of *P.a.* that triggers signaling (Abl-)-dependent and independent steps of the invasion process to promote efficient bacterial penetration of host cells.

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**Presentation:** Wednesday, September 30, 2015 from 14:00 – 14:15 in room Congress Saal.

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**383/MPV**

**Mycobacterial resuscitation promoting factor (RPF) - new insights into the mechanism of action**

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One third of the world's population is infected by the latent form of TB. The latent state of TB may persist for a lifetime, but it may reactivate into the active state under the influence of different factors (weakening of the immunity, social factors, etc.) causing infection development. According to the prevailing viewpoint, latent form of TB is connected with the natural ability of MTB to transform into the special state of dormancy similar to the process of sporulation. Supposedly the dormant forms reactivate in the tissues of a host, forming actively-replicating cells. A family of proteins, controlling the process of the dormant mycobacteria transformation, has been found (Mukamolova et al, 1998). These proteins promoted actinobacteria resuscitation (including MTB) from the dormant into the active state. In spite of the progress that has been made, intrinsic mechanisms, underlying the bases of reactivation are still unclear. Enzymatic activity of the proteins has been suggested (Cohen-Gonsaud, 2004, 2005) - when it was found that the proteins of this family are homologous to lysozyme. We have found, that mycobacterial peptidoglycan (PG) treatment by Rpf leads to small-molecular weight molecules (3 kDa) - muropeptides formation. Application of MALDI-TOF allowed us to determine chemical structure of the forming product. On the next step we applied the collected fraction of muropeptides in resuscitation procedure (Shleeva et al, 2004). The number of the reactivated cells was comparable with the number of the cells, undergone treatment by Rpf. Obviously, muropeptides, indeed, are responsible for signal transduction during resuscitation. Although, the same effects were observed before, on the example of *Bacillus subtilis's* endospores germination (Shah et al, 2008). The receptors responsible for muropeptides action were stated to be Ser/Thr kinases of PrkC. Drawing parallels between two processes - *Bacillus subtilis's* spores germination and *Mycobacterium tuberculosis's* resuscitation enables us to contemplate plausible

metabolic pathway of dormant mycobacteria transformation (Fig. 1). The knowledge of the exact mechanism of mycobacteria reactivation, would allow us to find new ways of tuberculosis treatment.

**Presentation:** Wednesday, September 30, 2015 from 14:15 – 14:30 in room Congress Saal.

**Figure 1**

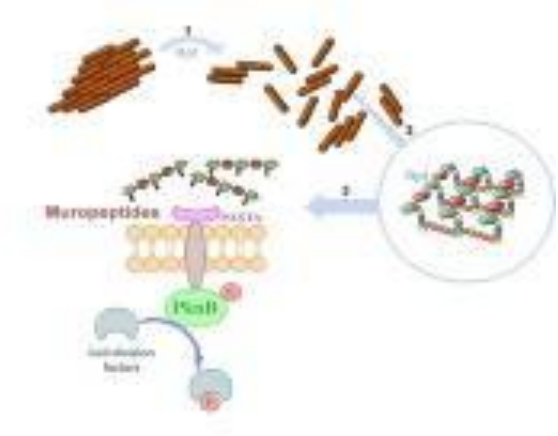


*Fig. 1. Supposed scheme of the dormant Mycobacteria reactivation.*

1.Rpf causes cellular aggregates deconstruction;
2.Rpf effect mycobacterial peptidoglycan, releasing muropeptides;
3.Muropeptides interact with a cellular receptor (presumably PASTA domain of a Ser/Thr kinase PknB);
4.Triggering of the intracellular mechanisms, controlling cell division

## MOLECULAR EPIDEMIOLOGY OF MICROORGANISMS (MSV)

**384/MSV**

**Anticipating the second-line antibiotic era: drug resistant tuberculosis strain drives epidemic in Central Asia**

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**Question**: Resistant-, multidrug resistant- (MDR) and even extensively drug resistant- (XDR) *Mycobacterium tuberculosis* complex (MTBC) strains have emerged worldwide and represent a serious challenge for global tuberculosis (TB) control. Interestingly, high rates of MDR-TB have been associated with particular phylogenetic lineages of the MTBC such as the Beijing lineage and the strong clonal expansion of particular MDR strains in Eastern Europe. However, a systematic investigation on the association of pathogen genetic and treatment-related factors contributing to an enhanced trans-regional transmission capacity of MDR strains in Eastern Europe has not been performed so far.
**Methods**: To address this question, we applied whole genome sequencing on 277 MDR-TB strains from Uzbekistan covering the years 2001 to 2006 for an in depth analysis of the population structure and to precisely assess the extent of ongoing transmission networks.
**Results**: Our results showed that transmission success is not equally distributed among the MDR strain population and can differ even among closely related outbreak strains. Analysis of genome characteristics allowed the identification of particular beneficial combinations of mutations conferring resistance to multiple first and second-line TB antibiotics and mutations potentially compensating for the fitness defect of the dominating MDR/XDR strain types. Moreover, we show that one particular highly resistant strain type, already existed before the introduction of programmatic second-line treatment in the region of Nukus, Uzbekistan and was most likely selectively promoted during the introduction of WHO endorsed DOTS strategies (directly observed treatment, short-course) in 1998 and 2003. This highly successful pre-XDR clone caused up to 75% of all MDR-TB cases in 2005/2006 in the study region.
**Conclusion**: We conclude that ineffective second-line drug regimens most likely created a diversity of different highly resistant TB strains and strongly argue for a rapid and precise molecular resistance diagnosis to prevent further selection for extremely transmissible XDR variants and thus, further complicate TB control.

**Presentation:** Monday, September 28, 2015 from 8:45 – 9:00 in room Roter Saal 2.

**385/MSV**

**Microevolution of ancestral Escherichia coli unravels niche-specific traits of successful pathogenic lineages**

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The ancestral Escherichia coli sequence type (ST) 10 harbors commensal and pathogenic isolates, thus presenting a valuable population for studying E. coli microevolution. Whole-genome sequencing and functional analyses of a representative ST10 E. coli collection defined three major groups. Uropathogenic E. coli (UPEC) and neonatal meningitis causing E. coli (NMEC) were of unique evolutionary origin, and functional analysis revealed two distinct metabolic groups, corroborating their habitat specific microevolution. Similarly, metabolic differences were unraveled between UPEC and commensals sharing a common genetic background. Host as well as pathotype-specific recombination events were identified in isolates from pigs and humans, and two further defined groups were associated with multidrug resistance. The identification of habitat specific metabolic pathways and recombination events paves the way for understanding infection biology and defining pathotype-specific biomarkers, possibly enabling future new anti-infective strategies.

**Presentation:** Monday, September 28, 2015 from 9:00 – 9:15 in room Roter Saal 2.

**386/MSV**

***Staphylococcus aureus* prevalence, antibiotic resistance and molecular diversity in the general population in Northeast Germany - results of the Study of Health in Pomerania (SHIP)**

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N. Haasler[4], K. Henselin[4], K. Kuehn[2], S. Nowotny[1], D. Radke[4]
K. Schulz[2], S. Schulz[1], C. Hai Vu[1], S. Westphal[4], H. Voelzke[4]
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**Question:** Asymptomatic carriage of *Staphylococcus aureus* in the nasal cavity is common and one of the most important risk factors for subsequent infections. We here report the prevalence, resistance and molecular diversity of *S. aureus* in the general population in Western Pomerania, Germany. Nasal swabs were obtained from 5884 adults in a large-scale population-based study: the Study of Health in Pomerania (SHIP). SHIP is one of the most comprehensive population-based studies worldwide, including functional tests for several organs, blood examinations, a whole-body MRI, OMICs analyses of body fluids as well as extensive questionnaires.

**Methods:** Nasal *S. aureus* colonization density was semi-quantified. Isolates were characterized using *spa* genotyping, as well as antibiotic resistance and virulence gene profiling.

**Results:** A cross-sectional analysis demonstrated that 26.2 % (1604/6130) of the adult population was colonized with *S. aureus*. A total of 1.2 % of the isolates were MRSA (18 HA-MRSA, 1 ST398-LA-MRSA). Most HA-MRSA isolates (12/18) belonged to the pandemic European HA-MRSA-ST22 clone. *Spa* typing revealed a diverse but highly clonal *S. aureus* population structure. The major seven CCs (CC30, 45, 15, 8, 7, 22 and 25) included ca. 75% of all isolates. Virulence gene patterns were strongly linked to the clonal background.

A longitudinal analysis revealed that 10.2 % (155/1512) of probands were persistent carriers, i.e. colonized with closely related or identical strains on both sampling occasions, and 20.9 % (n=316) were intermittent carriers, i.e. colonized with different strains at both sampling occasions or only once positive. Persistent carriers had significantly higher colonization densities than intermittent carriers. However, it was not possible to stratify subjects according to the type of carriage with this parameter because of the large variance in both groups.

**Conclusions:** In the future, the SHIP study will allow us to address some long-standing questions in *S. aureus* research, such as risk factors for nasal carriage as well as carriage-associated morbidities and mortality.

**Presentation:** Monday, September 28, 2015 from 9:15 – 9:30 in room Roter Saal 2.

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**387/MSV**

**Antimicrobial Susceptibility and Molecular Epidemiology of *Neisseria gonorrhoeae* from the Cologne Metropolitan Area**

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**Background**: Recent reports from around the world point to a dramatic increase in antibiotic-resistant *Neisseria gonorrhoeae*. However there are scarce data regarding the situation in Germany. We therefore sought to investigate the antimicrobial susceptibility of *N. gonorrhoeae* and their molecular epidemiology in the Cologne metropolitan area.

**Methods**: Twenty isolates were collected from patients with symptoms of urethritis reporting to one of the hospitals or doctors offices serviced by the participating laboratories between 11/2013 - 03/2014. With one exception all patients were male, with a median age of 30 years. All *N. gonorrhoeae* isolates cultured from these samples were included in this study. Susceptibilities to ciprofloxacin (CIP), ceftriaxone (CTX), azithromycin (AZT) penicillin G (PEN), and tetracycline (TET) were determined using CLSI agar dilution and interpretation guidelines. Molecular epidemiology was investigated by multi locus sequence typing (MLST).

**Results**:MIC results revealed that 6/20 (30%), 0/20 (0%) and 3/20 (15%) isolates were susceptible to TET, PEN, and CIP, respectively. However, all isolates recorded MICs of $\leq 0.25$ µg/ml and $\leq 0.5$ µg/ml to CTX and AZT, respectively. MLST analysis revealed 8 sequence types (ST), of which two (ST1901 and ST7363) were predominant with 6 isolates each. Furthermore, clonal complex (CC) 1901 (N=7) and CC7363 (N=10) accounted for 85% of the isolates. There was no correlation between ST, CC and antimicrobial susceptibility, with the exception of two CIP-susceptible isolates that were ST1594.

**Conclusions**: We have found evidence for the existence of two MDR clonal complexes in the Cologne metropolitan area. While resistance rates to CIP, PEN and TET was high, CTX and AZT resistance was not observed suggesting that these agents remain as empirical treatments of *N. gonorrhoeae*.

**Presentation:** Monday, September 28, 2015 from 9:30 – 9:45 in room Roter Saal 2.

**388/MSV**

**Map African TB - Population structure and evolution of *Mycobacterium tuberculosis* complex in Africa**

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D. Kombila[8], A. Alabi[8], S. Janssen[9], B. Lell[8], M. P. Grobusch[9]
T. Dlamini[10], T. A. Kohl[1], S. Ruesch-Gerdes[11], F. Ntoumi[12]
M. Frank[3,4], M. Bonnet[6], B. C. de Jong[5], A. Rachow[13,14]
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**Question**: According to the World Health Organization an estimated number of 2.8 million people were newly infected by tuberculosis (TB) and 690,000 died from TB in Africa (2013). Even more worrisome is the emergence of drug resistant *Mycobacterium tuberculosis* complex (MTBC) isolates in Africa. Since 2011 the number of TB patients infected with multidrug-resistant (MDR), MTBC strains, strains resistant to at least the two most effective antibiotics isoniazid and rifampicin, has doubled. Especially in Sub-Saharan Africa with its high rate of HIV co-infected people, the effective transmission of MDR TB is a threatening scenario.

**Methods**: To get an insight in transmission dynamics of TB in Africa we analyze the population structure of the MTBC in Africa using molecular typing methods (spacer oligonucleotide typing (spoligotyping), 24-loci mycobacterial interspersed repetitive units - variable number of tandem repeats (MIRU-VNTR) typing). These methods are ideally suited to analyze the population structure and chains of transmission of MTBC isolates. So far 2,900 African MTBC isolates from 14 countries, originating from Western Africa (420), Central Africa (1,140), Eastern Africa (930) and Southern Africa (450) have been genotyped. In addition to classical genotyping the genome of selected isolates is analyzed by whole-genome sequencing (WGS).

**Results**: The first analysis of the population structure revealed that 58% of MTBC isolates belong to a cluster of strains. Clustering of strains indicates patient-to-patient transmission of TB; this can be seen in all investigated countries. In addition to that cross-border transmission of antibiotic susceptible MTBC isolates in Kenya and Tanzania as well as Gabon and Cameroon was observed. Furthermore, analysis of isolates from Mozambique, Gabon and Swaziland showed active transmission of clonal MDR strains within the local population. At a first glance, we had an in-depth look into isolates from Gabon and Swaziland. We further analyzed those isolates by WGS and could confirm effective patient-to-patient transmission of MDR strains in two African countries. To prevent further spreading of the MDR strains in Gabon we established a molecular test assay to rapidly detect the MDR strains in Gabon. Therefore we identified, based on the WGS data, a specific single nucleotide polymorphism (SNP) for the MDR cluster and designed a real-time-PCR based assay to detect this SNP.

**Conclusion**: In conclusion, our study identified transmission of different MTBC lineages all over the African continent as well as spreading of already MDR strains in some countries. These results give a first impression of the population structure and transmission of MTBC isolates in Africa. To prevent further spreading of TB, especially MDR TB in Africa we will continue our study and aim to establish a database of the population structure of the MTBC in Africa.

**Presentation:** Monday, September 28, 2015 from 9:45 – 10:00 in room Roter Saal 2.

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**389/MSV**

**Whole Genome Analysis of African Methicillin-resistant *Staphylococcus aureus* with USA300-related Genotypes**

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**Question:** The USA300 clone is a hypervirulent methicillin-resistant *Staphylococcus aureus* (MRSA) lineage, which is highly prevalent in North America but rarely isolated elsewhere. Some MRSA with USA300 characteristics (Panton-Valentine leukocidin [PVL] and ACME positive, MLST Sequence Type [ST] 8) were reported from sub-Saharan Africa. However, due to differing *spa* types they were not regarded as true USA300. Our objective was to clarify the relation of these USA300-related isolates with typical USA300 strains using comparative genomics.

**Methods:** In total, 58 ST8 *S. aureus* isolates were collected in Australia (6), Cameroun (1), Côte d'Ivoire (10), DR Congo (10), Gabon (21), Ghana (2), Madagascar (1), and Nigeria (7) between 2005 and 2013. Whole genome sequencing (WGS) was performed to type all isolates according to the MLST$^{+}$ scheme (gene-by-gene comparison) using the Ridom SeqSphere$^{+}$ software v. 2beta (Ridom GmbH, Muenster, Germany). Moreover, USA300 characteristic genes for ACME (*arc* operon, *speG*) PVL (*luk*F-PV, *luk*S-PV), SaPI5 (*sek, seq*), mutated capsular polysaccharide genes (*cap5D, cap5E*), and SCCmec IV (*mecA, ccrA2, ccrB2,* IS431 and IS1272 transposases, *ΔmecR1*) were inferred from the sequences. A neighbour-joining tree based on MLST$^{+}$ data was constructed including all currently available ST8 NCBI RefSeq genomes (n=4) and an USA500 NCBI RefSeq genome.

**Results:** MLST$^{+}$ typing based on 1530 core genome genes present in all isolates resulted in three distinct clades. Sixteen isolates from Australia, Ghana, Gabon and Cameroun clustered with the USA300 reference strain, shared all USA300-specific traits (ACME, PVL, SCCmec IV, SaPI5, mutated cap5), and differed in ≤34 MLST$^{+}$ genes. Twelve isolates from Gabon and Nigeria clustered with USA500, mainly sharing the same *spa* type (t064) and cap5D mutations characteristic for USA500, but different virulence and resistance traits. The third cluster consisted mainly of ST8 methicillin susceptible *S. aureus* from Côte d'Ivoire, DR Congo, Gabon and Ghana, including two ST8 MSSA NCBI RefSeq genomes, which were all PVL- and ACME-negative. Isolates with *spa* type t008 characteristic for USA300 were found in different topological branches.

**Conclusion:** WGS data suggests that the USA300-related MRSA found in West and Central Africa belong to the real USA300 clonal lineage.

**Presentation:** Monday, September 28, 2015 from 8:30 – 8:45 in room Roter Saal 2.

**390/MSV**

**Microevolution of Sorbitol-Fermenting Enterohemorrhagic *Escherichia coli* O157:H⁻**

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**Introduction**: Enterohemorrhagic *Escherichia coli* (EHEC) cause diarrhea that may progress to the hemolytic uremic syndrome (HUS). Apart from the globally predominant strain O157:H7, sorbitol-fermenting (SF) O157:H⁻ has evolved as a serious pathogen in the last decades. First isolated in 1988, SF O157:H⁻ caused several outbreaks, whose infectious sources and ways of transmission could not be entirely elucidated.

**Objectives**: Here we utilize the high resolution of whole genome sequencing (WGS) to investigate the microevolution of SF O157:H⁻ since 1988 and during an outbreak.

**Material & Methods**: A total of 26 SF O157:H⁻ strains isolated between 1988 and 2013 were investigated, among them the first SF O157:H⁻ from 1988, 9 isolates related to the German outbreak in 1995/96, the first isolate from outside Germany (from the Czech Republic) and 15 randomly chosen clinical isolates from the German National Consulting Laboratory for HUS, Muenster. WGS was done on Illumina platforms (Illumina Inc., San Diego, USA); after quality trimming and de novo assembly, gene sequences for subsequent analyses were extracted using Ridom SeqSphere$^+$ software v. 2.4 beta (Ridom GmbH, Muenster).

**Results**: Within a set of genes (n=3494) that were present in all SF O157:H⁻ without insertions, deletions or recombinant segments, we determined in total 570 single-nucleotide polymorphisms (SNPs) among the SF O157: H⁻. The mean SNP-variation among all SF O157:H⁻ was 46.72 SNPs. Of the 9 outbreak associated strains, 4 shared the same genotype, the remaining clustered quite closely. Two isolates, varying in 225 and 217 SNPs, respectively, differed more than the rest. General phylogenetic analysis with Bayesian Evolutionary Analysis Sampling Trees (BEAST) revealed that the common ancestor of the analyzed SF O157:H⁻ has developed about 400 years ago.

**Conclusion**: The 4 isolates with the same genotype were definitively part of the outbreak in 1995/96. For the remaining isolates a clear-cut differentiation is difficult to make as the SF O157:H⁻ prove to be very closely related. Still WGS gave important insight as it also clustered strains very near to the outbreak genotype that by epidemiological assumptions were not thought to be part of the outbreak emphasizing the need for further research about the quite young SF O157:H⁻ clone.

**Presentation:** Monday, September 28, 2015 from 17:15 – 17:30 in room Roter Saal 2.

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**391/MSV**

**Towards NGS as frontline tool for routine typing of *S. aureus* at the National Reference Center for staphylococci and enterococci?**

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**Objectives:** The rapid progress of Next generation sequencing (NGS) technology has dramatically changed our vision of clinical microbiological diagnostics, strain characterization and typing during recent years. Although the constant decrease in cost nowadays enables the implementation of NGS technology in molecular surveillance activities, detailed bioinformatic analysis of genome data still remains a challenge. Therefore this study was initiated to evaluate feasibility and benefit of NGS for the staphylococcal molecular typing service provided by the National Reference Center (NRC) for staphylococci and enterococci in comparison to typing tools used to date.

**Materials and methods:** We selected a total of 282 *S. aureus* isolates sent to the NRC throughout February 2014. All isolates passed the routine typing workflow at our laboratory including phenotypic susceptibility testing, *spa*-typing (Sanger sequencing) and PCR-based *mecA*-detection. Depending on the initial strain report additional resistance and virulence determinants were investigated by phenotypic and genotypic methods, respectively.

Whole genome libraries were generated using the Nextera XT DNA Sample Prep Kit (Illumina). Sequencing was carried out on a MiSeq instrument and performed in paired-end mode using a v3 chemistry-based cartridge and aiming at a theoretical coverage of app. 100-fold.

Initial data analysis was performed using the Ridom SeqSphere 2.4 pipeline including Velvet as assembler. Based on the finished assemblies SeqSphere extracted *spa*-type and MLST from the whole genome data for each isolate. For in-depth-typing a predefined whole genome MLST scheme (SeqSphere MLST$^+$) was used which analyzes sequence diversity of 1861 *S. aureus* core genome loci. To extract further information concerning the presence of a variety of relevant resistance and virulence determinants, user-defined sets of corresponding genetic loci were added to the SeqSphere queries.

**Results:** Out of 282 genome datasets 275 yielded satisfactory results (≥95% of the 1861 MLST$^+$ target genes with good quality) in the SeqSphere pipeline, thus highlighting the importance of high quality raw data and an average coverage of at least 60-fold.

Results extracted from NGS data were overall concordant with those generated by currently used genotypic (*spa*-typing, MLST) and phenotypic methods (e.g. antibiotic resistance). Molecular typing based on whole genome MLST data, as expected, yielded significantly higher discrimination, especially for endemic *S. aureus* lineages and enabled the support of outbreak investigations in several institutions.

**Conclusion and outlook:** Whole genome sequencing was successfully established for molecular typing purposes at the NRC. Future work will focus on the clarification of contradictory results and on the comparison of results obtained from the combination of different available bioinformatic tools.

**Presentation:** Monday, September 28, 2015 from 17:30 – 17:45 in room Roter Saal 2.

**392/MSV**

**Implementation of NGS for outbreak investigations at the German National Reference Center for *Salmonella* exemplified by the 2013/14 *S*. Derby outbreak in Berlin and Brandenburg**

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From November 2013 till January 2014 a *Salmonella* (*S.*) Derby outbreak occurred among elderly people in Berlin and surrounding regions of the Brandenburg Federal State. Mainly hospitalized individuals and residents of nursing homes were affected, in total 145 cases had been reported. Microbiological analyses defined the outbreak strain as *S.* Derby phage type PT53 with $PFGE_{XbaI}$ pattern 16a (internal nomenclature). Additional outbreak-related isolates were obtained from food and asymptomatic carriers. Epidemiological investigations identified raw pork sausage as the suspected infection source.

The high number of isolates from patients, food and carriers as well as the availability of excellent epidemiological and microbiological data made this outbreak highly suitable for retrospective investigation by NGS. Forty-two *S.* Derby isolates (24 outbreak and 18 epidemiologically unrelated strains) had been sequenced using an Illumina MiSeq benchtop sequencer. The index case isolate was subjected to PacBio sequencing (GATC Biotech AG, Konstanz, Germany) and served as reference genome for subsequent mapping analyses. The MLST type, based on seven housekeeping gene loci, was extracted from the mapped sequence data and revealed ST682 for all outbreak isolates, thus supporting the epidemiological and microbiological data. However, classical MLST has limited discriminatory power for outbreak investigations. Since there is no internationally agreed core genome MLST scheme available yet for *Salmonella*, we applied a SNP-based approach to disclose the outbreak in high resolution.

This work describes the 1st application of NGS for a retrospective *Salmonella* outbreak investigation at the German National Reference Center for *Salmonella*. The aim was to establish this highly discriminatory method and to compare it to traditional typing methods in order to provide a powerful tool for real time genome-based outbreak investigations and molecular surveillance of enterobacterial pathogens in the future.

**Presentation:** Monday, September 28, 2015 from 17:45 – 18:00 in room Roter Saal 2.

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**393/MSV**

**Comparison of six commercial kits to extract bacterial chromosomal and plasmid DNA for MiSeq sequencing**

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Next generation sequencing is about to be incorporated into routine practice in clinical microbiology laboratories. Independent from specific applications and sequencing methods, robust DNA extraction methods are required. Plasmids may contribute to the genetic content of a bacterial strain and may differ from the bacterial chromosome with respect to their size, structure, copy number, and efficiency of extraction and sequencing.

In 2011, a difficult-to-control outbreak on a neonatal ward was caused by an extended-spectrum-betalactamase-producing *Klebsiella pneumoniae* strain (1). We recently sequenced to completeness the genome from one reference isolate from this outbreak. The outbreak strain carried one large (362 kb) and two smaller plasmids (4 and 5 kb). Difficulties in sequence reconstruction of the large plasmid raised the question to what extent the sequencing coverage of plasmid DNA was influenced by the choice of DNA extraction kit. Thus, we compared the ability of six commercially available DNA extraction kits that rely on different extraction principles, including silica-membranes or salting-out-protocols, to extract DNA from the ESBL-producing *K. pneumoniae* outbreak isolate. For this purpose, bacterial DNA from the same liquid culture was extracted with the different kits. The obtained DNA was compared in terms of yield, purity, and fragment length. Furthermore, hands-on-time and costs per extraction were compared. Sequencing libraries were prepared using the Nextera XT kit (Illumina) and sequenced on a MiSeq machine using v3 reagents with 2 x 300 cycles. Sequencing reads were aligned to the reference genome sequence by using BWA-SW. To evaluate the extraction efficiency, we compared the sequencing coverage of plasmid and chromosomal DNA by calculating the reads-per-kilobase-per-million-reads (RPKM) values. We observed that the coverage of bacterial genes was influenced by DNA extraction kit and type of replicon. While all extraction kits yielded reproducible and satisfactory results, extraction costs and time requirements varied widely.

1. Haller S, Eller C, Hermes J, Kaase M, Steglich M, Radonic A, Dabrowski PW, Nitsche A, Pfeifer Y, Werner G, Wunderle W, Velasco E, Abu Sin M, Eckmanns T, Nuebel U. 2015. What caused the outbreak of ESBL-producing *Klebsiella pneumoniae* in a neonatal intensive care unit, Germany 2009 to 2012? Reconstructing transmission with epidemiological analysis and whole-genome sequencing. BMJ Open 5:e007397.

**Presentation:** Monday, September 28, 2015 from 18:15 – 18:30 in room Roter Saal 2.

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**394/MSV**

**Separation of foreground and background reads in mixed NGS datasets**

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**Introduction**: NGS is a valuable technology for rapid and in-depth analysis of clinical samples, as it allows sequencing of a pathogen's whole genome directly from patient material within as little as 26 hours. However, the follow-up analysis is severely slowed down by the abundance of reads originating from the host. Thus, in order to exploit the full potential of the technology for rapid diagnostics, a method for rapid in silico removal of host reads is necessary.

**Aims**: Commonly, a mapping-based approach is used to separate reads: either reads mapping to a background reference or reads not mapping to a foreground reference are discarded. However, while the former approach is highly specific in discarding only true background reads and the latter is highly sensitive in only keeping foreground reads, neither offers a good balance. Hence we have aimed at developing a novel tool specifically geared towards both specific and sensitive separation of foreground and background reads.

**Materials and Methods**: In order to determine whether a read belongs to the foreground or the background, we train markov chains of an order k from 4 to 12 on user-provided sets of foreground and background reference sequences, where each state is a k-mer of length k and each transition is one of the four possible bases A, C, G and T. We then calculate the difference of log likelihoods of each transition observed within a read with regards to the foreground and the background markov chains. This difference is then used as a score for the separation of reads, with scores smaller than 0 indicating a background read and scores larger than 0 indicating a foreground read.

**Results**: We have tested our tool on several datasets, including Cowpoxvirus sequenced from a human host. In all cases, our tool was faster than any competing tool (achieving speeds of up to 10 Megabases/second using 4 CPUs), including Kraken and mapping

via bowtie2. At the same time, we consistently achieved the best F-Score of all tested tools.
Our tool is developed in python and java and available for download from
http://sourceforge.net/projects/rambok/
**Conclusion**: We have developed a freely available, easy to use, rapid and both highly sensitive and specific tool for the separation of foreground and background reads in mixed NGS datasets. We believe that this will be highly useful as an initial filtering step for anyone analyzing viral sequences via NGS.

**Presentation:** Monday, September 28, 2015 from 17:00 – 17:15 in room Roter Saal 2.

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**395/MSV**
**Modern bioinformatic applications for genome sequence-based identification and classification of pathogens and other microorganisms**
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**Background**: Since the end of the 19th century, when scientists succeeded in the first descriptions of pathogens, methods for the identification and classification of microorganisms have been continuously augmented. In the genomic age, with its ongoing decrease in sequencing costs, researchers can apply *in silico* approaches for whole-genome sequence-based identification and classification.
**Methods**: The Genome-to-Genome Distance Calculator (GGDC) is a web service for genome-based species and subspecies delineation freely available at http://ggdc.dsmz.de. It uses the fast and reliable Genome-Blast Distance Phylogeny (GBDP) method, which calculates intergenomic distances between pairs of (complete or incomplete) genomes. Branch support in phylogenetic trees inferred from these distances can be assessed using (pseudo-)bootstrapping. The method was, e.g., applied to *Escherichia coli*, the *Bacillus cereus* group and the genus *Brucella*.
**Results**: In the phylogenomic *E. coli* tree, the pathogenic *E. coli* strain S88 was revealed to be the closest neighbor of the type strain DSM 30083$^T$, which was well apart from the lab strain K-12. Whereas the *E. coli* phylotypes were largely confirmed, only five subspecies were distinguished, the largest one including all *Shigella* species. The *Bacillus* tree revealed many genomes misnamed in GenBank. *B. weihenstephanensis* and *B. mycoides* appeared as heterotypic synonyms. *B. anthracis* contained strains previously assigned to *B. cereus* or *B. thuringiensis*. *Brucella* was confirmed to contain only a single species and to not be divisible into subspecies, according to the usually applied criteria. However, the phylogenomic tree revealed distinct subgroups of strains belonging to *Br. abortus*, *Br. melitensis*, *Br. canis*, *Br. ovis* and *Br. ceti*, indicating a promising bioinformatics approach for a reliable identification of novel strains.
**Conclusions**: The application of our *in silico* approaches to datasets of several pathogenic groups clearly underlines their suitability regarding both a fast and reliable genome sequence-based identification and classification not only of pathogens but of microorganisms in general. These methods are easy-to-use for scientists and instantly allow for a plethora of analyses. Hence, these tools can quickly provide taxonomic insights about novel strains and thus also have potential regarding rapid clinical diagnostics and genomic surveillance.

**Presentation:** Monday, September 28, 2015 from 18:00 – 18:15 in room Roter Saal 2.

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# ANTIMICROBIAL RESISTANCE AND DRUGS, INFECTION PREVENTION (PRV)

**191/PRV**
**Interventions to stop the transmission of highly resistant microorganisms in a Dutch intensive care unit**
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**Introduction:** Our intensive care (ICU) faced a cumulative incidence of highly resistant microorganisms (HRMOs) in the past 3 years. Clusters of identical HRMOs, *E cloacae and Citrobacter spp.*, were found in different patients and in different ICU rooms. In particular sinks were contaminated.
**Objectives:** The objective of this study was to stop HRMO transmission between patients and eliminate environmental sources of reservoirs for bacteria.
**Patients & Methods:** The clinical setting was a 30-bed mixed level III ICU, 4 units with all single rooms. Weekly testing was conducted on sinks and twice weekly and on indication on patients to assess for the presence of HRMOs. A before after comparison of HRMO transmission was made between 274 patients in the baseline period, January 2014-May 2014 and 311 patients in the intervention period, September 2014-February 2015. All patients included in the study spent ≥ 48 hours on the ICU. A bundle of interventions included new staff education on hand hygiene, contact isolation for all patients, no longer polluting sinks with trash and cleansing each patient with 2% chlorhexidine gluconate (CHG) cloths daily instead of water and soap. All sinks were replaced. Compliance with hand hygiene and use of 2% CHG cloths was monitored throughout the study period. The study was approved by the ethics committee.
**Results:** Weekly testing of the sinks revealed a mean contamination of 0,39 ± 0,19 sinks before and 0 ± 0,01 sinks after introduction of the bundle; paired samples T-test: t = 6,09, p = 0,00. The before-after comparison of ICU patients is presented in Table 1. Results of cultures in patients are presented in table 2. No clusters of identical HRMOs could be detected in cultures of patients and sinks in the intervention period. Compliance in hand hygiene averaged from 47% at the start to 80% at the end of the intervention period, $X^2$ 22,5 p = 0,00. Compliance to cleansing with 2% CHG cloths was during the whole intervention period almost 100%.
**Conclusion:** Transmission of HRMOs on the ICU was eliminated by introduction of a bundle of interventions. Of interest, number of patients with HRMO positive cultures did not change between time periods, further confirming the similarities of the before-after patient population.

**Presentation:** Monday, September 28, 2015 from 18:18 – 18:30 in room Weißer Saal 2.

**Figure 1**
Table 1. Patient characteristics and outcome

| | Baseline period | Intervention period | P |
|---|---|---|---|
| Study population, n | 274 | 311 | |
| Male n (%) | 140 (51%) | 172 (55%) | 0,37 |
| Age | 59 | 61 | 0,10 |
| Apache II | 18 | 19 | 0,29 |
| Patient category, n<br>Medical<br>(neuro)surgical | <br>198<br>76 | <br>237<br>74 | 0,28 |
| LOS ICU, days | 9,3 | 9,5 | 0,79 |
| ICU mortality, n (%) | 34 (12%) | 53 (17%) | 0,24 |
| HRMO, patients, n (%) | 11 (0,04%) | 11 (0,04%) | 0,75 |

**Figure 2**

Table 2. HRMO species

| | Baseline period | Intervention period |
|---|---|---|
| **Enterobacteriaceae:** | | |
| *E.coli* | 3 | 4 |
| *Citrobacter spp.* | 5 | 1 |
| *E.cloacea* | 2 | 0 |
| *Klebsiella spp.* | 1 | 2 (1 blood culture) |
| **Non-fermenting gram-negatives:** | | |
| *Pseudomonas spp.* | 0 | 1 |
| **Gram positives:** | | |
| *S.Pneumoniae* | 0 | 2 |
| *MRSA* | 0 | 1 (wound culture) |
| Cultures | All sputum cultures | 1 blood culture<br>1 wound culture<br>5 sputum cultures<br>4 rectal cultures |

**396/PRV**

**Economic impact of MRSA admission screening in hospitals**

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**Introduction**: Hospital infections with multiresistant bacteria e.g. Methicillin-resistant Staphylococcus aureus (MRSA) cause heavy financial burden worldwide. As with all nosocomial infections, prolonged hospital stays are the main cost driver. Additionally, presumptive patient isolation while awaiting laboratory results is also costly. Rapid and precise identification of MRSA carrier ship in combination a target hygienic management are proven to be effective but cost incurring measures. Therefore health care providers have to decide which MRSA screening strategy (universal or risk-based target) and which diagnostic technology (PCR, Culture, Point-of-care) should be applied according to economic criteria.
**Aim**: Aim of this study was to determine which MRSA admission screening and infection control management strategy causes the lowest expected cost for a hospital.
Methods: A decision tree analytic cost model was developed, primary based on data from peer-reviewed literature. In addition, univariate sensitivity analyses of the different input parameters were conducted to study the robustness of the results. This allows the simulation of scenarios for hospitals with different structure and patient profiles (primary care vs. specialist care) or a departmental analysis (ICUs vs. general wards).
**Results**: In basis analysis risk-based Point-of-Care screening showed the highest mean cost savings per admission in comparison to no screening. At high MRSA prevalence rapid universal screening methods became favorable. Turns at low MRSA transmission rates may be favored the omission of screening.
**Conclusion**: The early detection of MRSA by rapid PoC or PCR technologies and the consistent implementation of appropriate hygienic measures result in a high economic efficiency of MRSA management. Whether general or target screening is more efficient depends mainly on epidemiological and infrastructural parameters. Based on the model, instructions for dealing with MRSA patients can be derived ones for treating physicians as well as for the hospital management.

**Presentation:** Monday, September 28, 2015 from 8:45 – 9:00 in room Weißer Saal 2.

**397/PRV**

**No evidence for transmission of cephalosporin and gyrase inhibitor resistant Escherichia coli in a hemato-oncology unit under weekly and admission screening and isolation of positive patients**

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**Introduction**: According to the recommendations of the Commission for Hospital Hygiene and Infectious Disease Prevention at the Robert Koch-Institute, patients infected or colonized with cephalosporin and gyrase inhibitor resistant Escherichia coli (3MRGN Ec) should be treated in single rooms in facilities taking care of patients at high risk of infection. There is little evidence regarding the nosocomial transmission rates of 3MRGN Ec. At the University Hospital of Wuerzburg, patients at the hemato-oncology unit are screened for multiresistant gramnegative enterobacteria (MRE) on admission, weekly during their stay and upon discharge. Isolation procedures are initiated if MRE are detected in rectal or anal swabs.
Aims: To use genetic typing by MLST to assess whether strains found on admission are more diverse than strains isolated during the course of the stay, which would provide indirect evidence for nosocomial transmission. To assess whether patients colonized by the same sequence type (ST) shared a room prior to the identification of the strain and implementation of isolation procedures.
**Materials and Methods**: 3MRGN Ec were typed by MLST (http://mlst.warwick.ac.uk/mlst/). The study period was February 2013 to January 2015.
**Results**: 3809 of 5498 patients were screened at least once. The screening compliance was 69.3%. Median and maximum numbers of stays were 1 and 40, respectively. Median and maximum duration of stays were 10 and 636 days. 223 patients were positive for 3MRGN Ec (5.9%). We found 62 STs. ST131 was dominant with 58 strains. The Simpson´s index of diversity was 0.9138. There was no difference between the ST-diversities of the admission dataset and that of the strain collection compiled from patients who became positive during their stay (0.90 vs. 0.93). Overlapping room occupancy of patients harboring 3MRGN Ec of identical STs was observed on very few occasions only.
Discussion: The prevalence of 3MRGN Ec was 5.9% (95% CI: 5.2-6.7%), which was within the range reported recently for comparable clinical units (Vehreschild MJ et al. J Antimicrob Chemother. 2014). Our study design was limited as it was observational and retrospective. Nevertheless, typing by MLST revealed no evidence for nosocomial transmission under enhanced screening and isolation of patients in a hemato-oncology unit.
**Conclusions**: Controlled multicentre studies are needed to determine the contribution of screening and isolation to the prevention of nosocomial transmission reported herein and to justify adjustment of hygiene precautions.

**Presentation:** Monday, September 28, 2015 from 9:00 – 9:15 in room Weißer Saal 2.

**398/PRV**

**Drains from patient's rooms sinks, showers and toilets as an environmental reservoir for carbapenem-resistant *Pseudomonas aeruginosa* on haematology-oncology wards.**

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**Introduction**: Haemato-oncology patients undergoing high-dose chemotherapy are at risk of prolonged neutropenia and they often have to receive antibiotics for therapeutically or prophylactic reasons. Thus, these patients are more likely to acquire severe infections caused by multiresistant bacteria. Personal protective equipment for the health-care workers and visitors, air filtration and filtration of drinking water are widely accepted infection prevention measures, such as disinfection of the patient's rooms including medical equipment among the patients. However, bacteria which may colonize the sinks are not removed by the standard surface cleaning procedures. A cross-transmission of MRGN from previously hospitalized patients being hospitalised in the same room is possible (1, 2, 3).
**Aim of this Study**: After a series of meropenem-resistant *Pseudomonas aeruginosa* infections in 2013, we started to perform microbiological testing of swabs from sinks, showers and toilets.
**Methods**: Setting: tertiary care centre in southwestern Germany, time period from January 2014 to Mai 2015.
Swabs were first cultured in enrichment medium (CASO bouillon) and than plated on Cetrimide agar (Oxoid), ESBL-Chromagar (MAST) and chromIDP.aeruginosa agar (Biomérieux), with

meropenem disc for agar-diffusion test. Resistant isolates were confirmed by MALDI-TOF MS and Vitek2 system (Biomérieux).
**Results**: Totally 1350 swabs were drawn with n = 47 (3,5%) positive for meropenem resistant PSAE (Mero-R-PSAE). Out of the 22 patient rooms on the two haematology wards, 5 have been at least once positive for Mero-R-PSAE in sink drains. The shower drains of 8 rooms and the toilets of 9 rooms have been at least once positive for Mero-R-PSAE.
**Conclusion**: Sink drains may be contaminated with PSAE even if athermo-disinfectant system (MoveoMed®) is installed. Shower drains in this hospital are difficult to disinfect (because of their construction) and are more often contaminated. Rim-free toilets have been also found to be occasionally PSAE contaminated. The incidence of PSAE infections of haematology patients is under surveillance and more evidence on the impact of this environmental monitoring on the patients outcome is needed.

**References**
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(2) Ferranti G., Marchesi I., Favale M., Borella P., Bargellini A. (2014): Aetiology, source and prevention of waterborne healthcare-associated infections: a review. J. Med. Microbiol. 63 (10):1247-59.
(3) Loveday H.P., Wilson J.A., Kerr K, Pitchers R. (2014): Association between healthcare water systems and Pseudomonas aeruginosa infections: a rapid systematic review. J. Hosp. Infect. 86: 7-15.

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**399/PRV**
**Investigation of colistin resistance in *Acinetobacter baumannii* from Spain and Greece isolated as part of the MagicBullet clinical trial**
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**Introduction**: Multidrug resistant (MDR) *Acinetobacter baumannii* has emerged worldwide with increasing frequency. The lack of antimicrobial agents effective against MDR *A. baumannii* strains led to the reintroduction of the polymyxin antibiotic colistin. However, resistance to colistin has been reported among clinical *A. baumannii* isolates, complicating the treatment of infections significantly.
**Objectives**: This study aimed to investigate the mechanisms of colistin (COL) resistance in *A. baumannii* obtained from patients hospitalized in Spain and Greece between 2012 and 2014 as part of the MagicBullet clinical trial in patients with ventilator-associated pneumonia (VAP).
**Methods**: Resistance to COL was determined by Etest and interpreted as >2 mg/l using EUCAST resistance breakpoints for *Acinetobacter* spp. *A. baumannii* isolates were investigated as isolate pairs (n=5), which were defined as a pair of isolates from the same hospital, usually but not exclusively from the same patient. Members of isolate pairs displayed a susceptible-to-resistant phenotype shift and were identical by rep-PCR. The isolate pairs were subjected to whole-genome sequencing (WGS) by MiSeq using 2 x 250 bp paired-end run. We compared the genes encoding PmrABC, RND-type efflux pump systems and the porins CarO, Omp25 and OprD as well as genes involved in the lipid A biosynthesis.
**Results**: COL-resistance was associated with either a 12 nucleotide deletion in *pmrB* or the amino acid substitution A28V in PmrB in two resistant isolates. Moreover, another resistant isolate revealed the combination of amino acid substitutions I232T in PmrB and T749R in the RND-pump AdeG. No other mutations were found in porins, other RND-type efflux systems or genes involved in lipid A biosynthesis. Further investigation revealed the amino acid substitutions G575V and I381N in the guanosine polyphosphate pyrophosphohydrolase/synthetase (SpoT) in two isolate pairs, respectively. SpoT synthesizes guanosine tetraphosphate, which has previously been described to be involved in antibiotic resistance.
**Conclusion**: Mutations in *pmrB* and *adeG* were found to be associated with COL-resistance. Amino acid substitutions in the enzyme guanosine polyphosphate pyrophosphohydrolase/synthetase (SpoT) were also associated with COL resistance.

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**400/PRV**
**Missense mutations of PBP2a are associated with reduced susceptibility to ceftaroline and ceftobiprole in African methicillin-resistant *Staphylococcus aureus***
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**Introduction**: Ceftaroline and ceftobiprole are new cephalosporins, which are active against methicillin-resistant *Staphylococcus aureus* (MRSA) by inhibiting PBP2a. A recent study from Ghana reported a high ceftaroline resistance rate (20%) among MRSA isolates, which predominantly belonged to ST247 (Egyir et al. 2015, J. Glob. Antimicrob. Resist.). This finding is surprising, as ceftaroline is not distributed in Ghana.
**Objectives**: The objective was therefore to assess the resistance rates to ceftaroline and ceftobiprole in a large collection of MRSA isolates from Africa and to investigate the underlying mechanism of resistance.
**Material and Methods**: The MRSA isolates were collected in Côte d'Ivoire (n=17), DR Congo (n=6), Gabon (n=21) and Nigeria (n=16). All isolates were *spa* typed and multilocus sequence typing (MLST) was performed exemplarily for one isolate per *spa* type. The minimum inhibitory concentrations (MICs) of ceftaroline and ceftobiprole were determined using the broth microdilution method and interpreted applying EUCAST breakpoints. The *mecA* gene was sequenced to assess potential missense mutations.
**Results**: In total, MICs above the susceptibility breakpoints were detected for ceftaroline (16.7%) and ceftobiprole (15%). All isolates carrying the *mecA* wildtype (n=28) as well as the missense mutations S225R (n=11) and G246E (n=10) were fully susceptible. However, eleven isolates from Nigeria carried a triple mutant of *mecA* (N146K-N204K-G246E) which was associated with high resistance rates to ceftaroline (90.9%) and ceftobiprole (81.8%). These isolates belonged to ST15 (n=1) and ST241 (n=10).
**Conclusion**: Ceftaroline and ceftobiprole resistance is present in Africa and associated with the ST241 MRSA lineage which is widespread in North and West Africa. Although ceftaroline resistance has been reported for ST241 MRSA in Switzerland, these isolates differ in the mutation pattern (Switzerland: N146K-E150K-G246E).

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**401/PRV**

**Approved Drugs containing Thiols as Inhibitors of Metallo-β-Lactamases:**
**a Strategy to Combat Multidrug-Resistant Bacteria**

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**Introduction**: The increasing occurrence of multidrug-resistant bacteria is one of the major global threats to human health. An alarming trend is the spread of metallo-β-lactamases (MBL) among gram-negative pathogens that confer resistance against almost all β-lactams including carbapenems. Hence, the development of new anti-infective agents remains one of the most significant demands in modern medicine. This work aims to identify already approved drugs, containing a zinc chelating thiol group, as inhibitors of MBL in order to restore the bactericidal activity of common β-lactam antibiotics against multidrug-resistant gram-negative pathogens.
**Materials and Methods**: Carbapenemase genes encoding New-Delhi-Metallo-β-Lactamase-1 (NDM-1), Verona-Integron-Encoded-Metallo-β-Lactamase-1 (VIM-1) and Impenemase-7 (IMP-7) were overexpressed in *E. coli* to yield polyhistidine-tagged proteins. With purified MBLs a fluorescence based assay platform was applied for studying inhibitors of ß-lactamases. We tested 11 approved drugs containing a thiol moiety in the fluorescent assay and $IC_{50}$ values were determined. In order to discriminate between zinc-withdrawing and direct binding to the enzyme, a thermal shift assay was conducted. Compounds showing inhibition in the fluorescence assay and binding in the thermal shift assay were further evaluated for their potential to inhibit bacterial β-lactamase activity by means of antimicrobial susceptibility testing.
**Results**: We established a novel sensitive fluorescence-based assay platform for studying inhibition of β-lactamases using the commercially available substrate fluorocillin. Remarkably, not all approved compounds inhibited MBL, although every compound carried a thiol group. To ensure that inhibitory effects result from binding of the compounds to the proteins but not from withdrawing zinc from the active site, we chose thermal shift measurement as secondary assay. Strong shifts could be observed for selected compounds, thereby confirming the results of the functional assay. Most promising compounds were passed to antimicrobial susceptibility testing using laboratory strains and patient isolates. Results showed that some of our compounds partially restored the efficacy of imipenem against pathogenic bacteria.
**Conclusion**: Overall, we found four approved drugs with non-anti-infective indications, which inhibit three clinically important MBL, namely Captopril, Thiorphan, Dimercaprol and Tiopronin. This result yields a good starting point for the development of potent MBL inhibitors, with the primary optimization goal being the uptake and activity in pathogens.

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**402/PRV**

**When bacteria get the "flu" - use of phages for combating multi-drug resistant pathogens**

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**Introduction**: Multidrug-resistant pathogens from the ESKAPE-group (i.e. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*) pose an increasing threat in hospital settings. The scarce prospects of newly introduced antibiotics in the future, has re-vitalized the concept of bacteriophages (phages) as potential alternative therapeutics. However, the full breath of phage diversity suitable for treatment of bacterial infections is largely unexplored.
**Aim**: In this study we evaluated the efficacy with which novel phages with therapeutic potential against ESKAPE can be isolated and we devised strategies for their rapid characterization and classification at phenotypical and molecular level.
**Material and Methods**: Environmental samples were collected from 16 different aquatic sources (e.g. from general and hospital wastewater, activated sludge samples from sewage plants, streams, rivers, ponds and lakes). Samples were pre-incubated with current clinical isolates of multidrug-resistant strains (i.e. five strains each from the ESKAPE-group). Phages were subsequently obtained by the double layer plaque assay and purified through multiple cultivation steps. For phenotypic characterization the host spectrum and morphology via electron microscopy was determined. Genomic comparison of phages was performed based on digitized fluorescent restriction-fragment-length-polymorphism-analysis (fRFLP).
**Results**: For all strains of *E. faecium*, *P. aeruginosa, and E. cloacae* lytic phages could be found. Conversely, phages against *K. pneumoniae* and *A. baumannii* were found for some but not all strains and no phages at all were isolated for *S. aureus*. Hospital wastewater contained the highest number of phages compared to natural sources. Electron microscopy revealed in most cases the typical morphology of dsDNA-phages of the group Myoviridae and Podoviridae. fRFLP-profiles indicated that each phage genome was unique which demonstrates the overall high phage diversity in the tested samples.
**Summary**: Phages with therapeutic potential can be easily and rapidly isolated against most nosocomial pathogens with the prime source being the immediate hospital vicinity.

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**403/PRV**

**The NF-κB inhibitor LG-ASA exhibits anti-pathogenic activity against influenza A virus and *S. aureus* co-infection *in vitro* and *in vivo***

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Infections with influenza A viruses (IAV) are still amongst the major causes of highly contagious severe respiratory diseases, not only bearing a devastating burden to human health, but also significantly affecting the economy. Another problem concerns increased fatality rates, linked to secondary bacterial pneumonia, caused by pathogens such as *Staphylococcus aureus* (*S. aureus*). Besides vaccination that represents the best option to get protected from IAV infections, only two classes of anti-influenza drugs, inhibitors of the viral M2 ion channel and the viral neuraminidase, have been approved. Furthermore, seasonal and pandemic IAV show a rapid development of resistant variants against the currently licensed therapeutics. Similarly, for antibacterial intervention highly effective antibiotics are available, but there is a frightening increase in resistant strains. Thus, an urgent need for novel anti-infective strategies targeting both pathogens is obvious.
In different studies we have identified virus-supportive cellular functions as potential targets for antiviral intervention. Among these, the cellular IKK/NF-κB signalling pathway was shown to regulate the viral ribonucleoprotein export out of the nucleus. Inhibition of NF-κB signalling results in reduced expression of cytokines, chemokines, and pro-apoptotic factors and subsequent inhibition of caspase activation and block of caspase-mediated nuclear export of viral ribonucleoproteins. In consequence, the production of progeny viruses is reduced.
Here we examined the anti-pathogen potential of the NF-κB inhibitor LG-ASA against IAV and/or *S. aureus* infection.
We established *in vivo* and *in vitro* co-infection models using serial infection with IAV and *S. aureus*. The effect of LG-ASA was

determined on viral and bacterial load and inflammatory responses *in vitro* and *in vivo*. Furthermore, survival of co-infected mice was examined in presence and absence of LG-ASA.
Our data indicate that targeting NF-κB signaling by LG-ASA inhibits IAV replication and intracellular bacterial load upon singular as well as co-infection. Interestingly, we were able to show that LG-ASA is able to block *S. aureus* internalisation. Furthermore, we provide evidence that treatment of mice with LG-ASA results in reduced pathogen load and enhanced survival during IAV/*S. aureus* coinfection.
Thus, the NF-κB inhibitor LG-ASA may serve as a potential agent against IAV and/or *S. aureus* infection.
Note: These data were presented in part at different meetings before**.**

**Presentation:** Tuesday, September 29, 2015 from 9:30 – 9:45 in room Weißer Saal 2.

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**404/PRV**

**Evaluation of *in vitro* activity of recombinant chimeric bacteriophage endolysin HY-133 against *Staphylococcus aureus* by time-kill curves in comparison to daptomycin and mupirocin**

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**Introduction**: Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity and mortality. The nasal cavity represents the microorganism´s principal habitat. Nasal eradication of MRSA is a major challenge for the patient management and sometimes exhausting and unsuccessful. The emergence of resistant strains and pharmacokinetic drawbacks are disadvantages of current decolonisation therapy by mupirocin. Thus, new therapeutic options are needed.
**Objectives**: The application of bacteriophage endolysins could represent a future strategy for improved MRSA decolonization. First experiments revealed low minimum inhibitory concentrations (MICs), comparable to other antistaphylococcal agents. Therefore, we characterized the *in vitro* bactericidal activity of the recombinant chimeric bacteriophage endolysin HY-133 against *S. aureus* by time-kill curves over two days in comparison to daptomycin and mupirocin.
**Methods**: The activity of the endolysin HY-133 (Hyglos GmbH, Bernried, Germany) was evaluated by time-kill curves in accordance to the CLSI guideline M26-A. The detailed bactericidal activity was determined for 1-, 2-, 4- and 16- fold MIC of HY-133 and, additionally, for daptomycin and mupirocin. The killing kinetics of one clinical methicillin-susceptible *S. aureus* (MSSA) and one MRSA isolate was determined by plating of serial dilutions at time-points 0 h, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h, and 48 h.
**Results**: HY-133 had a high bactericidal activity against MRSA and MSSA with the effect being maximal within four hours and similar to daptomycin. Already low concentrations of HY-133 nearly completely eradicated *S. aureus* and a bactericidal concentration was achieved for 2-, 4- and 16- fold MIC after one hour. There was no significant difference in the activities of HY-133 to MRSA and MSSA strains. However, an in vitro phenomenon of re-growth was observed after a few hours of incubation applying low endolysin concentrations, which was also found if daptomycin was tested.
**Conclusion**: This study confirmed a high bactericidal activity of HY-133 against MRSA and MSSA, especially in the first hours of application and also in low concentrations. Recombinant chimeric bacteriophage endolysins may offer an alternative option for rapid MRSA decolonization strategies.

**Presentation:** Tuesday, September 29, 2015 from 9:45 – 10:00 in room Weißer Saal 2.

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**406/PRV**

**Neisseria gonorrhoeae: Situation of antibiotic resistance in Germany**

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**Background**: The development of antimicrobial resistance in *N. gonorrhoeae* is a serious problem for treatment and control of gonorrhoea. Numerous formerly effective therapeutic agents are no longer appropriable.
The third generation cephalosporins are amongst the last agents to remain effective. Reduced susceptibility or resistance to these cephalosporins is increasingly common. There is a severe concern that multidrug resistant *N. gonorrhoeae* strains will spread globally (MDR-NG).
**Methods**: In 2014 GORENET (Gonococcal Resistance Network) a new *N. gonorrhoeae* surveillance programme for Germany was implemented as a non-selected collection from all regions of Germany. The isolates were tested for ceftriaxone, cefixime, azithromycin, ciprofloxacin and penicillin by E-test and interpreted according to EUCAST 4.0.
**Results**: 307 isolates were tested in 2014.
These results were compared to AMR-data from german isolates tested in EURO-GASP in period 2010-2013.
More than 11% of the isolates displayed resistance to azithromycin, which demonstrates a massive increase in comparison with EURO-GASP-data from previous years.
Nearly 2 % of all isolates showed a decreased susceptibility or resistance to cefixime (MIC>0,12mg/L).
These results are lower than in previous years, but still concerning. No resistance to ceftriaxone was detected in 2014. Rates of resistance to ciprofloxacin (>70%) and penicillin (30%) are very high across Germany.
**Conclusions**: In Germany ceftriaxone is an appropriate treatment for gonorrhea at present. Resistance to azithromycin is increasingly common. Cefixime is no longer recommended as first line therapy agent due to a high number of isolates with decreased susceptibility. Rates of resistance to ciprofloxacin and penicillin are high across Germany. Intensified surveillance of antimicrobial resistance status of *N. gonorrhoeae* is mandatory.

**Presentation:** Tuesday, September 29, 2015 from 17:00 – 17:15 in room Weißer Saal 2.

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**407/PRV**

**Measles in North-Rhine Westphalia**

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Measles is a serious threat affecting both, individual and public health. The virus is highly communicable and no specific antiviral therapy exists. Nevertheless, immunization with the live attenuated measles vaccine is a safe and effective means to prevent measles.
Overall vaccination coverage of 95% in the population is needed, to achieve herd immunity, prevent outbreaks and eventually eliminate the disease.
For more than a decade, surveillance data on confirmed measles cases as well as data on the immunization status of schoolchildren have been recorded on the state level. They allow for insights in the dynamics of the disease in North Rhine-Westphalia (NRW) and indicate starting points for public health actions.
According to the WHO criteria for the elimination of measles, less than 18 cases per year may occur in NRW (incidence < 1 case/1M inhabitants) over a period of several years. Generally, total case numbers vary every year. A maximum of 1750 confirmed cases was reached in 2006 (and 251 in 2007) due to a large outbreak and a minimum of 18 cases was recorded in 2012 [www.lzg.nrw.de].

However, the intended goal of the elimination of measles could not be achieved until now.
Vaccination coverage among children at the age of school-entry in NRW constantly increased in the last decade. The rate of children who received at least the first of the two doses of measles vaccine increased from 90.1% in 2002 to 97.7% in 2013. However, the rate of fully vaccinated school-children on the state level (94.6% - regionally differing between 88.2 and 97.6%) is still not high enough to effectively prevent measles transmission [www.lzg.nrw.de].
Moreover, immunization gaps exist in several population groups. The virus is easily passed on between unvaccinated (or not fully vaccinated) individuals and may thus lead to extended outbreaks of the disease. Measles cases and transmission often occur among young adults, refugees or children before receiving their first measles vaccine.
Consequent implementation of two basic strategies is needed in order to stop measles transmission in North Rhine-Westphalia. These are on the one hand, accurately timed immunization in all age groups according to the recommendations of the German Standing Committee on Vaccination (STIKO). Thus, in order to further reduce the number of susceptible individuals, information campaigns on the measles vaccination for the public (e.g. parents) or educational activities for medical staff could support closure of immunization gaps.
On the other hand active surveillance of the disease, combined with quick onset of public health measures like e.g. consequent case and outbreak management by the local health authorities, is needed in order to stop transmission of the disease.

**Presentation:** Tuesday, September 29, 2015 from 17:15 – 17:30 in room Weißer Saal 2.

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**408/PRV**

**Antibiotic consumption in hospitals: A new system for collection, automated calculation and reporting in Germany**

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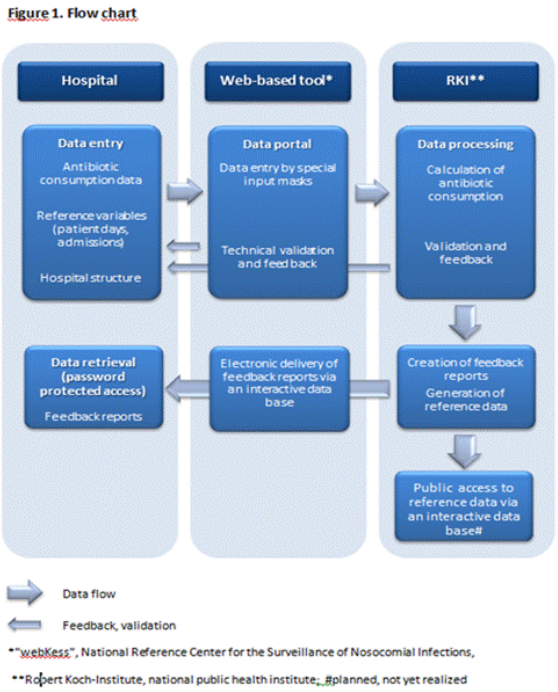
**Introduction**: Since 2011, German hospitals are legally obliged to monitor antibiotic consumption. In cooperation with the National Reference Center for the Surveillance of Nosocomial Infections, the national public health institute (Robert Koch Institute) built up a system for data collection, calculation and reporting.
**Aims:** The aims of the project are to support the hospitals in fulfilling the legal requirements and local antibiotic stewardship efforts and to provide benchmark data.
**Methods**: In order to enable standardized data provision and efficient data processing an electronic web-based tool has been developed. For saving resources, an already existing web-based system ("webKess"), which serves for the collection of data in the German Krankenhaus Infektions Surveillance System (KISS) has been extended in order to allow for the entry of antibiotic consumption data and the consecutive transfer to the Robert Koch Institute. In addition, this construct paves the way for future crosslinking of data from the different surveillance systems. Methodological basis is the WHO-ATC (Anatomical Therapeutic Chemical/DDD (Defined Daily Dose) method. The target measure is the quantity of DDD in relation to 100 bed days and admissions, respectively, calculated for the different medical specialities and ward types.
**Results**: The data flow can be divided into three major steps, which are schematically outlined in Figure 1.
1. Upload and transfer: Data upload takes place via a web-based tool, which allows manual data entry as well as the bulk import of whole data sets. Three different data files are required: One data set containing data on hospital structure, a second and third dataset containing data on antibiotic consumption and hospital activity data. Structurally correct datasets are transferred to the Robert Koch Institute for further data processing.
2. Data analysis: After undergoing validity checks concerning content and technical aspects, the data are merged in order to calculate the quantities of antibiotic consumption standardized by bed days and admissions, respectively.
3. Feedback: Reports can be retrieved by password-protected access via an interactive database, which allows a specification and tailoring of the request (e.g. concerning ward type, speciality, time period and antiinfectives to be presented) according to the needs and preferences of the user. The system offers different report types comprising a basic report containing data from successive time periods in order to allow the analysis of trends, a ranking list and reports comparing data of the individual hospital and aggregated data of reference hospitals.
**Conclusion**: An electronic system for data upload, automated processing and reporting has been built up providing a suitable instrument for antibiotic stewardship and allowing for the establishment of a nation-wide surveillance of antibiotic consumption.

**Presentation:** Tuesday, September 29, 2015 from 17:30 – 17:45 in room Weißer Saal 2.

**Figure 1**



**409/PRV**

**Mandatory reporting of carbapenem-resistant Gram-negative bacteria in Hesse, Germany, suggests rising trends and a decreasing role of international travel**

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[7]*Public Health Office, Gießen, Germany*
[8]*Hesse Ministry of Health, Wiesbaden, Germany*

**Background**: Carbapenems are potent broad spectrum β-lactam antibiotics that are used as the last resort treatment for many Gram-negative bacteria. Carbapenem-resistant gram-negative bacteria (CR-GN) have emerged as a global threat. In November 2011, mandatory reporting of CR-GN was introduced in Hesse, one of the German federal states with a population of 6.1 million.
**Methods**: The case definition includes isolates resistant to the four major antibiotic classes acylureidopenicillins, third- and fourth-generation cephalosporins, carbapenems and fluoroquinolone. For Pseudomonas aeruginosa isolates from blood or cerebrospinal fluid and for all other CR-GN isolates from any specimen are notifiable. We defined as possibly travel-associated patients with non-German residency or any stay outside Germany during the 12 preceding months. We extracted from the Hessian CR-GN database all patients notified between 1 January 2012 and 30 April 2015. We counted as CR-GN isolates notifications of the first isolate, identified at species level, per patient.
**Results**: Of the 865 patients reported during the study period, 135 patients were associated with a foodborne outbreak and excluded from further analysis. Of the remaining 730 patients 152 were notified in 2012, 199 in 2013, 277 in 2014 and 102 in the first four months of 2015. Information on travel history was available for 501 patients. From 2012 to 2015, 54.6%, 47.2%, 38.1% and 39.3% of patients were possibly travel-associated. Proportion of possibly travel-associated isolates was highest for Acinetobacter baumannii (67.7%; 113/167) and lowest for Pseudomonas aeruginosa (27.8%; 13/35) and Enterobacter spp. (12.9 %; 9/70).
**Conclusion**: In Hesse, preliminary data suggest rising trends of CR-GN notifications and decreasing associations with international travel, in line with ongoing autochthones transmission. Good surveillance and infection control measures are urgently needed to contain this spread.

**Presentation:** Tuesday, September 29, 2015 from 17:45 – 18:00 in room Weißer Saal 2.

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**410/PRV**

**MALDI-TOF MS used for Rapid Detection of Tobramycin resistant Gram-negative Bacteria**

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**Background:** The prevalence of resistant bacteria has been increasing in the last years. For hygienic and therapeutic measures, quick and cost-efficient methods are required to facilitate rapid resistance evaluation. In addition to quick species identification, MALDI-TOF MS provides meanwhile also resistance detection. Recently, quantitative MALDI-TOF MS has been employed to detect resistance in Meropenem-resistant *K. pneumoniae* based on bacterial growth. Here, we applied this approach for the detection of Tobramycin-resistant *K. pneumoniae, P. aeruginosa,* and *A. baumannii*.
**Methods:** 20 *K. pneumoniae,* 20 *P. aeruginosa, and 20 A. baumannii* strains were analyzed by an MS-based resistance test (MBT-ASTRA) and Etest. MBT-ASTRA employs quantitative MALDI-TOF MS to compare the growth of bacteria in BHI medium containing different concentrations of Tobramycin to the growth of the same strain in BHI medium without antibiotic after a respective incubation at 37°C. After growing, the cells were lysed in the presence of an internal standard. Lysates were spotted on a MALDI target and overlaid with HCCA matrix. After drying, MS profile spectra were acquired on a microflex LT/SH benchtop mass spectrometer (Bruker Daltonik GmbH). The relative protein amount was calculated using the internal standard. The ratio of the protein content of the BHI plus Tobramycin setup and of the BHI only setup was calculated (relative growth, RG). A species dependent cutoff for the RG was defined to achieve separation between sensitive and resistant strains.
**Results:** For *K. pneumoniae*, titration revealed a breakpoint concentration of 32 µg/ml Tobramycin necessary for discrimination between resistant and sensitive strains after 2 h incubation and a threshold for the RG of 0.4. Due to the their slower growth, the incubation time was increased to 3 h and 3.5 h for *P. aeruginosa* and *A. baumannii*, respectively, to achieve reliable evaluations. The RG threshold of 0.4 and the required Tobramycin concentration of 32 µg/ml could be directly transferred. For *A. baumannii*, even 16 µg/ml Tobramycin would be sufficient for a clear separation. Each strain represented its individual breakpoint concentration comparable to the MIC.
**Conclusions:** The comparison between the outcome of the MS-based assay providing results within a few hours and the routine assay revealed concordance.

**Presentation:** Tuesday, September 29, 2015 from 18:00 – 18:15 in room Weißer Saal 2.

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**411/PRV**

**Bacterial contamination of water samples in Gabon, 2013**

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**Question:** Unsafe drinking water is a risk factor for enteric diseases [1]. We therefore analysed the bacterial contamination of water from improved and unimproved sources in Gabon, Central Africa.
**Methods:** Within the course of a cross sectional study, 200 water samples were screened for coliform bacteria in Gabon in 2013. Species identification was performed for each isolate by standard procedures. Susceptibility testing was done by Vitek-2 automated systems (bioMérieux, Marcy l'Etoile, France) or agar diffusion test according to EUCAST guidelines (version 4.0).
**Results:** The proportion of contaminated samples was significantly higher in unimproved vs. improved water sources (43.8 vs. 10.9%, p=0.02). One extended spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* ($bla_{\text{TEM-1}}$ and $bla_{\text{CTX-M 15}}$) isolate was found in a rural river site. Three samples from improved hospital water contained isolates of reptile-associated *Salmonella enterica* subsp. *salamae* (II 42:r:-).
**Conclusions:** Contamination rates even in improved water sources are high in Gabon but lower than contamination rates of improved sources investigated in other studies in urban (27%) and rural (58%) settings in Africa [2]. However, the contamination of improved water sources with coliform bacteria yields the potential to spread enteric diseases. The detection of ESBL-producing *K.*

*pneumoniae* in river water suggests that open water bodies could be a reservoir of multi drug resistant bacteria. This could be a driving factor for high carrier rates of ESBL-producing *Enterobacteriaceae* in the community setting in Gabon (community associated carriage: 33.6%) [3].


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**Presentation:** Tuesday, September 29, 2015 from 18:15 – 18:30 in room Weißer Saal 2.

## NATIONAL REFERENCE LABORATORIES AND CONSILIARY LABORATORIES (RKV)

**412/RKV**

**Retrospective investigation of the largest German *Legionella pneumophila* outbreak to date in Warstein (2013) by core genome MLST revealed two different clones as causative agents**


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**Objectives**: In 2013 the city Warstein faced the hitherto largest *L. pneumophila* outbreak in Germany (78 laboratory confirmed Legionnaires' disease (LD) cases including one fatality). The epidemic strain, recovered from seven patients and several environmental sources was characterized as serogroup 1, monoclonal antibody (mAb) subtype Knoxville and MLST sequence type (ST) 345 [Maisa A., et al., *Eurosurveill.*, 2015 (in press)]. We further studied the outbreak strain using a core genome MLST (cgMLST) approach [Moran-Gilad J., et al. *Eurosurveill.*, 2015 (in press)].

**Methods**: Sequencing libraries of 30 outbreak isolates (ST345) and nine non-related ST345 and non-ST345 strains were prepared using the Nextera XT kit for 250bp paired-end sequencing with a minimum coverage of 100-fold run on a MiSeq Illumina sequencer followed by *de novo* assembling using Velvet. The cgMLST scheme consisting of 1,521 core genome targets was used to analyze all isolates with the Ridom SeqSphere$^+$ software (Muenster, Germany). The allelic profiles of each isolate served as basis to calculate a minimum spanning tree (MST) to examine the epidemiological relationship between outbreak and unrelated isolates.

**Results**: In total, 1,475 gene targets of the cgMLST scheme were common to all isolates. The MST revealed a clear distinction between the unrelated isolates and the outbreak isolates. Surprisingly, the outbreak isolates itself formed two distinct clusters differing by 39 alleles. The patient isolates as well as the majority of ST345 environmental isolates were found in both clusters.

**Conclusion**: cgMLST proved superior to standard typing approaches and was able to discriminate between related and unrelated isolates belonging to the same ST and mAb-subtype. Furthermore, cgMLST assigned outbreak isolates into two distinct clusters thereby implicating two intermixed ST345 clones in the Warstein outbreak.

**Presentation:** Wednesday, September 30, 2015 from 8:30 – 9:00 in room Roter Saal 1.

**413/RKV**

**Diagnostics and antifungal susceptibility testing by the National Center for Invasive Mycoses (NRZMyk)**


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[4]*Medizinisches Versorgungszentrum Dr. Engelschalk, Dr. Schubach, Dr. Wiegel und Kollegen, Passau, Germany*

[5]*Universitaet Wuerzburg, Institut fuer Hygiene und Mikrobiologie, Wuerzburg, Germany*


Two principal tasks of the German National Reference Center for Invasive Fungal Infections (NRZMyk) are diagnostics of fungal pathogens exceeding the routine spectrum by species identification tools that also consider rare and/or recently described species and antifungal susceptibility testing combined with the detection and the monitoring of mutations responsible for antifungal resistance.

Here we present exemplary cases of rare fungal pathogens that were identified by the NRZMyk. In one case, biopsy material of a cerebral abscess from a patient with acute myeloid leukemia (AML) after allogeneic hematopoietic stem cell transplantation was studied. *Emericella quadrilineata* (syn. *A. tetrazonus*), the sibling species of *Aspergillus nidulans* was identified as the etiological agent. In contrast to the more frequent *A. nidulans*, *E. quadrilineata* is susceptible against Amphotericin B but resistant against echinocandins. In second case, a *Fusarium* species was isolated from blood of a patient with acute lymphoblastic leukemia (ALL) that was identified molecularly as *Fusarium musae*. This species was described in 2011 from banana in Central and South America. Its occurrence in Europe and its clinical relevance have not been published yet. In a third case, *Trichosporon* sp. was isolated from bronchial exudate of a patient suffering from cystic fibrosis (CF) and identified as *Trichosporon mycotoxinovorans*. This species was recently recognized to cause infections in CF-patients and needs special attention because it is resistant against Amphotericin B, echinocandins and some isolates also against azoles.

In addition to identification of invasive fungal pathogens, susceptibility testing has become more and more important. Since December 2014 the NRZMyk has obtained 14 strains of *Candida* species including *C. albicans*, *C. dubliniensis*, *C. glabrata*, and *C. krusei* that were resistant against one or more antifungals. In a high proportion of *Candida* strains with proven resistance against echinocandins we could detect mutations of the fks genes that resulted in changes of amino acids.

Taken together, reference diagnostics are important to correctly identify rare causative agents of fungal infection. In some cases correct species identification will have direct impact on the choice of therapy. Our data support the need for systematic analyses regarding the development of resistance against echinocandins in *Candida* spp.

**Presentation:** Wednesday, September 30, 2015 from 9:00 – 9:15 in room Roter Saal 1.

**414/RKV**

**Meningococcal antigen typing system (MATS) based coverage estimates for Bexsero® on invasive MenB strains isolated in 6 years from infants, toddlers and adolescents in Germany**

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**Introduction and aims**: Bexsero® was approved for vaccination against invasive meningococcal serogroup B (MenB) disease from two months of age in Europe in 2013. Meningococcal Antigen Typing System (MATS) prediction of coverage by Bexsero® in 222 German MenB strains isolated from all ages in the period July 2007 to June 2008 was 82% (95% coverage interval: 69-92%) (Vogel et al., 2013). An unpublished subset analysis of this strain collection suggested lower coverage of strains isolated from infants. Moreover, data on strain coverage of Bexsero® over longer time periods are lacking. Thus, we estimated coverage of strains isolated over 5 additional years for age groups with high MenB incidence.

**Methods**: German MenB strains isolated from infants (n=148), 1 year olds (yo's) (n=83) and adolescents aged 12-17 years (n=107) from July 2008-June 2013 were analysed by MATS in addition to the 222 strains already tested. Strains are considered covered when the level of expression in MATS ELISA for at least one of the three antigens tested is above the positive bactericidal threshold (PBT), shown to be predictive of killing by vaccine-induced bactericidal antibodies, and/or they have a PorA VR2=4 (Donnelly et al. 2010). 95% coverage intervals (CI) were calculated based on observed intra-laboratory variation of the PBT (Plitaykis et al. 2012).

**Results**: Estimated mean coverage from July 2007 to June 2013 was 67% (95% CI: 56-82%) for infants (annual range: 61-83%), 74% (95% CI: 68-87%) for 1 yo's (range: 56-81%) and 84% (95% CI: 76-90%) for adolescents (range: 53-95%). For the three age groups combined, coverage was 74% (95% CI: 65-85%) and more stable overtime (range: 66% (2012/13) to 80% (2008/09)). Mean coverage of strains from infants increased from 59% (95%CI: 45-78%) in <6 month-olds to 73% (95%CI: 63-84%) in 6-11 month-olds (p=0.049). Estimated coverage in 2007/08 was 95% (95%CI: 89-100%) in 2-11 yo's, 82% (95%CI: 72-83%) in 18-49 yo's and 77% (95% CI: 57-80%) in ≥50 yo's. Strain coverage was similar in fatal and non-fatal cases in all age groups (p>0.5).

Conclusions: The data provide a more solid estimate of MenB coverage by Bexsero® in Germany and confirm lowest coverage for strains isolated from infants, the main target group for vaccination. This may be related to higher diversity of membrane proteins in this age group, as shown for PorA/FetA. The observed temporal variation in coverage underlines the need to monitor expression of vaccine antigens over time.

**Presentation:** Wednesday, September 30, 2015 from 9:15 – 9:30 in room Roter Saal 1.

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**415/RKV**

**Molecular discrimination of morphological similar tissue-invasive parasites reveals different epidemiology behind the scene**

D. Tappe*[1]
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The definitive diagnosis of unusual tissue-invasive helminth parasites is a challenge for both clinicians and diagnostic laboratories. Due to increasing travel, migration, global climate and land use change, more uncommon infections are likely to be seen. Depending on the complexity and integrity of the organisms, as well as the **Presentation:** the tissue section, the correct identification of the parasites may be complicated and is easily confused. However, therapy, prognosis, recognition of possible parasite reservoirs and vectors and thus future prevention all depend on the specific diagnosis. With the application of molecular tools the identification is greatly facilitated, also from sophisticated material such as formalin-fixed and paraffin-embedded tissue samples. Here, several diagnostic examples from the National Reference Center for Tropical Diseases of morphologically similar parasites in human tissues are presented, that have a totally different ecology, epidemiology, and prognosis.

**Presentation:** Wednesday, September 30, 2015 from 9:30 – 9:45 in room Roter Saal 1.

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**416/RKV**

**Human Adenovirus (HAdV) type 70: A novel, multiple recombinant species D adenovirus isolated from diarrheal faeces of a haematopoietic stem cell transplantation recipient**

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**Introduction:** Since the isolation of a multiple recombinant species D HAdV (HAdV-D), which was later designated as type 53, from a keratoconjunctivits outbreak in 2005, the definitions for novel HAdV types were updated. Novel HAdV types can now be designated by genomic cirteria, e.g. recombinations in their phylogeny, in addition to classical neutralization typing. 26 new HAdV types were published in the last decade due to this new definition.

Recently, a HAdV-D isolated from diarrhoeal faeces of an allogenic haematopoietic stem cell transplant (SCT) recipient was found to be non typeable by micro neutralization. In contrast to species HAdV-C, HAdV-D infections are rarely observed in SCT patients. Therefore, this isolate was analysed in detail.

**Methods**: The whole genome of the isolate was sequenced by a next generation sequencing method (MiSeq). Phylogenetic analysis of the whole genome, major capsid proteins and gene regions, as well as a search for recombination events was performed.

**Results**: A complete genomic sequence of 35.2 kb in length with a GC content of 57% was obtained and found to be distantly related to HAdV-D27 (96.25% identity). Imputed serology (sequence analysis of loops 1 and 2 of the hexon main neutralization epitope) implicated a new type with a nucleotide sequence identity of only 96.11% to HAdV-D37 (loop 1) and 95.76% to HAdV-D30 and -D37 (loop 2). The penton base gene showed a novel sequence clustering with HAdV-D38, but bootscan analysis indicated an intra-penton recombination event with HAdV-D60. Another recombination event was detected within the early genes region E3 with the 12.2kDa and the CR1-alpha genes derived from HAdV-D58. Moreover, the E4 region was derived from HAdV-D13 but all these genes had evolved significantly from their ancestors. By contrast, the recombinant fiber gene was almost 100% identical to HAdV-D29.

**Conclusion**: The genomics of this novel human adenovirus, designated as the HAdV-D70 [P70H70F29] prototype, supported the significance of multiple recombinations in the phylogeny of species D human adenoviruses.

**Presentation:** Wednesday, September 30, 2015 from 9:45 – 10:00 in room Roter Saal 1.

## ANTIMICROBIAL RESISTANCE IN DIFFERENT HOSTS (ZOV)

### 417/ZOV

**Linezolid-resistant *Enterococcus faecium* clinical isolates harbor a novel *cfr* methyltransferase from *Clostridium difficile***

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**Objectives**: Linezolid (LZD) represents one antibiotic of last resort for the treatment of infections with multidrug-resistant Gram-positive bacteria. As stated by the German antibiotic resistance surveillance network (ARS), resistance frequencies to LZD of *E. faecium* isolates from German hospitals remain below 1%. However, the National Reference Centre for Staphylococci and Enterococci in Germany received an increasing number of clinical LZD-resistant *E. faecium* in recent years (9% in 2014, in total n=251). A few isolates harbored a *cfr*-like gene locus and thus were studied in more detail.
**Methods**: *E. faecium* isolates were examined with respect to mutations in the 23S rDNA alleles and/or ribosomal protein-encoding genes *rplC*/*rplD* or the presence of *cfr*. Five isolates were subjected to whole genome sequencing by means of Illumina technology. Cloning and expression of the *cfr* locus from *Enterococcus* and *Clostridium* was performed to determine the Cfr-mediated phenotype. *In vitro* transfer experiments are currently in progress.
**Results**: Five of 251 LZD-resistant *E. faecium* strains investigated produced a PCR product for amplification of *cfr*. Detailed investigation of the *cfr*-encoding region revealed that *cfr* of German clinical enterococci is almost identical to *cfr* from *C. difficile* Ox3196. A Cfr-dependent LZD resistance phenotype could be demonstrated for isogenic *C. difficile* strains. Whole genome sequencing disclosed that *cfr* of the *E. faecium* isolates is located on transposon Tn*6218*. Cloning and expression analyses of *C. difficile* as well as of *E. faecium* derived *cfr* in both *E. faecium* and *E. faecalis* laboratory strains verified gene transcription, but MIC determination failed to detect evidence for Cfr-mediated LZD, chloramphenicol and streptogramin A resistance.
**Conclusion**: Although the methyltransferase Cfr was previously shown to confer LZD resistance in staphylococci, data concerning the involvement of Cfr in LZD resistance development in enterococci are inconsistent. Nevertheless, the potential to obtain and disseminate a resistance module from a gut commensal by means of horizontal gene transfer requires special attention and thus further investigation.

**Presentation:** Monday, September 28, 2015 from 17:45 – 18:00 in room Roter Saal 1.

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### 418/ZOV

**Comparison of resistance data from commensal and ESBL/AmpC producing *E. coli* isolated from the same samples**

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[2]*European Food Safety Authority, Parma, Germany*

**Introduction**: In food and food-producing animals monitoring of antimicrobial resistant bacteria is mostly conducted on commensal *E. coli*. From 2015 on in the EU also the selective isolation and subsequent resistance testing on ESBL/AmpC-producing *E. coli* is mandatory (2013/652/EU).
**Aim**: In our study we compared resistance data of non-selectively (commensal) and selectively (ESBL/AmpC-producing) isolated *E. coli* from identical samples of food and food-producing animals in order to assess the impact of the selection criteria for isolates included in the monitoring on the results (as regards resistance frequency and patterns).
**Material & Methods**: During 2014 more than 2000 *E. coli* isolated from food and food producing animals by the German federal state laboratories were included in the German monitoring of antimicrobial resistance in zoonotic and *commensal* bacteria (ZoMo-Nat2014) conducted at the Federal institute for Risk Assessment (BfR). At the same time voluntarily these laboratories selectively tested samples for ESBL/AmpC-producing *E. coli*, which subsequently were also sent to and investigated by the BfR (n=247). The antimicrobial resistance of 141 pairs (commensal and ESBL/AmpC) of *E. coli* isolated from identical samples was compared.
**Results**: In 7/141 (4.96%) non-selectively isolated strains an ESBL/AmpC resistance pattern was found. In six of these samples the corresponding selectively isolated *E. coli* showed the same (±1 dilution step) minimal inhibitory concentrations, in one sample the sulfamethoxazole value was higher in the ESBL/AmpC isolate. In 94 (66.67%) of the samples commensal *E. coli* susceptible to all of the tested antimicrobials were found alongside with the selectively-isolated ESBL/AmpC *E. coli.* Resistance to agents of one/two/three/four/five/six/seven antimicrobial families was expressed by 18/15/7/2/4/1/0 of the commensal and 1/36/42/43/15/3/1 of the ESBL/AmpC producing *E. coli* respectively. Resistance against ciprofloxacin was found in 18 (12.77%) of the commensal and 31 (21.99%) of the ESBL/AmpC producing *E. coli.*
**Conclusion**: In our study 4.96% of the non-selectively isolated *E. coli* from ESBL/AmpC producer positive samples showed a resistance pattern typical for ESBL/AmpC production. Also resistance to other important antimicrobial agents like e.g. fluoroquinolones, are more likely to be found along with the ESBL/AmpC producers. Multi-drug resistance (>2 antimicrobial families) was observed in 9.29% of the commensal *E. coli* and in 73.76% of the ESBL/AmpC producing isolates. This shows the necessity for the selective isolation of ESBL/AmpC-producing *E. coli* (as now required by EU), not only to reliably detect these particular resistance mechanism.

**Presentation:** Tuesday, September 29, 2015 from 9:00 – 9:15 in room Weißer Saal 1.

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### 419/ZOV

**Characterization and Disease Association of Shiga Toxin-producing *Escherichia coli* O91 using Whole Genome Sequencing (WGS)**

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**Introduction**: Shiga toxin-producing *Escherichia coli* (STEC) cause human diseases ranging from mild diarrhea to hemorrhagic colitis and the hemolytic uremic syndrome (HUS). At least one third of infections are caused by non O157 serogroups including O91. In adult patients and in food samples O91 is among the most commonly identified serogroups in Germany. Our previous analyses already indicated an association between disease severity and O91 H-type.
**Objectives**: To determine the responsible markers and the exact clonal structure of STEC O91, we used here the high resolution of whole genome sequencing (WGS).
**Material & Methods**: STEC O91 strains (comprising six different H-antigens and 11 multilocus sequence types [MLST STs]) were isolated between 1985 and 2013 from epidemiologically unrelated patients in Germany (n=103 strains), Austria (2), Finland (1), Argentina (1), Canada (3), and USA (1). All STEC O91 were subjected to WGS using different Illumina platforms (Illumina Inc., San Diego, USA). After sequencing and denovo assembly, coding regions were compared in a gene-by-gene approach (MLST[+]) using the SeqSphere[+] software version 2.0 beta (Ridom GmbH,

Muenster, Germany). Clusters were generated using the minimum spanning tree (MST) algorithm.
**Results**: In total, 111 STEC O91 patient isolates were analyzed by WGS. MST analysis of these isolates was based on 4671 genes, pairwise ignoring missing values, and resulted in five clusters (< 300 alleles differing) and six singletons. MLST$^{+}$-based clustering was concordant with classical MLST and serotyping. Most common ST were ST33 (67 samples), associated with serotypes O91:H14, H$^{-}$ and Hnt and ST442 (28 samples), associated with serotype O91:H21. These two serotypes represent two main clusters in MST with a distance of at least 2379 alleles among each other. One sample out of the O91:H14 cluster was isolated from a patient suffering from HUS, while eight samples out of the O91:H21 cluster were associated with HUS. The chance to develop severe illness and HUS manifestation is significantly higher in patients infected with the cluster representing serotype O91:H21 (Odds Ratio: 37.3).
**Conclusion**: WGS allows a detailed clustering of STEC O91 and prediction of severity of clinical disease (symptoms). Current investigations address a deeper evolutionary analysis and subclustering of O91 serotypes to elucidate their phylogenetic origin.

**Presentation:** Monday, September 28, 2015 from 17:00 – 17:15 in room Roter Saal 1.

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**420/ZOV**

**Human pathogenic *Clostridium difficile* strains in companion animals detected in a Germany-wide survey.**

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**Introduction**: In humans *Clostridium* (*C.*) *difficile* infections (CDI) can vary from symptomless carriage to life-threatening intestinal disease. The recent changes in epidemiology of CDI with an increasing incidence and severity are of particular concern. Although virulent strains affecting humans have also been isolated from various animal species, epidemiological data on *C. difficile* in companion animals are scarce, limiting the risk assessment of possible interspecies transmission.
Objectives: This study aimed to collect first national data on occurrence and genotypic variation of *C. difficile* in dogs, cats and their owners and define risk factors associated with colonization or CDI.
**Materials & Methods**: From July 2012 to August 2013, a Germany-wide survey was conducted sampling companion animals and their owners. Capillary gel electrophoresis based PCR ribotyping, Multilocus VNTR Analysis (MLVA) and PCR detection of toxin genes A, B and the binary toxin were used to characterise isolated *C. difficile* strains.
Results: A total of 1,435 faecal samples could be acquired from 415 different households with 40.7% of human and 59.3% of animal origin. The *C. difficile* isolation rates were 2.91% (17/584) and 2.94% (25/851) for human and animal samples, respectively. Typing revealed twelve resp. eight different PCR ribotypes in isolates from humans resp. companion animals. Three of the animal ribotypes could also be isolated from human samples (014/0, 010 and the highly virulent ribotype 078). Moreover ribotypes 027 and 078 were isolated in dogs. These ribotypes are considered highly virulent in humans. Within two households identical ribotypes were isolated from two partner animals (in both cases 014/0), whereas no *C. difficile* pair from owner and pet sharing the same household could be detected. The risk assessment revealed known risk factors for colonization or CDI in humans (antibiotic intake and age). In companion animals risk factors positively associated with *C. difficile* colonization/CDI were the contact to a human suffering from diarrhoea, intake of antibiotics or proton pump inhibitors, age, acute disease, inappetence, and diarrhoea.
**Conclusion**: *C. difficile* isolation rates are low in companion animals and their owners in Germany. Well known human ribotypes including virulent ribotypes 027 and 078 also occur in dogs and suggest a common infection source, zoonotic transmission or both. Future case-control studies should be implemented to get insight into the risk of zoonotic *C. difficile* infections.

**Presentation:** Monday, September 28, 2015 from 17:30 – 17:45 in room Roter Saal 1.

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**421/ZOV**

**A combined case control and MLST source attribution study of human *Campylobacter* infections in Germany**

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**Background:** *Campylobacter* infection is the most commonly reported bacterial gastroenteritis in Germany, but its epidemiology remains incompletely understood. To investigate the risk factors for *Campylobacter* infections and the routes of transmission in Germany, we have performed a case control study combined with molecular strain typing (MLST) and source attribution analysis in the framework of the FBI-Zoo network.
**Methods:** We conducted the study in selected districts of four German federal states. Case patients were recruited through local health authorities (Nov 2011-Feb 2014). Controls were randomly selected from population registries. All participants completed a questionnaire. *Campylobacter* isolates of a subset of case patients were cultured and further analyzed by MLST of seven housekeeping genes. Using the questionnaires, we conducted univariate logistic regression analyses (SVA), adjusted for age group, sex, and federal state, and multivariate logistic regression analyses (MVA).
**Results:** In the SVA, travelling abroad was determined to be a statistically significant risk factor (OR 1.11 (95% CI: 1.06-1.17; p-value <0,001; study population: 1814 cases, 3983 controls). For further source-directed analyses, we excluded cases that had travelled abroad. Consumption of chicken meat, preparation of poultry meat in the household, eating out, contact to chickens or ducks/geese, and the use of antacids were identified, among others, as risk factors in SVA. Being a vegetarian, consumption of beef, fresh fruit, vegetables, or herbs were negatively associated with disease. For children <5 years contact with animals (dogs, birds, chickens, ducks/geese), playing in a sand box, and using a pacifier were additional risk factors. Results of MVA will also be presented.
MLST was performed for 614 patient isolates and 543 isolates from animals (338) and food sources (205) that had been collected in the geographical and temporal context of the study. Several new *Campylobacter* sequence types (STs) were uncovered and currently dominant STs in humans, animals and food were identified.
MLST data combined with detailed source information of current animal and food strains were then combined for a source attribution analysis of the human isolates using Bayesian inference on an asymmetric island model. The main sources attributed to human *Campylobacter* infection overall, and specifically with *C. jejuni*, were consumption of chicken and contact with pets. The main source attributed to human *C. coli* infection was consumption of pork meat.
**Conclusions:** This first analysis of *Campylobacter* cases and controls in Germany in combination with molecular typing and source attribution confirmed that chicken meat consumption and its

preparation in the household are the most important risk factors for *Campylobacter* infections in Germany. Further risk factors and sources that were revealed by both approaches were pet contact (in particular for children) and pork meat for *C. coli* infection. To protect consumers, efforts should be intensified to reduce the *Campylobacter* load on chicken meat and to inform consumers how they can minimize risk of infection.

**Presentation:** Tuesday, September 29, 2015 from 8:30 – 8:45 in room Weißer Saal 1.

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**422/ZOV**

**Novel flagellin-like protein FlaC in unsheathed intestinal *Helicobacter* and *Campylobacter* species modulates the immune response and microbiota in vivo**

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**Question:** Bacterial microorganisms which colonize the intestinal tract have to deal with several unique characteristics specific for this habitat: a high density of resident microbiota of various species and an immunological environment primed by the resident microbiota. Little is known about how *Helicobacter* and *Campylobacter* ssp. interact with the innate immune systems of their hosts and with the major pattern recognition receptors (PRR) such as TLR and NLR receptors. It has been reported that *C. jejuni* or the closely related gastric pathogen *H. pylori* are restricted in their abilities to activate the innate immune system via TLR5 and also via TLR4. We have addressed the role of a novel immunomodulatory protein of *Campylobacter*, FlaC, in vitro and in a chicken model.

**Methods and Results:** In addition to the classical flagellin molecules, we identified the unusual flagellin-like protein FlaC and potential orthologues to be conserved in various *Campylobacter*, *Helicobacter* and one *Wolinella* species. FlaC is a secreted protein, and its amino acid sequences appear to be chimeras between TLR5-stimulating and non-stimulating, flagellins. We hypothesized that FlaC might be involved in the modulation of the host intestinal environment and microbiota. *Campylobacter* FlaC was used as a model protein in cell culture and in vivo in a chicken application experiment, either in the presence or the absence of *Campylobacter*. Ultrapure FlaC was able to activate different cell types of different host species, inducing the production of cytokine mRNA. Additionally, FlaC was shown to directly interact with TLR5. Preincubation with FlaC reduced the responsiveness of chicken and human macrophages towards bacterial LPS. We applied FlaC to two-weeks' old chicken via the cloacal route, with and without *Campylobacter* infection. In this setting, FlaC was able to modulate the immune response and the intestinal microbiota of chicken.

**Conclusions:** We conclude that intestinal pathogens, which possess flagella without a sheath, including various *Helicobacter* and *Campylobacter* spp., have evolved the novel host-stimulatory chimeric flagellin-like molecule FlaC in order to specifically modulate host responses, particularly towards other bacterial PRR ligands. We propose that these proteins act predominantly as a homeostatic or tolerogenic signal in the intestinal tract in the presence of the resident microbiota and can modulate the intestinal microbiota composition.

**Presentation:** Monday, September 28, 2015 from 8:30 – 8:45 in room Weißer Saal 1.

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**423/ZOV**

**Cell-penetrating Effector Proteins (CPE) of the LPX Subtype as Potential (Immune-) Therapeutics**

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Over millions of years (pathogenic) bacteria have developed sophisticated strategies to manipulate host immune responses for their own benefit. Many Gram-negative bacteria use a type III secretion system (T3SS) to inject effector proteins into the host cell cytoplasm. As several of these effectors modulate host immune signaling, they might be applicable as innovative therapeutic tools to attenuate excessive and detrimental immune reactions. This novel possibility of exploiting 'bacterial knowledge' has become even more interesting as the YopM protein of *Yersinia enterocolitica* was identified as a cell-penetrating effector (CPE) that is able to translocate into host cells in a T3SS-independent manner. Moreover, YopM remains functional upon autonomous internalization and efficiently downregulates the expression of several pro-inflammatory cytokines. Recently, also SspH1 of *Salmonella typhimurium* was shown to exhibit CPE-properties and anti-inflammatory effects.

Both YopM and SspH1 belong to bacterial effectors of the LPX subtype of leucine-rich repeat (LRR) proteins which further comprises different IpaH proteins of *Shigella* as well as the *Salmonella* proteins SspH2 and SlrP. Due to significant homology in sequence and structure, we suggested a general concept for T3SS-independent uptake of LPX effector proteins. In this study, we confirmed the ability of several recombinantly expressed LPX effector proteins of *Shigella flexneri* and *Salmonella typhimurium* to autonomously translocate into eukaryotic cells. For this we used different approaches including cell fractionation, immunofluorescence microscopy, and FACS analyses. Moreover, our results point to a major contribution of endocytosis to T3SS-independent cellular uptake. The function of the recombinant CPE as ubiquitin E3-ligases was proven by *in vitro* ubiquitination assays. The interaction with putative (new) interactions partners *in vivo* is part of current research.

Taken together, we provide further evidence for a general concept of T3SS-independent translocation of bacterial LPX effectors. Along with their capacity to modulate and suppress host immune signaling pathways, e.g. by ubiquitination of cellular targets, these bacterial effectors might serve as potential 'self-delivering' biological therapeutics in the future.

**Presentation:** Monday, September 28, 2015 from 8:45 – 9:00 in room Weißer Saal 1.

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**424/ZOV**

**Microbial inactivation of pancreatic proteases protects epithelial barrier integrity in the large intestine**

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**Question**: Antibiotic (AB) therapy has been shown to increase the risk for inflammatory bowel diseases (IBD) but the underlying mechanisms are unknown. Interestingly, specific ABs have been found to increase the proteolytic activity (PA) of pancreatic proteases in the large intestine. We aimed to unravel the impact of this acute AB-mediated increase in PA on the large intestinal barrier and the development of colitis. In addition, we aim to identify bacterial strains that mediate the physiological inactivation of pancreatic proteases in the large intestine.

**Methods and Results**: Vancomycin/Metronidazole (V/M) treatment of wildtype and $TNF^{deltaARE/+}$ (ARE) mice resulted in strong increase of the PA in the large intestine (>10x), whereas Ampicillin treatment did not. Protease assays and LC-MS/MS analysis revealed that the level and activity of pancreatic proteases in V/M treated mice is comparable to the one in germfree (GF) mice, demonstrating that V/M abrogated the majority of bacteria that are capable to inactivate pancreatic proteases. A correlation analysis of the abundance of specific OTUs and the respective

large intestinal PA in V/M and untreated mice revealed several bacterial species that may be relevant for the inactivation of pancreatic proteases. Transwell experiments using PTK6 cells and Ussing chamber experiments using cecal tissue from wildtype mice showed that the apical application of cecal water (CS) from V/M-treated or GF mice significantly reduced transepithelial electrical resistance (TEER) and increased epithelial permeability, whereas CS from SPF mice or PMSF-inactivated CS from V/M-treated or GF mice did not. A four week V/M intervention in wildtype and ARE mice did not result in macroscopic epithelial cell damage or increased inflammation in the large intestine, suggesting that the large intestinal epithelium is able to adapt to the exposure towards high proteolytic activity via yet unknown protective feedback mechanisms. However, cecal inflammation in ARE mice was significantly increased shortly after discontinuation of the AB treatment.

**Conclusion**: The present findings demonstrate that AB treatment can increase pancreatic protease activity in the large intestine to a level that strongly impairs the epithelial barrier function. The increase in large intestinal PA during AB treatment might therefore be of pathophysiological relevance for the development of IBD.

**Presentation:** Monday, September 28, 2015 from 9:00 – 9:15 in room Weißer Saal 1.

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**425/ZOV**

**Inhibition of host immune responses by an effector protease ofenteropathogenic *E. coli***

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Enteropathogenic Escherichia coli (EPEC) rapidly inhibit host innate immune responses upon infection of gastrointestinal epithelial cells. Several effector proteins translocated by the bacterial type III secretion system have been indicated in this process. While the bacterial zinc metalloproteases NleC and NleD specifically cleave NF-κB subunits or the MAPKs JNK and p38, another effector, NleE, inhibits activation of the TAB-TAK1 complex by transferring a methyl-group to a conserved cysteine residue in the zinc finger domains of TAB2 and 3. Recently it was shown that the effector protein NleB attaches a single GlcNAc to a conserved arginine residue in the death domain of cell signalling proteins including FADD, interfering with the formation of death receptor complexes, thereby inhibiting apoptosis in infected cells. Recently, we identified another effector protein to play a role in the inhibition of host innate immune responses. When expressed ectopically or translocated into host cells by the bacteria, the effector induced the degradation of the receptor interacting protein kinases (RIPK) 1 and 3 by a process that is independent of the proteasome or caspase-8 cleavage. Secondary structure analysis suggests that the protein is similar to the family of clan CA cysteine proteases, which also includes the bacterial effector protein YopT of Yersinia. Mutation of the amino acids of the catalytic triad abolished its ability to degrade its target proteins. Furthermore, in these mutants, RIPK1 or 3 can be co-immunoprecipitated, suggesting an interaction between the proteins. As a downstream effect of the degradation of both RIPK1 and 3, the effector protein inhibits caspase- independent cell death (necroptosis) in addition to the host inflammatory response, making EPEC the first pathogen ever described to inhibit inflammation, apoptosis and necroptosis.

**Presentation:** Monday, September 28, 2015 from 9:15 – 9:30 in room Weißer Saal 1.

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**426/ZOV**

**Blocking CERT-dependent ceramide transport leads to species-specific reduction of *Chlamydia* progeny formation.**

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**Background and Aim:** *Chlamydia* spp. represent a diverse group of gram-negative obligate intracellular bacteria causing different diseases in humans and animals. While *C. trachomatis*, the most common sexually transmitted bacterial pathogen worldwide strictly infect humans, infections with the zoonotic pathogen *C. psittaci* can be found in a large spectrum of host-species, including humans and birds. Interestingly, clinical pictures of *C. psittaci* in humans are very diverse covering fatal cases of pneumonia contrasted by latent asymptomatic infections. The molecular mechanisms of the different host tropisms and associated disease are not well understood.

Productive infections with *Chlamydia* rely on the interactions with the host cell to acquire nutrients such as lipids. We reasoned that there will be conserved and species-specific interactions with the cellular lipid transport machinery. The aim of the study was to compare the interactions of *C. trachomatis* and *C. psittaci* with the intracellular transport machinery in human epithelial cells focusing on factors of the cellular sphingolipid transport.

**Methods and Results:** First, we compared the localizations of proteins involved in different cellular trafficking pathways in HeLa cells infected with both *Chlamydia* spp. by confocal immunofluorescence microscopy. We confirmed the associations of a large subset of Rab proteins to the *C. trachomatis* inclusion. Surprisingly, only a reduced subset of Rab proteins was recruited to *C. psittaci* inclusions. Next, we analyzed the localization of the Golgi matrix protein golgin-84 (COPI marker) and the ceramide transport protein CERT (non-vesicular ceramide transport) in the different infection models. We observed a clear recruitment of CERT to *C. trachomatis* and *C. psittaci* inclusions whereas golgin-84 was only recruited to *C. trachomatis* but not to *C. psittaci* inclusions. Inhibition of CERT-dependent ceramide trafficking by HPA-12 treatment specifically reduced *C. psittaci* plaque formation in a dose-dependent manner. Morphological studies by electron microscopy supported the species-specific effect of CERT inhibition. Furthermore, sphingolipidomic analysis of purified infectious bacteria demonstrated that *C. psittaci* accumulated about 10 times more sphingolipids than *C. trachomatis*.

**Conclusions:** We showed that *C. trachomatis* and *C. psittaci* hijack distinct sphingolipid trafficking pathways and that infectious *C. psittaci* bacteria accumulate significantly more sphingolipids than *C. trachomatis* bacteria. These results indicate that CERT-dependent non-vesicular transport is essential for optimal growth of *C. psittaci* in human cells.

Future studies will address the role of CERT-mediated ceramide transport and sphingolipid accumulation as factors for host tropism and pathogenicity.

**Presentation:** Tuesday, September 29, 2015 from 17:00 – 17:15 in room Congress Saal.

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**427/ZOV**

**Conidia of the Fungal Pathogen *Aspergillus fumigatus* Interfere with the Maturation of Macrophage Phagolysosomes**

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**Introduction:** Aspergillosis is one of the most occurring fungal infections in immunocompromised patients. *A. fumigatus (Af)* conidia are inhaled and invade the lung tissue. They encounter the resident alveolar macrophages that exert an immune response consisting of phagocytosis, cytokine and chemokine production. Previously, we showed that *Af* conidia are able to evade the intracellular killing by inhibiting the acidification of the phagolysosome (PL). The conidial pigment consisting of dihydroxynaphthalene (DHN)-melanin was identified as the virulence determinant to interfere with the PL maturation[1,2].
**Aims:** This project aims at identifying the mechanisms of *Af* to inhibit PL acidification.
**Materials and Methods:** For this purpose, a protocol was developed to purify conidia-containing PLs from cell extract by coupling FACS separation and purification based on magnetic labeling[3]. The proteome of PLs was analyzed by nano-LC-MS to detect important players in the maturation process. As a reference, conidia-containing PLs of avirulent pigmentless *pksP* mutant conidia were investigated.
**Results:** A number of differentially produced proteins were identified in PLs containing WT conidia compared to PL containing pigmentless *pksP* mutant conidia. These proteins included Rab proteins but also proteins required for transport of ions. Furthermore, immunofluorescence staining indicated differences in the PL membrane and reduced assembly of the vATPase on the PL membrane of WT conidia-containing PL, explaining the lack of acidification.
**Conclusion:** DHN-melanin on the surface of *Af* conidia interferes with the maturation of the PL into a microbiocidal compartment. This is due to inhibition of vATPase assembly and interference with the lipid composition of PL membranes. Further analysis of the proteome and lipidome will help elucidating more detailed knowledge about *Af* immune evasion strategy.

**Reference**

[1] Heinekamp et al (2012), Front Microbiol **3**: 440
2 Thywißen et al (2011), Front Microbiol**2**: 96
[3] Steinhauser et al (2013), Traffic **14**: 321

**Presentation:** Tuesday, September 29, 2015 from 17:15 – 17:30 in room Congress Saal.

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**428/ZOV**

**Two novel *Chlamydia pneumoniae* effector proteins recruit SNX9 to the invading bacteria.**

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**Introduction**: *Chlamydia pneumoniae*, an obligate intracellular bacterium with an unique biphasic life cycle, is one of the most frequent causes of community-acquired pneumonia. The chlamydial developmental cycle is characterized by an alteration between the non-infectious but replicative RB form (reticulate body) and the infectious EB form (elementary body). The entire chlamydial infection cycle depends on an extensive, as yet incompletely understood, molecular crosstalk between pathogen and host, most of which is probably mediated by secreted bacterial effector proteins. Secretion of effector proteins starts with the first contact of the chlamydial EBs with the human host cell.
**Aim of the study**: Identification and understanding the function of *Chlamydia* proteins with relevance in early stages of infection in order to obtain a deeper understanding of the chlamydial pathogenicity mechanisms.
**Methods and results**: In our screen for novel chlamydial proteins with relevance for early aspects of the infection we identified the hypothetical proteins CPn0677 and CPn0678. Both proteins were found to be expressed late in the infection as determined by realtime PCR and Western blot analysis. Subcellular localization revealed for both proteins a colocalization with bacteria late in infection and an association with attached bacteria during early infection. Moreover differential permeabilization assays indicated an extrachlamydial localization of CPn0678 at early stages of infection. The hypothesis that these proteins could act as effector proteins was supported by our finding that both of them showed heterologous Type-3 dependent secretion in the *Shigella flexneri* system. In order to identify potential interaction partners bioinformatic analysis revealed several proline-rich-repeats in both proteins, which share limited but notable similarity to repeats in EspF, an enteropathogenic *Escherichia coli* (EPEC) protein, which evidently interacts with the human sorting nexin 9 (SNX9). SNX9 itself is involved in several endocytosis-related processes of the human host cell like membrane curvature, cytoskeletal reorganisation and vesicle fission. Indeed, biochemical assays revealed that CPn0677 and CPn0678 interact with human SNX9. In transfection experiments CPn0678 exhibited a high affinity to the human plasma membrane and induced an impressive recruitment of SNX9. Moreover, at early stages of infection an accumulation of SNX9 at the site of entry was observed. Importantly we could show that in SNX9 knock-down cells the chlamydial infection is reduced by more than 30%, indicating a relevance of SNX9 in the *C. pneumoniae* infection.
**Summary**: We have identified two novel *Chlamydia pneumoniae* effector proteins with relevance for the early infection. Most likely they interact with the human SNX9 protein to affect endocytotic processes relevant for host cell entry.

**Presentation:** Tuesday, September 29, 2015 from 17:30 – 17:45 in room Congress Saal.

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**429/ZOV**

**Adaptation of Staphylococcus aureus during long-term persistence in the lungs of a cystic fibrosis patient using differential RNA-Sequencing**

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**Introduction**: Many cystic fibrosis (CF) patients suffer from chronic pulmonary *Staphylococcus aureus* infections in their airways. Previous studies showed already that adaptation is necessary to survive in this hostile niche; however the mechanisms enabling this adaptation are not well-studied yet. We hypothesize that mainly local triggers like the variation of transcription start sites (TSS) and non-coding RNAs are responsible for the adaptation of the pathogen to the CF lung habitat.
**Objectives**: Therefore, we determined here the primary transcriptome using the differential RNA-Seq (dRNA-Seq) approach.
**Materials & Methods**: An isogenic pair of *S.aureus* isolates taken 13.2 years apart from the airways of the same CF patient was used for this analysis. The isolates were called “early” and “late” and were cultivated in RPMI 1640 Medium (Sigma) until the mid-log phase ($OD_{600}$ of 0.5). The total RNA was isolated with the Trizol protocol provided by Life technologies (Carlsbad, USA). The generation of the TEX+ and TEX- libraries was conducted by

Vertis (Freising, Germany) and sequenced on an Illumina MiSeq platform using 75 bp paired-end sequencing protocol. The data output was processed with the program READemption and calling of TSS was performed using TSSPredator.

**Results**: First analyses of the primary transcriptomes of the isogenic isolates revealed an extensive set of 1602 TSS for the "early" isolate and preliminary 1080 TSS for the "late" isolate. Both isolates have 929 TSS in common. One-hundred fifty-one TSS are only present in the "late" isolate but not in the "early" isolate indicating potential adaptational processes on the level of the primary transcriptome. Categorization of TSS led to 920 primary TSS (pTSS) in the "early" isolate and 687 pTSS in the "late" isolate. pTSS are within 300 nucleotides upstream of a gene having the highest expression values. All additional TSS upstream of this gene are considered secondary TSS (sTSS). sTSS account for 137 and 81, respectively. TSS located within annotated genes on the same strand (internal TSS [iTSS]) were 330 in the late and 185 in the early isolate; 179 and 109 TSS, respectively, could not be assigned to any annotated gene and were rated as orphan TSS (oTSS). Furthermore 234 (145 in the late isolate) antisense TSS (asTSS) were detected indicating antisense transcription.

**Conclusion**: In summary, our data provide a comprehensive overview of the primary transcriptome of *S. aureus* and will help to elucidate adaptation processes during long-term persistence in the human host. Future experiments will comprise the identification of TSS only present in the "early" isolate, 5'/3'-RACE and qRT-PCR experiments to verify the dRNA-seq results and phenotypic tests to determine the functional impact of the adaptations.

**Presentation:** Wednesday, September 30, 2015 from 8:30 – 8:45 in room Weißer Saal 1.

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**430/ZOV**

**Characterization of the adhesiom of *Salmonella enterica* serotype Typhimurium**

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*Salmonella enterica* serotype Typhimurium encodes for a large repertoire of adhesins including 13 fimbrial adhesins, three autotransported adhesins (ShdA, SadA and MisL) and two T1SS-secreted adhesins (SiiE and BapA) [1]. But research of adhesins in *Salmonella* has long been hampered because most of them cannot be expressed under *in vitro* growth conditions. Nonetheless, most fimbrial and non-fimbrial adhesins are expressed in the intestinal tract in animal models[2]. Therefore especially for fimbrial adhesins, only a small subset has been characterized yet.

To overcome this burden, we developed a set of strains where we exchanged the natural promotors of all known adhesins in *S.* Typhimurium with a tetracycline-inducible promotor cassette. We could demonstrate that under induction with the tetracycline-derivate anhydrotetracycline (AHT), most fimbrial and non-fimbrial adhesins in *S.* Typhimurium could be successfully expressed. Furthermore, we verified by a flow cytometry-based approach and by atomic force microscopy (AFM) that the adhesins are successfully assembled on the surface of the bacterium. To overcome possible redundancy between different adhesins of *S.* Typhimurium, we additionally generated a mutant, in which all known adhesins were deleted. This mutant was then complemented with individual operons encoding for adhesins either under control of their native promotor or under control of the tetracycline-inducible promotor element. To characterize novel adhesins in a heterologous background, we introduced the operons encoding for different adhesins under control of the tetracycline-inducible promotor cassette into a non-fimbriated *E. coli* strain by a plasmid-based approach. By this way, we were able to heterologously express most fimbrial and non-fimbrial adhesins in *E. coli*.

This collection of strains allows for the first time the study of the complete adhesiom of *S.* Typhimurium. This way we have the opportunity to specifically express and characterize novel adhesins with previously unknown function. We will present functional characterization of novel, previously uncharacterized fimbrial adhesins in *S.* Typhimurium. The long term goal of the study is the characterization of the complete adhesiome of *Salmonella* and to answer the question why *Salmonella spp.* encode for such a large repertoire of fimbrial and non-fimbrial adhesins.

**Reference**

1 Wagner, C.& Hensel, M.Adhesive mechanisms of *Salmonella enterica*. *Adv Exp Med Biol* **715**,17-34, doi:10.1007/978-94-007-0940-9_2 (2011).

2 Humphries, A. D. *et al.* The use of flow cytometry to detect expression of subunits encoded by 11 *Salmonella enterica* serotype Typhimurium fimbrial operons. *Mol Microbiol* **48**, 1357-1376, doi:3507 [pii] (2003).

**Presentation:** Wednesday, September 30, 2015 from 8:45 – 9:00 in room Weißer Saal 1.

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**431/ZOV**

**Hypochlorite-induced oxidative stress activates c-di-GMP synthesis and biofilm formation in *Pseudomonas aeruginosa***

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**Introduction**: The opportunistic human pathogen *Pseudomonas aeruginosa* is able to survive under a variety of often harmful environmental conditions due to a multitude of intrinsic and adaptive resistance mechanisms, including biofilm formation as one important survival strategy.

**Objectives**: In this study, we investigated the adaptation of *P. aeruginosa* towards hypochlorite (HOCl), a phagocyte-derived host defense compound which is used to kill invading bacteria and which can be found in chronically inflamed host tissue, e.g. in the lungs of CF patients.

**Methods & results**: In static biofilm assays, we observed a significant enhancement in initial cell attachment in the presence of sublethal HClO concentrations. Subsequent LC-MS analyses revealed a strong increase in cyclic-di-GMP (c-di-GMP) levels suggesting a key role of this second messenger in HClO induced biofilm development. Using DNA microarrays, we identified a 26-fold upregulation of ORF PA3177 coding for a putative diguanylate cyclase (DGC), which catalyzes the synthesis of the second messenger c-di-GMP - an important regulator of bacterial motility, sessility and persistence. This DGC PA3177 was further characterized in more detail demonstrating its impact on *P. aeruginosa* motility, adherence to biotic and abiotic surfaces, biofilm formation, antibiotic resistance and persistence using the respective *in vitro* and *in vivo* model systems. In addition, cell culture assays attested a role for PA3177 in response of *P. aeruginosa* to human phagocytes. Using a subset of different mutants, we were able to show that both Pel and Psl exopolysaccharides are effectors in the PA3177-dependent c-di-GMP network and are involved in the observed phenotypes.

**Conclusions**: Our results demonstrate that host-derived antimicrobials are sensed by invading bacteria and exert a huge impact on bacterial pathogenesis and adaptive antimicrobial defense mechansim including biofilm formation and antibiotic resistance.

**Presentation:** Wednesday, September 30, 2015 from 9:00 – 9:15 in room Weißer Saal 1.

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**432/ZOV**

**Outer membrane protein P1 is the CEACAM-binding adhesin of *Haemophilus influenzae***

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**Background:** *Haemophilus influenzae* (*Hinf*) is a Gram-negative pathogen colonizing the upper respiratory tract mucosa. *Hinf* belongs to a group of human-restricted bacteria, which bind to carcinoembryonic antigen related cell adhesion molecules (CEACAMs) on epithelial cells. Adhesion to CEACAMs is thought to be mediated by the *Hinf* outer membrane protein (OMP) P5 promoting establishment of the pathogens in the human nasopharynx.
**Question:** Aiming at preventing *Hinf* colonization, we sought to identify the molecular requirements for *Hinf* binding to CEACAMs.
**Methods:** Binding assays with soluble receptor ectodomains, followed by flow cytometric analysis or Western Blotting, was used to characterize CEACAM-binding profiles by wildtype and mutant *Hinf*. These analyses identified OMP P1, and not OMP P5, as the *bona fide* CEACAM-binding adhesin of a panel of typeable and non-typable Hinf strains. Multiple amino acid sequence alignment of OMP P1 as well as heterologous expression of wild-type, chimeric, or mutated OMP P1 in *E. coli* identified the molecular details of the OMP P1-CEACAM interaction.
**Results:** To our surprise, P5 mutants of *Hinf* still avidly bind to CEACAMs and *Hinf* P5 expressed in *E.coli* fails to mediate CEACAM binding. Instead, a genetic screen identifies *Hinf* OMP P1, a homologue of *E. coli* FadL, as the CEACAM-binding adhesin of Hinf. Deletion of P1 in *Hinf* as well as heterologous expression in *E. coli* demonstrates that P1 is necessary and sufficient to bind several human CEACAMs. Concordantly, when expressed on the surface of *E. coli*, P1 mediates adhesion to and invasion into epithelial cells. Interestingly, OMP P1 selectively recognizes human CEACAMs, but not homologs from other mammals and this binding preference is preserved upon expression in *E. coli.* Besides, detailed structure-activity relationship investigations with OMP P1 mutants demonstrate that several flexible extracellular loops allow OMP P1 to engage human CEACAMs.
**Conclusion:** Together, our data identify OMP P1 as the bona-fide CEACAM-binding invasin of *Hinf.* This is the first report providing evidence for an involvement of the major outer membrane protein P1 of *Hinf* in pathogenesis.

**Presentation:** Wednesday, September 30, 2015 from 9:15 – 9:30 in room Weißer Saal 1.

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**433/ZOV**

**The membrane-associated role of LIPP during the *Chlamydia pneumoniae* infection**

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**Introduction:** *Chlamydia pneumoniae* (*C. pn.*) is a Gram-negative human pathogen which causes infections of the respiratory tract. All *Chlamydiae* share a dimorphic life cycle with an infectious form, the elementary body (EB), and a metabolic active, non-infectious form, the reticulate body (RB). As an obligate intracellular pathogen the adhesion and internalization are crucial steps in the chlamydial life cycle. The first interaction with the host cell is thereby accomplished by the Outer membrane complex protein B (OmcB) binding to heparan sulfate moieties on the host cell surface, followed by the interaction of the chlamydial polymorphic membrane proteins (Pmps) with host cell receptors. Specifically, the interaction of the Pmp21 adhesin and invasin with its human interaction partner, the EGF receptor (EGFR), results in receptor activation, down-stream signaling and finally internalization of the bacteria. Blocking both, the OmcB and Pmp21 adhesion pathways, did not completely abolish infection, suggesting the presence of additional factors relevant for host cell invasion.
**Objectives:** Characterization of the novel lipid-binding adhesin LIPP from *C. pneumoniae.*
**Methods:** Adhesion of soluble recombinant Protein to human cells and artificial membranes/Infection of eukaryotic cells with *C. pn.*/ PS Staining by Annexin and LactC2/
**Results:** In a search for additional adhesins and invasins, the novel chlamydial protein LIPP was identified. LIPP is surface localized on infectious EBs, and pre-incubation of EBs with specific anti-LIPP antibodies reduced subsequent infection. Adhesion assays using soluble recombinant LIPP (rLIPP) and rLIPP-coupled fluorescent latex beads revealed that the protein is able to adhere to human cells. Interestingly, soluble rLIPP is able to bind to the negatively charged phospholipid phosphatidylserine (PS) and to a lesser extent to phosphatidic acid (PA) in artificial membranes. PS, usually restricted to the inner leaflet of human cells, becomes externalized upon *Chlamydia pneumoniae* adhesion to the host cell. This process is accomplished by the activity of LIPP, based on the findings that binding of rLIPP to human cells externalizes PS. Furthermore, infectious EBs pre-coated with rLIPP induced an increased PS externalization compared to untreated EBs; correspondingly, EBs pre-treated with anti-LIPP antibodies showed a reduction in PS externalization. Interestingly pretreatment of epithelial HEp-2 cells with rLIPP did not reduce subsequent infection, but instead strongly promoted EB internalization, confirming the relevance of LIPP for chlamydial infection.
**Conclusions:** Overall, the results show that LIPP is a novel *C. pn.* adhesin involved in membrane modulation and is thereby the first cell surface protein found in Gram-negative bacteria, directly interacting with PS in the host cell plasma membrane to promote the uptake process.

**Presentation:** Wednesday, September 30, 2015 from 9:30 – 9:45 in room Weißer Saal 1.

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**434/ZOV**

**Wall teichoic acid glycosylation governs *Staphylococcus aureus* nasal colonization**

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Nasal colonization by the human pathogen *Staphylococcus aureus* is a major risk factor for hospital- and community-acquired infections. A key factor required for nasal colonization is a cell surface-exposed zwitterionic glycopolymer, termed wall teichoic acid (WTA). However, the precise mechanisms that govern WTA-mediated nasal colonization have remained elusive. Here, we report that WTA GlcNAcylation is a pivotal requirement for WTA-dependent attachment of community-acquired methicillin-resistant *S. aureus* (MRSA) and emerging livestock-associated MRSA to human nasal epithelial cells, even under conditions simulating the nutrient composition and dynamic flow of nasal secretions. Depending on the *S. aureus* strain, WTA O-GlcNAcylation occurs in either α- or β-configuration, which have similar capacities to mediate attachment to human nasal epithelial cells suggesting that many *S. aureus* strains maintain redundant pathways to ensure appropriate WTA glycosylation. Strikingly, lack of WTA glycosylation significantly abrogated MRSA to colonize cotton rat nares *in vivo*. These results indicate that WTA glycosylation modulates *S. aureus* nasal colonization and may help to develop new strategies for eradicating *S. aureus* nasal colonization in the future.

Winstel V, Kuehner P, Salomon F, Larsen J, Skov R, Hoffmann W, Peschel A, Weidenmaier C. 2015. Wall teichoic acid glycosylation governs Staphylococcus aureus nasal colonization. mBio 6(4):e00632-15. doi:10.1128/mBio.00632-15.

**Presentation:** Wednesday, September 30, 2015 from 9:45 – 10:00 in room Weißer Saal 1.

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**435/ZOV**

**Low oxygen tensions found in *Salmonella*-infected gut tissue boost *Salmonella* replication in macrophages by impairing antimicrobial activity and augmenting *Salmonella* virulence**

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**Introduction**: In *Salmonella* infection, the *Salmonella* Pathogenicity Island-2 (SPI-2)-encoded type three secretion system (T3SS2) is of key importance for systemic disease and survival in host cells. In the streptomycin-pretreated mouse model SPI-2-dependent *Salmonella* replication in lamina propria macrophages (MΦ) is required for the development of colitis. In addition, containment of intracellular *Salmonella* in the gut depends on the antimicrobial effects of the phagocyte NADPH oxidase (PHOX), and possibly type 2 NO synthase (NOS2). For both antimicrobial enzyme complexes oxygen ($O_2$) is an essential substrate. However, the amount of available $O_2$ upon enteroinvasive *Salmonella* infection in the gut tissue and its impact on *Salmonella*-MΦ interactions was unknown.
**Methods**: We measured the gut tissue $O_2$ levels in the streptomycin-pretreated mouse model of *Salmonella* enterocolitis using luminescence-2D-*in vivo* $O_2$ imaging. Intracellular *Salmonella* replication and survival was monitored with Gentamicin protection assays and bacterial fluorescent reporter assays in peritoneal and bone marrow-derived MΦ. NO and cellular ROS production was detected by the Griess reaction and a ROS-sensitive dye. *Salmonella*-perceived ROS was detected by an OxyR-dependent reporter system. SPI-2 activity and translocation of SPI-2 virulence proteins were monitored with reporter plasmids.
**Results**: We found that gut tissue $O_2$ levels dropped from ~78 Torr (~11% $O_2$) to values of ~16 Torr (~2% $O_2$) during infection. Since *in vivo* virulence of *Salmonella* depends on the *Salmonella* survival in MΦ, *Salmonella*-MΦ interaction was analysed under such low $O_2$ values. These experiments revealed an increased intracellular survival and replication of wild type and *t3ss2* non-expressing *Salmonella*. These findings were paralleled by blunted NO and ROS production and reduced *Salmonella* ROS perception. In addition, hypoxia enhanced SPI-2 transcription and translocation of a SPI-2-encoded virulence protein. Only if *t3ss2* non-expressing *Salmonella* were used in a PHOX and NOS2-deficient situation, hypoxia did not further enhance *Salmonella* recovery.
**Discussion**: These data suggest that hypoxia-induced impairment of antimicrobial activity and *Salmonella* virulence cooperate to allow for enhanced *Salmonella* replication in MΦ. Analysis of *Salmonella*-host interaction under low $O_2$ conditions might unravel important features of *Salmonella* pathogenesis and might lead to refined treatment strategies.

**Presentation:** Wednesday, September 30, 2015 from 13:00 – 13:15 in room Roter Saal 1.

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**436/ZOV**

***Neisseria meningitidis* causes a G1 arrest in the human epithelial cell line Detroit 562**

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Microbial pathogens have developed several mechanisms to modulate and interfere with host cell cycle progression. Recent work from our group has shown that infection of brain endothelial cells with *Neisseria meningitidis* results in an accumulation of cells in the S phase and involves p21 and cyclin G2. In this study, we now analysed the effect of the human pathogen *N. meningitidis* on the cell cycle of two epithelial cell lines, NP69 and Detroit 562. Two pathogenic serogroup B isolates as well as a set of carrier isolates were employed and tested for their ability to adhere to and invade into both cell lines and to modulate the cell cycle.
We found that bacteria adhered equally well to both NP69 and Detroit 562 cells, whereas the commensal strains were significantly less invasive. Using propidium iodide staining and 5-ethynyl-2'-deoxyuridine (EdU) pulse-labeling, which precisely establishes the cell cycle pattern based on both cellular DNA replication and nuclear DNA content, we provide evidence that meningococcal infection arrested cells in the G1 phase of the cell cycle at 24 hrs post-infection. In parallel a significant decrease of cells in the S-phase was observed. Interestingly, G1 phase arrest was only induced after infection with live bacteria but not with heat-killed bacteria. In addition, using Western blot analysis we demonstrate that bacterial infection resulted in a decreased level of the cell cycle regulatory gene cyclin D1. Furthermore, *N. meningitidis* infection induced an increase in the expression of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ that was accompanied by a re-distribution to the cell nucleus as shown by immunofluorescence analysis.
In summary, we present data that *N. meningitidis* can interfere with the processes of host-cell cycle regulation that might favor effective bacterial colonization and invasion of epithelial cells.

**Presentation:** Wednesday, September 30, 2015 from 13:15 – 13:30 in room Roter Saal 1.

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**437/ZOV**

**Pneumococcal adherence and virulence factor B (PavB) interacts with human glycoprotein vitronectin**

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**Introduction:** *Streptococcus pneumonia*e is endowed with a diverse repertoire of proteinaceous colonizing and virulence factors. Pneumococcal adherence and virulence factor B (PavB) is a typical sortase-anchored surface protein and characterized by repetitive sequences referred to as Streptococcal Surface Repeats (SSURE). PavB has the ability to interact with human fibronectin, plasminogen and thrombospondin, respectively. In this study we dissected the interaction of PavB and its SURRE domains with human glycoprotein and complement inhibitor vitronectin.
**Methods:** Recruitment of vitronectin by *S. pneumoniae* and its isogenic mutants devoid of *pavB* was assessed by flow cytometry. To study the interaction of PavB/SSURE fragments with human vitronectin, far western blots and ELISA were performed. To narrow down the specific binding domain of PavB in vitronectin, a series of C-terminally truncated vitronectin fragments were employed in binding assays.
**Results:** Flow cytometric analysis shows that *S. pneumoniae* recruits human multimeric vitronectin in a dose-dependent manner while isogenic mutants devoid of PavB showed a marked decrease in binding to vitronectin. Far western blots further prove the ability of PavB to interact with both soluble and immobilized forms of vitronectin. ELISA assays confirmed the ability of PavB to interact with vitronectin but also revealed that this interaction is a charge

and heparin dependent interaction. Far western blots, ELISA and surface plasmon resonance indicated the critical role of the heparin binding domain three (HBD3) of vitronectin for the interaction with PavB.
**Conclusions:** *S. pneumoniae* interacts specifically with human glycoprotein and complement inhibitor vitronectin via the SSURE domains of PavB. The SSSURE domains are critical for this interaction and similar to other pneumococcal adhesins like PspC and Hic the SURRE domains bind to the C-terminal part of vitronectin with a pivotal role of the HBD3.

**Presentation:** Wednesday, September 30, 2015 from 13:30 – 13:45 in room Roter Saal 1.

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**438/ZOV**
**Surface exposed glycolytic proteins of *Mycoplasma pneumoniae* are able to bind human vitronectin**
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**Introduction:** *Mycoplasma pneumoniae* (M.p.) is a common agent of atypical pneumonia in humans. Because of the strongly reduced genome this species exhibits a minimal metabolism and expresses a limited number of virulence factors. Glycolysis is the major pathway to produce ATP taking place in the cytosol of the cells. However, many studies reported for various microorganisms that different glycolytic enzymes can occur as well at the cell surface and are possible binding partners of human extracellular matrix (ECM) proteins, like plasminogen. In previous reports, we have this confirmed for GAPDH, enolase and the PDH cluster of M.p..
**Materials and Methods:** In this study, we produced the glycolytic enzymes of M.p.(n=19) as full-length recombinant proteins in *E.coli*, further these proteins were used to obtain polyclonal monospecific antisera. Analysis of the localization was carried out by different immunological approaches like immunofluorescence and colony blot. Furthermore different methods to separate total M.p. proteins into membrane and cytosolic proteins were used. In particular, the interactions between surface-localized recombinant proteins and the human ECM protein vitronectin were investigated.
**Results:** A surface-association could be confirmed for eight of the nineteen glycolytic enzymes of M.p.. Additionally, an interaction between M.p. cells and human vitronectin was observed. Further, analysis of concentration-dependent binding to human vitronectin revealed that a high proportion of surface-associated proteins like lactate dehydrogenase, transketolase and PDH subunit A are able to interact with this ECM protein; but there are differences in the strength of the interaction. All interactions can be inhibited by addition of specific sera against the recombinant proteins.
**Conclusions**: In conclusion, this is the first investigation of the interaction of surface-displaced proteins of a mycoplasma species with human vitronectin. The results of the study show the binding of human vitronectin to many glycolytic enzymes of M.p.. These are the preconditions, that the interactions are possibly important in the host-microbe interaction. In future experiments, the degradation of vitronectin in the presence of glycolytic enzymes will be investigated to confirm a direct influence on this important host factor.

**Presentation:** Wednesday, September 30, 2015 from 13:45 – 14:00 in room Roter Saal 1.

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**439/ZOV**
**The interplay of HIF-1α induced host metabolic changes and mitochondrial dysfunction promotes *Chlamydia pneumoniae* growth under hypoxia**
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The obligate intracellular bacterium *Chlamydia pneumoniae* depends on host cell metabolism and energy supply. Cellular oxygen availability is a key regulator of host metabolism affecting pathogen growth. Enhanced growth and infection rate of *C. pneumoniae* have been observed under hypoxia (2% $O_2$). Under low oxygen conditions, hypoxia-inducible factor-1α (HIF-1α) regulates the host cell metabolism causing a switch from oxidative phosphorylation to anaerobic glycolysis. Metabolic interaction of *C. pneumoniae* and its host cell under hypoxia were analyzed to clarify the role of HIF-1α and other hypoxia-mediated factors in promoting *C. pneumoniae* infection.
Two-photon microscopy was used to analyze the metabolic activity of *C. pneumoniae*-infected HEp-2 cells under hypoxia. By using dimethyloxallyl glycine (DMOG), the impact of HIF-1α stabilization under normoxia was analyzed. Metabolic changes were further elucidated by the Seahorse XF analyzer, calculating mitochondrial respiration and glycolytic function. As model for hypoxia, mitochondrial dysfunction under normoxia was mimicked by targeting the ATP synthase using oligomycin and siRNA.
Enhanced host cell metabolic activity under hypoxia was indicated by an increased NAD(P)H intensity. To analyze if the enhanced metabolic activity is responsible for the beneficial growth of *C. pneumoniae* under hypoxia, HIF-1α was stabilized by DMOG. An increased glycolytic and reduced mitochondrial activity was induced by HIF-1α stabilization. This metabolic switch promoted the growth and progeny of *C. pneumoniae*. But hypoxia has apparently an additional effect. By analyzing the mitochondrial function, it was shown that hypoxia caused a mitochondrial hyperpolarization and an increase of reactive oxygen species (ROS). The cellular redox-system was enhanced in *C. pneumoniae*-infected cells under hypoxia and might thereby lead to the observed ROS reduction compared to hypoxic control cells. Dysfunctional mitochondria, induced by hypoxia or inhibition of the ATP synthase, promoted the infection.
We could show that *C. pneumoniae* growth is highly influenced by host metabolic changes. The interaction of mitochondrial dysfunction with HIF-1α regulated genes promoting an anaerobic glycolysis are key elements in supporting chlamydial growth under hypoxia.

**Presentation:** Wednesday, September 30, 2015 from 14:00 – 14:15 in room Roter Saal 1.

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**440/ZOV**
**Type I interferon-mediated signaling is inhibited upon influenza A virus and *Staphylococcus aureus* coinfection**
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Influenza A viruses (IAV) are the causative agents of severe respiratory diseases. The majority of disease fatalities are linked to secondary bacterial pneumonia, caused by pathogens such as *Staphylococcus aureus* (*S. aureus*). One major problem of the increased pathogenicity is the dysregulation of the cellular immune response. While this problem is known for a long time, there is only scarce knowledge about the interplay of IAV with *S. aureus* during infection on a molecular level.
Thus we assessed the regulation of type I interferon (IFN) response in an IAV/*S. aureus* co-infection model *in vitro*.
To investigate cellular signal transduction processes in the human lung epithelial cell-line (A549) upon IAV/*S. aureus* co-infection we established a complex infection procedure. For co-infection various IAV subtypes, the *S. aureus* strain 6850 and heat killed *S. aureus* (HKSA) were employed. Viral titers were analysed by standard plaque assays. Regulation of pathogen-induced type I IFN-mediated signaling was investigated on mRNA and protein levels via qRT-PCR, Western Blot analysis and co-immunoprecipitation assays.
Coinfection of IAV/*S. aureus* resulted in enhanced type I interferon (IFN) expression in comparison to singular infection. Although the innate immune response was increased, we observed higher viral titers in presence of *S. aureus*. Interestingly, mRNA levels of strictly IFN-stimulated genes, such as *MxA* or *OAS*, were rather

decreased, correlating with reduced IFN-induced protein expression. Based on these results, we hypothesized a block of type I IFN signaling provoked by the bacteria.
In fact, we were able to show that independent of the initial stimulus that drives IFN beta up-regulation, metabolically active intracellular *S. aureus* inhibits type I IFN-mediated STAT1 phosphorylation and subsequently STAT1-STAT2 dimerization.
In the presence of *S. aureus* the first line of defence against IAV is interrupted, resulting in a boost of viral replication, which may lead to enhanced pathogenicity.
Note: These data were in part presented at previous meetings and partly published in Warnking *et al.* (Cell Microbiol.; 2014, 17(3):303-317)

**Presentation:** Wednesday, September 30, 2015 from 14:15 – 14:30 in room Roter Saal 1.

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**441/ZOV**

**Cytotoxic effects of the A-subunit of subtilase cytotoxin of Shiga toxin-producing *Escherichia coli***

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The SubAB subtilase is an AB-type protein toxin from Shiga toxin-producing *Escherichia coli* (STEC), zoonotic pathogens, which cause a variety of symptoms in humans ranging from mild diarrhea to hemorrhagic colitis and the life threatening hemolytic uremic syndrome. The SubAB cytotoxin is found in STEC isolates from domestic ruminants and from food and acts as an enzyme inside mammalian cells. To this end, the pentameric $SubB_5$-subunit binds to a sialic acid receptor (Neu5Gc) on the cell surface, triggers endocytotic uptake of SubAB and mediates the retrograde transport of the enzymatically active SubA-subunit into the endoplasmic reticulum (ER). SubA acts as a subtilisin-like serin protease in the ER and cleaves the ER-chaperone GRP78/BiP. This results in an accumulation of misfolded proteins, ER stress and finally cell death. When we characterized the formation of biologically active SubAB complexes from recombinant SubA and SubB subunits on cultured cells, we made the unexpected observation that SubA alone caused morphological changes, caspase activation and cell death in human epithelial cells (HeLa) in the absence of SubB, when applied in higher concentrations to the cells. Moreover, SubA alone was able to bind to the surface of HeLa cells in a concentration-dependent manner (1). Although the molecular mechanisms underlying the observed SubA-effects are still under investigation, the new findings essentially contribute to a better understanding of the mode of action of these clinically relevant toxins from foodborne pathogens.

**Reference**

(1) Funk, J.[§], Biber, N.[§], Schneider, M., Hauser, E., Enzenmueller, S., Foertsch, C., Barth, H.*, Schmidt, H.* (2015). Cytotoxicity of the recombinant subtilase variant SubAB2-2 of shiga toxin-producing *Escherichia coli* strain LM14603/08 and comparison with other SubAB variants. *Infect. Immun.* 83: 2338-2349.
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**442/ZOV**

**Characterization of Prophage-Encoded Esterases of *Escherichia coli* O157:H7 and their Role in Substrate Utilization**

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Enterohemorrhagic *Escherichia coli* (EHEC) are food-borne pathogens that can cause hemorrhagic colitis and the life-threatening hemolytic-uremic syndrome. EHEC strains can use mucus-associated carbohydrates as energy source for growth. One of them is the 5-*N*-acetyl-9-*O*-acetyl neuraminic acid ($Neu5,9Ac_2$) which is a component of the mucus glycoprotein mucin. This carbohydrate can also be catabolized by other *E. coli* strains. Responsible for this process is the $Neu5,9Ac_2$ esterase NanS, which cleaves the acetate residue from C9 of $Neu5,9Ac_2$.
In non-pathogenic *E. coli*, deletion of the corresponding *nanS* gene resulted in the loss of growth capability on $Neu5,9Ac_2$. However the deletion of *nanS* in EHEC O157:H7 did not influence growth.
In addition to *nanS*, seven *nanS*-homologues open reading frames (ORFs) are present in EHEC O157:H7 strains EDL933. These ORFs are located on different Shiga toxin (Stx)-encoding - and non-Stx encoding prophages. ORF Z1466 was characterized recently by our group and encodes an esterase with similar function as NanS [Nuebling et al., 2014].
The presence of multiple *nanS*-homologues prophage-encoded genes raises the question on their functionality and their impact for the growth of EHEC strains.
In this study, we want to clarify the question whether the other ORFs are biologically active and encode functional $Neu5,9Ac_2$ esterases, and whether these esterases work under different conditions.
In addition to Z1466, three further ORFs with homologies to *nanS* of *E. coli* O157:H7 strain EDL933 were cloned, expressed, and purified as His-tagged proteins. The temperature and pH optimum as well as the substrate specificity were investigated. The recombinant esterases were added to cultures of an *E. coli* C600Δ*nanS* deletion mutant in M9 minimal medium with $Neu5,9Ac_2$ as sole carbon source.
It could be shown that the three expressed esterases are enzymatically active and can also cleave $Neu5,9Ac_2$. Moreover an acetic acid release from bovine submaxillary gland mucin could be measured. The temperature optima were 30 and 50 °C and the pH optima 9 and 8, respectively. *E. coli* C600Δ*nanS* in contrast to C600 could not grow in M9 minimal medium with $Neu5,9Ac_2$. However if one of the $Neu5,9Ac_2$ esterases was added to the medium the mutant could grow, indicating that the NanS function could be complemented by externally added esterases.
The results show that EHEC bacteria encode several $Neu5,9Ac_2$ esterases with different enzymatic characteristics. That lead to the hypothesis that these enzymes can operate in different environmental milieus. We hypothesize that $Neu5,9Ac_2$ plays a role as a carbon source for EHEC in the gut. Future work is necessary to clarify the role of these multiple enzymes in the metabolism of EHEC strains.

**Presentation:** Monday, September 28, 2015 from 9:45 – 10:00 in room Weißer Saal 1.

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**443/ZOV**

**Diversity of *Coxiella burnetii* Genotypes in the context of an outbreak amongst goats and sheep**

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**Introduction**: In 2009 a goat and sheep flock on an experimental station on the Swabian Alb, Germany was affected by a Q fever outbreak, resulting in animal losses due to abortion and weak offspring (goats: 25%, sheep: 18%). *Coxiella burnetii* was identified in numerous materials, e.g. birth products, abortion material. A screening survey for the next two years was initiated to study excretion and serology of *Coxiella burnetii*. Further molecular typing of positive samples was initiated to identify involved genotypes and their dynamics over time.

**Material and Methods**: Swabs, blood-, milk and fecal samples were taken regularly and examinded by a *C. burnetii* specific real-time PCR assay using the IS1111-element as a target. Positive materials were genotyped with two different methods: determination of IS1111-elements and a 14 marker Multi-Locus-VNTR-analysis (MLVA).

**Results**: 8% resp. 3 % of the blood samples form goats and sheeps were C. burnetii-DNA positive. Nearly all investigated animals shed DNA through vaginal mucus (100%), milk (goats 97%, sheep: 78%) and faeces (100%). We could identify two different MLVA-genotypes and seven IS1111-genomic variations. Combining both methods eight different genotypes were identified. One MLVA-genotype was predominant covering all studied materials except one. Also a predominant IS1111-pattern was seen in 83% of the samples.

**Conclusions**: The outbreak was caused likely by one prevalent genotype. Whereas the MLVA method identified only one genotype variant in one material, the IS1111 assay was able to show 6 additional types, showing a very close relationship to the main genotype. The number of these microvariants are increasing over time, but some are existing at the beginning of the outbreak too. Maybe differences in virulence could be an explanation for this observations, but variations in the host immunity are also possible. Identical genotypes in goats and sheep support the hypothesis of transferring infection between the flocks. IS1111 and MLVA are suitable methods for monitoring the intraherd dynamics of Q fever infected herds, with IS1111 showing a better discriminatory power. The observed genotypes match to known variants seen in southern Germany in the recent years. This study reveals a genotype diversity in an outbreak situation which is similar to observations made in the Dutch Q fever epidemic from 2007 to 2010. A whole genome sequencing approach will further elucidate the genomic dynamic of *C. burnetii* in the future.

**Presentation:** Monday, September 28, 2015 from 17:15 – 17:30 in room Roter Saal 1.

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**444/ZOV**

**Methicillin resistant *Staphylococcus aureus* from infections in horses in Germany : significance for infections in humans**

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**Objective:** MRSA infections in equine clinics are obviously common, also nasal colonization of veterinary staff was reported by several studies. Previous studies in Europe and in Northern America revealed a dynamics of clonal complexes and lineages associated with these infections. The isolates attributed to CC8 (*spa*-type t008 and t036) declined and were replaced by isolates which represent an equine clinic specific clade of CC398. In this context the question of significance for infections in humans is of particular interest.

**Materials and methods**: In order to get an actual overview MRSA from infections in horses (n=272, from 17 equine hospitals and 39 veterinary practices all over Germany) and from veterinary staff (n= 349, working at 5 equine clinics and 3 large animal practices) were subjected to *spa*-typing, SCC*mec* grouping, to PCR for canonical SNP- detection of the equine clinic specific subpopulation of CC398, and antibiotic resistance profiles (MIC, PCR for selected resistance genes). The results obtained were compared to typing characteristics of MRSA-isolates from infections in humans at the German National Reference Center for Staphylococci and Enterococci from 2006 until 2014.

**Results**: A total of 272 methicillin resistant *Staphylococcus aureus* from equine infections as well as 67 isolates from veterinary staff were subjected to molecular typing. The majority of isolates from horses were attributed to clonal complex (CC) 398 (82,7%), and 66% to the subpopulation (clade) of CC398- which is associated with equine clinics. MRSA attributed to CC8 (ST254, t009, t036, SCC*mec*IV and ST8, t064, SCC*mec*IV) are less frequent (16,5%). Single isolates were attributed to ST1, CC22, ST130, and ST1660. The emergence of MRSA CC22, ST130 and ST1660 in horses was not reported so far worldwide.

Nasal MRSA colonization was found in 18,4% of veterinary personnel and veterinarians with professional exposure to horses. The typing characteristics of these isolates corresponded to those of isolates from infections in horses.

Based on the comparison to typing characteristics of a substantial number of isolates from infections in human the proportion of isolates -which exhibit characteristics of MRSA from equine medicine- is assessed to be very low (0,12%). For the equine clinic associated subpopulation (clade) of MRSA CC398, the presence of blood culture isolates in this sample suggests the zoonotic potential: equine clinic associated MRSA CC398 are able to cause invasive infections in humans!

**Conclusion**: As this low proportion was also found among MRSA originating from screening on nasal carriage at hospital admission (n= 6029) transmission of MRSA from equine clinics to the community seems to be rare so far. Especially the emergence of MRSA ST22, SCC*mec*IV in equine clinics needs attention in further surveillance.

**Presentation:** Monday, September 28, 2015 from 18:00 – 18:15 in room Roter Saal 1.

**445/ZOV**

**Occurrence and molecular characterization of extended-spectrum β-lactamases producing *E. coli* from migratory avian species in Pakistan**

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**Introduction:** Emergence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* in wildlife has been recently reported in several parts of the world. It has been hypothesis that wildlife might become infection sources or even reservoirs, contributing to the zoonotic spread of these bacteria.
**Objectives:** With focus on environmental dissemination of the antimicrobial resistance bacteria, we investigated the occurrence and molecular clonality of ESBL producing *E. coli* in wild migratory avian species in Pakistan.
**Methods:** A total of 100 migratory birds were screened for ESBL-producing *E. coli* during migratory season (Oct-Mar) 2013-2014. Initial screening was done on selective plates i.e. ChromAgar-ESBL followed by phenotypic detection of ESBL using double disk synergy tests. PCR confirmation for ESBL-resistance genes like blaCTX-M, blaTEM and blaSHV was done and in case of blaCTX-M, sequencing was performed to determine CTX-M types. Carbapenem resistance was screened with PCR for blaVIM, blaKPC, blaNDM-1 and blaOxa-48. Furthermore, to determine clonal lineages, multilocus sequence typing (MLST) was employed.
**Results:** Overall, 26 % (26/100) of birds carried ESBL producing *E. coli.* These positive birds include Eurasian coot followed by Mallard, common pochard, Eurasian wigeon, shovelers and starlings. PCR showed blaCTX-M as the most frequent ESBL found in all 26/26 (100) avian isolates. Combinations of blaCTX-M and blaTEM was found in 19/26 strains. Among CTX-M types, twenty-one (80%) harbored CTX-M-15 type whereas four carried CTX-M-1 and one CTX-M-3. None of the *E. coli* carried genes for carbapenemase. MLST led to detection of 16 STs including 6 new STs. Among these, we also observed clinical relevant sequence types like ST224, ST617 and ST354.
**Conclusion:** Migratory birds in Pakistan carried high numbers of ESBL producing *E. coli.* CTX-M-15 is the most common CTX-M genotype found in this study. MLST revealed that some of the STs from migratory birds also shared clinically important sequence types with human indicating environmental pollution and potential zoonoses risk. Due to migratory behavior, these birds could be involved in transboundary spread of antibiotic resistant bacteria.

**Presentation:** Monday, September 28, 2015 from 18:15 – 18:30 in room Roter Saal 1.

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**446/ZOV**

**Surveillance of decontamination measures directed against ESBL-E and MRSA on a pig farm**

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**Introduction and Objective**: A potential risk for entry of multidrug-resistant pathogens into the food chain is the colonization of livestock. Therefore novel concepts for the eradication of drug resistant commensals on farms are necessary.
**Material and Methods**: In this report we evaluated the decontamination measures taken on an MRSA and ESBL-E contaminated farm, which preceded the conversion from piglet breeding to gilt production. Sampling of pigs, the environment (air, dust, water) and humans was performed before and after decontamination of the old and in a newly constructed stable.
**Results**: Microbiological surveillance demonstrated that the hygiene and management measures (complete removal of pigs, cleaning and disinfection of the stable and construction of an additional stable meeting high quality standards) eliminated MRSA and ESBL-E strains detected on the farm. After restart of pig production, ESBL-E remained undetectable over twelve months, whereas MRSA was recovered from pigs and environment within the first two days after purchase of new pigs. However, *spa* typing uncovered acquisition of a MRSA strain (t034) not found before decontamination. Interestingly, we further observed a delayed shift in *spa* types of MRSA strains colonizing a farm worker (from t2011 to t034) two months after decontamination process.
**Conclusions**: Altogether, the report demonstrates that eradication and hygiene procedures similar to those used in this report can lead to successful elimination of contaminating MRSA and ESBL-E in pigs and stable environment. Nevertheless, they could not prevent acquisition of a new MRSA strain. In conclusion, eradication of resistant bacteria from a pig farm is costly, but possible and conveys benefits. However, re-introduction of colonized new animals must be avoided by very intensive screening shortly before purchase and transfer.

**Presentation:** Tuesday, September 29, 2015 from 9:00 – 0:00 in room Weißer Saal 1

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## ADDENDUM

**INV12**

**Interaction of Salmonella enterica with the intestinal mucosa: Role of the giant non-fimbrial adhesin SiiE**

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The intestinal pathogenesis of the food-borne pathogen Salmonella enterica requires the interaction with the apical membrane of enterocytes. We found that S. enterica requires the function of the giant non-fimbrial adhesin SiiE for the efficient adhesion to, and invasion of polarized epithelial cells. SiiE is substrate of the type I secretion system (T1SS) encoded by Salmonella Pathogenicity Island 4 (SPI4). SiiE has a remarkable domain structure of 53 repetitive bacterial immunoglobulin (BIg) domains. Structural and functional analyses revealed that Ca2+ binding sites formed within, as well as between, BIg-domains are required for the secretion process and the proper surface expression of SiiE. SiiE is retained on the bacterial surface in a growth phase of highest expression of invasion genes. Retention and later release from the surface is controlled by two non-canonical subunits of the T1SS. The characterization of binding properties of SiiE revealed interaction with host cell glycostructures and lectin blockade studies identified N-acetyl-glucosamine and 2,3-sialic acid as parts of the glycostructures required for binding of SiiE. We will present our recent findings on the structure-function relationship of SiiE and discus current models for the cooperation of a giant adhesin with invasion factors during interaction of Salmonella with polarized epithelia.

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