ABSTRACTBAND

70. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) e. V.

ISBN 978-3-9816508-6-0

Abstracts

ORAL PRESENTATIONS

Monday, 19 February 2018

Tuesday, 20 February 2018

Wednesday, 21 February 2018

POSTER PRESENTATIONS

Monday, 19 February 2018 • 19.00-21.00

Postersession 01

Tuesday, 20 February 2018 • 17.00-18.30

Postersession 02

AUTHOR INDEX

KEY

Fachgruppe (FG), Ständige Arbeitsgemeinschaft (StAG)

Sections **Presentation/Poster-ID**

HAUPTSYMPOSIUM 01
Health Care associated Infections (HAI)
19 Febr. 2018 • 11.30–13.00

001/INV
Infection Prevention and Control Past, Present, and Future: The US Perspective?
L. Herwaldt
University of Iowa Hospitals and Clinics, Internal Medicine, Iowa City, IA/US)

The Abstract has not been submitted.

Presentation: Monday, February 19, 2018 from 11:30 – 11:50 in room Audimax Saal.

002/INV
Hospital infection prevention in Germany: Current state and the most important challenges
P. Gastmeier
Charité – Universtätsmedizin Berlin, Institut für Hygiene und Umweltmedizin, Berlin, Germany

The prevalence of nosocomial infections (NI) has not changed during the last 20 years. However, the incidence of antimicrobial resistance (AMR) has increased substantially. This will be illustrated using the data of the national prevalence studies and the data of SARI (Surveillance system of antibiotic usage and resistance in intensive care units). In addition, the patients have a much higher awareness for HIP and AMR.
The most important challenges in the field of hospital infection prevention (HIP) can be distinguished in three groups: 1. Changes in the German health care system; 2. General problems in the field of HIP, microbiology and antibiotic stewardship (ABS); 3. Research problems in HIP.
1. The demographic development leads to an increase of older people and a shortage of the work force. Together with a less attractive occupational image, this leads to a shortage of nurses. Understaffing is a well-known risk factor for NI. In addition, economical incentives lead to an extension in volume of medical procedures. This means more patients as justified have a risk for NI. Furthermore, understaffing leads to less communication between physicians and nurses which is relevant for all quality management activities, but also in the field of HIP.
2. HIP and prevention of AMR requires a good interaction of HIP and ABS experts as well as microbiologists on the hospital level. Due to the 'Landeshygieneverordnungen' the number of physicians and nurses in the field of HIP has increased substantially during the last 8 years. The number of ABS experts is also increasing. The number of microbiologists available in the hospitals has been dramatically reduced during the last 20 years. Meanwhile only 25% of hospitals have a microbiologist available in the hospital. Because of the necessary interaction of all three disciplines, we need local experts with skills and experience in all three subjects or at least a very close cooperation, which is often not existing.
3. We still have a lot of research questions in the field of HIP. We need multicentre randomized trials. However, it is difficult to get funding for these studies. In addition, studies to investigate measures to influence resistance should have a cluster randomized design. But this often requires a waiver of informed consent which is very difficult to receive from the local institutional review boards. Furthermore we need more research in the field of implementation science.

Presentation: Monday, February 19, 2018 from 11:50 – 12:10 in room Audimax Saal.

003/INV
Infection Control to Prevention of health Care associated Infection: the World perspective
J. Storr
Infection Prevention & Control/Quality Systems & Resilience, Universal Health Coverage and Health Systems, World Health Organization, Genf/CH

The Abstract has not been submitted.

Presentation: Monday, February 19, 2018 from 12:10 – 12:30 in room Audimax Saal.

WORKSHOP 01
Microbial Pathogenesis (FG MP/FG EK)
19 Febr. 2018 • 11.30–13.00

004/MPV
Von Willebrand Factor Promotes Adhesion of *Streptococcus pneumoniae*
H. Jagau*[1], K. Lahme[1], V. Elspass[1], I. K. Behrens[1], M. Rohde[2], M. Fulde[3], M. A. Brehm[4], T. Obser[4], G. König[4], R. Schneppenheim[4], T. Kohler[5], S. Hammerschmidt[5], W. Tegge[6], R. Köster[7], M. Steinert[1], S. Bergmann[1]
[1]*TU Braunschweig, Institute for Microbiology, Braunschweig, Germany*
[2]*Helmholtz Center for Infection Research, Central Facility for Microscopy, Braunschweig, Germany*
[3]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[4]*University Medical Center Hamburg Eppendorf, Department of Pediatric Oncology and Haematology, Hamburg, Germany*
[5]*Ernst-Moritz-Arndt Universität Greifswald, Department Genetics of Microorganisms, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany*
[6]*Helmholtz Center for Infection Research, Department of Chemical Biology, Peptide Synthesis, Braunschweig, Germany*
[7]*Technische Universität Braunschweig, Zoological Institute, Department of Cellular and Molecular Neurobiology, Braunschweig, Germany*

Introduction: *Streptococcus pneumoniae* is a main causative agent for community acquired pneumonia, which is associated with a high amount of cardiovascular complications such as major adverse cardiac events[1]. These complications are associated with an imbalanced coagulation and characterized by a severe, life threatening disease progress. The detailed pathomechanism that induce the severe dysregulation of hemostasis in response to a pneumococcal lung infection remains unknown so far.
Objectives: We investigated the interaction of pneumococci with VWF to detail with the aim to elucidate the function of VWF in the infection process. As second major objective, we aimed to identify and to characterizes the VWF binding protein of *S. pneumoniae*.
Materials and Methods: Recruitment of VWF to the bacterial surface was determined by binding analyses with iodinated VWF and flow cytometry studies with different serotypes and capsule-deficient mutant strains. After identification of the bacterial enolase as VWF binding protein, kinetic parameters were determined by surface plasmon resonance and microscale thermophoresis. A peptide spot array of enolase enabled the detection of putative VWF binding sites. The impact of VWF recruitment on pneumococcus infection was further analysed by cell culture infection analyses with primary endothelial cells followed by differential immunofluorescence staining and confocal microscopy. A microfluidic system enabled the simulation of endothelial infection in circulating fluids mediating high shear stress. At high flow rates, the mechanosensitive VWF multimerizes to strings of high molecular weight (HMW). Recruitment of zebrafish VWF to pneumococci was further visualized by immunomicroscopy after *in vivo* infection of *Danio rerio* larvae (5 dpf).
Results: Globular VWF is recruited to the surface of capsulated and non-capsulated pneumococci. We identified the ubiquitously expressed, surface-displayed enolase of *S. pneumoniae* as high affinity VWF-binding protein, predominantly interacting with the VWF domain A1. Display of the enolase molecule structure revealed the formation of four VWF binding pockets by the peptide 195YGAEIFHALKKILKS210. Furthermore, in static cell culture infection, exogenously added VWF serves as bridging molecule

mediating pneumococcal adherence to primary endothelial cells in heparin-sensitive manner. Microfluidic studies further demonstrate that multimerized VWF-strings provides bacterial attachment sites at high flow rates. Likewise, we demonstrate a VWF-pneumococcus colocalization *in vivo* in 5-day old zebrafish larvae.
Conclusion: *S. pneumoniae* is recruiting VWF and subverts this interaction for adhesion to the vascular endothelium even at high flow rates. The use of VWF as adhesion cofactor might support vascular colonization by pneumococci thereby promoting the reported hemostatic imbalance leading to severe cardiovascular complications.

Reference
[1] Rae N. et al., (2016), PMID 26886878

Presentation: Monday, February 19, 2018 from 11:30 – 11:45 in room Hörsaal 1.

005/MPV
Escherichia coli Nissle 1917 is resistant to Shiga toxin-, lambda- and T4-phages, inhibits stx-phage production by EHEC, reduces phage titer and protects *E. coli* K-12 strains against phage infection.
S. Bury[1], M. Soundararajan[1], T. Oelschlaeger*[1]
[1]*University of Würzburg, Institut für Molekulare Infektionsbiologie, Würzburg, Germany*

E. coli strain Nissle 1917 (EcN) might be a suitable preventive / therapeutic medication for EHEC infections due to its property to inhibit Stx production by EHEC strains (Reissbrodt et al. 2009; Rund et al. 2013). However, since Shiga toxin (Stx) is encoded by prophage(s) in EHEC, EcN might be turned into a Stx-producer after application in EHEC patients. The aim of this study was to test EcN for stx-phage sensitivity. For that purpose, EcN and *E. coli* K-12 strains were incubated with stx-, lambda and T4-phages and subsequently phage titer (plaque assay) and lysogeny (PCR) was determined. Phage titer was also determined for EHEC alone and together with EcN. Furthermore, transcriptome analysis was employed for identification of EcN genes upregulated in the presence of phages. Highest upregulated genes were cloned into *E. coli* K-12 strains to demonstrate protectivity against phage infection. EcN surface structure mutants were employed to find those components involved in phage resistance and phage inactivation. Results obtained showed EcN to be completely resistant to all phages employed i.e. EcN was not lysed by phages nor became EcN lysogenic. We further noticed EcN to inihibit stx-phage production by EHEC and to inactivate the phages during coincubation. In addition, EcN protected K-12 strains against phage infection when both bacteria and phages were coincubated. Trancriptome analysis of EcN after coincubation with lambda phages revealed strong upregulation of genes encoding superinfection exclusion protein and a phage repressor encoded by a not inducible prophage in EcN. Expression of the phage repressor in K-12 strains resulted in partial protection against lambda and stx-phage infection. An EcN mutant lacking the K5 capsule was partially sensitive to lambda and stx-phages, could no longer protect K-12 strains and inactivate these phages showing an important role of the capsule in phage resistance and inactivation. In summary, we could not convert EcN into a Stx producer and found EcN to be completely resistant to stx-, lambda- and T4 phages. Furthermore, EcN is able to protect K-12 strains against phage infection. These properties are based on an EcN phage repressor and EcN's K5 capsule. Taking all this and its GRAS status into account we believe EcN to be a safe drug for the prevention and treatment of EHEC infections.

References
[1] Reissbrodt et al. 2009. Inhibition of growth of Stx-producing E. coli by nonpathogenic E. coli. FEMS Microbiol Lett 290: 62 – 69.
[2] Rund et al. 2013. Antagonistic effects of probiotic EcN on EHEC strains of serotype O104:H4 and O157:H7. IJMM 303: 1-8.

Presentation: Monday, February 19, 2018 from 11:45 – 12:00 in room Hörsaal 1.

006/MPV
A highly sensitive assay for high-throughput and real-time monitoring of the function of bacterial type III secretion systems
S. Westerhausen*[1], C. Torres-Vargas[1], I. Grin[1], S. Wagner[1]
[1]*Institut für Mkrobiologie und Hygiene, Zelluläre und molekulare Mikrobiologie, Tübingen, Germany*

Gastrointestinal infections occur upon ingestion of contaminated food and can be caused by a wide range of pathogens. Among these, *Salmonella* are the major causes for food-borne illness. *Salmonella* employ type III secretion systems (T3SS) as their major virulence determinants *[1,2]*. These nanomachines, that span the inner and outer bacterial membranes, deliver different types of effectors for manipulating the host cell.
Several assays have been developed to the activity of T3SS. However, these assays are often labor intense, low in throughput, time consuming and semi-quantitative. Our aim was to develop a quick and robust assay to characterize T3SS-dependent secretion and host cell injection of effector proteins by *Salmonella*.
To this end, we have tested several luciferases of different origins utilizing different types of substrates and coupled them to two well-characterized *Salmonella* T3SS effectors, SipA and SopE [3,4]. The luciferase Nanoluc coupled to effector SipA showed the best results. Further tests revealed the suitability of this assay for high-throughput screening, kinetic measurements of secretion and assessment of host cell injection of effector proteins by *Salmonella*.

References
[1] A. Haraga, M. B. Ohlson, and S. I. Miller, "*Salmonellae interplay with host cells*," Nature Reviews Microbiology, vol. 6, no. 1, 2008.
[2] A. Fabrega and J. Vila, "*Salmonella enterica serovar Typhimurium skills to succeed in the host: Virulence and regulation*," Clinical Microbiology Reviews, vol. 26, no. 2, 2013.
[3] D. Zhou, M. S. Mooseker, and J. E. Galan, "Role of the S. typhimurium Actin-Binding Protein SipA in Bacterial Internalization," Science, vol. 283, no.5410, 1999.
[4] K. Ehrbar, A. Friebel, S. I. Miller, and W. D. Hardt, "Role of the Salmonella Pathogenicity Island 1 (SPI-1) Protein InvB in Type III Secretion of SopE and SopE2, Two Salmonella Effector Proteins Encoded Outside of SPI-1," Journal of Bacteriology, vol. 185, no. 23, 2003.

Presentation: Monday, February 19, 2018 from 12:00 – 12:15 in room Hörsaal 1.

007/EKV
Cross-kingdom synergism between *Proteus mirabilis* and *Candida albicans* enhances enterocyte damage
M. J. Niemiec*[1,2], M. Kapitan[1,2], G. Samesch[1], I. Auge[1], T. Monsen[3], S. D. Himpsl[4], H. L. T. Mobley[4], I. D. Jacobsen[1,2,5]
[1]*Leibniz Institute for Natural Product Research and Infection - Hans Knöll Institute, Research Group Microbial Immunology, Jena, Germany*
[2]*Center for Sepsis Control and Care, Jena, Germany*
[3]*Norrlands Universitets Sjukhus (NUS), Clinical Microbiology, Umeå, Sweden*
[4]*University of Michigan Medical School, Ann Arbor, United States*
[5]*Friedrich Schiller University, Jena, Germany*

The human gut, as the organ harboring the highest density of microbes, is a relevant source of life-threatening infections. Numerous opportunistic bacteria and fungi colonize the gut and can disseminate into the bloodstream (BS) upon immunosuppression or impairment of mucosal barrier function. While the mechanisms promoting translocation of *Candida albicans* into the BS are not fully understood, recent studies estimate about one third of *Candida* BS infections to be in fact polymicrobial – implying a role for *Candida*-bacteria interactions. To understand the causality between co-colonization and coinfection, we investigate the interplay of *C. albicans* and the gram-negative opportunist *Proteus mirabilis*, an emerging cause of bacterial BS infections.
In the absence of host cells, fungal numbers were reduced during long-term coincubation with *P. mirabilis* while bacterial survival

was prolonged, suggesting antagonistic interactions favoring *P. mirabilis.* Similarly, *Candida* survival was reduced in mixed biofilms in human urine. In contrast, we observed significantly increased damage of enterocytes during *in vitro* coinfections with *C. albicans* and four different *P. mirabilis* laboratory strains compared to summed-up single-species damage. This synergistic effect was dependent on the presence of *P. mirabilis* hemolysin HpmA. In contrast, *C. albicans* filamentation and candidalysin-mediated cell damage were dispensable for synergistic interactions. Interestingly, less virulent yeasts, *e.g.*, *Saccharomyces cerevisiae* did also promote synergistic host cell damage with *P. mirabilis*. Furthermore, synergistic interactions were observed with several primary *Proteus* clinical isolates from urine and blood cultures, independent of the site of isolation or the ability to swarm on agar plates.
Experiments aimed to determine (i) whether *C. albicans* affects hemolysin production of *P. mirabilis*, (ii) which fungal factors are relevant for this, and (iii) if synergistic virulence occurs *in vivo*, namely in nematodes and mice, are currently ongoing.

Presentation: Monday, February 19, 2018 from 12:15 – 12:30 in room Hörsaal 1.

008/EKV

Aspf2 from *Aspergillus fumigatus* recruits human plasma regulators Factor H, FHL-1, FHR1, and plasminogen and mediates immune evasion and cell damage

P. Dasari*[1], I. Shopova[2], D. Wartenberg[2], H. M. Dahse[1], N. Beyersdorf[3], P. Hortschansky[2], S. Dietrich[4], Z. Cseresnyés[4], M. Thilo Figge[4], M. Westermann[5], C. Skerka[1], A. A. Brakhage[2,6], P. F. Zipfel[1,6]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Infection Biology, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Jena, Germany*
[3]*Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany*
[4]*Leibniz Institute for Natural Product Research and Infection Biology, Research Group Applied Systems Biology, Jena, Germany*
[5]*Electron Microscopy Center of the University Hospital, Jena, Germany*
[6]*Friedrich Schiller University, Institute for Microbiology, Jena, Germany*

The opportunistic fungal pathogen *Aspergillus fumigatus* can cause life-threatening infections, such as hypersensitivity pneumonitis, allergic asthma (AA), and allergic bronchopulmonary aspergillosis (ABPA) as well as invasive aspergillosis, particularly in immune-compromised patients. When *A. fumigatus* invades the human host, conidia are the first fungal cells that are immediately confronted by the human complement system. Conidia activate the complement system of all three pathways. To evade host-mediated complement attack, Aspergillus recruits host soluble plasma complement regulators, like Factor H, FHL-1, C4BP, and plasminogen. However, the fungal protein(s) that bind human complement regulators to the fungal surface has not yet been identified. Here, we identify Aspf2 as the first *A. fumigatus* Factor H binding protein. Aspf2 recruits several human plasma regulators, Factor H, FHL-1, FHR1, and plasminogen. Factor H binds to Aspf2 via two regions located in SCRs6–7 and 19–20. FHL-1 bound via SCRs6–7, and FHR1 via SCRs3–5. Factor H and FHL-1 bound to Aspf2 retained cofactor activity for C3b inactivation. A Δaspf2 knockout strain was generated which bound Factor H with 29% and FHL-1 with 45% lower intensity. Similarly, when challenged with complement active NHS, Δaspf2 conidia had substantially more C3b (>40%) deposited on their surface and were more efficiently phagocytized by human neutrophils (up by 16%) compared to wild-type conidia. Furthermore, Aspf2 recruited human plasminogen and, tPA activated plasmin cleaved the chromogenic substrate S2251 and degraded fibrinogen. Plasmin attached to conidia damaged human lung epithelial cells, induced cell retraction, and caused matrix exposure. Thus, Aspf2 is a central immune evasion protein and plasminogen ligand of *A. fumigatus*. By blocking host innate immune attack and by disrupting human epithelial cell layers, Aspf2 assists in early steps of fungal infection and likely allows tissue penetration.

Presentation: Monday, February 19, 2018 from 12:30 – 12:45 in room Hörsaal 1.

009/IIV

Conidial surface proteome analysis reveals the highly abundant CcpA protein essential for *Aspergillus fumigatus* virulence

O. Kniemeyer*[1], V. Voltersen[1], S. Hermann[2], M. Blango[1], T. Heinekamp[1], M. Strassburger[3], T. Krüger[1], P. Bacher[4], E. Weiss[5], K. Hünniger[6], H. Liu[7], P. Hortschansky[1], A. Scheffold[4], J. Löffler[5], S. Krappmann[8], O. Kurzai[6], H. Einsele[5], S. G. Filler[7], U. Reichard[2], A. A. Brakhage[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany*
[2]*University Medical Center, Institute for Medical Microbiology, Göttingen, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Transfer Group Anti-infectives, Jena, Germany*
[4]*Clinic for Rheumatology and Clinical Immunology, Charité - University Medicine, Department of Cellular Immunology, Berlin, Germany*
[5]*University Clinic Würzburg, Medical Clinic and Policlinic II, Würzburg, Germany*
[6]*University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*
[7]*Biomedical Research Institute at Harbor-UCLA Medical Center, Los Angeles, United States*
[8]*University Hospital Erlangen and Friedrich Alexander University of Erlangen-Nürnberg, Microbiology Institute - Clinical Microbiology, Immunology and Hygiene , Erlangen, Germany*

Questions: *Aspergillus fumigatus* is a common airborne fungal pathogen of humans and a significant source of mortality in immunocompromised individuals. Conidia (asexually produced spores) are the first form of *A. fumigatus* to come in contact with lung epithelial and immune cells. Thus, the composition of the conidial surface has the potential to directly influence the outcome of an *A. fumigatus* infection. The surface of *A. fumigatus* conidia is composed of polysaccharides, proteins, dihydroxynaphthalene (DHN)-melanin pigment, and other secondary metabolites. The polysaccharides that form the cell wall of *A. fumigatus* conidia have been extensively studied, but less is known about conidial surface proteins with immune-modulatory functions. The only well-studied protein is the RodA hydrophobin that forms a hydrophobic surface layer on dormant conidia. Here, we aimed to identify additional conidial surface proteins which play a role during host pathogen interaction.
Methods: By hydrogen flouride-pyridine extraction of condial proteins and subsequent LC-MS/MS analysis we characterised the *A. fumigatus* spore proteome.
Results: We identified 116 proteins in the conidial cell wall. One protein, designated conidial cell wall protein A (CcpA) was nearly as abundant as the hydrophobin layer-forming protein RodA. CcpA peaks in expression during sporulation on resting conidia. Despite a high surface abundance, the cell surface of Δ*ccpA* resting conidia appeared normal. However, trypsin shaving of Δ*ccpA* conidia revealed novel surface-exposed proteins normally masked in the wild-type strain. Interestingly, swollen Δ*ccpA* conidia led to higher activation of neutrophils and dendritic cells than wild-type conidia and caused significantly less cell damage to epithelial cells *in vitro*, suggesting a role for CcpA as a stealth protein in immune evasion. In addition, virulence was highly attenuated when cortisone-treated mice were infected with Δ*ccpA* conidia. Although CcpA played an important function in overcoming the innate immune response, recombinant CcpA protein was unremarkable when presented to the adaptive immune response, where it was neither immunosuppressive nor immunologically inert.
Conclusions: Together, these data suggest that CcpA is a major structural spore protein that may hide immunogenic structures of the conidial surface from the host innate immune system.

Presentation: Monday, February 19, 2018 from 12:45 – 13:00 in room Hörsaal 1.

WORKSHOP 02
Eukaryotic Pathogens incl. DMykG Lecture (FG EK)
19 Febr. 2018 • 13.30–15.00

010/EKV
Evolutionary adaptations of *Candida species* to a hostile host
S. Brunke[1]
[1]*Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*

Many niches of the human host are extremely hostile environments for microbes. Pathogens invading these niches have to face a strictly limited supply of important nutrients like iron or zinc, constant assault with antimicrobial effector molecules and adverse physical conditions like high body temperature. Still, a small range of microbes, including some fungi, have adapted to these conditions and developed often complex strategies to successfully deal with host-imposed stresses.
We use *Candida* species as models to investigate the evolutionary background of these strategies both retrospectively by interspecies comparisons and prospectively by experimental evolution. Especially *Candida albicans* and *C. glabrata*, both common commensals of humans, exhibit many species-specific adaptations to their host. We therefore compare these two (and more) *Candida* species using comprehensive transcriptional profiling and screening of mutants under infection-like *ex vivo* and *in vitro* conditions. We found surprising differences among the species in their responses to host-imposed stresses, and specific adaptations, for example in the regulation of their iron acquisition systems. This indicates that the evolution towards pathogenicity followed often independent trajectories in theses different species, with many species-specific solutions.
In a different approach we have shown that exposure to selected host stresses, like engulfment by immune cells, can lead to surprising evolutionary adaptations in the laboratory: For example, we observed the *de novo* appearance of filament-driven escape mechanisms in both *C. albicans* and *C. glabrata* in long-term experiments. Such adaptations seem to often require surprisingly few genetic changes. For example, a striking re-wiring of complex signalling networks was found to depend only on a single nucleotide mutation. With such approaches, we are able to use experimental evolution to better understand the genetic basis of pathogenicity without relying on prior hypotheses.
Overall, an analysis of the evolutionary adaptations to the host is central to our understanding of pathogenicity mechanisms, and for finding novel ways to combat pathogenic fungi in their host habitat.

Presentation: Monday, February 19, 2018 from 13:30 – 14:00 in room Audimax Saal.

011/EKV
Asymptomatic *Giardia* sp. infections of Caco-2 monolayers: Do we need a better *in vitro* model to study giardiasis?
M. Kraft*[1,2], C. Klotz[1], R. Bücker[2], J. Schulzke[2], T. Aebischer[1]
[1]*Robert Koch-Institut, Division 16 Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany*
[2]*Charité CBF, Institute of Clinical Physiology, Berlin, Germany*

Background: The protozoan parasite *Giardia duodenalis* is responsible for more than 280 million cases of gastrointestinal complaints ("giardiasis") every year, worldwide. One proposed pathomechanism is the induction of epithelial barrier dysfunction by apoptosis or alterations of the tight junction complex, which increase epithelial permeability and may impact nutrient uptake and normal gut function, and can lead to infiltration of luminal antigens or invasion of pathogens. However, enigmatic to giardiasis is the range of medical conditions from severe chronic enteritis to complete asymptomatic courses in most patients. We investigated whether *G. duodenalis* trophozoites can compromise epithelial barrier function by using an *in vitro* co-culture system with Caco-2 monolayer. Complementary experiments using human small intestinal cells derived from organoids are currently being carried out to compare both models.
Materials and methods: Measurements of trans-epithelial electric resistances (TEER) on *Giardia*-infected Caco-2 transwell-monolayers were used to indicate epithelial permeability and alterations of tight junctions were analyzed via immunofluorescence assays (IFAs). Additionally, the releases of several cytokines were investigated via Luminex® assay. Epithelial intestinal cell monolayers were derived from established human intestinal organoids, *Giardia* trophozoites were clinical isolates from symptomatic patients and grown axenically until infection.
Results: Testing 11 various clinical *G. duodenalis* isolates (4x A, 6x B, 1x E assemblage) revealed a reproducible dose-dependent and attachment-independent TEER-increase in parasite-Caco-2 cell co-cultures. This effect was uniform irrespective of the culture conditions tested (aerobic/anaerobic, various incubation time, virus-infected isolates) or the applied infection doses (up to MOI 100). Furthermore, we determined the absence of degradation or delocalization of tight junction proteins (ZO-1, occludin and claudin-1). Also, cytokine levels were unchanged upon *G. duodenalis* infection. Primary human intestinal epithelial cell culture monolayers derived from intestinal organoids have been set up to perform complementary ongoing experiments.
Conclusion: Our investigations suggest that the widely used *G. duodenalis*/Caco-2 co-culture model recapitulates the course of asymptomatic infections that may require additional, yet unknown factors to induce barrier dysfunction. We currently test the hypothesis that organoid-derived primary cell culture monolayers will represent a more reliable *in vitro* model to depict the mechanisms of *G. duodenalis* pathogenesis.

Presentation: Monday, February 19, 2018 from 14:00 – 14:15 in room Audimax Saal.

012/EKV
HIF-1α drives fungal immunity in human macrophages by abrogating autophagy
D. Friedrich*[1], D. Zapf[1], B. Lohse[1], G. S. Deepe[2], J. Rupp[1]
[1]*University of Lübeck, Department of Infectious Diseases and Microbiology, Lübeck, Germany*
[2]*University of Cincinnati College of Medicine, Division of Infectious Diseases , Cincinnati, United States*

Histoplasma capsulatum is the causative agent of endemic mycoses in immunocompromised patients. The pathogen is able to switch from environmental mold form into pathogenic yeast phase once it is inhaled. Resident phagocytes such as alveolar macrophages take up the pathogen in the alveolar space. Since macrophages (MΦ) are not able to contain *H. capsulatum* growth prior to activation by cell-mediated immunity, *H. capsulatum* replicates inside their phagosomes leading to granuloma formation. *H. capsulatum*-induced granulomas were shown to be areas of low oxygen where MΦ stabilize hypoxia-inducible factor (HIF)-1α. This transcription factor ensures cell survival by modifying host cell metabolism and promoting innate immune activation. In previous studies, we could show that enhanced HIF-1α protein drives the glycolytic phenotype of human MΦ during *H. capsulatum* infection.
We aimed to elucidate the further impact of HIF-1α on fungal immunity. Therefore, we investigated the effect of HIF-1α on cytokine release, autophagy and pathogen survival in human MΦ during *H. capsulatum* infection.
Human monocytes were isolated from peripheral blood of healthy patients and subsequently differentiated into MΦ. Afterwards, cells were infected with *H. capsulatum* (MOI 5:1) and analyzed up to 24 h post infection (hpi). The abundance of HIF-1α protein was enhanced by pharmacological stabilizer (IOX2). The release of TNF-α, IL-1β and IL-10 was assessed by Luminex assay. Protein levels of HIF-1α and autophagy marker LC3-II were investigated by Western blot. Further, cellular localization of LC3-II protein was visualized using ImageStream. Pathogen survival was assessed by isolating and plating *H. capsulatum* from infected cells 24 hpi and counting colony forming units after 7 days.
MΦ stabilized HIF-1α during infection with *H. capsulatum* 24 hpi ($p<0.05$, $n=3$). The release of TNF-α and IL-1β was enhanced 2

and 24 hpi respectively, but additional HIF-1α stabilization did not further enhance the release of both pro-inflammatory cytokines ($p<0.05$, n=3). In contrast to that, *H. capsulatum* led to an increase of IL-10, which was reversed by elevated HIF-1α levels ($p<0.05$, n=3). Infection with *H. capsulatum* increased LC3 II protein levels, while elevated HIF-1α protein levels abrogated LC3-II ($p<0.01$, n=4). Remarkably, additional HIF-1α stabilization decreased survival of *H. capsulatum* 50 +/- 5% 24 hpi ($p<0.05$, n=3).
Our findings highlight a pivotal role of HIF-1α in fungal immunity. HIF-1α drives the pro-inflammatory phenotype and abrogates autophagy leading to *H. capsulatum* growth inhibition. Autophagy might facilitate nutrient acquisition ensuring pathogen survival. In future, we will characterize the phagosomal environment of *H. capsulatum* in order to elucidate HIF-1α dependent changes hampering pathogen survival in MΦ.

Presentation: Monday, February 19, 2018 from 14:15 – 14:30 in room Audimax Saal.

013/EKV

The expression of *ECE1* in *Candida albicans* is regulated by the transcription factor Ahr1

S. Ruben*[1], R. Martin[2], O. Kurzai[2,3]
[1]*Septomics Research Center, Friedrich Schiller University and Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute, Fungal Septomics, Jena, Germany*
[2]*University of Würzburg, Insitute for Hygiene and Microbiology, Würzburg, Germany*
[3]*National Reference Center for Invasive Fungal Infections, Leibniz Institute for Natural Product Research and Infection Biology –Hans Knoell Institute, Jena, Germany*

Introduction: The dimorphic growth of *Candida albicans* is an important virulence factor of this opportunistic pathogen. Its transition from yeast to hyphal growth form is accompanied by the upregulation of several core filamentation response genes. *ECE1* is one of them and encodes for the newly described Candidalysin, the first cytolytic peptide toxine in a human fungal pathogen. The exact regulation of *ECE1* transcription is still unknown. Several transcription factors (TF) involved in hyphal morphogenesis can theoretically bind to the *ECE1* promoter. Among them is Ahr1, which was recently shown to be involved in processes like white-opaque-switching and biofilm formation.
Objectives: The aim of this study was to analyze the regulation of *ECE1*, encoding for Candidalysin, in *Candida albicans*. Therefore, we took a closer look at the role of the different TFs with known binding-sites in the *ECE1* promoter. Further analyses focused on the TF Ahr1 concerning morphology and *ECE1* expression.
Material and Methods: We examined the promoter of *ECE1* for binding-sites of known TFs involved in regulation of hyphal formation. Deletion mutants of these TFs were screened for *ECE1* expression using a GFP-reporter system, where GFP was under the control of the *ECE1* promoter (pECE1-GFP) and qRT PCR. Since findings for *ahr1*Δ arose interest, the effect of a hyperactive *AHR1* allele, where the gene was fused to a Gal4 activator domain, was studied using qRT PCR and microscopy.
Results: In contrast to other tested TF deletion mutants, the *C. albicans ahr1*Δ mutant formed phenotypically normal hyphae in serum-containing medium, but showed decreased *ECE1* expression and no GFP signal (pECE1-GFP) when compared to the wild type. A hyperactive *AHR1* allele on the other hand, induced high levels of *ECE1* expression even under yeast growth conditions. Additionally, it significantly induced *ECE1* expression and hyphal growth in *cph1*Δ*/efg1*Δ mutants, which are usually only able to form yeast cells even under hyphae inducing conditions.
Conclusions: The transcription factor Ahr1 is required for high level transcription of the *ECE1* gene. Surprisingly, a hyperactive allele of this regulatory gene can bypass the absence of hyphal growth stimulation to induce *ECE1* transcription. This novel mechanism of *ECE1* regulation seems to be independent from the well-known regulators of hyphal growth, Cph1 and Efg1.

Presentation: Monday, February 19, 2018 from 14:30 – 14:45 in room Audimax Saal.

014/EKV

How to turn an embryo into a cancerous monster

K. Brehm*[1], U. Koziol[1], M. Spiliotis[1], R. Harrmann[1], R. Duvoisin[1]
[1]*University of Würzburg, Institute of Hygiene and Microbiology, Würzburg, Germany*

Introduction: The metacestode larval stage of the fox-tapeworm *Echinococcus multilocularis* grows infiltratively, like a malignant tumor, into the intermediate host liver, causing the lethal disease alveolar echinococcosis (AE). The molecular basis of this unusual growth behavior has not been elucidated so far.
Aims: The study aimed at characterizing proliferative parasite cell populations and parasite signaling pathways which direct cancerous growth.
Materials and Methods: We made use of the *Echinococcus* genome project, functional genomic methodology, and sophisticated *in vitro* cultivation systems for *Echinococcus* larvae and stem cells.
Results: We show that *Echinococcus* metacestode proliferation is decisively driven by the parasite"s germinative cell population, which displays homologies to human stem cells, which is the parasite"s only mitotically active cell type, and which gives rise to all differentiated parasite cells. We also show that cancerous parasite growth involves a modification of anterior-posterior axis formation and modulation of *wnt*-signalling, as it also often occurs in human cancer. More specifically, the invading embryonic oncosphere larva transiently shuts down the anterior (head) pole of the animal, resulting in the cyst-like metacestode which grows as posteriorized tissue under control of *wnt*-family cytokines. After extensive cancerous growth, the anterior pole is established again at numerous sites within the metacestode, giving rise to multiple protoscoleces. Within the metacestode, *wnt*-signalling factors are produced by muscle cells, explaining their presence in the immobile metacestode. RNAi against beta-catenin, the central component of *Echinococcus wnt*-signalling, or general inhibition of *wnt*-signalling, resulted in aberrant tissue formation and severely hampered parasite development. Interestingly, albendazole, the current drug against AE proved to be ineffective against the parasite"s germinative (stem) cells, thus explaining their limited efficacy against the metacestode.
Conclusions: We explain for the first time why the *Echinococcus* metacestode grows like cancerous tissue and does not, like typical tapeworms, directly develop from the oncosphere into scoleces. Our analyses place the parasite"s stem cells and the *wnt* signaling pathway at a central position for the development of new drugs against AE. These are urgently necessary since the parasite"s stem cells are inherently resistant against currently used drugs. Our data also establish an interesting connection between the *Echinococcus* metacestode and human cancer.

Presentation: Monday, February 19, 2018 from 14:45 – 15:00 in room Audimax Saal.

WORKSHOP 03
Microenvironmental Factors in Infection - Effects on Pathogen and Host Response (FG II)
19 Febr. 2018 • 13.30–15.00

015/IIV

Lactobacilli protect intestinal cells against *Candida albicans*-mediated cytotoxicity in an *in vitro* commensal gut model

R. Gratz*[1], K. Graf[1], B. Hube[1,2,3]
[1]*Hans-Knöll-Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*University Hospital, Center for Sepsis Control and Care, Jena, Germany*
[3]*Friedrich Schiller University, Jena, Germany*

The main reservoir for systemic *Candida albicans* infections is the gut, where the fungus normally exists as a harmless commensal that interacts with the host and the microbiota. However, removal or imbalance of the bacterial microbiota by antibiotic treatment, for instance, can facilitate fungal overgrowth - a significant predisposing factor for disseminated *Candida* infections.
Our aim is to investigate the commensal-to-pathogen shift of *C. albicans* induced by the removal of protective bacteria, as well as to elucidate how these non-pathogenic microbes keep the fungus in a commensal stage. Thus, we established a two cell line based commensal *in vitro* gut model in which we were able to show a time-, dose-, and species-dependent protective effect of different lactobacilli strains against *C. albicans*-induced cytotoxicity. This effect required viable bacteria, the presence of host cells and was not related to a competition for adhesion sites. Still, lactobacilli affected hyphal elongation, ramification and induced shedding of hyphae from the host cell surface at later stages. Most likely, this loss of contact leads to the damage reduction observed in presence of bacteria. Indeed, while invading hyphal cells of *C. albicans* induced transcription of the damage-related transcription factor *cFOS* in human cells no such regulation was measurable upon bacterial-fungal co-infection.
Next, similar to the clinical situation, protective lactobacilli were removed by an antibiotic treatment in order to induce the commensal-to-pathogen-shift of the fungus - a state that is characterized by elevated host cell damage. Via transcriptional profiling of fungal and host cells we want to identify genes involved in the commensal-to-pathogen-shift of *C. albicans* and to elucidate the unerlying protective mechanism of lactobacilli.
In short, using a reductionism approach we experimentally dissect the complex interactions of the human-associated pathogenic fungus *C. albicans*, the bacterial microbiota and the human host in order to investigate their impact on disease development.

Presentation: Monday, February 19, 2018 from 13:30 – 13:45 in room Hörsaal 1.

016/IIV

Strain-level gut microbiota dynamics in patients with recurrent *Clostridium difficile* infection following Fecal Microbiota Transplantation

D. Podlesny[1], E. Dörner[1], S. Dutta[2], W. F. Fricke*[1,3]
[1]*University of Hohenheim, Nutrigenomics, Stuttgart, Germany*
[2]*Sinai Hospital, Baltimore, United States*
[3]*University of Maryland School of Medicine, Institute for Genome Sciences, Baltimore, United States*

Introduction: Fecal Microbiota Transplantation (FMT), the infusion of human feces from healthy donors into the gastrointestinal tract of recipient patients, has the potential to serve as a therapeutic treatment for microbiota-associated infectious and inflammatory problems. FMT efficacy, attributed to the restoration of gut microbiota homeostasis, has been demonstrated for recurrent *Clostridium difficile* Infection (rCDI) and suggested for metabolic syndrome. Yet the underlying mechanisms have only been insufficiently investigated and the long-term consequences of FMT remain largely unknown.
Objective: We used fecal metagenomics to study microbiota dynamics in rCDI patients, before and after FMT, and their corresponding donors at the individual strain level.
Methods: A total of 35 fecal samples from twelve FMT cases, including multiple rCDI patient samples collected before and up to one year after single allogenic FMT, were analyzed by metagenomic whole-genome shotgun (WGS) sequencing. Taxonomic profiling of microbial communities on the strain-level was performed based on analysis results obtained with StrainPhlAn, which relies on the identification of single nucleotide variants (SNVs) in species-specific marker genes.
Results: Microbiota engraftment following FMT was found to be dependent on bacterial taxonomy, as evidenced by cases where donors and rCDI patients carried the same or distinct strains before and after FMT, depending on the bacterial species. Strain-level microbiota dynamics were also affected by patient-donor relationships, with evidence for shared bacterial strains in husbands-wife pairs even before FMT.
Conclusion: FMT holds considerable promise as a therapy for rCDI and other microbiota-associated disorders, yet short and long-term effects need to be fully understood to ensure patient safety. Metagenomic strain-level sequence analysis may help disentangle the role of specific members of the microbiota for gut homeostasis and efficacy of FMT.

Presentation: Monday, February 19, 2018 from 13:45 – 14:00 in room Hörsaal 1.

017/IIV

Synthetic oligosaccharide-based vaccines protect mice from *Clostridium difficile* infections

F. Broecker[1], E. Wegner[2], P. Kaplonek[1], A. Ensser[3], P. Seeberger[1], J. Mattner*[2]
[1]*Max Planck Institute of Colloids and Interfaces, Department of Biomolecular Systems, Berlin, Germany*
[2]*Klinische Mikrobiologie Erlangen, Erlangen, Germany*
[3]*Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Virologisches Institut , Erlangen, Germany*

Introduction: *Clostridium difficile* is the cause of emerging nosocomial infections that have risen in incidence, morbidity and mortality worldwide. Thus, the development of a vaccine to prevent this devastating disease is highly desirable, as commonly used frontline antibiotics become less effective and frequently induce recurrent disease by disrupting the intestinal microbiota. Several recently identified bacterial surface glycans such as lipoteichoic acid (LTA) and polysaccharide I and II (PS I and II) have been suggested as promising vaccine candidates to preclude *C. difficile* infection and colonization1-2.
Aims: We conjugated PS-I, PS-II and LTA to CRM197, a carrier protein used in commercial vaccines in order to assess their potential as colonization-preventing vaccines against *Clostridum difficile* infections.
Materials and Methods: Stool and serum samples of patients with *Clostridium difficile* infections were evaluated for IgG and IgA antibodies against PS I, PS II and LTA. Mice were vaccinated with glycoconjugate vaccines against PS I, PS II and LTA. The composition of the intestinal microbiota, the replication and dissemination of *Clostridium difficile* as well as the extent of intestinal inflammation was assessed using conventional plating assays, 16S rRNA sequencing, immunohistochemistry and histopathological analyses.
Results: The analysis of stool and serum samples obtained from patients with *C. difficile* infections using glycan microarrays of synthetic oligosaccharide epitopes revealed robust humoral immune responses to PS-I, PS II and LTA and their related oligosaccharide substructures. Furthermore, PS I-, PS II- and LTA-glycoconjugate vaccines protected mice from *C. difficile* infections. PS I-, PS II- and LTA glycoconjugates induced glycan-specific antibodies in mice and substantially limited *C. difficile* colonization after infection without disrupting the intestinal microbiota. The glycoconjugates were even superior to a toxin-targeting vaccine candidate in preventing disease.
Summary: Glycoconjugate vaccines against *C. difficile* are a complimentary approach to toxin-targeting strategies and are advancing through preclinical work. In addition to the clinically most advanced vaccine approaches which target secreted clostridial

cytotoxins, these glycoconjugates prevent also asymptomatic carriage.

References

[1] Martin, C. E. *et al.* Immunological evaluation of a synthetic *Clostridium difficile* oligosaccharide conjugate vaccine candidate and identification of a minimal epitope. *J. Am. Chem. Soc.* **135**, 9713–9722 (2013).

[2] Broecker, F. *et al.* Synthetic lipoteichoic acid glycans are potential vaccine candidates to protect from *Clostridium difficile* infections. *Cell Chem. Biol.* **23**, 1014–1022 (2016).

Presentation: Monday, February 19, 2018 from 14:00 – 14:15 in room Hörsaal 1.

018/IIV

***Heligmosomoides polygyrus* infection in colitis-associated colon cancer: intestinal immune modulation by helminths promotes carcinogenesis.**

E. Pastille*[1], A. Frede[1], H. J. McSorley[2], R. M. Maizels[3], A. M. Westendorf[1]

[1]*University Hospital Essen, Institute of Medical Microbiology, Essen, Germany*

[2]*University of Edinburgh, Edinburgh, United Kingdom*

[3]*University of Glasgow, Glasgow, United Kingdom*

Question: Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract, associated with an increased risk of colorectal cancer development. Since the etiology of UC is still unknown, current therapies are able to alleviate the acute inflammation but fail to cure the patients. The exposure of IBD patients to helminths appears to be a novel promising approach as helminths provoke an immunosuppressive state in the host by releasing immunomodulatory molecules and inducing regulatory T cells (Tregs). On the contrary, specific parasite infections are well known to be directly linked to carcinogenesis. In the present study, we aimed to unravel the apparently controversial function of helminths in a mouse model of colitis-associated colon cancer (CAC).

Methods: CAC was induced in mice by a single i.p. injection of the procarcinogen Azoxymethan followed by the administration of dextran sulphate sodium (DSS) via the drinking water. By administration of DSS alone an acute colitis was induced. At different time points during CAC mice were infected orally with 200 stage 3 larvae of the intestinal helminth *Heligmosomoides (H.) polygyrus*.

Results: We could demonstrate that the treatment of mice with *H. polygyrus* at the onset of colitis and CAC does not ameliorate colonic inflammation but further facilitates tumor development. More precisely, *H. polygyrus* infection before the induction of DSS-mediated colitis exacerbates pathology in the colon which was accompanied by increased levels of IL-6 and CXCL1, and strong activation of CD4+ effector T cells. Furthermore, *H. polygyrus* infection induced an expansion of highly activated CD4+ Foxp3+ Tregs and a prolonged reduction of CD8+ effector T cells frequencies in the colon.

Conclusion: Together, our results demonstrate that the therapeutic application of helminths during CAC might have tumor-promoting effects and therefore should be well-considered.

Presentation: Monday, February 19, 2018 from 14:15 – 14:30 in room Hörsaal 1.

019/IIV

Development of murine models mimicking key symptoms of diarrhea-positive haemolytic-uraemic syndrome

S. Dennhardt*[1,2,3], W. Pirschel[1,2], B. Wissuwa[1,2], C. Daniel[4], F. Gunzer[5], S. Lindig[1], W. W. Rudolph[5], K. Amann[4], S. M. Coldewey[1,2,3]

[1]*Jena University Hospital, Department of Anaesthesiology and Intensive Care Medicine, Jena, Germany*

[2]*Jena University Hospital, Septomics Research Centre, Jena, Germany*

[3]*Jena University Hospital, Center for Sepsis Control and Care, Jena, Germany*

[4]*Friedrich-Alexander University Erlangen-Nürnberg, Department of Nephropathology, Erlangen, Germany*

[5]*Dresden University of Technology, Institute of Medical Microbiology and Hygiene, Dresden, Germany*

Introduction: Infections with enterohaemorrhagic *Escherichia coli* (EHEC) can cause diarrhea-positive haemolytic-uraemic syndrome (HUS), a severe systemic complication clinically characterised by thrombotic microangiopathy, haemolytic anaemia, and acute kidney injury. Shiga toxins (Stxs) as main virulence factors of EHEC play a key role in HUS development.

Objectives: This work aimed to develop murine models of HUS suitable to serve as preclinical tools to further elucidate molecular mechanisms and test novel pharmacological interventions in this condition.

Methods: Male C57BL/6J mice were subjected to vehicle or Stx2 derived from an *E. coli* O157:H7 patient isolate. To establish a model with fast disease progression a single high Stx2 dose was administered on day 0 and mice were sacrificed on day 3 (acute model). To establish a model with moderate disease progression three low Stx2 doses were administered on day 0, 3 and 6 and mice were sacrificed on day 7 (subacute model). Biochemical indicators of kidney dysfunction and morphological as well as immunohistochemical alterations of kidneys were analysed. Changes in renal signaling pathways were assessed by gene expression analysis.

Results: Compared with sham mice, mice challenged with Stx2 developed kidney dysfunction and injury indicated by significant rises of urea, creatinine and neutrophil gelatinase-associated lipocalin in the plasma as well as by occurrence of severe tubular injury combined with a loss of endothelial cells and thrombotic microangiopathy. We observed a significant increased haematocrit in mice challenged with Stx2 indicating dehydration that was less pronounced in mice subjected to the 7-day regime. However, compared to sham mice, the degree of complement activation, immune cell invasion, apoptosis and proliferation was significantly increased in kidneys of mice subjected to the 7-day regime, but not in kidneys of mice subjected to the 3-day regime. Microarray analysis revealed distinct gene expression patterns in both models but a total of 91 overlapping genes regulated by Stx2-challenge.

Conclusions: We established and extensively characterised two distinct murine models of HUS. The subacute model appears to better reflect human HUS as kidney dysfunction, thrombotic microangiopathy and renal endothelial damage are accompanied by renal complement activation and invasion of immune cells. However, as injured kidneys in HUS patients may additionally be affected by prerenal mechanisms, such as dehydration, the acute model may allow studying certain pathophysiological aspects. Thus, both models together will serve as suitable tools to study HUS pathology.

S.D. and W.P. contributed equally

Presentation: Monday, February 19, 2018 from 14:30 – 14:45 in room Hörsaal 1.

020/IIV

Dynamics and trafficking of mGBP protein complexes within membranous compartments

N. Steffens*[1], L. Legewie[1], E. Kravets[1], D. Degrandi[1], K. Pfeffer[1]
[1]*Universitätsklinikum Düsseldorf, Institute of Medical Microbiology, Düsseldorf, Germany*

Introduction: Toxoplasmosis is a disease that results from infection with the protozoan parasite *Toxoplasma gondii* (*T. gondii*). After invasion of target cells, *T. gondii* creates a specific membranous subcellular compartment, the parasitophorous vacuole (PV). Members of the mGBP (murine guanylate binding protein) family, in particular mGBP2 and mGBP7, assemble at the cytoplasmatic side of the PV and interact with membrane compartments via as yet uncharacterized mechanisms[1,2].
Objectives: The aim of our studies is the analysis of the molecular mechanisms by which mGBP7 impairs the vital functions of the PV-membrane of *T. gondii.*
Method: Stable cell lines expressing fluorescent mGBP7 (and other members of the mGBP family) were generated and infected with GFP-/mCherry-tagged *T.gondii* to analyze the recruitment, kinetics and dynamics of mGBP7 translocation via Confocal Live Cell Imaging. In addition, super-resolution technology such as structured illumination microscopy (SIM), stimulated emission depletion microscopy (STED) and Airyscan was used to analyze the different structures of the doubly transduced cell lines during *T. gondii* infection in detail. Furthermore, giant unilamellar vesicles technology (GUV) will be employed to investigate the binding capacity of GFP-tagged mGBP2 and/or mGBP7 to membranes and their modulation of membrane integrity. Also, cytosolic compartments of mGBPs (so called vesicle-like structures) of Interferon-γ activated cells and the PV of *T. gondii* will be characterized by immuno electron microscopy.
Results: Previous experiments showed that some mGBPs are able to form heteromers and most of them are located in vesicle-like structures (VLS). Our first results indicated that mGBP7 also forms heteromers and is located in VLS. Furthermore, mGBP7 colocalizes with mGBP3 (in VLS and at the *T. gondii* PV) and with mGBP6 (at *T. gondii* PV) but virtually does not colocalize with mGBP2. This suggests an interaction hierarchy within the mGBP family members. In addition, mGBP7 and mGBP3 are able to accumulate directly at the plasma membrane of *T. gondii*, subsequently leading to parasite death.
Conclusion: In summary, mGBPs belong to a family of GTPases that can interact with the *T. gondii*-PV and also some mGBPs directly attack the parasite membrane. The analysis of the mechanisms of immunity will help to understand Toxoplasmosis and to find new treatment opportunities in the long term.

References

[1] Kravets E, Degrandi D, Ma Q, Peulen TO, Klümpers V, Felekyan S, Kühnemuth R, Weidtkamp-Peters S, Seidel CA, Pfeffer K. Elife. 2016 Jan 27;5. pii: e11479. doi: 10.7554/eLife.11479
[2] Degrandi D, Konermann C, Beuter-Gunia C, Kresse A, Würthner J, Kurig S, Beer S, Pfeffer K. .J Immunol. 2007 Dec 1;179(11):7729-40

Funding: SFB 1208 – Identity and Dynamics of Membrane Systems – DFG

Presentation: Monday, February 19, 2018 from 14:45 – 15:00 in room Hörsaal 1.

WORKSHOP 04
Molecular Epidemiology of VRE and Multidrug-Resistant Gram-Negatives (FG PR)
19 Febr. 2018 • 13.30–15.00

021/PRV

Vancomycin-resistant *Enterococcus faecium* isolates in hospitals from 2012 to 2016, data from the German Antibiotic Resistance Surveillance System (ARS)

A. Reuss*[1], U. Koppe[1], I. Noll[1], M. Feig[1], M. Schneider[1], M. Abu Sin[1], T. Eckmanns[1]
[1]*Robert Koch Institute, Berlin, Germany*

Question: In many European countries, an increase in vancomycin-resistant *Enterococcus faecium* (VRE) isolates has been observed and the spread of VRE represents a significant public health problem. Bloodstream infections with VRE are associated with a higher mortality. We investigated the extent of VRE in Germany.
Methods: We analysed data from the German Antimicrobial Resistance Surveillance (ARS) System which contains routine data of antimicrobial susceptibility testing from voluntarily participating German laboratories. The analyses were restricted to hospitals which participated continuously from 2012 to 2016. The first clinical *E. faecium* isolate per quarter per hospital patient was included. Screening isolates were excluded. *Enterococcus* isolates are not always differentiated down to species level; this depends on clinical severity and sample material. To ensure similar lab procedures with regards to species identification, we determined the proportion of vancomycin resistance for a) isolates from laboratories where *Enterococcus* species were identified in more than 95% of isolates and b) blood cultures where *Enterococcus* species were always identified. The data set of 01.10.2017 was used.
Results: In total, 29,554 isolates from 136 hospitals were analysed. The median age of patients was 73 years (IQR: 62-81 years). 48.9% of the isolates were from women, 43.0% from men, and for 8.1% the sex was not specified. Most isolates were from urine (44.2%), followed by swabs (16.2%), wounds (16.0%) or blood cultures (8.4%). Susceptibility testing against vancomycin was performed in almost all of the isolates (99.5%). The overall number of *E. faecium* isolates increased from 4,396 in 2012 to 6,485 in 2016. In laboratories where *Enterococcus* species were identified in more than 95% of isolates (17 hospitals), the proportion of VRE isolates was 14.8% (95%-CI: 9.2%-22.9%). A decrease from 14.3% (95%-CI: 7.9%-24.5%) in 2012 (n=966 isolates) to 10.2% (95%-CI: 7.1%-14.5%) in 2013 (n=1,079 isolates) was observed, followed by an increase to 18.1% (95%-CI: 8.5%-34.3%) in 2016 (n=1,161 isolates). In blood cultures (111 hospitals), the overall proportion of VRE isolates was 13.6% (95%-CI: 9.7%-18.8%) and fluctuated between 14.7% (95%-CI: 10.4%-20.3%) in 2012 (n=415 isolates) and 12.0% (95%-CI: 8.1%-17.4%) in 2014 (n=509 isolates). 0.9% of VRE isolates were also resistant to linezolid (95%-CI: 0.6%-1.3%; 98.8% tested) and 0.5% to tigecyclin (95%-CI: 0.2%-1.1%; 92.8% tested).
Conclusions: Vancomycin resistance in *E. faecium* isolates is common in Germany with fluctuating proportions over the years. Our results show that the overall number of clinical *E. faecium* isolates has increased by almost 50% from 2012 to 2016. This highlights an increasing impact on the clinical routine and a growing public health problem and emphasizes the need to control further spread of vancomycin resistance.

Presentation: Monday, February 19, 2018 from 13:30 – 13:45 in room Hörsaal 3.

022/HYV

Molecular epidemiology of VRE in a University Hospital in Germany from 2004-2009

W. Schneider-Brachert*[1], J. Peterander[1], V. Bayerl[1], V. Meier[1], C. Seisenberger[1], F. Hitzenbichler[2], B. Salzberger[2], T. Holzmann[1]
[1]*University Hospital Regensburg, Inst. for Clinical Microbiology and Hygiene, Regensburg, Germany*
[2]*University Hospital Regensburg, Internal Medicine, Regensburg, Germany*

We have witnessed a dramatic increase in VRE isolation from 2004 to 2014. To understand the epidemiology of this process it is necessary to understand the molecular epidemiology which has driven this local evolution.
We therefore analysed all available VRE isolates from our strain bank to dissect the year to year increase and ward to ward spread.
VRE isolates from clinical origin and screening bullions were recultured, re-identified by MALDOTOF and MIC were determined. MLST was performed by standard protocols and ST determination was done by pubmlst.org.
Nearly all of the VRE isolates were E. faecium strains and from 233 strain bank isolates 217 were recultured and used fro further analysis. In addition, espA and hyl were amplified by PCR and used as virulence markers and vanA and vanB were determined.
In 2004 only two VRE isolates were recorded, one was recultured and determined as vanB-positive ST 2008, espA and hyl positive. In 2005 10 VRE isolates were available 1 vanA and 9 vanB, and four different ST were identified: ST 17 (1), 186 (1), 192 (1), and 203 (7). In 2006 12 VRE were all vanB positive and 6 MLST determined: ST 17 (3), 186 (1), 192 (1), 203 (19, 208 (1), and 780 (3). In 2007 there was a dramatic VRE increase due to a VRE outbreak in an ICU. 72 VRE strains were characterised, all vanB positive, and St 17 (18) and 192 (42) were the most prominent ST types. In 2008 a further increase was observed with 86 VRE isolates, all but one vanB positive and again ST 17 (28) and 192 (40) were the most abundant ST. In 2009 the VRE load decreased to 36 isolates with 3 vanA and 33 vanB genotype, and at a lower level ST 17 (10) and 192 (13) the most prominent ones.
In conclusion, after an outbreak (2007) at a medical ICU we observed a dramatic increase of two VRE ST, ST 17 and ST 192, other ST were less abundant. We are currently mapping our ST to the different clinical centres and their wards to determine local transmission as a reason for the enormous spread from 2004 to 2009.

Presentation: Monday, February 19, 2018 from 13:45 – 14:00 in room Hörsaal 3.

023/HYV

Phylogenetic analysis of 773 Vancomycin-resistant *Enterococcus faecium* isolates from a seven-year period

J. Liese*[1], L. Schüle[1], P. Oberhettinger[1], L. Tschörner[1], M. Marschal[1], I. B. Autenrieth[1], M. Willmann[1], S. Peter[1]
[1]*University Hospital Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany*

Question: Vancomycin-resistant enterococci (VRE) are a frequent cause of nosocomial outbreaks and colonization of patients with VRE bears the risk of subsequent infections that are difficult to treat. In the beginning of 2016 we noted a sudden increase in VRE incidence at our university hospital. To gain insights into the local epidemiology and transmission routes before and during the outbreak we performed whole genome sequencing (WGS) and phylogenetic analysis on 773 Vancomycin-resistant *Enterococcus faecium* isolates recovered from patients between 2010 and 2016.
Methods: VRE isolates were collected from routine and screening samples and the first strain from each patient was sequenced using the Illumina MiSeq and NextSeq platforms. Sequence assembly, extraction of MLST sequence types and phylogenetic reconstruction were performed using an in-house bioinformatic pipeline and a commercial software package. Epidemiological patient data was retrieved from the electronic hospital data repository.
Results: SNP analysis revealed that 568 (73.5%) VRE strains could be grouped into one of nine major phylogenetic clusters, which comprised between 15 and 275 isolates. The outbreak was dominated by van-B positive isolates belonging to MLST types 17, 80, and 117, representing five phylogenetic clusters. The earliest detection of cluster member isolates occurred several months before an increase in detection rates was noticed, which suggests unnoticed reservoirs and/or adaptive processes of the strains in the hospital. Initial epidemiological analysis of hospital occupancy data suggested a large number of possible transmissions, but distinct cluster transmission events could be identified, when phylogenetic data was combined with epidemiological data.
Conclusions: WGS and phylogenetic SNP analysis of clinical VRE isolates revealed a greater heterogeneity of strains than initially suggested by epidemiological data or typing by MLST. This lead to the exclusion of putative transmission events, and enabled better matching of epidemiological and typing data. Intra-hospital transmissions as well as increased import by patients colonized outside our hospital were responsible for the observed sharp rise in VRE detection during the outbreak.

Presentation: Monday, February 19, 2018 from 14:00 – 14:15 in room Hörsaal 3.

024/MSV

Colistin resistance in *Enterobacteriaceae* isolates from human patients in Germany – a silent spread of *mcr-1*?

Y. Pfeifer*[1], M. Pietsch[1], C. Woelke[2], G. Werner[1]
[1]*Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany*
[2]*MVZ Diamedes GmbH, Bielefeld, Germany*

Background: Since the first description of the novel plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* from China in 2015 nearly 300 reports on its worldwide emergence - mainly from livestock animals - were published. In human medicine colistin is one of the few remaining substances for treatment of infections with multidrug-resistant gram-negative pathogens (4MRGN), and resistance to colistin is tested in most cases only if a 4MRGN isolate is detected. Thus, the assessment of spread of colistin resistance and *mcr-1* in Germany is difficult. Here we report the results of *mcr-1* screening of *Enterobacteriaceae* isolates from one clinical diagnostic laboratory in Germany.
Materials and Methods: The laboratory - that tests colistin susceptibility routinely for all *Enterobacteriaceae* - sent in all detected colistin-resistant isolates (February 2016 – October 2017) for *mcr-1/mcr-2*-screening by PCR. Antimicrobial susceptibility testing was performed by microbroth dilution and Etest. Presence of further resistance genes and other mechanisms that are involved in colistin resistance were analysed by PCR and sequencing. Bacterial strain typing was performed by enzymatic macrorestriction and subsequent pulsed-field gel electrophoresis (PFGE). Resistance gene transfer was tested in conjugation assays.
Results: During the 22nd-month sampling period 75 colistin-resistant isolates were analysed (49 *E. coli*, 19 *Klebsiella spp.*, 7 *Enterobacter cloacae*). We found *mcr-1* in 19 *E. coli* that were isolated from urine (n=12), blood culture (n=3), wound swabs (n=3) and one pharyngeal swab. Using PFGE-typing and phylogenetic PCR-typing we differentiated 16 *E. coli* clones with *mcr-1* (including one ST131). Additional resistance to ampicillin (100%) and ciprofloxacin (26%) was observed due to production of TEM-1 beta-lactamase and QnrS1, respectively. All 19 *mcr-1*-positive *E. coli* harboured an IncX plasmid of 30-35kb size, similar to previously described plasmids in poultry. In contrast, none of the colistin-resistant *Klebsiella spp. and E. cloacae* (n=24) produced Mcr-1. Sequence analyses of the intrinsic *mgrB* gene of *Klebsiella pneumoniae* showed various modifications that are most probably associated with the loss of functionality of this gene and contribution to colistin resistance.
Conclusion: Our data indicate a low occurrence of colistin resistance in different enterobacterial species. In only 40% of the colistin-resistant *E. coli* isolates *mcr-1* was the mechanism for colistin resistance. However, the spread of this resistance gene is probably underestimated due to its plasmid-location and the diagnostic lack for colistin in human medicine.

Presentation: Monday, February 19, 2018 from 14:15 – 14:30 in room Hörsaal 3.

025/PRV

Analysis of OXA-48-carrying plasmids causing a multi-species outbreak in a German tertiary care hospital

F. Lange*[1], M. Korte-Berwanger[1], H. von Wulffen[2], C. Moecke[3], S. G. Gatermann[1]

[1]*Ruhr-Universität Bochum, Medizinische Mikrobiologie, Bochum, Germany*
[2]*MEDILYS Laborgesellschaft mbH, Hamburg, Germany*
[3]*Asklepios Kliniken, Hamburg, Germany*

Introduction: OXA-48 is the most prevalent carbapenemase among *Enterobacteriaceae* in Germany. It confers resistance against aminopenicillins and carbapenems and strains carrying OXA-48 often co-express an ESBL conferring resistance against third generation cephalosporins. Therefore, nosocomial infections with OXA-48 expressing *Enterobacteriaceae* are often difficult to treat. Occasionally, outbreak situations with carbapenemase-carrying *Enterobacteriaceae* occur, most of which are due to clonal spread of bacteria. In the case at hand, however, OXA-48-carrying isolates of several different Enterobacteriaceae species were found at unusually high frequency in a tertiary care hospital involving more than 80 inpatients. These isolates belonged to several different species and, in some cases, several different clones within one species as determined by PFGE. Therefore, clonal spread could not account for this unusal accumulation of OXA-48 expressing strains.
Aims: To determine epidemiological relationship between the OXA-48 bearing isolates in this setting.
Methods: Plasmid DNA was extracted from 18 OXA-48-carrying isolates of different species obtained from the hospital and was transformed in *E. coli* NEB-10-Beta. Plasmid DNA was extracted from the transformants, digested with *Eco*RI and *Hind*III and analyzed by gel electrophoresis. Non-identical plasmids were then sequenced by Illumina MiSeq and Oxford Nanopore MinION. Plasmid sqeuences were assembled using hybridSPAdes. PCR Primers were deduced from the sequences in order to identify the different plasmid types in the remainder of the isolates.
Results: Subsequent gel electrophoresis showed that 10 of 18 transformants contained a plasmid showing an identical restriction fragment pattern, while 4 more transformants showed patterns similar to the prior. Two transformants contained non-related plasmids. PCRs were designed to identify strains involved in the outbreak for further epidemiological management.
Discussion: Transforming and restriction-typing of plasmids is not viable for rapid identification of outbreaks, sequencing-based methods are required. WGS of isolates in question, however, does not usually allow for complete assembly and identification of the carbapenemase-bearing plasmids. For reliable and rapid plasmid identification, WGS reads need to be mapped on known plasmid sequences. Therefore, sequences of the most prevalent plasmids carrying resistance genes need to be investigated in a systematic way to allow for rapid detection of plasmid-outbreaks spreading resistance among multiple species in a hospital setting.

Presentation: Monday, February 19, 2018 from 14:30 – 14:45 in room Hörsaal 3.

026/PRV

Incidence of 3rd generation cephalosporin resistant *Enterobacteriaceae* infections - a prospective multicenter cohort study in 6 German university hospitals

A. Rohde*[1], F. Schwab[1], J. Zweigner[2], M. Wiese-Posselt[1], H. Seifert[2], W. Kern[3], P. Gastmeier[1]

[1]*Charité - Universitätsmedizin Berlin, Institute of Hygiene and Environmental Medicine, Berlin, Germany*
[2]*Universitätsklinikum, Köln, Germany*
[3]*Universitätsklinikum, Freiburg i. Br., Germany*

Introduction and Aim: Hospital-acquired infections (HAI) are an increasing problem, especially those caused by third generation cephalosporin resistant *Enterobacteriaceae* (3GCRE) are of concern. We aim to describe the occurrence of infections with 3GCRE and the proportion of additional carbapenem resistance in German university hospitals.
Methods: In 2014 and 2015 we performed surveillance for 3GCRE in 6 German university hospitals in clinically relevant specimens. 3GCRE infections were counted as HAI when the symptoms began after the third day of stay (admission day = day 1) and were otherwise classified as community-acquired (CAI).
Results: In total, 578 420 patients with 3 385 112 patient days were under surveillance. Among those patients 3681 3GCRE infections were monitored. We observed a CAI incidence of 0.27 and a HAI incidence of 0.31 per 100 patients. *E. coli* caused the highest incidence of CAI (0.15 per 100 patients) and HAI (0.12 per 100 patients). The most frequent infections caused by 3GCRE were urinary tract infections (UTI) among CAI (0.15 per 100 patients) and HAI (0.11 per 100 patients).
A threat to patient safety are *Enterobacteriaceae* with additional resistance mechanisms. The overall incidence of additional fluoroquinolone resistance was 0.14 per 100 patients and that of carbapenem resistance was 0.008 per 100 patients. The highest proportion of carbapenem resistance among 3GCRE was observed in CAI caused by *Klebsiella* spp.: 27.6% of the 3GCR *Klebsiella* spp. causing community-acquired lower respiratory tract infections (CA-LRTI) and 20.0% of the 3GCR *Klebsiella* spp. causing community-acquired bloodstream infections (CA-BSI) were additionally resistant to carbapenems.
Comparing the data to the nationwide surveillance system for multidrug-resistant organisms (KISS) shows that the incidences of university hospitals are comparable to those of intensive care units of regular hospitals. Probably, this is due to their position at the end of the treatment chain. On admission, the patients most likely have received previous treatment to a high extent and have higher co-morbidity scores.
Summary. Our analysis shows that German university hospitals have a high incidence of 3GCRE CAI and HAI. Antibiotic management of patients with CAI should take local antibiotic resistance epidemiology into account and antibiotic stewardship measures should also be extended to transferring institutions and outpatient care.

Presentation: Monday, February 19, 2018 from 14:45 – 15:00 in room Hörsaal 3.

Hauptsymposium 02
Antimicrobial Agents and Infectious Diseases (AAID)
19 Febr. 2018 • 16.00–16.30

027/INV
Antibiotic resistance profiling
S. Häußler
Helmholtz Centre for Infection Research, Dept. of Molecular Bacteriology, Braunschweig, Germany

The emergence and spread of resistance limits the use of antibiotics in the treatment and management of microbial diseases. With the aim to develop a molecular tool to identify antimicrobial resistance in bacterial pathogens, we use ultra-deep transcriptome sequencing and combine quantitative data on gene expression profiles with qualitative information about sequence variations. The acquired data serve as a valuable tool for global correlations of resistance phenotypes with the genomic make-up of the clinical multi-drug resistant Gram-negative bacteria. Resistance towards the clinically most important antibiotic classes, β-lactams, aminoglycosides and fluoroquinolones, can be explained in the great majority of the clinical isolates due to sequence variations in target genes, acquisition of antibiotic inactivating enzymes, inactivation of porins and/or overexpression of enzymes that confer antibiotic resistance.

Presentation: Monday, February 19, 2018 from 16:00 – 16:30 in room Audimax Saal.

028/INV
EUCAST – current issues in phenotypic susceptibility testing
G. Kahlmeter
Central Hospital, Department of Clinical Microbiology, Växjö, Sweden

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) continues to determine breakpoints for new agents together with EMA and the pharmaceutical companies seeking license for new antimicrobial agents.
For organisms lacking breakpoints and often AST methods, these are being developed. In the meantime, there is advice on the website on what to do when there are no breakpoints. The current definition of the "intermediate" category is confusing – clinical colleagues avoid it and to resurrect the respect for intermediate the definition needs revision. As resistance and multi-resistance (MDR) increase all over the world, empiric therapy fails increasingly often. This is particularly serious in life-threatening infections such as blood stream infections. While waiting for new methods to speed up both species diagnostics and AST we need to improve the performance of what is available to all of us – phenotypic AST directly from blood culture bottles (BCB). EUCAST is preparing recommendations for direct inoculation of disk diffusion plates from BCBs and has just completed a field trial in 40 laboratories. As part of the problems caused by MDR, accurate colistin AST has grown in importance. Only few techniques are available to us when gradient tests, disk diffusion and agar dilution fail and the quality afforded by the semi-automated machines is in doubt. EUCAST has recently published guidance on how to and how to not perform colistin AST. There are ongoing investigations on the quality of antibiotic disks from different manufacturers and in 2018 we start evaluating Mueller Hinton agars used in disk testing. Finally, betalactam resistance in Haemophilus influenza used to caused mainly by betalactamase production. Now this has changed and the most common mechanisms for resistance towards betalactams, including cephalosporins and carbapenems, are due to mutations in PBP3. The detection of these and the evaluation of their clinical significance is important and EUCAST is trying to develop appropriate techniques for routine AST.

Presentation: Monday, February 19, 2018 from 16:30 – 17:00 in room Audimax Saal.

DGHM-LECTURE: Bacterial toxins - How to outsmart the host
19 Febr. 2018 • 17.00–17.40

029/INV
DGHM Lecture: Controlling nosocomial spread of antibiotic resistance and hospital-acquired infections: what works and why?
M. Bonten
UMC Utrecht, Utrech, Netherlands

During the lecture the dynamics of antibiotic resistant bacteria in healthcare settings will be addressed, both from a theoretical perspective (using mathematical models) and from an applied science perspective (using clinical trials). The focus will be on MRSA and multidrug-resistant Enterobacteriaceae (such as ESBL- and carbapenemase-producing species), and on the most widely propagated infection control measures, including hand hygiene, pre-emptive isolation, screening and decontamination. The motivation for these studies is to define the most (cost-)effective approach to prevent the occurrence of healthcare associated infections and to minimize the transmission of resistant bacteria and resistance genes.

Presentation: Monday, February 19, 2018 from 17:00 – 17:40 in room Audimax Saal.

WORKSHOP 06
From Host Colonization to Invasion (FG MP)
19 Febr. 2018 • 16.00–17.30

030/MPV
RNA-based Infection Research
N. V. Littwin*[1]
[1]*Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*

The Helmholtz Institute for RNA-based Infection Research (HIRI) is a joint venture between the Helmholtz Centre for Infection Research (HZI) in Braunschweig and the Julius Maximilian University of Würzburg (JMU). Located on the Würzburg medical campus, it will be the first research institution worldwide bridging the areas of RNA research and infectious diseases. Scientists at the HIRI will develop innovative RNA-based approaches to elucidate the complex mechanisms of pathogens and the host immune system, down to the single-cell level, and to investigate their translational potential. This talk will introduce the major topics and goals of research at the HIRI.

Presentation: Monday, February 19, 2018 from 16:00 – 16:07 in room Hörsaal 1.

031/MPV
Gene copy number variation allows phenotypic diversification of *S. aureus* lineages of the same clonal population
S. Heilbronner*[1], M. Holden[1], A. Peschel[1]
[1]*University of Tübingen, Infection Biology, Tübingen, Germany*

Introduction: Bacteria can regulate protein expression to adapt to changing environments. However, changes of growth conditions can require upregulation of protein expression that is far beyond of regulatory control. Gene duplication and amplification (GDA) represents a RecA driven mechanism allowing protein overexpression due to gene dosage effects and the mechanism has frequently been described to transfer antibiotic resistance. The detection of GDAs in clinical isolates is technically challenging and the potential of this mechanism to optimize the evolutionary adaption to natural selective pressures such as immune defences is poorly understood.
Aims:
1. Identification of occurrence and frequency of GDAs in *Staphylococcus aureus* USA300 clinical populations.
2. Tracing of GDA development in *in vitro* evolution experiments.
3. Phenotypic characterisation of *S. aureus* copy number variants
Material and Methods: Next generation sequencing (NGS) datasets of USA300 isolates were investigated to identify genomic loci showing alterations in the coverage scaffold, indicating GDAs. Molecular techniques were used to verify copy number variations and to trace GDA development *in vitro*. Cell culture and *in vivo* models were used to identify phenotypic consequences of GDAs.
Results: The analysis of NGS scaffolds indicated that the number of genes within the lipoprotein locus *csa1* varied amongst USA300 clinical isolates. The observation was confirmed using qPCR. Furthermore, *csa1* copy number variation was shown to occur *in vitro* and bacterial culturing resulted in the creation of strains with up 120 copies of *csa1*. Many antibiotics induce SOS-responses, thereby increasing cellular RecA levels. We found that antibiotic pressure increased the amplification frequency tenfold suggesting that GDAs are induced by environmental stress. Amplification of *csa1* had severe phenotypic consequences. The microbial membranes were shown to be heavily loaded with Csa1 proteins resulting in a strongly increased immunostimulatory capacity. Whether this phenotypic switching results in different abilities to overcome immune defences or to colonize different environmental niches is under current investigation.
Summary: We showed that gene copy number variation (i.e. in the *csa1* locus of *S. aureus)* is a common but neglected phenomenon. Investigation of NGS coverage scaffolds can be used to get primary evidence about GDAs. We developed molecular techniques to trace GDAs *in vitro* and showed that their frequency is influenced by antibiotic pressure. Finally we showed that GDAs can have drastic phenotypic consequences that might influence the success of individual lineages arising from a shared clonal background.

Presentation: Monday, February 19, 2018 from 16:07 – 16:21 in room Hörsaal 1.

032/MPV
Colonizing and invasive *Staphylococcus epidermidis* populations: inter- and intra clonal diversity
A. Both*[1], L. Christian[2], A. Zahar[2], G. Kroll[1], H. Büttner[1], H. Rohde[1]
[1]*UK Hamburg-Eppendorf, Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany*
[2]*Helios ENDO Klinik, Hamburg, Germany*

Staphylococcus epidermidis is ranked under the most important pathogens causing hospital-acquired infections. Typically, these infections occur after implantation of medical devices, and association of *S. epidermidis* infections with implanted foreign material relates to the species biofilm forming ability. Contamination of a device during implantation is thought to be the most relevant route of infection. Infecting *S. epidermidis* isolates are usually derived from the patient"s skin microbiome, while transmission from hospital staff is less common. The major aim of this study was to analyze phenotypic and genotypic diversity of colonizing and infecting *S. epidermidis* from patients with prosthetic joint infections. To this end, nasal swabs and intraoperative tissues were prospectively collected from patients with suspected or proven PJI. 15/55 cases revealed growth of *S. epidermidis* from tissues or joint fluids and were included in the analysis. In 15/15 patients *S. epidermidis* was identified in nasal swabs. Per patient, 6 – 15 (mean 13.8) randomly picked *S. epidermidis* colonies grown from primary plates were subjected to pulsed field gel electrophoresis analysis. Here, 1 – 5 different PFGE types were identified per patient. Intriguingly, in 4/15 cases comparative PFGE demonstrated presence of the infecting *S. epidermidis* isolate within the patient´s nose microbiota, indicating an endogenous infection source. Nasal *S. epidermidis* isolates exhibited marked heterogeneous biofilm forming phenotypes. To test if *S. epidermidis* isolated from infections also display population heterogeneity, 10 colonies from primary plates were randomly picked and analyzed. All isolates per patient belonged to an identical PFGE type, demonstrating a monoclonal infection. Further analysis, however, found that isolates from individual patients differed dramatically with regard to their biofilm forming capacity under different growth conditions, susceptibility to antimicrobials and expression of various virulence associated factors. These initial findings suggest that invasive *S. epidermidis* evolve into a heterogeneous population, which could support the survival within hostile environments and establishment of chronic infections. Work is in progress to nail down genetic basis of observed phenotypic patterns and to describe their importance for *S. epidermidis* pathogenesis.

Presentation: Monday, February 19, 2018 from 16:21 – 16:35 in room Hörsaal 1.

033/MPV
How commensal staphylococci adapt to our immune system
M. T. Nguyen*[1], J. Uebele[2], H. Nakayama[3], I. Bekeredjian-Ding[2], F. Götz[1]
[1]*University of Tübingen, Microbial Genetics, Tübingen, Germany*
[2]*Paul-Ehrlich-Institut, Federal regulatory agency for Vaccines and Biomedicines, Langen, Germany*
[3]*Biomolecular Characterization Unit, RIKEN Center for Sustainable Resource Science, Saitama, Japan*

In Gram-positive bacteria, lipoproteins (Lpp) are major players in alerting our immune system. The question is whether commensal staphylococcal species differ in immune activation from a non-commensal species. Here we show that the TLR2 response of *S. aureus* and *S. epidermidis* is almost ten times lower than that of *S carnosus*. The major reason for this is the different modification of the lipid moiety of the Lpp. In *S. aureus* and *S. epidermidis* the N-terminus of the lipid moiety was acylated with a long-chain fatty acid (C17), while in *S. carnosus* it was acylated with a short-chain

fatty acid (C2). The long-chain *N*-acylated Lpp, recognized by TLR2-TLR1 receptors, silenced both innate and adaptive immune response, while the short-chain *N*-acetylated Lpp of *S. carnosus*, recognized by TLR2-TLR6 receptors, boosted it. Here, we unraveled a new mechanism of immune adaptation by commensal staphylococci based on long-chain fatty acid *N*-acylation of Lpp.

References

[1] **Minh-Thu Nguyen,** Julia Uebele, Kumari Nimerta, Hiroshi Nakayama, Lena Peter, Anne-Kathrin Woischnig, Mathias Schmaler, Nina Khanna, Naoshi Dohmae, Bok Luel Lee, Isabelle Bekeredjian-Ding, and Friedrich Götz. How commensal staphylococci adapt to our immune system. (*Revision in Nature Communication*)

[2] Shideh V. Shahmirzadi#, **Minh-Thu Nguyen#** and Friedrich Götz. Evaluation of *Staphylococcus aureus* Lipoproteins: Role in Nutritional Acquisition and Pathogenicity. ***Front. Microbiol.*** 7:1404. doi: 10.3389/fmicb.2016.01404. (#equally contributed).

[3] **Minh Thu Nguyen** and Friedrich Götz. Lipoproteins of Gram-Positive Bacteria: Key Players in the Immune Response and Virulence. ***Microbiol Mol Biol Rev.*** 2016 Aug 10;80(3):891-903. doi: 10.1128/MMBR.00028-16.

Presentation: Monday, February 19, 2018 from 16:35 – 16:49 in room Hörsaal 1.

034/MPV

Quorum sensing "cheaters" within *Staphylococcus aureus* populations are selected under aerobic growth

S. E. George*[1], J. Hrubesch[1], I. Breuing[1], T. Hertlein[2], K. Ohlsen[2], J. Schade[1], C. Weidenmaier[1], C. Wolz[1]

[1]*Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany*

[2]*Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany*

Staphylococcus aureus wields an arsenal of virulence factors to facilitate infection of diverse host environments. Long term infections have been known to display a high degree of heterogeneity within the bacterial population. The driving force behind this adaptive capacity is only partially understood. We hypothesize that the recombination/mutation rate is accelerated during infection in part due to the activation of the SOS response which is induced by antibiotics and host-related factors. We employed evolution and competition experiments to understand the development of genetic variants under varying oxygen environments and subsequently, the implication for the entire population.

We find that non-hemolytic population variants develop rapidly in response to SOS inducing antibiotics. These variants possess a fitness advantage and were found to be defective in function of the quorum sensing system Agr. Despite the increased fitness of Agr variants, a stable heterogeneous community is established over time. The Agr system comprises the secretion and sensing of a small peptide pheromone to form a positive feedback loop which regulates the expression of two main effectors, RNAIII and AgrA. While RNAIII orchestrates the expression of most virulence factors including hemolysins, AgrA directly regulates the expression of small leukolytic peptides, the Phenol-soluble-modulins (PSMs). We therefore evaluated RNAIII and PSM mutants. The PSMs were found to account for the fitness cost associated with Agr; however both were implicated in the selection of Agr variants. Under hypoxia, Agr variants are not selected although general mutation rates are higher and *agr* regulated genes were found to be up-regulated compared to normoxic growth. Furthermore, while the Agr variant has the fitness advantage under aerobic growth, conditions of limited oxygen favor the ancestor. *In vivo*, the maintenance of such diversity could be valuable by increasing the fitness of the bacterial community as a whole.

Presentation: Monday, February 19, 2018 from 16:49 – 17:03 in room Hörsaal 1.

035/MPV

The role of *Salmonella* Pathogenicity Island 2 (SPI2) in the course of neonatal non-typhoidal *Salmonella* infections

K. van Vorst*[1,2], A. Dupont[3], K. Zhang[3], C. Pfarrer[4], U. Repnik[5], G. Griffiths[5], M. Hensel[6], P. Valentin-Weigand[2], M. Nornef[3], M. Fulde[1]

[1]*Freie Universität Berlin, Department of Veterinary Medicine, Institute of Microbiology and Epizootics, Berlin, Germany*

[2]*Institute of Microbiology/ University of Veterinary Medicine Hannover, Hannover, Germany*

[3]*Institute of Medical Microbiology/ RWTH University Hospital, Aachen, Germany*

[4]*Department of Anatomy/ University of Veterinary Medicine Hannover, Hannover, Germany*

[5]*Department of Biosciences/ University of Oslo, Oslo, Norway*

[6]*Institute of Microbiology/ University of Osnabrück, Osnabrück, Germany*

Non-typhoidal *Salmonella* (NTS), such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium, are among the most prevalent causative agents of infectious diarrheal disease in humans and animals worldwide and, in addition, also contribute to invasive infections in human infants. Especially in developing countries with poor hygiene conditions, NTS are frequently isolated from cases of neonatal sepsis and meningitis. The pathogenicity of *Salmonella* is conferred by horizontally acquired chromosomal regions, called Salmonella pathogenicity islands (SPIs). SPIs encode sets of effector proteins, which are delivered into the host cell cytosol via SPI-specific type-three secretion systems. The role of SPI effectors in host-pathogen interactions has been extensively studied *in vitro* and it is widely accepted that SPI2 is crucial for the establishment of an intracellular compartment, the *Salmonella* containing vacuole (SCV), which allows the bacteria to survive and replicate inside the host cell. However, knowledge about pathogenesis and especially contribution of effector proteins to invasive NTS infections *in vivo* is scant. Previous studies rely on adult mouse models and include an antibiotic treatment prior to the infection to deplete the intestinal microbiota. We recently established a neonatal mouse model that allows mucosal barrier penetration and subsequent bacterial dissemination without prior antibiotic treatment. In contrast to adult mice, invasion of the neonatal intestinal epithelium mainly depends on effector molecules encoded by SPI1, as demonstrated earlier. In the present study, we used this infection model to extend our knowledge on the role of individual SPI2 effector proteins in establishment and progression of systemic *Salmonella* infections in the neonate host. Oral infection of neonates with wildtype and SPI2-deficient *Salmonella* resulted in similar bacterial loads of the gastrointestinal tract, but re-isolation rates of SPI2 mutants from systemic organs, such as liver and spleen, were significantly decreased. Interestingly, in contrast to the general understanding of SPI2 as prerequisite for SCV formation *in vitro*, mutants were able to establish and maintain SCVs *in vivo*. In fact, SPI2 deficient bacteria grow to high numbers inside SCVs without harming their respective host cell. By evaluating in total 15 isogenic SPI2 effector protein deficient *Salmonella* strains, we demonstrate that SifA, a SPI2 effector, which is anchored to the SCV´s membrane and allows interaction with the host cell microtubule network, significantly contributes to the SPI2-dependent phenotype *in vivo*. Our results suggest that its lack prevents SCV transmigration from the apical to the basolateral site of the neonate enterocytes and, finally, systemic spread of *Salmonella in vivo*.

Presentation: Monday, February 19, 2018 from 17:03 – 17:17 in room Hörsaal 1.

036/MPV

Multiphoton intravital microscopy (MP-IVM) of *Helicobacter pylori*-leukocyte interaction in the murine stomach

B. Busch*[1], H. Ishikawa-Ankerhold[2], U. Harrison[1], P. Palamides[1], S. Massberg[2], R. Haas[1]
[1]*Max von Pettenkofer Institute, Medical Microbiology and Hospital Epidemiology, München, Germany*
[2]*Hospital of the Ludwig-Maximilians-University, Walter Brendel Centre of Experimental Medicine, München, Germany*

Helicobacter pylori is a human-associated pathogen, that is only found in the human gastric mucosa and colonizes approximately 50% of the world population. The infection with this gram-negative, microaerophilic, spiral-shaped bacterium leads to a chronic bacterial gastritis that may lead to peptic ulcer disease, duodenal ulcer, MALT (mucosa-associated lymphoid tissue) lymphoma or gastric cancer.[1] *H. pylori* is supposed to be acquired in the early childhood and the transmission probably occurs via the oral-oral or fecal-oral route within families.[1],[2] Despite a strong immune response upon infection, *H. pylori* is able to persistently colonize the human gastric mucosa. Therefore *H. pylori* employs various strategies that either help to avoid a proper recognition by the human immune system via pattern recognition receptors (PRR) or that lead to immune tolerance via the induction of regulatory T-cells that suppress an *H. pylori* specific memory T-cell response.[3],[4]
Besides mounting evidence that *H. pylori* actively modulates the human immune system to its own benefit the direct interaction of *H. pylori* with immune cells in vivo is only poorly understood. The aim of this study was to investigate the *H. pylori* leukocyte interaction directly in the living organism. Therefore we established a novel murine stomach model that will allow us to directly observe *H. pylori* leukocyte interactions via the multiphoton intravital microcopy (MP-IVM). We used several mouse reporter strains where specific subsets of leukocytes (neutrophils, macrophages or dendritic cells) are fluorescently labeled. These mice strains were infected with *H. pylori* PMSS1 wt mRFP strain[5] for different time periods (3 h up to 12 weeks). Besides the imaging of a chronic *H. pylori* infection (3-12 weeks infection time), this new approach allows us for the first time to directly observe the initial steps of an *H. pylori* infection *in vivo*.

References

[1] D. B. Polk, R. M. Peek, Jr., *Nature reviews. Cancer* **2010**, *10*, 403-414.
[2] X. Didelot, S. Nell, I. Yang, S. Woltemate, S. van der Merwe, S. Suerbaum, *Proc Natl Acad Sci U S A* **2013**, *110*, 13880-13885.
[3] I. C. Arnold, I. Hitzler, A. Muller, *Frontiers in cellular and infection microbiology* **2012**, *2*, 10.
[4] M. Oertli, M. Sundquist, I. Hitzler, D. B. Engler, I. C. Arnold, S. Reuter, J. Maxeiner, M. Hansson, C. Taube, M. Quiding-Jarbrink, A. Muller, *J Clin Invest* **2012**, *122*, 1082-1096.
[5] D. Keilberg, Y. Zavros, B. Shepherd, N. R. Salama, K. M. Ottemann, *MBio* **2016**, *7*.

Presentation: Monday, February 19, 2018 from 17:17 – 17:30 in room Hörsaal 1.

WORKSHOP 07
Pathogenesis of Gastrointestinal Infections (FG MP/FG GI)
20 Febr. 2018 • 08.30–10.00

037/MPV

Integrating Conjugative Element ICE*Hptfs4* of *Helicobacter pylori* is efficiently transferred in the absence of site-specific integration

C. Spicher[1], E. Weiss[1], R. Haas[1], W. Fischer*[1]
[1]*Max von Pettenkofer Institute, Chair of Medical Microbiology and Hospital Epidemiology, München, Germany*

The human gastric pathogen *Helicobacter pylori* colonizes approximately 50% of the world population, frequently causing chronic gastritis, gastric or duodenal ulcers, but also gastric cancer and MALT lymphoma. While development of gastric cancer has been associated with the activity of the Cag type IV secretion system (T4SS), duodenal ulcers might be associated with the presence of another T4SS, the TFS4 system. The genes encoding this system, including the duodenal ulcer-promoting gene *dupA*, are located on a genome island termed ICE*Hptfs4*, which is frequently present in strains from all *H. pylori* populations [1]. Integration sites of this genome island are highly variable, but short flanking sequence motifs are consistently found, suggesting that horizontal transfer between different *H. pylori* strains is common.
We have previously shown that ICE*Hptfs4* forms a circularized product after excision from the chromosome (depending on the site-specific recombinase XerT), and can be transferred from donor to recipient cells by a conjugation-like mechanism [2]. The aim of this study was to further characterize excision and transfer mechanisms of ICE*Hptfs4*. To do so, we used isogenic donor or recipient strain mutants for mating experiments, and we established a reporter system for ICE excision.
Our results indicate that exchange of ICE fragments by homologous recombination is much more efficient than site-specific integration of the complete element, which seems to be extremely rare under laboratory conditions. Transfer is independent of the ICE*Hptfs4* type IV secretion system in the donor strain, but strictly depends on the DNA uptake and recombination proteins ComEC and RecA in the recipient. Excision of ICE*Hptfs4* does not only depend on XerT, but additionally on a promoter region upstream of the *xerT* gene. Intriguingly, transfer of ICE*Hptfs4* does not require excision, underscoring the highly efficient homologous recombination system.
In conclusion, we have shown that ICE*Hptfs4* is a genome island which can be horizontally transferred by a conjugation-like mechanism which nevertheless depends on the recipient cell transformation and recombination machinery. Although the island is constantly excised from the chromosome, excision is not a requirement for subsequent transfer. Thus, the impact of ICE*Hptfs4* excision on *H. pylori* pathogenicity remains to be shown in future studies.

References

[1] Fischer, W., Breithaupt, U., Kern, B., Smith, S. I., Spicher, C. & Haas, R. (2014). *BMC Genomics* **15**: 310.
[2] Fischer, W., Windhager, L., Rohrer, S., Zeiller, M., Karnholz, A., Hoffmann, R., Zimmer, R. & Haas, R. (2010). *Nucleic Acids Res.* **38**: 6089-6101.

Presentation: Tuesday, February 20, 2018 from 8:30 – 8:45 in room Audimax Saal.

038/MPV

Widely distributed novel type of pili associated with a Shiga-toxigenic and enteroaggregative *E. coli* hybrid strain is essential for autoaggregation and aggregative adherence to epthelial cells

C. Lang*[1], R. Prager[1], A. Fruth[1], E. Tietze[1], G. Holland[2], M. Laue[2], A. Flieger[1]

[1]*Robert Koch Institut, FG11 Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*

[2]*Robert Koch Institut, ZBS4 Special light and electron microscopy, Berlin, Germany*

Introduction: A large outbreak 2011 in Germany associated with the so far highest number of haemolytic uremic syndrome was caused by an enterohemorrhagic *E. coli* (EHEC) of the rare serotype O104:H4. The outbreak strain harboured genes characteristic for both EHEC and enteroaggregative *E. coli* (EAEC). Such seldom described EHEC/EAEC hybrids show a high pathogenicity potential due to the combination of virulence genes. After analysing the EHEC strain collection of the German National Reference Centre for Salmonella and other Enteric Bacterial Pathogens (NRC) for clinical isolates sent to NRC between 2008 and 2012, we found two novel strains exhibiting both EHEC and EAEC marker genes, specifically *stx2* and *aatA*. One of the strains, isolated from a patient with diarrhea in 2012, harboured *stx2b*, was typed as Orough:H-, and belonged to MLST ST26. Interestingly, no aggregative adhesion fimbria (AAF) I-V genes were detected although the strain revealed aggregative adherence similar to the 2011 outbreak strain[1].

Aims: This study aimed to analyse the genome of the Orough:H- EHEC/EAEC strain and to identify genes involved in aggregative adherence. Detected genes were characterized by mutagenesis and phenotypic assays.

Methods: PacBio and Illumina MiSeq whole genome sequencing and sequence analysis including BLAST approaches were performed. Genes of interest were deleted and assays for bacterial autoaggregation, adherence to HEp2 cells, light- and scanning electron microscopy (SEM) were performed. PCR was used to screen for the new genes in AAF/I-V-negative and *aatA*-positive *E. coli* strains.

Results: Genome sequencing confirmed the EHEC/EAEC hybrid nature of the strain and revealed a chromosome of 5.2 Mb and three plasmids, among them an uncommon *aatA*-coding plasmid of 108kb. Instead of AAF/I-V genes, we found genes for a potential novel type of fimbriae, designated as aggregate-forming pili (AFP). The genes were arranged in an operon structure and most coded for proteins with some homology to the bundle forming pilus apparatus of enteropathogenic *E. coli*. Deletion of the *afp*-operon lead to decreased adhesion to HEp2 cells and loss of bacterial autoaggregation. SEM analysis of bacterial aggregates revealed fimbria-like structures largely absent in the *afp*-mutant. Additionally, a variety of *aatA* positive but AAF/I-V negative *E. coli* strains also comprised the novel AFP operon.

Conclusion: The novel EHEC/EAEC strain harboured genes for a new type of fimbria coding on a plasmid together with the *aat* operon typical for EAEC. These genes were essential for bacterial autoaggregation and aggregative adherence to HEp2 cells. Furthermore, they were present in a wide variety of *aatA*-positive intestinal pathogenic *E. coli* from human infections indicating wide distribution of the new fimbria type.

Reference

[1] Prager R, Lang C, Aurass P, Fruth A, Tietze E, Flieger A, 2014. Two novel EHEC/EAEC hybrid strains isolated from human infections. PLoS One.

Presentation: Tuesday, February 20, 2018 from 8:45 – 9:00 in room Audimax Saal.

039/MPV

Oral EPEC infection of the newborn mouse

A. Dupont*[1], A. Pütz[1], A. Rosinski[1], U. Repnik[2], I. Rosenshine[3], M. Hornef[1]

[1]*University Hospital Aachen, Institute of Medical Microbiology, Aachen, Germany*

[2]*Oslo University, Section for Physiology and Cell Biology, Oslo, Norway*

[3]*Hebrew Univeristy of Jerusalem, Institute for Microbiology and Molecular Genetics, Jerusalem, Israel*

Enteropathogenic *Escherichia coli* (EPEC) is a major causative agent of infantile diarrhea in developing countries. Despite extensive *in vitro* studies, the investigation of the host-pathogen interaction *in vivo* has been hampered by the lack of a suitable small animal model. Recently, we developed a neonatal mouse model to investigate EPEC infection *in vivo*. Infection of newborn mice led to spontaneous gut colonization with intimate attachment of EPEC microcolonies to the intestinal mucosa (A/E lesions). Here, we further describe the host response (cell death, signal transduction and inflammasome activation) to EPEC infection and characterize the role played by the intestinal epithelial and hematopoietic cell compartments using specific knockout mouse lines.

Reference

[1] Dupont A, Sommer F, Zhang K, Repnik U, Basic M, Bleich A, Kühnel M, Bäckhed F, Litvak Y, Fulde M, Rosenshine I, Hornef MW, 2016, Age-dependent susceptibility to Enteropathogenic Escherichia coli (EPEC) infection in mice, *PLoS Pathog*, 12:e1005616.

Presentation: Tuesday, February 20, 2018 from 9:00 – 9:15 in room Audimax Saal.

040/MPV

In search for the factors contributing to *Escherichia coli* O104:H4 exceptional pathogenicity

N. Haarmann*[1], P. Berger[1], I. Kouzel[1], M. Berger[1], H. Karch[1], A. Mellmann[1]

[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*

Introduction: *Escherichia coli* O104:H4 (*E.coli* O104:H4) was identified as the pathogen causing the largest outbreak of bloody diarrhea and haemolytic syndrome (HUS) in Germany in 2011. It is hypothesized that the exceptional pathogenicity of this strain derives from its hybrid character with virulence determinants originating from enterohemorrhagic *E. coli* (EHEC; Shiga toxin) and enteroaggregative *E. coli* (EAEC; aggregative adherence fimbriae I), respectively.

Objectives: Here, we address the question if *E. coli* O104:H4 displays virulence traits superior to the ones of regular EAEC strains, which could thus contribute to its exceptional pathogenicity.

Materials and Methods: In this study, we compare the *E. coli* O104:H4 strain C227-11 Φcu (cured of the Shiga toxin-encoding phage) with the prototypical EAEC strains 55989, 042, 17-2. The strains were characterized using adherence, biofilm, motility, acid resistance, ELISA IL-8 and cytotoxicity assays. Furthermore, the production of virulence factors in different growth media was measured using semi-quantitative Western Blot analysis.

Results: The outbreak strain C227-11 Φcu and the EAEC strain 17-2 adhere strongest to epithelial cells. EAEC 042 is the best Biofilm producer of the characterized strains, as well as displays the strongest motility and triggers the strongest IL-8 response. C227-11 Φcu and EAEC 55989 behave similarly in most phenotypical assays, which is in agreement with their close genotypic relationship. The expression of the majority of analysed virulence factors is triggered by SCEM medium which mimics the conditions in the colon.

Summary: Our phenotypical characterization lead us to the conclusion that *E. coli* O104:H4 displays strong but not exceptional virulence traits when compared to the analyzed EAEC strains. Thus our data indicate that the synergistic effect of EHEC and EAEC virulence factors rather than a superior EAEC

phenotype contribute to the exceptional pathogenicity of the 2011 outbreak strain.

Presentation: Tuesday, February 20, 2018 from 9:15 – 9:30 in room Audimax Saal.

041/MPV

Arginase promotes acute intestinal inflammation

M. Gänsbauer[1], J. Baier[1], A. Ensser[2], J. Mattner*[1]
[1]*Klinische Mikrobiologie Erlangen, Erlangen, Germany*
[2]*Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Virologisches Institut, Erlangen, Germany*

Introduction: The enzymes arginase 1 (Arg1) and the inducible nitric oxide synthase (iNOS) compete for the common substrate L-arginine. Whereas Arg1 converts arginine to ornithine and urea, iNOS produces citrulline and nitric oxide (NO). NO itself exhibits anti-microbial functions, but nitrate can also be used by distinct bacteria as substrate for their nitrate respiration.
Aims: Patients with inflammatory bowel diseases (IBD) unexpectedly exhibit both a higher Arg1 and iNOS activity and expression in the intestinal submucosa and the intestinal vasculature in comparison to non-IBD patients, likely due to an unlimited availability of L-arginine in the gut. As alterations in the composition of the intestinal microbiota are characteristic for IBD patients and animal models of acute and chronic colitis, we aimed to characterize in the present study the mutual interactions between Arg1 and iNOS and the intestinal microbiota and to identify the cell compartment, which mediates arginase-dependent effects on intestinal inflammation.
Materials and Methods: The expression of Arg 1 and iNOS, the composition of the intestinal microbiota, bacterial replication and dissemination as well as the extent of intestinal inflammation was assessed in IBD patients and in mouse models of acute and chronic dextran sodium sulfate (DSS) and *Salmonella typhimurium* induced colitis using immunohistochemistry, conventional plating assays, 16S rRNA sequencing, high resolution endoscopy and different conditional Arg1-knockout mice.
Results: Tie2-Cre x Arg1fl/fl mice that lack Arg1-expression in hematopoietic and endothelial cells unexpectedly developed less severe colitis than wild type littermate controls upon DSS application or infection with *Salmonella typhimurium*. The protection from colitis correlated with alterations in the composition of the intestinal microbiota. Furthermore, the endothelial permeability, bacterial dissemination, leukocyte adhesion and the inflammatory immune response of myeloid cells were significantly reduced in Tie2-Cre x Arg1fl/fl mice compared to wild type littermate controls. Fecal transfers into broad-spectrum antibiotic-treated B6 recipient reconstituted this phenotype suggesting that an altered microbiota in Arg1-deficient Tie2-Cre x Arg1fl/fl mice decreases the susceptibility of mice to DSS- or infection induced intestinal damage.
Summary: In summary these data suggest that Arg1 promotes an accumulation and a systemic dissemination of intestinal bacteria and subsequent inflammation due to the induction of endothelial dysfunction and dysregulated myeloid cell responses.

Presentation: Tuesday, February 20, 2018 from 9:30 – 9:45 in room Audimax Saal.

042/MPV

Exploring Oligo-MM12 mice to study precision microbiome reconstitution against *Clostridium difficile* infections

N. Smit*[1], E. Galvez[1], B. Stecher[2], T. Clavel[3], T. Strowig[1]
[1]*Helmholtz-HZI, Microbial Immune Regulation Research Group, Braunschweig, Germany*
[2]*Max von Pettenkofer Institute, Medical Microbiology and Hospital Epidemiology, München, Germany*
[3]*Uniklinik RWTH Aachen, Functional Microbiome Research Group, Aachen, Germany*

Introduction: *Clostridium difficile* is gram-positive, spore forming bacteria, which causes severe diarrhea and life-threatening complications in infected individuals. In many developed countries, *C. difficile* infection (CDI) is now the major cause of antibiotic-associated nosocomial infections. The standard treatment for CDI are antibiotics, however, up to 30% of patients are suffering from recurrent CDI. Transfer of fecal material from healthy individuals is able to cure CDI, demonstrating that the microbiota serves an important role in preventing CDI. Recent studies have showed that conversion of primary to secondary bile acids (secBA) by commensals is important for this colonization resistance (CR).1 Moreover, restoration of secBA production by microbiota engineering is sufficient to reduce susceptibility to CDI in mice. However, restoration of secBA may not be sufficient to recover CR, but rather delays CDI2.
Objectives: We want to explore gnotobiotic mouse models to investigate the interplay of secBA-producers (secBAP) with other commensals. Specifically, we aim to identify bacteria that cooperate with secBAP to inhibit the germination and growth of *C. difficile.*
Material and Methods: We are using gnotobiotic mouse model harboring twelve mouse-microbiota derived bacteria (Oligo-MM12: OMM12), but lacking bacteria that produce secBA.3,4 Additional commensals are derived from the MiBC and our internal strain collection.5 OMM12 mice were infected with *C. difficile* (VPI 10463). CDI was monitored by plating on selective agar and ELISA for inflammatory markers.
Results: OMM12 mice infected with *C. difficile* do not show any CR, even without antibiotic pretreatment. To examine the effect of specific bacteria on the CDI, the OMM12 mice were in addition colonized: group 1 received secBAP, group 2 received a consortium of eight bacteria and group 3 received both. Upon infection, the mice from group 3 showed the highest protection against CDI 3 d.p.i. as demonstrated by reduced colonization and no body weight loss. While group 1 did have a better protection than the OMM12, it was higher colonized than group 3 on 1 d.p.i. The protection in group 1 was further decreased 3 d.p.i. This suggests that supplementing secBAP with additional commensals results in improved and extended protection against CDI.
Conclusion: In our study we implemented a gnobiotic mouse model that will aid in the search for bacteria that confer a heightened CR against CDI. Even though secBAP were shown in our study to provide partial protection similar to what other (pre)clinical studies have demonstrated, their sole transfer does not provide an extended protection against CDI. This leads to our working hypothesis that secBAP need other bacteria to keep the colonization stable, or that other bacteria additionally target *C. difficile*.

References

[1] Winston, *Anaerobe,* 2016
[2] Buffie, *Nature,* 2015
[3] Brugiroux, *Nat.Microbiol.* 2016
[4] Studer, *Front.Cell.Infect.Microbiol.* 2016
[5] Lagkouvardos, *Nat.Microbiol. 2016*

Presentation: Tuesday, February 20, 2018 from 9:45 – 10:00 in room Audimax Saal.

WORKSHOP 08
Implementation Science and Experiences (StAG HY)
20 Febr. 2018 • 08.30–10.00

043/PRV
Contributions of psychology and behaviour change to implementing nosocomial infection prevention: via hand hygiene "only"? A narrative review of reviews

T. von Lengerke*[1], B. Lutze[2], I. Tomsic[1], I. F. Chaberny[2]
[1]*Hannover Medical School, Centre for Public Health and Healthcare, Medical Psychology Unit , Hannover, Germany*
[2]*Leipzig University Hospital, Department of Diagnostics, Institute of Hygiene, Hospital Epidemiology and Environmental Health, Leipzig, Germany*

One main reason for nosocomial infections (NI) are human factors.[1-2] Conceptualizing compliance with NI prevention measures as behaviour is crucial in order to base implementation interventions on psychology as *the* science of human behaviour.[3] The measure to which psychological theory/behaviour change have been applied mostly is hand hygiene.[3-4] We explore whether other measures have received such scientific scrutiny as well.
For 1990-99, 2000-09 and 2010-17(Oct), we searched PubMed for reviews on NI to which (cross infection OR infection prevention), (psychology OR behaviour), (compliance/adherence OR implementation), and (implementation interventions) applied as conditions, operationalized by appropriate MeSH terms/text strings. In the first analysis reported here, reviews were categorized by title focus on hand hygiene, antibiotic prescribing/stewardship (p./s.), other measures, or general topics (e.g. organization).
Six reviews were included for 1990-99, 47 for 2000-09, and 44 for 2010-17(Oct; by extrapolation, 56 reviews may be expected until 2019). As Fig. 1 shows, while the rate of reviews with general topics remained relatively stable (since 2000), that of reviews on hand hygiene declined (1990-99: 67%, 2000-09: 45%, 2010-17[Oct]: 30%). In contrast, rates of reviews on antibiotic p./s. and other topics, while on lower levels of up to 14% (antibiotic p./s. in 2010-17(Oct)), have increased.
Psychology/behaviour change are increasingly recognized in NI prevention research and implementation. While initially confined to hand hygiene, reviews have increasingly focused on antibiotic p./s. and other topics, e.g. vaccination and catheter insertion. This may also stimulate and feed into a general psychological theory of guideline adherence in the prevention of NI.[5] Also, further analyses on the evidence for specific psychological and behaviour change approaches reported in the reviews is needed, and under progress.

References

[1] Geffers C, Rüden H, Gastmeier P. Nosokomiale Infektionen. Berlin: Robert Koch-Institut; 2002
[2] Paitoonpong L, Wong CKB, Perl TM. Healthcare-associated infections. In Nelson KE, Masters Williams C, eds. Infectious disease epidemiology: theory and practice. 3rd ed. Burlington, MA: Jones & Bartlett; 2014. p. 369-463
[3] Edwards R, Charani E, Sevdalis N, et al. Optimisation of infection prevention and control in acute health care by use of behaviour change: a systematic review. Lancet Infect Dis 2012;12:318-29
[4] Srigley JA, Corace K, Hargadon DP, et al. Applying psychological frameworks of behaviour change to improve healthcare worker hand hygiene: a systematic review. J Hosp Infect 2015;91:202-10
[5] von Lengerke T, Lutze B. Application of psychological theories to organisational behaviour: the case of professional adherence to guidelines in health care. In Körner M, Kowalski C, eds. Organizational behaviour in health care institutions in Germany: theoretical approaches, methods and empirical results. in press

Figure 1

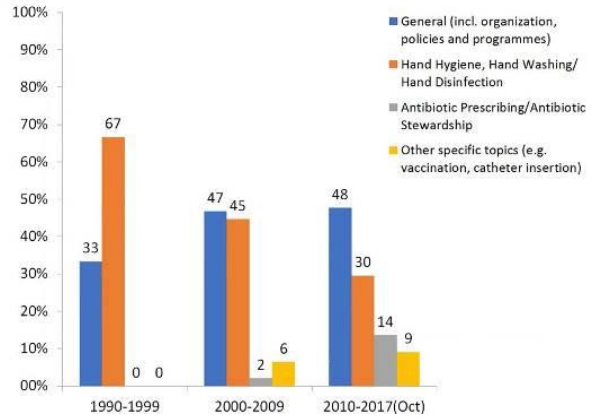


Fig. 1: Proportions of reviews related to nosocomial infection prevention which include psychology and behaviour change but differ in focus (by title), 1990-2017

Presentation: Tuesday, February 20, 2018 from 8:45 – 9:00 in room Hörsaal 1.

044/PRV
Goal setting and performance feedback as components of a successful intervention to improve hand hygiene

S. Diefenbacher*[1], P. Fliß[2], J. Tatzel[3], J. Wenk[2], J. Keller[1]
[1]*Ulm University, Social Psychology, Ulm, Germany*
[2]*Bode Chemie GmbH, BODE SCIENCE CENTER, Hamburg, Germany*
[3]*Hospitals Heidenheim, Institute for Infection Control, Heidenheim, Germany*

Introduction: Hand hygiene (HH) plays an important role in infection prevention but is often suboptimal. New approaches to improve HH are needed.
Aims: To test the potential of goal setting and performance feedback in improving HH.
Materials and Methods: A prospective controlled intervention study was conducted at four non-intensive care units at a German hospital. Before, new dispensers allowing for electronic counting of dispenser usage were installed on all four units and staff was informed about the upcoming study. The study involved four phases: habituation (T1), baseline (T2), intervention (T3) and post-intervention (T4). Dispenser usage was recorded continuously. In addition, randomly sampled direct observation of hand hygiene compliance (HHC) by trained external observers was conducted on 10 days during each phase. The main outcome measure was the phases" mean dispenser usage per day and patient room.
Each unit was assigned to one of four conditions: goal setting, performance feedback, both goal setting and performance feedback or none (control).
The goal-setting procedure consisted of a guided team meeting at both units in which HCWs were informed about the unit"s observed HHC rate in T2 and the importance of achieving at least 80% HHC in order to effectively reduce nosocomial infections. Then staff discussed the HHC goal they wanted to achieve during the next four weeks, before everyone indicated a goal proposal. As final HHC goal the mean of all individual proposals was used, noted on a poster (see Fig. 1) and put up in the staff room.
Performance feedback was provided at staff rooms on a newly installed monitor (see Fig. 2) and presented as the 7-days mean HHC rate. The rate was estimated combining dispenser usage and the data collected during direct observation in T2.
Results: In the goal setting condition dispenser usage significantly increased from baseline to intervention phase, however, the effect did not last after the end of the intervention (M_{T1} = 6.9, M_{T2} = 7.2, M_{T3} = 9.6, M_{T4} = 7.5). In the performance feedback condition the increase from T2 to T3 was only marginally significant and was again not sustained in T4 (M_{T1} = 8.3, M_{T2} = 6.9, M_{T3} = 9.5, M_{T4} = 8.7). However, in the combined condition dispenser usage did not only increase significantly from T2 to T3, but was still significantly elevated in the post-intervention phase (M_{T1} = 8.6, M_{T2} = 7.9, M_{T3} = 17.0, M_{T4} = 12.9). The control condition experienced an

unexpected increase during the baseline phase (M_{T1} = 8.4, M_{T2} = 10.2, M_{T3} = 8.2, M_{T4} = 7.1).

Summary: This prospective controlled intervention study provides evidence for the relevance of goal setting and performance feedback in improving HH, particularly when both are combined. Specifically, elevated HH was observed during the intervention phase (compared to baseline) for all experimental conditions; in the combined condition, the improvement was sustained even after the intervention had ended.

Figure 1

Figure 2

Presentation: Tuesday, February 20, 2018 from 8:30 – 8:45 in room Hörsaal 1.

045/HYV

Self-reported guideline compliance for the prevention of surgical site infections: How does medical staff assess SSI-preventive implementation?

B. Lutze*[1], M. Vielstich[1], I. Hartlep[1], T. von Lengerke [2], A. Schachtrupp[3], I. F. Chaberny[1]

[1]*Leipzig University Hospital, Department of Diagnostics, Institute of Hygiene, Hospital Epidemiology and Environmental Health, Leipzig, Germany*

[2]*Hannover Medical School, Centre of Public Health and Healthcare, Medical Psychology Unit, Hannover, Germany*

[3]*B. Braun Foundation, Melsungen*

Surgical site infections (SSI) have been identified as the most common type of nosocomial infections.[1] A recent systematic review showed that the use of SSI-bundles significantly reduced SSI-risk after colorectal surgery by 40%.[2], However, guideline publication is not necessarily sufficient for implementing its measures.[3-4] Preventive measures often are known to medical staff, but implementation in clinical routine is more difficult. To our knowledge, there is no data in Germany on SSI-preventive compliance. Thus, we aimed to assess self-reported guideline compliance for SSI-prevention.

At a nursing congress in Kassel (2017), medical staff was surveyed via a self-administered questionnaire. Participants assessed implementation of preventive measures on their ward (e.g. checklists, hand hygiene observations, MRSA screening, dressing changes, drainage [5]) on 7-point Likert scales (1 "not at all"-7 "fully"). Due to the target group (no surgical staff), the survey focused exclusively on preventing SSI by pre- and postoperative measures.

N=255 questionnaires were analysed (nurses: 80%). Routine MRSA screening was assessed as most frequent (mean [M]=5.5). Respondents also reported that they change drainages in a timely manner after operations (M=4.9), and increased hand hygiene after operations (M=4.8). Information about dressing changes after operations were self-reported at a moderate level (M=4.6). Use of checklists and hand hygiene observations by external infection control specialists on wards were rated lowest (both M= 3.7).

Self-reported reported compliance regarding SSI-preventive measures varied systematically. Eventually, the present data may provide one basis for developing educational reflection tools (shown to be effective in hand hygiene promotion [6]) to enhance SSI-guideline compliance. That is, self-reported compliance should be contrasted with compliance data gathered by standardized observation in future research and practice.

References

[1] Behnke M et al. Nosocomial infection and antibiotic use—a second national prevalence study in Germany. Dtsch Arztebl Int 2013;110:627-33

[2] Zywot A et al. Bundles prevent surgical site infections after colorectal surgery: meta-analysis and systematic review. J Gastrointest Surg 2017;21:1915-30

[3] Francke AL et al. Factors influencing the implementation of clinical guidelines for health care professionals: a systematic meta-review. BMC Med Inform Decis Mak 2008;(8):38

[4] Flodgren G et al. Tools developed and disseminated by guideline producers to promote the uptake of their guidelines. Cochrane Database Syst Rev 2016;(8):CD010669

[5] Working Group 'Hospital & Practice Hygiene' of AWMF. Strategien zur Prävention von postoperativen Wundinfektionen. AWMF-Leitlinien-Registernr. 029/031. Available at http://www.awmf.org [6] von Lengerke T. et al. Promoting hand hygiene compliance: PSYGIENE—a cluster-randomized controlled trial of tailored interventions. Dtsch Arztebl Int 2017;114:29-36

Presentation: Tuesday, February 20, 2018 from 9:00 – 9:15 in room Hörsaal 1.

046/PRV

Evidence of the medical and economic benefits of implementing hygiene measures by hygienic physicians in trauma surgery/orthopaedics: study design for a multicentre prospective cohort study

M. Strybos*[1], R. Otchwemah[1,2], B. Bouillon[2], P. Recht[1], B. Marche[2], C. Kugler[3], C. Alefelder[4], D. Sauerland[5], H. Paul[6], U. Ligges[7], F. Mattner[1]

[1]*Kliniken der Stadt Köln gGmbH, Institut für Hygiene, Köln, Germany*

[2]*Kliniken der Stadt Köln gGmbH, Klinik für Orthopädie, Unfallchirurgie und Sporttraumatologie, Köln, Germany*

[3]*Albert-Ludwigs-Universität Freiburg, Institut für Pflegewissenschaften, Freiburg i. Br., Germany*

[4]*Helios Universitätsklinikum Wuppertal, Krankenhaushygiene, Wuppertal, Germany*

[5]*Universität Witten/Herdecke, Fakultät für Wirtschaftswissenschaft, Witten, Germany*

[6]*Kliniken der Stadt Köln gGmbH, Zentralapotheke, Köln, Germany*

[7]*TU Dortmund, Statistisches Beratungs-und Analysezentrum, Dortmund, Germany*

Background: The KRINKO recommends nominating one authorized medical specialists in every medical department as a hygiene physician. The function of the hygiene physician has been roughly described, but no detailed explanation about the tasks assumed can be found. Moreover, there are no data for their contribution to the prevention of infections or antibiotic resistance in Germany. Within the BMG-funded project "HygArzt" (ZMVI1-2516FSB111) the effectiveness, efficiency, and the associated medical and economic advantageousness, as well as the implementation of hygiene measures by hygienic physicians in trauma surgery/orthopaedics, are to be proven.

Methods/design: This multicentre prospective cohort study will compare the initial situation, before any intervention has been made, with the outcome after the interventions. The study period is three years, divided into eight study phases taking place at three different study locations. During this trial 97 Outcome-parameter including process-based (adherence measurement of the measures introduced e.g. antiseptic washing or use of antiseptic nasal ointment), aggregated and related data at departmental levels should be collected and investigated. This data collected from various sources or sub-studies and will be recorded in a newly created database. Patient-related data are used as primary and secondary data, which are routinely collected in accordance with HygmedVoNRW §8 and the §23 Protection against Infection Act. Furthermore, standardized protocols according to various guidelines (e.g. perioperative antibiotic administration, hand hygiene, dressing change, drainage removal and external fixator care) will also be considered. After the successful introduction and implementation of preventive measures in the pilot hospital, the concept (best practice model) will be transposed to the other two study hospitals to confirm the transferability. After that a further evaluation of the implementations, its success and the impact on the infection rates is planned. Finally, a subject-specific modular training program will be developed based on all study"s results and offered to hygiene physicians of other hospitals.
Discussion: The collected data are intended to provide evidence that, the implementation of specific preventive measures by hygienic physicians results in a reduction of nosocomial infections and multi-resistant pathogens as well as a reduction in the consumption of antibiotics. For the first time within a study focuses on the hygienic physician as key driver for the implementation of hygienic preventive measures. The applied Train-the-Trainer approach is intended to achieve a rapid implementation of preventive measures. Furthermore, a general best-practice model focusing adherence-based hygiene measures will be developed in order to be provided to other hospitals or medical disciplines.

Presentation: Tuesday, February 20, 2018 from 9:15 – 9:30 in room Hörsaal 1.

047/PRV

Infection control in burn units – experiences made during a MRSA outbreak

C. Baier*[1], R. Ipaktchi[2], E. Ebadi [1], A. Limbourg[2], T. R. Mett[2], P. M. Vogt[2], F. C. Bange[1]
[1]*Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[2]*Hannover Medical School, Department of Plastic, Aesthetic, Hand and Reconstructive Surgery, Burn Center, Hannover, Germany*

Introduction: Colonization and infection with Methicillin-resistant *Staphylococcus aureus* (MRSA) in burn patients is often described in literature, e.g. [1]. Moreover, nosocomial outbreaks with MRSA occur in burn units, e.g. [2]. We also experienced an outbreak including 8 burn patients [3]. Here we demonstrate the current infection control concept in our burn unit, which was enhanced during the MRSA outbreak [3].
Methods: We performed a molecular (PFGE and Spa-Typing) and epidemiologic investigation of our MRSA outbreak. Moreover we implemented several infection control measures during the outbreak and enhanced our existing infection control concept due to the experiences made.
Results: Our burn unit is a tertiary referral center with six separate single bed patient rooms. Preemptive barrier precautions were already in place before the outbreak occurred.
Molecular analysis (PFGE and Spa-Typing) revealed, as suspected, the monoclonal spread of a unique outbreak strain. Our investigation showed that most probably MRSA colonized patients themselves were the reservoir for the ongoing MRSA spread. We believed that MRSA transmission occurred from patient-to-patient.
For outbreak control we implemented (among others) the following interventions:

- Restriction in hydrotherapy use for MRSA positive patients and enhanced disinfection and cleaning procedure.
- Hand hygiene compliance measurements.
- Restrictions of admissions and short term unit closure.
- Weekly MRSA prevalence screening.
- Repeated Training sessions for the staff.

In addition, in the late phase of the outbreak continous (intact) skin and mucosa decolonization with octenidine was implemented for all patients.
Summary: Although nosocomial MRSA acquisition rates decrease in Germany in the recent years, MRSA still is a relevant challenge, especially in a high risk population as burn patients. We adapted our infection control due to experiences made in a MRSA outbreak. It now includes for instance routine admission screening and targeted weekly prevalence screening in case of a MRSA positive sample. This measure enables early detection of transmission events and advanced measures can be implemented quickly if necessary. As described in literature, we experienced as well that (short term) ward closure may be a very effective measure in outbreak termination.

References

[1] Bahemia IA., et al. Microbiology and antibiotic resistance in severe burns patients: A 5 year review in an adult burns unit. *Burns* 2015;41(7):1536–42.
[2] Garvey MI., et. al. Improved understanding of an outbreak of meticillin-resistant Staphylococcus aureus in a regional burns centre via whole-genome sequencing. *J Hosp Infect* 2016;94(4):401–4.
[3] C. Baier, et al. A multimodal infection control concept in a burn intensive care unit – lessons learnt from a meticillin-resistant Staphylococcus aureus outbreak, *J Hosp Infect*, 2017, Doi: 10.1016/j.jhin.2017.07.023.

Presentation: Tuesday, February 20, 2018 from 9:30 – 9:45 in room Hörsaal 1.

048/HYV

Bacterial contamination of central intravenous catheters and administration sets

J. Menger *[1], K. Rosin[1], M. H. Schulze[2], P. Fehling[1], M. Kaase[1], O. Moerer[3], S. Scheithauer[1]
[1]*University Medical Center, Department for Infection Control, Göttingen, Germany*
[2]*University Medical Center, Institute for Medical Microbiology, Göttingen, Germany*
[3]*University Medical Center, Department of Anaesthesiology, Göttingen, Germany*

Introduction: Catheter-related bloodstream infections (CRBSI) are important health-care associated infections. CRBSIs might emanate from the insertion site or from contaminated hubs. In addition, intravenous catheters might be colonized following bacteremia or fungemia. The impact of ascending bacterial or fungal contamination within the tubing is unclear and only few studies have examined the pattern of contamination in the administration set.
Aims: To characterize the extent of bacterial contamination in administration sets at the time of removal of central venous catheters.
Material and Methods: Two intensive care units participated in this study. Only catheters removed for suspected CRBSI were included. Blood cultures drawn around the time of suspicion for CRBSI and catheter tips were processed according microbiological-infectiological quality standards. For the administration set, qualitative culture was performed by flushing defined segments with tryptic soy broth, incubation for up to 7 days and cultivation on Columbia blood agar in case of turbidity. Species identification was done by MALDI-TOF or biochemical methods.
Results: Bacteria or fungi were found in 8 out of 44 catheter tips. In total 40 out of 879 cultured segments of the administration set (4.6%) and a total of 12 out of 45 administration sets (26.7%), respectively, showed bacterial growth. Bacteria were even found within the administration set, e. g. in 6.2% of the hubs and in 1.7% of the tubing. The same bacterial species in adjacent segments of the administration set was found in seven cases and up to four

adjacent segments were affected by species such as *Enterobacter cloacae* (n = 2), *Escherichia coli* (n = 1), *Klebsiella pneumoniae* (n = 2) or *Staphylococcus epidermidis* (n = 2).
Conclusion: As expected, unequivocal microbiological results suggesting a definite catheter-related infection were found only in a minority of episodes with catheter removal for suspected CRBSI. Irrespective of this, the rate of contaminated segments was high and the finding of identical bacterial species even in distal parts of the administration set in adjacent segments emphasizes the role of either ascending or descending spread of bacteria or fungi within the tubing. This study highlights the importance of aseptic techniques when accessing stopcocks to prevent contamination. Caution should be exercised when deciding not to replace intravenous medications after removal of a venous line, since ascending spread of contaminating bacteria cannot be excluded.

Presentation: Tuesday, February 20, 2018 from 9:45 – 10:00 in room Hörsaal 1.

WORKSHOP 09
From Hearts to Bones and into the Cell (StAG KM)
20 Febr. 2018 • 08.30–10.00

049/KMV
International Survey of Clinical Practice re Perioperative Antibiotic Prophylaxis in Orthopaedic Surgery in Europe
C. Berberich*[1], N. Renz[2], A. Trampuz[2]
[1]*Heraeus-Medical GmbH, Training/Med. Education, Wehrheim, Germany*
[2]*Charité - Universitätsmedizin, Berlin, Centrum für Muskuloskeletale Chirurgie, Berlin, Germany*

Introduction: Due to the easy bacterial colonization of implants, perioperative antibiotic prophylaxis (PAP) is considered mandatory in arthroplasty. However, increasing antibiotic resistancies & higher patient infection risks have raised concerns about the adequacy of current perioperative antibiotic prophylaxis (PAP) strategies.
Aim: To determine & compare the current clinical PAP practice before arthroplasty surgery in university & community hospitals across Europe.
Method: In an anonymous web-based survey, 18 questions were submitted to >2000 registered users of the PRO-IMPLANT Foundation (www-pro-impant-foundation.org) in Feb. 2017.
Results: A total of 358 questionnaires were received & analysed. Countrywise, the majority of answers came from Germany (n=141), Spain (n=38) and Switzerland/Austria (n=37). 249 (70%) questionnaires were filled out by surgeons, 85 (24%) by infectious disease specialists & microbiologists. Most participants work in university or larger community hospitals (>80%).
Key Observations:
1. Cephalosporins (1./2. gen.) are still most widely used in routine PAP (94%).
2. First alternative antibiotic of choice are glycopeptides (65%, in Spain 85%), followed by clindamycin (40%, in Spain 6%)
3. Single shot prophylaxis was reported by 62%. In Spain, however, most participants (70%) favor multiple doses (15% even beyond 24h).
4. Customization of PAP in response to bacterial resistance (MRSA, GNB) was reported by 33% (in Spain 53%).
5. Customization of PAP because of higher infections risks was reported by 72% (in Spain 84%). Main reasons were: septic revisions (80%), long duration of surgery (65%), unclear MRSA status (51%).
6. Dual PAP was considered in determined risk situations in 51% (in Spain 77%). A combination of glycopeptide & cephalosporin was then preferred (57%).
7. Use of a combination of systemic and local antibiotics was reported by 87%. Antibiotic-loaded bone cement (ALBC) with gentamicin was the preferred choice as local antibiotic carrier (92%). 50% use high dose ALBC in high risk patients & septic revisions.
Conclusions: The clinical practice reflects guideline recommendations in many countries (except UK) and a standard PAP prevails. However, deviations from standard (PAP customization/dual antibiotics) are frequently performed in response to antimicrobial resistance & infections risks. This trend was more pronounced in the South of Europe (e.g. Spain), which may be explained by the higher prevalence of multiresistant pathogens.

Presentation: Tuesday, February 20, 2018 from 8:30 – 8:43 in room Hörsaal 3.

050/KMV
Development of a database on periprosthetic joint infections
P. Rämer*[1], C. Suren[2], C. Querbach[3], R. Bernard[3], R. von Eisenhart-Rothe[2], H. M. L. Mühlhofer[2], D. Busch[1]
[1]*Technische Universität München, Institut für medizinische Mikrobiologie, Immunologie und Hygiene, München, Germany*
[2]*Klinikum rechts der Isar, Klinik und Poliklinik für Orthopädie und Sportorthopädie, München, Germany*
[3]*Klinikum rechts der Isar, Krankenhausapotheke , München, Germany*

Question: Periprosthetic joint infections (PJIs) pose a multidisciplinary challenge. In addition to the surgical difficulties faced, the need for long term antimicrobial therapy demands critical evaluation of the potential development of antimicrobial resistances, bio-availability at the site of infection and long-term tolerability. Moreover, in an ageing patient cohort drug-interactions with pre-existing medication need to be addressed carefully. Finally, once a multidisciplinary consensus on therapeutic modalities is reached, this consensus needs to be communicated to all parties involved in the care of PJI patients.
The address the aforementioned challenges the members of the PJI board review at the "Klinikum rechts der Isar" developed a database on PJIs.
Aims: The aims of the planned database were:
1) Prospective survey of all patients suffering from PJI that are/were treated at the Clinic and polyclinic for orthopedics and sports orthopedics at the "Klinikum rechts der Isar". This survey included relevant data on the implant, medical pre-conditions, risk factors and diagnostic as well as therapeutic procedures carried out before the consultation of the "Klinikum rechts der Isar".
2) Prospective survey of all microbial pathogens found during the diagnostic assessment oft he PJI.
3) Prospective survey of the antiinfective therapy during the multidisciplinary treatment of the PJI.
4) Documentation of the board review decisions based on 1) - 3) and (real-time) communication of these to all parties involved in the patient care.
5) Possibility to retrospectively amend PJI cases that were treated at the "Klinikum rechs der Isar".
Methods: Starting September 2016 the PJI board review (Clinic for orthopedics, Institute for Medical Microbiology, Pharmacy) had bi-weekly planning meetings which were joined by a member of IT services. In this initial phase we defined the clinical and scientific needs the database as well as the quality and quantity of the surveyed data. Thereafter each specialty designed a specific user interface for the respective data. User interfaces were presented and discussed in the board review. The database is SQL based and uses Microsoft Access interfaces. Analysis of the data is performed using QlikView (Radnor, PA, USA).
Results: During the first phase we designed 14 user interfaces that allow us to characterize the infection and its therapy. These interfaces allow us to generate a HTML file in real-time, that can be accessed by medical personnel. This ensures a continuous flow of information from the members of the PJI board review to the clinicians responsible for the patient"s care during the hospital stay.
Conclusion
We established a database that allows to document and communicate clinical decisions of the multidisciplinary PJI board review in real-time. In addition, the comprehensive survey of the data will allow scientific evaluation of diagnostic and therapeutic strategies.

Presentation: Tuesday, February 20, 2018 from 8:43 – 8:56 in room Hörsaal 3.

051/KMV

A retrospective long-term study of *Staphylococcus aureus* in the airways of cystic fibrosis patients with regard to dynamics in persistence, resistance and adaptive phenotypes

M. Schwerdt[1], C. Neumann[1], B. Schwartbeck[1], S. Kampmeier[2], S. Herzog[1], D. Görlich[3], A. Dübbers[4], J. Große-Onnebrink[4], C. Kessler[4], P. Küster[5], H. Schültingkemper[5], J. Treffon[1], G. Peters[1], B. C. Kahl*[1]
[1]*University Hospital Münster, Medical Microbiology, Münster, Germany*
[2]*University Hospital Münster, Institute for Hygiene, Münster, Germany*
[3]*University Hospital Münster, Institute of Biostatistics and Clinical Research, Münster, Germany*
[4]*University Hospital Münster, Department of Pediatrics, Münster, Germany*
[5]*Clemenshospital , Department of Pediatrics, Münster, Germany*

Questions: *Staphylococcus aureus* is one of the earliest and most prevalent pathogens in patients with cystic fibrosis (CF). To determine the dynamics with regard to persistence, resistance and adaptive phenotypes of *S. aureus* in the airways of CF patients on a long-term perspective, we conducted a retrospective study.
Methods: We used data from our microbiological database since 1994 until 2016 for patients treated at two centres in Münster, Germany, respectively. Furthermore, the resistance to selected antibiotics was determined for all patients" isolates and for 15 patients on a longitudinal basis. In addition, the prevalence of adaptive phenotypes such as small colony variants (SCVs) and mucoid *S. aureus* was assessed.
Results: For this study, 2867 patient years with respiratory specimens (mean of 9.3 years for every patient, range 0 - 22 years) were evaluated for 283 CF patients (median age of 7 years at the beginning of the observation period, range 0 - 57 years, 51% male). 18% of patients were rarely infected by *S. aureus* (≤ 24% of observation years), 20% of patients intermittently (25-49%) and 61% persistently (≥ 50% of observation period). Susceptibility testing for 12969 *S. aureus* isolates resulted in resistance to methicillin in 9%, trimethoprim/sulfamethoxazole in 10%, levofloxacin in 14%, gentamicin in 20%, erythromycin and/or clindamycin in 30% and penicillin in 80%. *S. aureus* isolates of 15 patients revealed dynamics of resistance with increase, decrease and loss of resistant isolates to the analysed antibiotics during the study period. SCVs were isolated at least once from 42% (n = 118) of patients and mucoid isolates from 2% (n = 7) of patients. In the last study year, 89 patients were infected by *S. aureus* only (59%), 44 patients by *S. aureus* and *P. aeruginosa* (29%) and 18 by *P. aeruginosa* only (12%). Patients infected by *S. aureus* only were younger and had better lung function compared to the other two groups.
Conclusions: We determined a high percentage of patients with persistent *S. aureus* infection. During persistence of isolates, mostly fluctuation of resistance against various antibiotics was observed in the isolates indicating acquisition and loss of resistance genes by *S. aureus*. The prevalence of adaptive phenotypes during long-term persistence was high for SCVs (42% of patients), but low for mucoid isolates (2% of patients), which might be underestimated for mucoid phenotypes due to the retrospective study design and the difficulty to detect mucoid isolates in primary cultures.

Presentation: Tuesday, February 20, 2018 from 8:56 – 9:09 in room Hörsaal 3.

052/KMV

Beta-lactam resistance of *H. influenzae* from invasive infections in Germany 2016

T. Lâm*[1], H. Claus[1], U. Vogel[1]
[1]*Würzburg University, Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction: Aminopenicillin resistance is well documented in invasive *H. influenzae*. The main resistance mechanism is based on expression of beta-lactamase. In addition, mutations in PBP3, which is coded by the *fts*I gene play a role. This resistance mechanism has also been described as the cause of resistance against other beta-lactam antibiotics. However, invasive isolates resistant against third generation cephalosporines or carbapenems have been found only occasionally.
Objectives: This study aimed at providing prevalence data for the beta-lactam resistance in invasive *H. influenzae* isolates in Germany 2016. Additionally, we analysed the role of *fts*I mutations found in resistant isolates.
Materials and Methods: *Haemophilus influenzae* isolates from blood or cerebrospinal fluid collected by the laboratory surveillance system in Germany 2016 were analysed prospectively for antibiotic susceptibility against ampicillin, imipenem, meropenem, and cefotaxime. MICs were determined by Etest. Interpretation was done according to EUCAST breakpoints 2016. The *fts*I sequence of *H. influenzae* strain Rd KW20 was taken as a reference to detect mutations in resistant isolates.
Results: Antibiotic resistance was tested for 474 isolates. Ampicillin resistance was found in 19% (n=91), 7% (n=31) was beta-lactamase negative ampicillin resistance (BLNAR). Three isolates showed cefotaxime resistance. Imipenem resistance was found in 16 % (n=76), however, all isolates were meropenem susceptible.
Sequencing of *fts*I revealed mutations in all imipenem resistant *H. influenzae*. Among these isolates, all phenotypic ampicillin susceptibility types were found. However, out of 30 imipenem resistant beta-lactamase negative ampicillin susceptible isolates, 28 showed borderline ampicillin minimal inhibitory concentrations of 0.75 or 1 µg/ml. Genetic analysis showed that all of these isolates were gBLNAR.
Summary: Imipenem resistance was remarkably high in invasive isolates in Germany and was correlated with *fts*I mutations. All isolates were susceptible to meropenem, which is the carbapenem of choice for infections in the central nervous system. Cefotaxime resistance was rare, but the cases found warrant continued surveillance.

Presentation: Tuesday, February 20, 2018 from 9:09 – 9:22 in room Hörsaal 3.

053/KMV

Fluorescence in situ hybridization for the visualization of microorganisms in patients with left ventricular assist devices

A. Moter*[1,2], F. Schönrath[3], L. Kursawe[1], K. Schönrath[3], J. Schulze[1], E. Potapov[3], T. Krabatsch[3], F. Kaufmann[3], V. Falk[3,4], A. Moter[1]
[1]*German Heart Center Berlin, Biofilmcenter, Berlin, Germany*
[2]*Charité - Universitätsmedizin Berlin, Institute for Microbiology, Berlin, Germany*
[3]*German Heart Center Berlin, Department of Cardiothoracic and Vascular Surgery, Berlin, Germany*
[4]*Charité - Universitätsmedizin Berlin, Department of Cardiovascular Surgery, Berlin, Germany*

Introduction: A major problem for the patients and challenge for their physicians are infections following left ventricular assist device (LVAD) implantation. Routine diagnostic culturing techniques often fail to detect all relevant pathogenic microorganisms, in particular, when microorganisms grow in sessile communities, so called biofilms. Moreover, growth of typical members of the skin flora in culture may raise the question if an infection or a contamination of the sample is detected.
Aims: Aim of this study was to investigate if Fluorescence in situ hybridization (FISH) in combination with pan-bacterial PCR and sequencing may improve diagnosis and therefore antimicrobial treatment of LVAD associated infections. FISH is a molecular biological microscopic tool that concomitantly visualizes, localizes and identifies bacteria and fungi in clinical samples in situ.
Materials and Methods: LVAD drivelines from 15 patients undergoing LVAD exchange were obtained during explantation for various reasons (thrombosis, death, recovery, etc.). The drivelines were subjected to FISH and 16S rRNA-gene PCR and sequencing. FISH/PCR results were compared to standard culture results from swabs and blood culture.
Results: FISH/PCR detected microbial colonization on LVAD drivelines in 11 of the 15 cases. These were bacterial (n = 9), yeast (n = 1) and mixed bacterial-yeast (n = 1) colonization. Microbial species comprised *Corynebacterium* spp., coagulase negative

staphylococci, *Pseudomonas* spp., *Enterococcus faecium*, and *Candida albicans*. The spatial formation of the microorganisms on the drivelines ranged from few single bacteria to multi-species biofilms close to the heart.

Summary: FISH/PCR successfully diagnosed LVAD driveline infections also in questionable cases. FISH/PCR identified the relevant key causative pathogen among several culture results and in culture negative infections. Microorganisms that are otherwise ignored or missed, were detected and the differentiation between contamination and infection was possible. Therefore, FISH/PCR may support individualized clinical therapy decision making in the future.

Presentation: Tuesday, February 20, 2018 from 9:22 – 9:35 in room Hörsaal 3.

054/KMV

Cytokine responses, antibody kinetics and clinical characteristics of imported and autochthonous typhus group rickettsioses, Germany, 2010-2016

J. Rauch[1], D. Tappe*[1]
[1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

Question: Typhus group rickettsioses are caused by *Rickettsia typhi* and *Rickettsia prowazekii*, two biosafety level 3 organisms of the family *Rickettsiaceae* that encompass obligate intracellular gram-negative zoonotic bacteria. *R. typhi* is responsible for endemic flea-borne murine typhus in predominantly tropical coastal regions, whereas *R. prowazekii* is the causative agent of epidemic louse-borne typhus in both temperate and tropical regions. Little is known about the immunology of human infection and kinetics of seroconversion for these two CDC category B bioweapon pathogens, however.

Material and Methods: Typhus group rickettsioses that had been diagnosed at the National Reference Centre for Tropical Pathogens 2010–2016 were retrospectively analyzed. Cases were re-investigated and serologically re-analyzed by indirect immunofluorescence tests for IgM, IgA, and IgG. Antibody kinetics were investigated from follow-up sera. Serum cytokine responses were measured by flow cytometry from all available sera. In addition, novel nested qPCRs targeting the *psrA* genes of *R. typhi* and *R. prowazekii* were employed from stored clinical material.

Results: Twenty-eight cases were retrieved in the database. Infections were most often acquired during travel in Southeast Asia, followed by Europe, Africa, and The Americas. In the majority signs and symptoms consisted of fever, followed by exanthema, headache, myalgia, cough, and splenomegaly. Hospitalization was necessary in more than half of the patients. Laboratory changes included anemia, leukocytosis, thrombocytopenia, increased levels of C-reactive protein and elevated activities of lactate dehydrogenase, creatin kinase, and liver enzymes. Seroconversion occurred with parallel detection of IgM, IgA, and IgG. The earliest day of illness with detection of specific antibodies was day 7. Molecular detection of *R. typhi* was successful in three patients, from full blood and a liver biopsy. *R. prowazekii* was detected in none of the subjects. Serum levels of chemokines that attract predominantly monocytes, neutrophils and/or T cells to the side of infection and mainly proinflammatory cytokines like IFN-gamma, IFN-alpha and IL-6 started to increase in the first week of illness, peaked in the second week, and then declined.

Conclusion: Except for the development of exanthema, the clinical presentation of the patients was unspecific. A high rate of hospitalization was recorded. Seroconversion occurred earlier than expected for rickettsioses, and involved all antibody classes simultaneously. PCR detection was rarely achieved. Most cytokine and chemokine elevations occurred in the second week of infection, coinciding with organ symptoms. Especially after travel to Southeast Asia, an infection with *R. typhi* should be considered when elevated levels of liver enzymes and lactate dehydrogenase, accompanied by leukocytosis and thrombopenia, are present.

Presentation: Tuesday, February 20, 2018 from 9:35 – 9:48 in room Hörsaal 3.

055/KMV

***C. trachomatis* (*Ctr*) uses its Major Outer Membrane Protein (MOMP) to inhibit mitochondrial apoptosis during infection**

C. Waguia Kontchou*[1], I. Gentle[1], G. Häcker[1]
[1]*Uniklinikum Freiburg, Center of Medical Microbiology and Hygiene, Freiburg i. Br., Germany*

Introduction: *C. trachomatis* (*Ctr*) is an obligate intracellular bacterial pathogen that is the leading cause of bacterial sexually transmitted disease and preventable blindness worldwide. *Ctr* has an intracellular biphasic developmental cycle, in which replication occurs within a specialized vacuole known as the inclusion. Infection with *Ctr* strongly inhibits apoptosis in human cells, which is likely required for survival of the pathogen.

Aim and Methods: Very strong anti-apoptotic activity in the mitochondrial pathway has been observed by many groups, and a number of contradictory models have been put forward. In this study we have tested the proposed models of apoptosis inhibition by *Ctr* and have found that none could explain the strong anti-apoptotic activity. We then conducted a fine-mapping of the point of inhibition of the mitochondrial apoptotic pathway and moved on to identifying the chlamydial factor involved.

Results: The point of action of *Ctr* was identified as the inhibition of the effector of cytochrome *c*-release during apoptosis, the Bcl-2-family protein Bak. Chlamydial major outer membrane protein (MOMP) was found specifically to interact with and to inhibit Bak only upon Bak-activation during apoptosis on mitochondria of infected cells, or in cells ectopically expressing MOMP.

Conclusions: Our findings propose the novel model that *Ctr* uses MOMP to inhibit apoptosis. As a porin, MOMP has structural similarity to the mitochondrial porin VDAC, a known regulator of Bak-activity. While during mitochondrial apoptosis VDAC dissociates from Bak and releases its inhibition, our results suggest that MOMP in this situation replaces VDAC as an inhibitor of apoptosis.

Presentation: Tuesday, February 20, 2018 from 9:48 – 10:00 in room Hörsaal 3.

HAUPTSYMPOSIUM 03
Host Susceptibility / Fungal Diseases (HS)
20 Febr. 2018 • 10.45–11.45

056/INV
Candidalysin is critical for candida albicans infection
J. Naglik
Mucosal & Salivary Biology Division, Dental Institute, King's College London, London, United Kingdom

Cytolytic proteins and peptide toxins are critical virulence factors of bacterial pathogens and play a major role in bacterial disease. Human pathogenic fungi were not known to possess such toxins. Recently, we discovered that the hyphal form of Candida albicans produces a cytolytic peptide toxin (Candidalysin) that is critical for pathogenesis and immune activation. Candidalysin is a 31 amino acid peptide derived from its parent protein, Ece1p. Upon secretion, Candidalysin intercalates into and destabilises the structural integrity of epithelial cell plasma membranes. As a result, alarmins are released, which leads to epithelial activation via the epidermal growth factor receptor (EGFR). EGFR activation triggers mitogen-activated protein kinase (MAPK) signalling and, ultimately, a strong inflammatory response, which helps clear the fungal infection.

Presentation: Tuesday, February 20, 2018 from 10:45 – 11:15 in room Audimax Saal.

057/INV
MELLEC: A new Reception for Fungal Infections
G.D. Brown
University of Aberdeen, Medical Research Council Centre for Medical Mycology, Aberdeen, UK

The last few decades has seen a tremendous increase in our understanding of the mechanisms underlying the development of protective anti-microbial immunity. Key among these discoveries is the identification of pattern recognition receptors (or PRRs) expressed by immune cells which recognise conserved microbial components, such as beta-glucans. Recognition of these structures by PRRs, particularly by members of the C-type lectin receptor (CLR) family, triggers intracellular signalling cascades that initiate a variety of cellular and inflammatory responses, and induce the development of pathogen specific adaptive immunity. We now understand that innate recognition by CLRs is essential for the development of protective antimicrobial immunity. In this presentation, I will cover our discovery of a novel CLR that is providing new insights into the function and roles of these receptors.

Presentation: Tuesday, February 20, 2018 from 11:15 – 11:45 in room Audimax Saal.

HAUPTSYMPOSIUM 04
Association of Microbiome and Diseases (MD)
20 Febr. 2018 • 13.45–14.45

058/INV
Control of Pathogen Colonization by Immunity and the Microbiota
G. Núñez
University of Michigan Ann Arbor, Department of Pathology and Comprehensive Cancer Center, Michigan, U.S.A.

The mechanisms that allow enteric pathogens to colonize the intestine in the presence of the microbiota and how host immunity and the indigenous microbiota regulate pathogen colonization remain poorly understood. Our laboratory is using *Citrobacter rodentium*, a mouse pathogen that models human infections by enteropathogenic *E. coli*, to understand the mechanisms that regulate the colonization and clearance of the pathogen in the gut. These studies have revealed how the pathogen colonizes and replicates successfully early during infection and how host immunity and the indigenous microbiota cooperate to eradicate the pathogen in the later stage of the infection. These studies have also revealed that Clostridia species protect the host from colonization by *C. rodentium* and *Salmonella enterica* in the intestine. Furthermore, these studies have shown that the intestine of mice after birth lack protective Clostridia species providing a mechanism to account for the enhanced susceptibility of mice and humans to enteric infection during the neonatal period.

References
[1] Kamada, N., Kim, Y.G., Sham, H.P., Vallance, B.A., Puente, J.L., Martens, E.C., Núñez, G. (2012). Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science 6086: 1325-1329.
[2] Kamada, N., Sakamoto, K., Seo, S.U., Zeng, M.Y., Kim, Y.G., Cascalho, M., Vallance, B.A., Puente, J.L., Núñez, G. (2015). Humoral Immunity in the Gut Selectively Targets Phenotypically Virulent Attaching-and-Effacing Bacteria for Intraluminal Elimination. Cell Host & Microbe. 17:617-27.
[3] Kim Y-G, Sakamoto K, Seo S-U, Pickard JM, Gillilland MG III, Pudlo NA, Hoostal M, Li X, Wang TD, Feehley T, Stefka AT, Schmidt TM, Martens EC, Fukuda S, Inohara N, Nagler CR, Núñez G. (2017). Neonatal Acquisition of Clostridia Species Protects Against Colonization by Bacterial Pathogens. Science 356:315-319

Presentation: Tuesday, February 20, 2018 from 13:45 – 14:15 in room Audimax Saal.

059/INV
Gut microbiota and colorectal cancer
P.W. O'Toole
University College Cork, School of Microbiology & APC Microbiome Institute, University College Cork, Cork, Ireland

Alterations in gut microbiota composition and function have been linked to various diseases including cancer. Colorectal cancer (CRC) could be caused by microbes affecting inflammation, cell death or by increasing mutation frequency in susceptible individuals. Thus, the microbiota would act as an additional environmental risk factor for cancer. Supporting this theory, CRC-associated microbes can accelerate cancer in animal models, but definitive causative links in humans have yet to be established.
Regardless of the mechanisms involved, CRC-associated microbiota composition differs from those in healthy subjects and we showed that they are linked with distinct mucosal gene-expression profiles. Furthermore, several labs have reported that the altered microbiota on colonic biopsies or in stool samples can be used to detect CRC. Because the CRC microbiota often contains bacteria from the oral cavity, we tested oral swabs for CRC detection. The oral microbiota data allowed us to identify people with polyps or cancer from healthy controls, and combining oral data with faecal microbiota data led to significantly improved sensitivity and specificity.
An exciting way of treating some cancer types is to administer immune checkpoint inhibitors, monoclonal antibodies or small molecules that prevent inhibition of T-cell activation, allowing the immune system to fight the cancer. Recent data from animal models and some human studies has shown that whether immune check-point inhibitors are clinically effective or not depends on the patient's gut microbiota composition. This relates to how the microbiota co-regulates T-cell activation. Furthermore, whether the patient experiences colitis or not, which happens in a minority of those treated and requires therapy withdrawal, also appears to be a feature of their gut microbiota composition.
Overall, the gut microbiota is a promising (and targetable) risk factor for colorectal cancer, a potential diagnostic for colorectal cancer, and a modifiable modulator of cancer immunotherapy.

Presentation: Tuesday, February 20, 2018 from 14:15 – 14:45 in room Audimax Saal.

WORKSHOP 11
Progress in Microbial Identification and Laboratory automation (StAG DV/FG DKM)
20 Febr. 2018 • 13.45–15.15

060/DVV

Characterization of Pyrazinamide Resistance in *M. tuberculosis* isolates in Germany

E. Richter*[1], U. Eigner[2], I. Frischmann[3], A. Veldenzer[3], M. Weizenegger[3], R. Schwarz[4]
[1]*MVZ Labor Limbach, TB-Labor, Heidelberg, Germany*
[2]*MVZ Labor Limbach, Studienabteilung, Heidelberg, Germany*
[3]*MVZ Labor Limbach, Molekulare Diagnostik, Heidelberg, Germany*
[4]*MVZ Labor Limbach, Infektionsdiagnostik, Heidelberg, Germany*

Introduction: Phenotypic drug susceptibility testing of Pyrazinamide (PZA) for *M. tuberculosis* is challenging. So far, no unequivocal reference method is available. Worldwide, the most commonly used method is the broth based Bactec MGIT 960 technique using a particular, acidified medium. However, false resistant results are known necessitating alternative or additional analyses. Repetition of the test is the most common way to overcome this problem. As alternative, mutation analysis of the *pnc*A gene, which encodes the enzyme pyrazinamidase, responsible for activation of the prodrug PZA, is recommended to confirm true resistance to PZA. We assessed the *pnc*A -gene sequences in all strains tested resistant to PZA by phenotypic drug susceptibility testing to estimate the presence and distribution of *pnc*A mutations in PZA-resistant *M. tuberculosis* strains isolated in a German TB laboratory.
Material and Methods: All strains tested resistant to PZA by phenotypic drug susceptibility testing (Bactec MGIT 960) as well as PZA susceptible isolates were included into this study. From every patient only one isolate was used. The *pnc*A gene sequences were analyzed either by Sanger sequencing of the complete *pnc*A gene or by Whole Genome Sequencing of the entire genome.
Results: Among 22 PZA resistant *M. tuberculosis* strains 17 different patterns of genetic variants scattered over the full length of the gene could be detected. Only in two strain, no genetic alteration was present (one of them was determined as intermediate resistant to PZA). Most alterations were single base mutations leading to amino acid changes, but also resulting in a stop codon. In some strains, an additional (lineage specific) silent mutation could be documented. Deletion of single nucleotides could be determined, leading to frame shifts. In one patient, a large genome fragment comprising nine genes including *pnc*A was deleted, which could only be confirmed by WGS. Most of the mutations seen in the isolates have already been published, however, few genetic alterations are novel, including deletion of a large fragment of the genome. More than 20 PZA-susceptible strains (first line drugs susceptible and resistant) were used as controls. None of the mutations detected in PZA resistant strains were seen in PZA susceptible isolates. However, a silent mutation specific for the Delhi/CAS lineage could be documented as well.
Summary: Analyzing strains, phenotypically resistant to PZA, we could confirm the high variation of genetic alterations in PZA-resistant strains isolated in Germany. Additional sequencing of the *pnc*A gene, at least in cases of phenotypic PZA drug resistance, can improve diagnostic validity of drug susceptibility testing of the still important first line drug PZA. However, sequence data have to be analyzed carefully to adequately appraise any mutation detected.

Presentation: Tuesday, February 20, 2018 from 13:45 – 14:00 in room Seminarraum 2.

061/DVV

Rapid detection of vancomycin-resistant enterococci by a luminescence-based assay

S. Hardt*[1], P. Ahmad-Nejad[1], B. Ghebremedhin[1]
[1]*Institute for Med. Laboratory Diagnostics, HELIOS University-Hospital Wuppertal, Medical Laboratory Diagnostics, Wuppertal, Germany*

Introduction: Enterococci colonize as regular commensals in the intestine and are well known for their intrinsic as well as acquired resistances against frequently used antibiotics. Due to the clinical relevance of vancomycin resistant enterococci (VRE) (e.g. increasing resistance rates among Enterococci), more efficient detection methods are needed in today"s health care system.
Objective: Here we present a new phenotypic-based assay to detect VRE, which is more rapid and cost-effective than common antimicrobial susceptibility testing (AST) systems for VRE detection.
Material and Methods: We developed the rapid luminescence-based VRE detection assay (LVA) as a confirmatory test detecting VRE growing on culture media (agar-LVA) or in enterococci positive blood cultures (BC-LVA). Overall, the present study included 372 strains of enterococci (mainly E. faecium). The BD PhoenixTM (Becton Dickinson, Heidelberg, Germany), the disc diffusion method and an epsilometer test (Etest) served as references.
Results: According to the given basic parameters a sensitivity of 99.57% and a specificity of 99.00% were obtained. The LVA also works with E. faecalis (data not shown). The total time required for a run of the agar-LVA is 4.3 hours (excluding prior gaining of pure cultures on a nutrient medium).
According to the LVA performed by means of blood cultures the specificity was 98.31% and the sensitivity 96.67%. The calculated elapsed time for receiving results with a sensitivity and specificity given above is 4.5 hours. The time for preparing artificially positive blood culture vials was deducted due to the fact that in clinical context this would not be necessary.
Conclusion: In conclusion, for the detection of VRE in infected, colonized or septic patients, the LVA is a fast (almost 20 hours faster than other conventional phenotypic-based methods for AST), safe and cost-effective method that can replace more time-consuming methods.

Presentation: Tuesday, February 20, 2018 from 14:00 – 14:15 in room Seminarraum 2.

062/DVV

Sepsis by Carbapenemase-producing enterobacteria: a full maldi-based approach

M. Cordovana*[1], M. Kostrzewa[2], J. Glandorf[2], S. Ambretti[1]
[1]*Azienda Ospedaliero-Universitaria di Bologna Policlinico Sant'Orsola-Malpighi, Microbiology and Virology, Bologna, Italy*
[2]*Bruker Daltonik GmbH, Bremen, Germany*

Introduction: The increasing occurrence of sepsis caused by carbapenemase-producing enterobacteria (CPE) observed in the last years represents the most worrying expression of the global spreading of those strains in hospital settings. As they are burdened by high morbidity and mortality, a prompt detection of the carbapenemase production strongly affects the patient"s clinical outcome.
Objectives: In this study, an innovative full MALDI-based approach to quickly detect CPE in positive blood cultures was evaluated, using a combination of the novel applications of the Biotyper system (Bruker Daltonik) directly on the bacterial pellet extracted from the positive blood culture bottles. KPC-producers were identified by an automated detection of the 11109 KPC-specific peak by the Biotyper software, while an imipenem hydrolysis assay was used to confirm KPC activity and to detect the other carbapenemases.
Methods: 77 blood cultures positive for *K. pneumoniae* (n=46 carbapenemase-producers, n=31 susceptible to carbapenems) were analyzed with this novel approach.
The bacterial pellet was extracted by Sepsityper® kit (Bruker Daltonik).

The automated detection of the 11109 m/z KPC-specific peak was performed by the Biotyper software simultaneously with the species ID, processing the mass spectra generated from the bacterial pellet.
The sensitivity of the automated detection of this specific peak was assessed after the evaluation of the prevalence of the KPC+ strains that presented such peak, performed by visual inspection of the spectra.
The same pellet was used to perform the STAR-CARBA imipenem hydrolysis assay (Bruker Daltonik), to verify the carbapenemase activity.
Results: The KPC-specific peak was detected by Biotyper software in 34/35 (97.1%) of the KPC+ strains in which this peak was present, corresponding to 34/37 (91.9%) of the total number of KPC+. The peak was detected in none of the other strains.
STAR-CARBA imipenem hydrolysis assay resulted positive for 46/46 carbapenemase-producing strains, and negative in the carbapenem-susceptible strains (31/31).
The novel approach provided a conclusive result in a time frame between 30 min (when the KPC-specific peak was detected – 73.9% of the positives) and 2 h (in all the other cases).
Conclusions: The full MALDI-based approach enabled the rapid detection of different kind of carbapenemases directly from the positive blood culture bottles, with absolute sensitivity and specificity, and allowing a significant shortening of potential reporting time in comparison with the actual routine (30 min-2 h).
Considering the analytical performance, and the user-friendly workflow, these promising results suggest the possibility of an efficient implementation in routine practice of these novel MALDI Biotyper applications.

Presentation: Tuesday, February 20, 2018 from 14:15 – 14:30 in room Seminarraum 2.

063/DVV

Mass Spectrometry-based PhyloProteomics (MSPP) of *Campylobacter coli*

M. Emele*1, S. Smole-Možina2, R. Lugert1, W. Bohne1, U. Groß1, O. Bader1, A. E. Zautner1
[1]*Universitätsmedizin Göttingen, Medizinische Mikrobiologie, Göttingen, Germany*
[2]*Department of Food Science and Technology, Chair of Biotechnology, Microbiology and Food Safety, Ljubljana, Slovenia*

Introduction: Besides *C. jejuni*, *C. coli* is the most common bacterial cause of gastroenteritis worldwide. *C. coli* can be subdivided into three clades, which are generally associated with sample source, as clade 2 and 3 strains are more commonly isolated from environmental waters.
Objective: Little is known about the evolution of these clades, their ecological significance and how they relate to clinical infections caused by *C. coli.* The confirmation of the isolate as well as the discrimination between the clades is commonly done using laborious MLST-typing. The aim of this study was to establish a typing scheme based on Mass-spectrometry-based-Phyloproteomics (MSPP) to replace time-consuming sequence-based methods.
Methods: A total of 30 *C. coli isolates* were analyzed by MALDI-TOF-based intact cell mass spectrometry (ICMS) and evaluated to establish a *C. coli*-MSPP scheme. MLST was used as reference method.
Results: Different isoforms of the detectable biomarkers resulting in biomarker mass shifts were associated with their amino acid sequences and included into the *C. coli*-MSPP typing scheme. In total we identified 15 biomarkers to differentiate C. coli into three clades and three subclades**.**
Conclusion: In this study the principle of MSPP-typing, previously demonstrated on *C. jejuni* ssp. *jejuni*, *C. jejuni* ssp. *doylei* and *C. fetus*, has been successfully adapted to *C. coli*. The clades and subclades can be sufficiently discriminated by MSPP. This further highlights that MSPP bears a high potential of a fast and easy-to-perform intra species typing**.**

Presentation: Tuesday, February 20, 2018 from 14:30 – 14:45 in room Seminarraum 2.

064/DVV

Processing of positive blood cultures using total laboratory automation – chances, challenges and limits

I. Burckhardt*[1], S. Winter[1], M. Weitnauer[1], S. Zimmermann[1]
[1]*Zentrum für Infektiologie, Medizinische Mikrobiologie und Hygiene, Heidelberg, Germany*

Introduction: Laboratory automation entails a substantial reorganization of the classic manual microbiology workflow. For screening and urine samples automation resulted in a reduced time to report and an increase in detection of gram-positive bacteria. Automation of blood cultures is challenging due to identical lab numbers for paired blood culture bottles, generation of gram slides and gram-morphology driven initial susceptibility testing. Additionally, processing blood cultures is already a highly optimised workflow in the manual setting.
Aims: We integrated our blood culture workflow into an existing automation system, which already processed up to 600 samples per day. We monitored and compared times to report and convenience of workflow with the manual workflow in order to decide whether a further optimization of blood culture processing was possible with our lab automation system.
Materials and Methods: We implemented a workflow incorporating the creation of gram slides, sub-culture plates and direct susceptibility testing as well as follow-up work (ID, AST) for each bottle type. Different imaging times for plates were assessed for suitability. We compared the data of 200 bottles of true blood cultures (aerobic and anaerobic bottles) processed either manually or with the automated workflow.
Results: Workflow programming successfully addressed the problem of identical sample IDs for aerobic and anaerobic blood culture bottles, integration of the gram slide, sub-culture and direct susceptibility testing. For validation purposes plates were imaged after 4, 5, 6 and 7h of incubation. In our setting a six-hour image was the best compromise between acceleration of workflow and presence of sufficient growth for further follow-up work. Gram slides showed fewer artefacts and were easier to interpret. Comparison of times to report (TTR) for gram slides was difficult because TTRs were not only dependent on the time to gram stain but also on time to telephone call. These two aspects could not be disentangled.
Summary: The development of an automated workflow for blood cultures was more complex than for urine or screening samples because of the required processing of a positive sample. The main limitation for workflow acceleration was the growth kinetic of bacteria on plates. Integrating blood cultures into automation had the advantage of giving standardised results for en-bloc samples, however we lost the flexibility of the manual workflow for singular positive samples during daytime. Automated processing of samples during the day was challenging and speed depended mainly on the occupancy of the system.

Presentation: Tuesday, February 20, 2018 from 14:45 – 15:00 in room Seminarraum 2.

065/DVV

Comparison of diagnostic β-D-glucan, galactomannan and mannan assays for detection of invasive fungal infections.

K. Dichtl*[1], J. Wagener[1]
[1]*Max von Pettenkofer Institute, Faculty of Medicine, LMU Munich, München, Germany*

Invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients with impaired immunological function. This group includes critically ill patients and patients undergoing immunosuppressive therapies, e.g., treatment of hematooncologic malignancies and hematopoietic stem cell transplantation. The most prevalent fungal pathogens in this population at risk belong to the genera *Candida* and *Aspergillus*. Laboratory diagnosis of invasive candidiasis and invasive aspergillosis (IA) is primarily based on cultivation and on the detection of fungal antigens such as β-D-glucan (BDG), galactomannan (GM) and mannan (Man) in specimens.

In this retrospective study, we compared the performance of a newly available turbidimetric BDG assay with GM and Man ELISAs. Two cohorts of well characterized IFI cases according to the EORTC/MSG consensus guidelines were included. The first cohort consists of 45 cases of proven IA, the second cohort of 121 cases of candidemia. Depending on the cohort, serum samples were analyzed for BDG and GM (aspergillosis) or BDG and Man (candidemia). Serum samples of the candidemia cohort were obtained in a period of seven days before sampling of subsequently positive blood cultures. Control groups comprise serum samples derived from ambulatory patients without suspected IFI and from patients with bacteremia.
The BDG antigen test had a significantly higher sensitivity in detecting candidemia and IA compared to the GM and Man antigen ELISAs. Besides this, positive BDG testing typically detected candidemia 1 – 3 days before blood cultures yielded positive results. To our knowledge, this is the first retrospective evaluation of a turbidimetric assay for detection of BDG with large patient cohorts. Based on our results we propose that testing for BDG in serum samples can be a helpful tool for early diagnosis and treatment of IFI caused by *Candida* and *Aspergillus*.

Presentation: Tuesday, February 20, 2018 from 15:00 – 15:15 in room Seminarraum 2.

WORKSHOP 12
Molecular Epidemiology of Infectious Diseases (FG MS/FG ZO/FG PR)
20 Febr. 2018 • 15.15–16.45

066/MSV

NGS outbreak analysis of non-toxigenic *Corynebacterium diphtheriae* clusters in Northern Germany

A. Dangel*[1], A. Berger[1,2], R. Konrad[1,2], A. Sing[1,2]
[1]*Bavarian Health and Food Safety Authority (LGL), Oberschleißheim, Germany*
[2]*German Consiliary Laboratory on Diphtheria, Oberschleißheim, Germany*

Both diphtheria and its causative pathogen *Corynebacterium diphtheriae*, have been drifting out of focus in western countries due to effective vaccination programs. However, the number of cases, submitted to the German Consiliary Laboratory on Diphtheria, is increasing continuously again since several years. Especially cases of non-toxigenic *C. diphtheria*, causing wound and blood stream infections are rising. In this study we investigated a recent accumulation of *C. diphtheriae* cases located in Hamburg, Berlin and further cities in Northern Germany with common social-economic characteristics, e.g. homelessness and drug abuse. In order to characterize the isolates in detail and to find out if an outbreak with a common strain is ongoing, next generation sequencing (NGS) analysis was used as tool to study phylogenetic relationship.
46 non-toxigenic *C. diphtheriae* cases, submitted from Northern Germany between 2016 and middle of 2017 were initially selected for analysis. They were routinely identified as *C. diphtheriae* by culture, API Coryne and MALDI-TOF. Presence of the toxin gene was analyzed by qPCR. MLST profiles were obtained and an accumulation of sequence type 8 (ST-8) identified. 76 samples were then analyzed by Nextera XT library preparation and MiSeq whole genome (wg) sequencing, including the MLST-identified ST-8 group, together with samples submitted before 2016, typed as ST-8 or yet untyped and current untyped samples. NGS data were analyzed for phylogenetic relationship by core genome (cg) MLST and wg SNP profiles. Furthermore, presence of specific virulence and acquired resistance genes was analyzed and compared.
By MLST and NGS 54% of the sequenced samples were classified as ST-8. cgMLST of the ST-8 samples revealed several outbreak clusters with a maximum of 5 alleles difference and different geographic focus. Remarkably, all ST-8 cases from Hamburg belonged to one cluster. All samples from Berlin organized separated from the Hamburg samples in two independent clusters. By inclusion of additional cgMLST targets or performing wg SNP-phylogeny, the Hamburg-associated cluster still showed a very close relationship, whereas the two clusters with Berlin participation were divided in smaller subclusters. Moreover, virulence factors and acquired resistance genes showed patterns of shared genes between the strains of common clusters.
In conclusion, non-toxigenic *C. diphtheriae* has the potential to become a public health threat in western countries, as strains can persist in risk groups and lead to outbreaks. A detailed relationship structure between outbreak isolates could be detected by NGS, which would not have been possible with MLST typing only. Furthermore, virulence and resistance profiles can be obtained from the same dataset using NGS. This shows that NGS can serve as a valuable tool in infectious disease surveillance, helping to identify outbreaks and transmission and giving detailed insights in isolate characteristics.

Presentation: Tuesday, February 20, 2018 from 15:15 – 15:30 in room Audimax Saal.

067/MSV

Bringing together what belongs together: Optimizing murine infection models by using mouse-adapted *Staphylococcus aureus* strains

P. Trübe[1], D. M. Mrochen[1], D. Schulz[1], S. Fischer[2], K. Jeske[2], T. Hertlein[3], D. Grumann[1], I. Jorde[1], K. Pritchett-Corning[4], S. Wiles[5], B. Bröker[1], R. Ulrich[2], K. Ohlsen[3], S. Holtfreter*[1]
[1]*University Medicine Greifswald, Department of Immunology, Greifswald, Germany*
[2]*Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany*
[3]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[4]*Charles River, Research and Professional Services, Wilmington, MA, United States*
[5]*University of Auckland, Department of Molecular Medicine and Pathology, Auckland, New Zealand*

Question: *Staphylococcus aureus* is a leading cause of bacterial infection world-wide. Despite extensive efforts, there is currently no vaccine available. Vaccine development relies heavily on clinically-relevant infection models. However, the suitability of mice for *S. aureus* infection models has often been questioned, because they were not considered to be natural hosts of *S. aureus*. Moreover, experimental colonization of mice with human-adapted *S. aureus* is usually transient, and routinely requires very high infection doses. We have previously reported an outbreak of *S. aureus* infections in a C57BL/6 colony. The causative strain, named JSNZ, is an excellent colonizer of laboratory mice[1]. This outbreak prompted us to investigate whether laboratory mice and wild small rodents are natural hosts of *S. aureus*. Moreover, we tested whether murine *S. aureus* strains show an enhanced virulence in mice as compared to human-adapted strains.
Methods: We genotyped (*spa*, multiplex PCRs) 230 *S. aureus* isolates from laboratory mice, provided by various vendor facilities around the globe, as well as university- or company-associated breeding facilities. In addition, we analysed 48 *S. aureus* isolates from wild small rodents and shrews caught in different remote locations in Germany and Czech Republic. Selected murine isolates were compared with the widely used human-adapted *S. aureus* strain Newman in murine pneumonia and bacteremia models.
Results: Laboratory mice were predominantly colonized with clonal complex (CC) 88 (47.0%), followed by several lineages commonly found in humans, e.g. CC15 (14.3%), and CC1 (13.5%). In contrast, wild small rodents and shrews were frequently colonized with unique lineages: CC49 (35.4%), CC1956 (29.2%), and ST890 (18.8%)[2]. *S. aureus* isolates from both cohorts lacked some properties commonly found among human isolates, e.g., a phage-encoded immune evasion cluster, superantigen genes on mobile genetic elements and penicillin resistance[2,3]. Notably, the mouse-adapted strain muCC49 was highly virulent in BALB/c mice in pneumonia and bacteremia models, allowing a reduction of the inoculation dose by one log.
Conclusions: Both laboratory and wild mice are natural hosts of *S. aureus* and hence provide better infection models than previously assumed. Some mouse-adapted *S. aureus* strains allow a

significant reduction of the inoculation dose and are hence a promising tool to develop clinically more relevant infection models.

References

[1] Holtfreter S, et al. PLoS ONE. 2013
[2] Mrochen DM, et al. Int J Medical Microbiol. 2017
[3] Schulz D, et al. Front Cell Infect Microbiol. 2017

Presentation: Tuesday, February 20, 2018 from 15:30 – 15:45 in room Audimax Saal.

068/MSV

Characterisation of Neisseria gonorrhoeae isolates using a core-genome MLST scheme

P. Higgins*[1,2], J. Ertel[1,2], M. Fabri[3], C. Lehmann[2,4], M. Parcina[2,5], C. Schwarze-Zander[2,6], H. Seifert[1,2]
[1]*University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[2]*German Centre for Infection Research, , Bonn-Köln, Germany*
[3]*University of Cologne, Department of Dermatology, Köln, Germany*
[4]*Uiversity Hospital Cologne, Department of Internal Medicine I, Köln, Germany*
[5]*University Hospital Bonn, Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany*
[6]*University Hospital Bonn, Department of Internal Medicine I, Bonn, Germany*

Introduction: Recent reports from around the world point to a dramatic increase in antibiotic-resistant *Neisseria gonorrhoeae*. However there are scarce data regarding this organism in Germany.
Objectives: We sought to investigate the antimicrobial susceptibility and molecular epidemiology of *N. gonorrhoeae* using a core-genome MLST scheme on strains isolated in the Cologne metropolitan area.
Materials and Methods: Thirty-five isolates were collected from patients with symptoms of urethritis reporting to the Department of Dermatology, University of Cologne, between 11/2015 - 05/2017. All patients were male, with a median age of 30 years. Susceptibilities to azithromycin, cefotaxime, ciprofloxacin, penicillin and tetracycline were determined by Etest. An *ad-hoc* core-genome MLST (cgMLST) scheme based on 1567 alleles was produced to investigate their molecular epidemiology. Genome sequences were also used to investigate the presence of antibiotic resistance genes and determine traditional 7-loci MLST.
Results: Eleven isolates were fully susceptible to all the antibiotics tested. Non-susceptibility to cefotaxime (n=1), tetracycline (n=5), azithromycin (n=8), ciprofloxacin (n=16) and penicillin (n=12) was found. Eleven isolates were non-susceptible to one antibiotic, eight were non-susceptible to two antibiotics, and four were non-susceptible to ≥ 3 antimicrobials and were considered multidrug resistant (MDR). At least one antibiotic retained activity against each isolate. Resistome analysis revealed that all isolates carried *penA* and three also had TEM. One isolate with a tetracycline MIC of 32mg/L harboured TetM. Ciprofloxacin non-susceptibility was associated with GyrA substitutions. Seven-loci MLST revealed 11 sequence types (ST), with ST-7363 represented by 5 isolates which were all ciprofloxacin resistant. Analysis from our cgMLST scheme using 1567 alleles allowed us to delineate isolates further. Each ST was separated from other STs by at least 203 allelic differences for single locus variants, and >306 allelic differences for double locus variants. The ST-8156 cluster comprised 6 isolates (≤ 63 allelic differences). A further five clusters were seen containing isolates with 2-56 allelic differences. We detected three possible transmission events, where isolates differed between 2 and 5 alleles. Isolates within these clusters shared the same resistome and exhibited similar antimicrobial susceptibility profiles.
Summary: Our cgMLST scheme allowed us to further differentiate between isolates with the same 7-loci sequence type. We detected at least three potential transmission events. Although the number of MDR isolates remains low, at least one of the transmission events involved MDR isolates. Cefotaxime showed good activity against *N. gonorrhoeae* from the Cologne area.

Presentation: Tuesday, February 20, 2018 from 15:45 – 16:00 in room Audimax Saal.

069/MSV

Import of pandemic, community associated methicillin-resistant *Staphylococcus aureus* through skin and soft tissue infection in intercontinental travellers into Europe 2011-2016

D. Nurjadi*[1,2], K. Heeg[1], P. Zanger[1,2]
[1]*Heidelberg University Hospital, Department of Infectious Diseases (Microbiology and Hygiene), Heidelberg, Germany*
[2]*StaphTrav Network, Heidelberg, Germany*

Objectives: Increased mobility, migration and intercontinental travel facilitate the spread of virulent and multi-resistant organisms. Of particular interest is the spread of virulent epidemic MRSA strains such as ST-8-IVa USA300 and ST8-IVc USA300 Latin American Variant causing an *S. aureus* epidemic on the American continent. The StaphTrav Network was initiated in 2011 as a collaborative research project on the surveillance of imported *Staphylococcus aureus* to Europe, comprising of 13 travel-medicine centres across Europe (www.staphtrav.eu). This study analyses all *S. aureus* strains collected from 2011-2016 for epidemic MRSA clones imported to Europe through intercontinental travel.
Methods: Consulting physicians at participating centres are requested to submit swabs of travel related skin and soft tissue infections SSTI together with detailed information on subject characteristics to the co-ordinating centre at Heidelberg University Hospital, Germany. Only patients, who acquired SSTI while abroad or within 30 days post-travel are eiligible. In related cases, only the index case is considered for the analysis. All MRSA strains submitted by participating centres were characterized by *spa* types, MLST, lukF/lukS encoding Panton-Valentine Leukocidin (PVL), SCCmec cassette, Arginin Catabolic Mobile Element (ACME). Epidemic MRSA clones were confirmed by whole genome sequencing (WGS).
Results: By July 2016, 564 eligible swab materials from participating centres have been received. *S. aureus* were cultured in 66% (n=375) of travelers with SSTI. 14% (n=51) of all isolated *S. aureus* were methicillin-resistant (MRSA), 84% (n=43) of all MRSA strains harboured PVL. The risk of acquiring MRSA vary by region and was highest in Latin America. Over one third of all imported MRSA belonged to the major clones circulating worldwide, such as ST8 USA300, ST30 South Pacific clone and ST772 Bengal Bay Clone, as confirmed by WGS. USA300 and USA300 Latin American variant were the most commonly imported MRSA. Over 50% of MRSA SSTI patients were colonized by the same strain in the nasal cavity. Moreover, over half of imported MRSA were resistant to two or more recommended alternative oral antibiotics.
Conclusion: SSTI in travellers fosters the global spread of MRSA. USA300 and USA300-LV are the most commonly imported MRSA to Europe and about one third of imports belong to major epidemic clones. Over half of MRSA SSTI patients were colonized with the same strain in their nasal cavity, highlighting the important role of nasal colonization in the chain of infection. These findings will inform hygiene and prevention recommendations and will thus be valuable in limiting further spread of epidemic MRS

Presentation: Tuesday, February 20, 2018 from 16:00 – 16:15 in room Audimax Saal.

070/MSV

Antimicrobial Resistance in Germany 2013 – 2016: Results from the European Antimicrobial Resistance Surveillance Network (EARS-Net)

I. Noll*[1], M. Feig[1], T. Eckmanns[1]
[1]*Robert Koch Institute, Infectious Disease Epidemiology, Berlin, Germany*

Introduction: The European Antimicrobial Resistance Surveillance Network (EARS-Net) is the main EU surveillance system for Antimicrobial Resistance (AMR) providing comparable data on Antimicrobial Resistance for all 28 EU Member States, Iceland and Norway. This allows to evaluate the national situation within the European context.

Objectives: To give an overview over key EARS-Net species–antimicrobial group combinations in Germany 2013-2016 within the European context

Methods: German data in EARS-Net originate from the Antimicrobial Resistance Surveillance (ARS) System. Antimicrobial susceptibility testing is performed by automated systems, results are (predominantly) evaluated according to EUCAST. Resistance for a specific species – antimicrobial class is based on invasive isolates only and is computed as the percentage of R isolates out of all isolates tested. Tests for trends are based on data of those laboratories with continuous participation over the whole period.

Results: The table shows results for the past four years in Germany compared to the European median as well as the population-weighted European mean percentage taken from the Annual report of the European Antimicrobial Resistance Surveillance Network 2016 (to be published mid-November 2017). In Germany, for most species-antimicrobial class-combinations under surveillance the situation has been stable over the past 4 years, with only one increasing trend for combined resistance in *Escherichia (E.). coli* and significant decreases for fluoroquinolones in *E. coli*, Ceftazidime in *Pseudomonas aeruginosa*, MRSA and High level Gentamicin in *Enterococcus faecalis*.

For many species-antimicrobial class-combinations under surveillance the resistance percentages in Germany in 2016 do not differ much from the European median percentage with a few positive exceptions: For *Klebsiella pneumonia* as well as for *Acinetobacter spp.*, resistance percentages for almost all antimicrobial classes under surveillance are more than ten percent lower than the European median or population-weighted mean (preliminary results!).

Summary: Over the past four years, the AMR situation in Germany shows a high degree of stability in invasive isolates with regard to key species–antimicrobial group combinations.

Figure 1

Percentage of resistant (R) resp. non-susceptible (RI) isolates of all isolates tested in Germany 2013 - 2016 compared to EARS-Net median and mean percentage 2015

Microorganism x antimicrobial class	German EARS-Net results 2013	2014	2015	2016	trend	EARS-Net participating countries median 2015	mean* 2015	trend
Escherichia coli								
Fluoroquinolones R	22,1	20,6	19,5	19,7	-	24,1	22,8	
3rd gen. Cephalosporins R	10,7	10,5	10,4	11,5		11,7	13,1	+
Aminoglycosides R	7,0	6,9	7,2	7,1		11,2	10,4	
Carbapenems R	0,1	0,1	< 0,1	< 0,1		< 0,1	0,1	
combined resistance	2,7	3,0	3,0	3,5	+	5,5	5,3	+
Klebsiella pneumoniae								
Fluoroquinolones R	15,1	12,7	9,6	12,2		28,4	19,7	+
3rd gen. Cephalosporins R	16,1	12,7	10,1	13,7		26,1	20,3	+
Aminoglycosides R	10,0	7,1	5,5	7,8		22,1	22,5	+
Carbapenems R	0,7	0,7	0,1	0,5		0,5	8,1	+
combined resistance	7,0	5,3	3,1	5,4		17,3	18,6	+
Pseudomonas aeruginosa								
Piperacillin/TAZ R	18,8	17,4	17,7	17,7		14,0	18,1	+
Fluoroquinolones R	16,4	13,0	14,4	12,5	-	16,2	19,3	-
Ceftazidime R	10,2	9,9	9,1	10,5		9,9	13,3	
Aminoglycosides R	7,6	5,9	7,3	6,9		9,2	13,3	-
Carbapenems R	15,4	17,0	15,0	15,0		15,5	17,8	
combined resistance	9,2	8,9	8,2	7,9		10,0	12,9	
Acinetobacter spp.								
Aminoglycosides R	6,1	4,1	5,5	2,9		41,9	nd	
Fluoroquinolones R	9,7	6,0	8,6	5,7		51,9	nd	
Carbapenems R	8,9	5,5	6,6	4,9		28,7	nd	
combined resistance	5,2	2,1	3,7	2,2		23,2	nd	
Staphylococcus aureus								
Oxacillin/Meticillin R	12,8	12,9	11,2	10,3	-	12,7	16,8	-
Enterococcus faecalis								
HL Gentamicin R	39,7	33,6	31,3	25,7	-	31,3	31,3	
Enterococcus faecium								
Vancomycin R	14,6	9,1	10,2	12,1		10,2	8,3	
Streptococcus pneumoniae								
Penicillin RI	6,9	4,4	6,2	4,0		10,1	nd	
Macrolides RI	10,6	7,1	8,1	7,8		13,5	nd	

mean* population weighted mean of all EARS-Net participating countries 201:nd not done

combined resistance

E. coli, K. pneumoniae : resistance to fluoroquinolones, 3rd gen. Cephalosporins and Aminoglycosides;
Acinetobacter spp .: resistance to fluoroquinolones, Carbapenems and Aminoglycosides;
P. aeruginosa : resistance to 3 or more antimicrobial groups listed

color codes

- < 1 %
- 1 % - < 5 %
- 5 % - < 10 %
- 10 % - < 25 %
- 25 % - < 50 %
- > 50 %

trends

- \+ significant increasing trend
- \- significant decreasing trend

calculation of trends is based on data from laboratories reporting consistently over the four year period

European results will be updated after publication of EARS-Net report on 18th November 2017

Presentation: Tuesday, February 20, 2018 from 16:15 – 16:30 in room Audimax Saal.

071/MSV

Clinical and molecular characteristics of community-associated MRSA clones from skin and soft tissue infections in Heidelberg, Germany 2012-2016

S. Klein*[1], M. Menz[1], K. Heeg[1], D. Nurjadi[1]
[1]*Universitätsklinikum Heidelberg, Department für Infektiologie, Heidelberg, Germany*

Question: Methicillin- resistant *Staphylococcus aureus* (MRSA) is of major concern worldwide due to its high pathogenicity and resistance to first-line therapy. It is associated with worse outcome and health care associated infections. Besides, community-acquired MRSA (CA-MRSA) has gained more attention as clones exhibiting high virulence have been shown to circulate in the community of specific regions, like USA300. However, in Germany these CA-MRSA clones have rarely been reported. Besides, knowledge of molecular epidemiology and evolution as well as clinical features or resistance profiles of CA-MRSA clones in Germany is sparse. We aimed to characterize CA- MRSA clones isolated from patients with skin and soft tissue infections, treated in the outpatient department at Heidelberg University Hospital between 2012 and 2016 retrospectively.

Methods: 94 clinical isolates were included in the analysis. Identification was done with Latex agglutination and presence of DNAse. Antimicrobial susceptibility testing was performed with Vitek2. Presence of *mecA* and *nuc* was confirmed by RT-PCR. Isolates were *spa*- typed, presence of Panton- Valentine-Leukocidin (PVL) as well as genotypic resistance to mupirocin and chlorhexidin was analyzed by PCR. Clinical information

concerning underlying disease, treatment, chronic infections, recurrent infections, travel history or immigration status was collected from the patient"s record. Data was analyzed with STATA.
Results: Resistance to clindamycin and macrolides was more than 50%, while co-trimoxazol, tetracyclin and fusidic acid resistance was low. All isolates were susceptible to rifampicin, fosfomycin and linezolid. Resistance to the decolonizing agents mupirocin and chlorhexidin was low. 40% were PVL positive. 43% of all isolates belonged to the *spa*-CC 045 cluster with t003 being the most frequent *spa* type (n=28). 18% (n=17) were *spa*-CC 024 cluster with n=12 t008 clones. Only 7.5% of the cluster *spa*-CC 045 was PVL positive, but 64% of the cluster *spa*-CC 024. Presence of PVL was associated with recent travel history or migration.
Conclusions: Our data shed light into the community-associated MRSA clones circulating in the region of Heidelberg. As expected, the cluster *spa*-CC 045 is the most frequent cluster with t003, the Rhine-Hessen clone being the most frequent *spa*-type. The abundance of the *spa*-CC 024 cluster is somewhat surprising, as it has not been reported by previous studies. The association between travel and migration and PVL- positivity indicates that clones of high virulence, such as ST8/t008 are imported into Germany and are already circulating in the community. The high percentage of PVL positive clones and multiple resistances to orally administered alternative agents raise much concern and should be monitored closely.

Presentation: Tuesday, February 20, 2018 from 16:30 – 16:45 in room Audimax Saal.

WORKSHOP 13
Surveillance: From Nosocomial Infections, Staphylococcus aureus to long term Facilities (FG PR)
20 Febr. 2018 • 15.15–16.45

072/PRV
Development of nosocomial MRSA infections in Germany from 2007 until 2016. Data from the german hospital infection surveillance system (KISS).
T. Kramer*[1], C. Schröder[1], C. Geffers[1], P. Gastmeier[1]
[1]Institut für Hygiene und Umweltmedizin, Charité Unisversitätsmedizin Berlin, Berlin, Germany

Introduction: During the past decade in Germany many efforts have been made in order to decrease the burden due to methicillin resistant *Staphylococcus aureus* (MRSA). In some European countries including Germany a decrease of nosocomial infections due to MRSA was observed in the recent past.
Aim: We tried to further determine the proportion of nosocomial *Staphylococcus aureus* infections in Germany that were accounted for by MRSA in the past nine years.
Methods: Data from the German Hospital Infection Surveillance System (*Krankenhaus-Infektions-Surveillance-System*, KISS) from the years 2007–2016 were analyzed. Data on nosocomial infections occurring on intensive care units (ICU) were registered: primary sepsis, lower respiratory tract Infection and urinary tract infections. In surgical wards, data on postsurgical wound infections were collected.
Results: The number of participating intensive care units varied from 465 to 985, while the number of participating surgical wards varied from 432 to 1139. Over the period 2007–2016, the percentage of nosocomial *Staphylococcus aureus* infections that were due to MRSA dropped significantly, from 32.78% to 19.97%. In primary sepsis the percentage of infections due to MRSA dropped from 37.11% to 21.79% (p=.0131) and from 38.71% to 19.22% for lower respiratory tract infections (p<.0001) on ICUs. There was only a minor reduction in nosocomial urinary tract infections on ICUs from 37.04% to 33.33% (p=.988). The proportion of MRSA in postsurgical wound infections caused by *Staphylococcus aureus* decreased from 21.11% to 7.39% (p=.0004). In ICUs (n=240) and surgical wards (n=183) that continuously participated from 2007 until 2016 a similar development was observed.
Conclusion: A significant reduction of the proportion of nosocomial *Staphylococcus aureus* infections due to MRSA in Germany was observed over the period from 2007 to 2016. The reasons for this development remain unclear and should further be elucidated.

Figure 1

	2007/08	2009/10	2011/12	2013/14	2015/16	total
intensive care wards	465	533	645	856	985	1218
surgical wards	432	558	681	972	1139	1556
nosocomial S. aureus infections	2654	2727	2856	3177	2994	14408
nosocomial MRSA infections (%)	870 (32.8)	836 (30.7)	73 (26.4)	800 (25.2)	598 (20.0)	3857
nosocomial S. aureus infections on intensive care wards	1913	1965	2072	2246	2441	10637
nosocomial MRSA infections on intensive care wards (%)	719 (37.6)	679 (34.6)	627 (30.3)	657 (29.3)	547 (22.4)	3229
nosocomial S. aureus infections on surgical wards	741	762	784	931	553	3771
nosocomial MRSA infections on surgical wards (%)	151 (20.4)	157 (20.6)	126 (16.1)	143 (15.4)	51 (9.2)	628

Figure 1: Development of nosocomial infections caused by *Staphylococcus aureus* during the study period.

Presentation: Tuesday, February 20, 2018 from 15:15 – 15:30 in room Hörsaal 1.

073/MSV
Metapopulation dynamics of nasal carriage *Staphylococcus aureus* within and among hosts
A. Gonzaga*[1], M. Steglich[1,2], C. Sievers[3], J. Mehraj[3], G. Krause[3], U. Nübel[1,2]
[1]Leibniz-Institute DSMZ, Braunschweig, Germany
[2]German Center of Infection Research (DZIF), Braunschweig, Germany
[3]Helmholtz Centre for Infection Research, Braunschweig, Germany

Staphylococcus aureus is a common constituent of the normal human body flora, and an important opportunistic pathogen residing on skin and mucosae of healthy people. Whole-genome sequencing has proved effective at identifying bacterial isolates as parts of the same transmission network. Moreover, genome sequencing may even enable the reconstruction of patient-to-patient transmission pathways. However, inference of transmission will require an improved understanding of bacterial diversity within individual hosts, which may fluctuate with time and vary among individuals.
Therefore we examined in detail the evolutionary dynamics of *S. aureus* from asymptomatic carriage hosts. Sequencing a total of 202 genomes from 15 colonized hosts, we investigated diversity, , population dynamics and transmission groups over a period of approximately 12 months.
With the aim of addressing its genetic lineages we have used a gene-by-gene approach (whole-genome multilocus sequence typing) as well as comparisons of the core genome to provide high resolution data across a group of related l isolates. To assess the relationship between the 202 isolates, we have visualized the distances between their allelic profiles in a minimum spanning network.
Results suggest that putative transmission events can be detected based on sequence data. Colonization was steady over time, each household showed usually several sequence types and most individuals were colonized by a single strain each. Rates of *S. aureus* transmission among family members living in the same household were unexpectedly low.

Presentation: Tuesday, February 20, 2018 from 15:30 – 15:45 in room Hörsaal 1.

074/HYV

Decolonization of health care workers carrying a methicillin-susceptible *Staphylococcus aureus* isolate

A. Richter*[1], I. Eder[2], B. König[2], B. Lutze[1], A. C. Rodloff[2], U. H. Thome[3], M. Weiss[4], I. F. Chaberny[1]
[1]*University Hospital Leipzig, Institute of Hygiene, Hospital Hygiene and Environmental Medicine, Leipzig, Germany*
[2]*University Hospital Leipzig, Institute of Medical Microbiology and Epidemiology of Infectious Diseases, Leipzig, Germany*
[3]*University Hospital Leipzig, Neonatology Unit, Leipzig, Germany*
[4]*University Hospital Leipzig, Central Institution of Occupational Medicine and Occupational Safety, Leipzig, Germany*

Question: In 2016, an increased rate of methicillin-susceptible *Staphylococcus aureus* colonization was detected on a neonatal intensive care unit at the Leipzig University. Typing results showed a predominant *spa*-type t091 which can cause bloodstream infections in children [1]. Considering nosocomial clustering, several infection prevention measures (e.g. intensified standard precautions, single-occupancy room, cohorted patients, continuing education of staff) were introduced, including staff screening followed by decolonization of colonized health care workers.
The interventions were initiated to reduce the rate of *S. aureus* colonization on the neonatal unit and for infection prevention.
Methods: Positively screened staff members performed a five days long decontamination scheme at home. Decolonization products containing octenidine as active ingredient were used first. At least 48 hours after completing the procedure, the success of the intervention was tested (three buccal and nasal swabs were taken on consecutive days). If two efforts of decolonization were not successful, staff members were provided with a mupirocin containing nasal ointment instead of octenidine.
Results: Of 128 employees examined, 43 (33.6%) were identified as carriers of *S. aureus*. In nine cases (20.9%; 9/43) the *S. aureus* matched with type t091. Two carriers (4.7%; 2/43) of methicillin-resistant *S. aureus* (MRSA) were detected as well. The first decolonization effort against t091 and MRSA failed all together. After a second decolonization period, three cases became negative. Finally, eight remaining staff members were decolonized successfully with mupirocin containing nasal ointment.
Conclusions: Various reasons may be responsible for difficulties of decolonization. For example the challenge of managing decolonization at home and inhibitory factors as well as inconsistencies in performance of measures of decolonization. Additionally, differences in consistencies between the preparations for the nasal decontamination may be considered.
For assessing the sustainability of the decolonization a follow-up one year after decolonization would be an interesting approach.

Reference

[1] Ilczyszyn WN, Sabat AJ, Akkerboom V, Szkarlat A, Klepacka J, Sowa-Sierant I et al. Clonal Structure and Characterization of *Staphylococcus aureus* Strains from Invasive Infections in Paediatric Patients from South Poland: Association between Age, *spa* Types, Clonal Complexes and Genetic Markers. PLoS One 2016; 11(3): e0151937

Presentation: Tuesday, February 20, 2018 from 15:45 – 16:00 in room Hörsaal 1.

075/HYV

Point-Prevalence Study on the Urinary Incontinence Management in Long Term Care Facilities

K. Hüppe[1], A. Koll[1], P. Bergen[2], C. Henke-Gendo*[2]
[1]*District of Hildesheim, Public Health Department, Hildesheim, Germany*
[2]*Governmental Institute of Public Health of Lower Saxony (NLGA), Hospital Epidemiology, Hannover, Germany*

Introduction: The most important measure to prevent Catheter-associated Urinary Tract Infections (CAUTI) is to reduce the use of indwelling urethral catheters. In order to estimate the potential of this approach and other preventive measures of CAUTI in long term care facilities (LTCFs), a district-wide point-prevalence study on urinary catheter use was performed in the district of Hildesheim.
Methods: In a regional network's project, all 53 LTCFs in the district of Hildesheim were invited to participate in a survey. They were asked about their use of indwelling urethral catheters, suprapubic catheters, condom catheters or diapers, and descriptive information on the facility (number of residents, availability and qualification of infection control experts, documentation of care standards) were additionally recorded. For each catheterized resident, date and place of first insertion, indication, numbers of urinary tract infections within the last four weeks as well as antibiotic treatment were recorded.
Results: In total, 1253 residents of 17 (32%) facilities could be included in the analysis. The overall prevalence of urinary catheter application was 10.3%, ranging from 0.0% to 22.0% in the single LTCFs. In 70% of cases, the catheter had been initially inserted at a hospital. Indications for catheter use were various. In 18 (14%) cases no appropriate indication for catheter usage could be found. The availability of internally employed personnel within the facility (manager or authorized person), who were particularly trained in infection control, was found to be positively correlated with the appropriateness of the indications. However, occupation of an external infection control nurse had no effect on the appropriate indication for catheter use. Prevalence of an antibiotic treatment due to CAUTI within the last four weeks was 9.6%. The predominantly used drug was ciprofloxacin.
Conclusions: The prevalence of urinary catheter usage showed extreme variation between the single LTCFs. The rate is certainly dependent on the medical conditions of the residents, but facilities with personnel which is particularly trained in the issues of infection control generally show a lower rate of unappropriated catheter use. Therefore, education and training of the nursing staff and management seems to be important. CAUTI is frequent indicating at a high disease burden in this population. Further studies, e.g. studies to highlight the hospitals" role as place of the initial catheter insertion, are necessary and might emphasize the importance of a more effective intersectoral cooperation.

Presentation: Tuesday, February 20, 2018 from 16:00 – 16:15 in room Hörsaal 1.

076/PRV

Outbreaks and colonization recorded by the control outbreak registry of the Deutsches Beratungszentrum für Hygiene (German consulting center for infection control and prevention)

R. Schaumann*[1], M. Reska[1], S. Schulz-Stübner[1]
[1]*Deutsches Beratungszentrum für Hygiene - BZH GmbH, Freiburg i. Br., Germany*

Question: Outbreaks of infectious diseases are an increasing burden on hospitals as well as the health system in general. Furthermore, the number of patients colonized "only" with possible pathogens also increases. Therefore, we analyzed our data for possible discriminations and prevention methods.
Methods: At the end of 2013 at our center we implemented a quality assurance registry of outbreak investigations including colonization of patients with possible pathogen microorganisms. Now we analyzed the registered outbreaks until June 2017 (n=63).
Results: Within 63 outbreaks we had 381 infected patients, 97 colonized patients and 186 infected health care workers (156 x norovirus, 22 x influenza, 8 x scabies) as well as two MRSA-colonized health care workers. Thus, norovirus was the leading causative microorganism, whereas gram-negative bacteria, including MDR *K. pneumoniae*, MDR *E. coli*, MDR *E. cloacae* and XDR *A. baumannii*, dominated the bacterial microorganisms, followed by *S. aureus*, including MRSA, and *C. difficile*. Outbreaks lasted between 6 and 185 days and were related to colonization only in about a quarter. No deaths were recorded. Risk factors and improvement potentials for future outbreaks could be identified.
Conclusion: Due to increasing screening recommendations and detection of resistant organisms as markers of potential transmission in general more "colonization only"-outbreaks are detected. Therefore, the determination of prevention methods to reduce transmissions of possible pathogen microorganisms is of great importance. I.e. compliance of the staff with respect to

vaccination as well as required time to do their work and use of protective equipment.

Presentation: Tuesday, February 20, 2018 from 16:15 – 16:30 in room Hörsaal 1.

077/PRV

Mandatory notification for carbapenem non-susceptibility of *Acinetobacter* spp. and *Enterobacteriaceae* in Germany

A. Reuss*[1], A. von Laer[1], M. Abu Sin[1], T. Eckmanns[1]
[1]*Robert Koch Institute, Berlin, Germany*

Question: Carbapenems are antibiotics of last resort for the treatment of patients with multi-drug resistant bacterial infections. Since May 2016, a national mandatory notification of carbapenem non-susceptibility in *Acinetobacter* spp. and *Enterobacteriaceae* has been implemented in Germany to prevent further spread. We describe the epidemiology of carbapenem non-susceptibility in *Acinetobacter* spp. and *Enterobacteriaceae* in Germany based on these mandatory notification data.

Methods: Notifications of carbapenem non-susceptible *Acinetobacter* spp. and *Enterobacteriaceae* from week 35/2016 (29/08/2016) to week 34/2017 (27/08/2017) in Germany were analyzed. Laboratories report to local public health authorities who notify data to federal states and from there to the Robert Koch Institute. The case definition included all notifications with *Acinetobacter* spp. or *Enterobacteriaceae* (culture or PCR) AND carbapenem non-susceptibility (phenotype or detection of a carbapenemase gene). Isolates tested resistant or intermediate susceptible to at least one carbapenem (imipenem (IPM), meropenem (MEM), and for certain *Enterobacteriaceae* ertapenem (ETP)) were classified as non-susceptible. Intrinsic resistance was excluded.

Results: During the one year period, 4,028 notifications fulfilled the case definition with an average of 78 notifications per week. Persons with carbapenem non-susceptible *Acinetobacter* spp. (n=690) were more often male (69%) than persons with carbapenem non-susceptible *Enterobacteriaceae* (n=3,338, 61%). Other characteristics were similar for *Acinetobacter* spp. and *Enterobacteriaceae*: median age was 67 years (IQR: 52 to 77 years), 90% were hospitalized and among 1,849 persons with available information, 64% were colonized and 36% infected. The mainly identified pathogens were *Klebsiella* spp. (1,392, 35%), *Enterobacter* spp. (888, 22%), *Acinetobacter* spp. (690, 17%) and *Escherichia* spp. (600, 15%). Specimen materials included mainly screening swabs (1,790, 44%), urine (770, 19%), wound swabs (447, 11%) and blood cultures (129, 3%). Among *Enterobacteriaceae* (n=1,870), susceptibility to IPM and MEM was tested more often than susceptibility to ETP (68%, 73% and 46%, respectively). When all three carbapenems were tested (n=767), 75% were not susceptible to IPM or MEM and 90% to ETP. A carbapenemase was identified in 433 *Acinetobacter* spp., mostly OXA-23 (72%). The mainly identified carbapenemases in *Enterobacteriaceae* (n=1,870) were OXA-48-like (53%), VIM-1 (13%), NDM-1 (11%) and KPC-2 (11%).

Conclusions: The national notification data, albeit incomplete, confirm the significance of carbapenem non-susceptible pathogens in Germany with a high proportion of colonizations. In *Enterobacteriaceae*, non-susceptibility testing to ETP was less often performed than to IPM and MEM - although almost 90% of notified *Enterobacteriaceae* were not susceptible to ETP.

Presentation: Tuesday, February 20, 2018 from 16:30 – 16:45 in room Hörsaal 1.

WORKSHOP 14
Mechanisms of Host-Mikrobiota Interactions (FG PW/FG GI)
20 Febr. 2018 • 15.15–16.45

078/PWV

Within host genome evolution of the Oligo-Mouse-Microbiota bacterial community

D. Garzetti*[1,2], S. Brugiroux[1,3], D. Ring[1], S. Hussain[1], B. Stecher[1,2]
[1]*Max von Pettenkofer Institute, Faculty of Medicine, LMU, München, Germany*
[2]*German Center for Infection Research, DZIF partner site, München, Germany*
[3]*University of Clermont Auvergne, Clermont-Ferrand, France*

Introduction: Bacterial genome evolution is mostly driven by small additive mutations and horizontal gene transfer (HGT), events which are normally selected for in the course of environmental changes. The functionality of the mammalian intestinal ecosystem is influenced by various host and external factors, affecting the composition, gene expression and evolution of the microbial community over time. Whole-genome sequencing is nowadays a powerful tool to investigate the functional evolution of microbiota communities and, therefore, understand the genome stability/flexibility of the whole bacterial population and its single members.

Objectives: We have previously established a defined consortium of 12 cultivable murine isolates (Oligo-MM^{12}) in a gnotobiotic mouse model to study the role of the gut microbiota and its association with diseases [1]. We now aim to use this model for investigating short- and long-term genome evolution of commensal bacteria, and for delineating microbiota-associated functions and metabolic potential in their natural host.

Materials and Methods: Whole-genome sequences of the 12 original bacterial strains were obtained by assembling Illumina and PacBio sequences [2]. Cecum content from mice colonized for 2 years over 4 generations with the Oligo-MM^{12} was used to generate an evolved metagenomics dataset and, when possible, single bacterial genomes of re-isolated bacteria. Shotgun-sequencing was performed either on Illumina MiSeq or HiSeq platforms for single genomes and for the metagenome, respectively. Read assembly was performed using SPAdes and Ray software, followed by automatic annotation with the RAST or MG-RAST servers, and whole-genome comparison with Mauve.

Results: Comparison between genome sequences of the same strain before and after long-term colonization of the mouse gut, without any disturbance or external stimulus, revealed high genome stability in some bacteria (e.g. *Blautia coccoides*) and HGT events in others (e.g. *Enterococcus faecalis*). Analysis of the evolved metagenome gave first hints on the driving forces influencing colonization ability and metabolic potential of the 12 bacteria.

Summary: Evolution of bacterial genomes within complex gut microbial communities can be efficiently studied using a small defined consortium of murine isolates as a reductionist approach.

References

[1] Brugiroux S, Beutler M, Pfann C, Garzetti D, Ruscheweyh HJ, Ring D, Diehl M, Herp S, Lötscher Y, Hussain S, Bunk B, Pukall R, Huson DH, Münch PC, McHardy AC, McCoy KD, Macpherson AJ, Loy A, Clavel T, Berry D, Stecher B. 2016. Genome-guided design of a defined mouse microbiota that confers colonization resistance against Salmonella enterica serovar Typhimurium. Nat Microbiol 2:16215.

[2] Garzetti D, Brugiroux S, Bunk B, Pukall R, McCoy KD, Macpherson AJ, Stecher B. 2017. High-quality whole-genome sequences of the Oligo-Mouse-Microbiota bacterial community. Genome Announc 5:e00758-17.

Presentation: Tuesday, February 20, 2018 from 15:15 – 15:30 in room Hörsaal 3.

079/PWV

Distinct microbial signatures are associated with varying susceptibility to *Citrobacter rodentium* infection in mice

L. Osbelt*[1,2], S. Thiemann[2], T. Strowig[2]
[1]*Otto von Guericke University, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Magdeburg, Germany*
[2]*Helmholtz-Zentrum für Infektionsforschung, Mikrobielle Immunregulation, Braunschweig, Germany*

Introduction: The gastrointestinal tract is colonized by complex microbial communities collectively called the gut microbiota. The metabolic activity of these microbes has an enormous impact on the host including influencing resistance or susceptibility to specific enteric pathogens. Even though it is widely accepted that varying composition among individuals impacts the outcome of enteric infections in human and mice, it is not well understood which members of these diverse communities contribute to disease severity.
Question: Do naturally occurring variations in the microbiota composition of mice impact the course of infections with *Citrobacter rodentium*, a model pathogen for enterohemorrhagic *Escherichia coli* (EHEC) infections in humans?
Methods: The impact of differences in microbiota composition on the outcome of infection with *C. rodentium* was assessed using isogenic mouse lines (C57BL/6N) obtained from different breeding facilities. For cohousing experiments mice were housed together in cages at 1:1 ratios for 4-6 weeks before infection experiments. All experiments were performed with a bioluminescence expressing *C. rodentium* strain (ICC180). For 16S rRNA gene analysis, the V4 region of the 16S rRNA gene was amplified. Statistical analyses and data visualization was performed with GraphPad Prism and the R statistical programming package phyloseq.
Results: We identified that isogenic mouse lines from different breeding facilities show highly varying disease kinetics after infection. Especially two mouse lines (SPF-1 and SPF-2) displayed a more than 1000-fold difference in *C. rodentium* colonization within the first days of infection. Transfer of SPF-1 and SPF-2 communities into germ-free mice verified that the varying susceptibilities are determined by microbiota composition. In susceptible mice *C. rodentium* rapidly colonized the cecum and this difference was also observed in *in vitro* experiments coculturing intestinal bacteria and *C. rodentium* suggesting that specific commensals in resistant SPF-2 mice inhibit growth of *C. rodentium* early on. We hypothesize that distinct bacterial-produced metabolites are responsible for the inhibition of *C. rodentium* in resistant mice. Preliminary analysis of the microbial composition using 16S rRNA gene sequencing revealed a microbial signature, which is associated with reduced pathogenic colonization in the first days of infection.
Conclusions: Genetically identical mice with distinct microbiota composition display varying susceptibility to *C. rodentium* infection. On-going experiments aim to identify the causative bacteria and/or their produced metabolites responsible for growth inhibition of *C. rodentium* during the early phase of infection. Identifying bacteria with protective properties against enteric pathogens has high potential for the development of novel preventive interventions against hospital-associated infections.

Presentation: Tuesday, February 20, 2018 from 15:30 – 15:45 in room Hörsaal 3.

080/PWV

CD101 maintains intestinal immune homeostasis due to the control of bacterial replication and the regulation of anti-inflammatory immune responses

M. Purtak[1], R. Schey[1], A. Arnold[1], K. Dettmer[2], A. Ensser[3], P. Oefner[2], J. Mattner*[1]
[1]*Klinische Mikrobiologie Erlangen, , Erlangen, Germany*
[2]*Institute for Functional Genomics, University of Regensburg, Regensburg, Germany*
[3]*Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Virologisches Institut, Erlangen, Germany*

Introduction: CD101 is a transmembrane, Ig-like domains containing glycoprotein, which exerts negative-costimulatory effects *in vitro*, but its function *in vivo* remains poorly defined. We have recently reported that an enhanced expression of CD101 is associated with less severe colitis and significant alterations in the composition of the intestinal microbiota in patients with inflammatory bowel disease (IBD) and mouse models of chronic colitis1.
Aims: Thus, we elucidated in this study the mechanism(s) by which CD101 interferes with distinct bacteria and mediates protection from IBD and enterocolitis.
Materials and Methods: The expression of CD101, the composition of the intestinal microbiota, bacterial replication and dissemination as well as the extent of intestinal inflammation was assessed in the dextran sodium sulfate (DSS) and *Salmonella*-induced mouse models of acute and chronic colitis using different conditional CD101-knockout mice. The expression of CD101 and the composition of the intestinal microbiota in patients with mild, moderate and severe Crohn´s disease and ulcerative colitis were compared to age- and sex-matched control individuals.
Results: We observed that an infection with *Salmonella typhimurium* led to a reduced recovery of *Lactobacilli* and *Clostridia spp*. in CD101-/- compared to wild type littermates. Similar results were obtained in DSS-treated CD101-/- mice, which revealed in addition an accumulation of *Enterobacteriaceae* in the gut. These alterations in the composition of the intestinal microbiota were critical for the protective effects mediated by CD101 as only a reconstitution of CD101+/+, but not CD101-/- mice with *Lactobacilli* ameliorated DSS- and *Salmonella*-induced colitis and inhibited the translocation of *Enterobacteriaceae* into extra-intestinal organs. While *Lactobacilli* adhered to CD101 without affecting its expression, myeloid cells revealed an accumulation of antimicrobial metabolites and eliminated *Enterobacteriaceae* more efficiently correlating with the extent of CD101 expression. Most importantly, in IBD patients a reduced CD101-expression on peripheral and intestinal CD11b+ monocytes and CD4+ T cells correlated with an enhanced intestinal permeability and disease activity.
Summary: CD101 exhibits a protective role in murine enterocolitis and human IBD. Our data also suggest that commensal bacteria require CD101 to protect from severe enterocolitis and IBD. How certain bacterial species (that might be missing in IBD patients) promote the function of CD101 and how CD101 restricts bacterial replication is part of our ongoing analyses.

Reference

[1] Schey, R. *et al.* CD101 inhibits the expansion of colitogenic T cells. *Mucosal Immunol.* **9**, 1205–1217 (2016).

Presentation: Tuesday, February 20, 2018 from 15:45 – 16:00 in room Hörsaal 3.

081/PWV

Evaluation of the safety of bacillus thuringiensis strains ABTS-351 and ABTS-1857 within a simulated gut environment

P. Van den Abbeele*[1]
[1]*ProDigest, Zwijnaarde, Belgium*

Bacillus cereus sensu stricto has been implicated in both beneficial and adverse effects on the human digestive system. Pathogenicity of individual species and strains varies and is little understood. The aim of the current project was to investigate the colonization potential of two commercial *Bacillus thuringiensis* (Bt) strains (ABTS-351 and ABTS-1857) and how this affects human gut microbes and host cells.

For this puprpose, the validated *in vitro* Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) that mimics the complete human gastro-intestinal tract, was applied. A worst-case scenario was mimicked by administering a large number of spores (4.9 x 108 CFU/d) to an antibiotic-disturbed gut microbiome, a standard procedure used to enable pathogenic infection by e.g. *C. difficile*.

Following gastric passage, spores of both strains were detected at lower levels than theoretical dose levels indicating that spores only survived to a limited extent. A ca. 1 log difference in total viable counts versus spore counts for Bt strain ABTS-351, which would normally indicate the presence of vegetative cells, was determined to be diminished thermal tolerance resulting in these spores being killed by the standard pasteurization procedure (20" at 80°C) to quantify them. Further, Bt treatments had minor beneficial effects on recovery of the microbiota upon antibiotic-induced dysbiosis, i.e. slower increase of health-detrimental metabolites related to protein fermentation and a strong stimulation of the health-related butyrate-producing *Faecalibacterium prausnitzii*. Finally, spore-free extracts of the model in contact with an in vitro human cell culture model, consisting of a co-culture of Caco-2 cells and THP-1 macrophages, revealed that neither treatment caused damage to cells or impeded the ability of cells to respond to stress. Both treatments improved cell integrity (TEER), opposite to what would be observed during a diarrheal event.

It could thus be concluded that the two commercial Bt strains under investigations did not show any indication as if they could be involved in foodborne infection or intoxication.

Presentation: Tuesday, February 20, 2018 from 16:00 – 16:15 in room Hörsaal 3.

082/PWV

***Chlamydia trachomatis* serovar D is recognized by TLR4 and MD-2 in epithelial cells of the human urogenital tract**

S. Albrecht*[1]
[1]*Universitätsmedizin Mannheim, Institut für Med. Mikrobiologie und Hygiene, Mannheim, Germany*

Introduction: *Chlamydia trachomatis* serovar D-K is the leading cause of female genital tract infections in industrialized countries. These infections are asymptomatic in over 70% of cases and, thus, occur undetected and untreated. In these cases, the infection will lead to chronic activation of the immune response and to prolonged inflammation resulting in possible complications such as hydrosalpinx, pelvic inflammatory disease, extra-uterine gravidity or tubal factor infertility. In female mice infected with *C. muridarum* we could show that inflammation-induced uterine tissue remodeling will not stop even after clearance of the bacterial load [1].

Epithelial cells recognize invading microorganisms through pattern recognition receptors presented on their surface, resulting in the release of inflammatory cytokines. For example, the Toll-like receptor (TLR) family member TLR4 in combination with MD-2 and CD14 has been identified as the principle signal transducer in lipopolysaccharide (LPS) recognition.

Purpose: In the past, the role of TLR4 in *Chlamydia* recognition in epithelial cells of the urogenital tract has been controversially discussed. Whereas in rat prostate epithelial cells endogenous TLR4 is recruited to the *C. muridarum* inclusion, the co-localization of YFP-labeled TLR4 to the *C. trachomatis* serovar L2 inclusion in human cervix cancer cells could not be monitored. With our studies of endogenous TLR4 in human cervix cancer and bladder cancer cells infected with *C. trachomatis* serovar D we want to give this discussion a new turn. Furthermore, we want to address the question, if the cellular recognition of *C. trachomatis* mediated by TLR4 is crucial for cytokine secretion.

Results: Immunofluorescence studies of endogenous TLR4 and MD-2 in human urogenital tract cells revealed that both, TLR4 and MD-2, are recruited to the bacterium as early as 6 h p.I. At later time points (24-30 h p.I.), TLR4 and MD-2 are still associated with the bacterial inclusion. To extend our studies, we analyzed the localization of the intracellular TLR4 adapters MyD88, TRIF and TRAM by immunofluorescence staining. We observed that MyD88 and TRIF, but not TRAM, is recruited to the *C. trachomatis* inclusion.

Conclusions: We found strong evidence that human TLR4 and MD-2 recognize chlamydial LPS as they change their cellular localization and are recruited to the bacterial inclusion. We assume that host cytokine secretion is processed via the MyD88-dependent pathway. As TRIF is a direct adaptor to TRAM and TLR3, we speculate that its co-localization with the inclusion might be not TLR4- but rather TLR3-dependent.

Next, we want to investigate if TLR4 combined with MD-2 results in cytokine release upon *C. trachomatis* recognition by using siRNA or specific inhibitors.

Reference

[1] Müller, T., et al., *Vaccination with the polymorphic membrane protein A reduces Chlamydia muridarum induced genital tract pathology.* Vaccine, 2017.

Presentation: Tuesday, February 20, 2018 from 16:15 – 16:30 in room Hörsaal 3.

083/PWV

Systems diagnostic of the human gut microbiome on inflammatory diseases using metaproteome analysis of fecal samples

T. Schlegel*[1,2], K. Schallert[1], R. Vilchez-Vargas[2], L. Bechmann[2], A. Link[2], C. Schulz[3], A. Canbay[2], D. Benndorf[1,4], U. Reichl[1,4], R. Heyer[1]
[1]*Otto von Guericke University, Chair of Bioprocess Engineering, Magdeburg, Germany*
[2]*Otto von Guericke University Clinic Magdeburg, Department of Gastroenterology, Hepatology and Infectious Diseases, Magdeburg, Germany*
[3]*Ludwig Maximilians University Clinic Munich, Department of Gastroenterology, Medical Clinic and Polyclinic II, München, Germany*
[4]*Max Planck Institute for Dynamics of Complex Technical Systems Magdeburg, Bioprocess Engineering, Magdeburg, Germany*

Introduction: The gut microbiome comprises about 1013 microorganisms. Among other functions the microorganisms produce and metabolize biomolecules, and interact directly with the host immune system. Crohn"s disease (CD) and Ulcerative Colitis (UC) are chronic inflammatory bowel diseases (IBD). They are associated with alterations in gut barrier function, mucosal inflammation and in the composition of the gut microbiome. The risk for developing a colon carcinoma is enhanced due to the chronic inflammation. However, the impacts of the microbiome on IBD and the development of cancer are poorly understood.

Objective: The aim of this study was to investigate the correlation between the microbial taxonomies in the gut and their metabolic functions with an increased inflammatory level of the gut and to identify marker proteins for the different diseases. Therefore, microbial and human proteins from fecal samples of patients with IBD (n_{CD}=11; n_{UC}=14), irritable bowel syndrome (IBS; n_{IBS}=13), healthy individuals ($n_{control}$=17) as well as patients with colon adenoma (n_A=7) and gastric carcinoma (GCA; n_{GCA}=8) were analyzed by a metaproteomic approach.

Methods: The workflow comprised protein extraction with liquid phenol in a ball mill, tryptic digestion of the complete sample and peptide separation by liquid chromatography coupled to an Orbitrap MS/MS. For protein identification, taxonomic and functional result interpretation the *MetaProteomeAnalyzer* software and a tailored protein database consisting UniProtKB/SwissProt as well as several metagenomes from human fecal samples were used.

Results: Analysis of the fecal samples identified up to 3,000 metaproteins in each sample from human as well as from bacteria, archaea and viruses. Dominant bacterial proteins were ribosomal proteins, elongation factors as well as compounds of the protein biosynthesis and glycolysis pathway belonging to the phyla Firmicutes, Proteobacteria and Bacteriodetes. In CD patients the amount especially of the order *Clostridiales* was decreased compared to the control group.
The most abundant identified human proteins were several immunoglobulins and digestive enzymes. Samples from patients with CD had significantly higher amounts of the cell adhesion molecule cadherin. In patients with active UC a higher number of cathepsin G involving in matrix degradation in inflammatory tissues and peptidoglycan recognition protein were identified. Various human antibacterial peptides such as azurocidin, defensin and lipocalin were increased in patients with CD and active UC. In contrast patients with UC in remission showed no significant differences regarding to the presence of these antibacterial peptides to healthy individuals.
Conclusion: Metaproteome analysis of gut proteins showed changed expression in IBD and cancer patients. Accordingly, metaproteomics may support the understanding of the pathogenesis and could be applied in non-invasive diagnosis of IBD.

Presentation: Tuesday, February 20, 2018 from 16:30 – 16:45 in room Hörsaal 3.

WORKSHOP 15
Bacterial Virulence – Mechanisms and their Regulation (FG MP)
21 Febr. 2018 • 08.30–10.00

084/MPV

Heterogeneous toxin production promotes success in two-strain bacterial competition

B. von Bronk[1], M. Weber[2], A. Mader[1], M. Lechner[2], E. Hebisch[1], E. Frey[2], M. Opitz*[1]
[1]*LMU, Faculty of Physics , München, Germany*
[2]*Arnold Sommerfeld Center for Theoretical Physics, LMU, München, Germany*

Bacterial interactions determine microbial community composition dynamics, which in turn affects human physiology and human health. One important type of bacterial interaction is the competition by toxin release. Here, we study competition by production and release of the bacteriocin ColicinE2 in *Escherichia coli*[1,4,5]. By quantitatively analyzing ColicinE2 expression dynamics in single cells[3], that are controlled by a complex gene regulatory network[2], we find that there a three different types of toxin expression in dependence of the external stress level. The degree of heterogeneous toxin production highly affects the competition of the toxin producing strain C with a strain sensitive towards the toxin. Furthermore, by combining experimental and theoretical approaches we demonstrate that stochasticity in toxin expression in the initial phase of competition determines competition outcome[1]. In particular we observe that competitive success of the toxin producing strain (C) is only found if (i) a C edge cluster hast formed at the end of the initial competition phase and (ii) the beneficial and detrimental effects of toxin production are balanced, which is the case at intermediate toxin producer fractions. Our findings highlight the importance of stochastic processes for bacterial competition, which might also be relevant for other microbial community interactions in which the random choice between phenotypes can have long-lasting effects for community fate.

References

[1] B. v. Bronk, *et al.*. Effects of Stochasticity and Division of Labor in Toxin Production on Two-strain Bacterial Competition in *Escherichia coli.* **Plos Biology, doi.org/10.1371/journal.pbio.2001457 (2017).**
[2] M. Lechner, *et al.*. Hierarchical Post-transcriptional Regulation of Colicin E2 Expression in *Escherichia coli*. **Plos comp. biol.12(12):e1005243 (2016).**
[3] A. Mader, *et al.*. Amount of colicin release in *Escherichia coli* is regulated by lysis gene expression of the Colicin E2 operon. **PlosOne, DOI:10.1371 (2015)**
[4] M. F. Weber, *et al.*. Chemical warfare and survival strategies in bacterial range expansions**. J.R.Soc. Interface, 11(96): 2014172 (2014)**
[5] E. Hebisch, *et al.*. High variation of fluorescence protein maturation times in closely related *Escherichia coli* strains. **PLOS ONE, 8(10): e75991 (2013)**

Presentation: Wednesday, February 21, 2018 from 8:45 – 9:00 in room Audimax Saal.

085/MPV

CREDIBLE – A *Cre*-med*i*ated dou*ble* reporter system to study penetration of physiological barriers by cell-penetrating effector proteins

M. L. Lubos*[1], T. Danyukova[1], B. Skryabin[2], C. Geyer[3], M. A. Schmidt[1], C. Rüter[1]
[1]*Institute of Infectiology, ZMBE, Münster, Germany*
[2]*TRAnsgenic animal and genetic engineering Models TRAM, Münster, Germany*
[3]*Preclinical Imaging eXperts, PIX, Institute of Clinical Radiology, Münster, Germany*

Bacterial pathogens including *Yersinia* and *Salmonella* express effector proteins that are translocated into the host cell via a type three secretion system (T3SS) during infection. Previous studies identified the effector protein YopM of *Yersinia enterocolitica* as a novel bacterial cell-penetrating effector (CPE) that is able to translocate across the host cell plasma membrane independently of *Yersinia*"s T3SS. The two N- terminal α-helices (2aH) have been identified as the protein transduction domain (PTD) of YopM, which mediates autonomous translocation and has the ability to intracellularly deliver molecular cargos such as GFP.
In order to further study and characterise the mechanisms of cell penetration by CPEs and further cell-penetrating peptides (CPPs) *in vivo*, we have generated a Cre-mediated double reporter ("CREDIBLE") system. Transgenic mice harbouring the "CREDIBLE" construct, express two reporter genes, namely near-infrared fluorescent protein (iRFP) and luciferase upon Cre/loxP-recombination.
The "CREDIBLE" system has been proven to be functional and both reporters are expressed upon recombination *in vitro*. Furthermore, crossing transgenic mice with PGK-Cre mice, expressing Cre-recombinase lead to recombination events, indicating the functionality of the system *in vivo*.
In order to analyse the distribution of CPP/CPEs *in vivo*, we have constructed different PTD-Cre fusion proteins, including 2aH-Cre. The recombinant proteins will be administered via different routes into the transgenic mice and their distribution can be analysed in real time by non-invasive live optical imaging.
In addition, the system described here can be applied in a variety of studies using drug delivery systems such as exosomes or targeted drug delivery in order to characterise their efficacy and distribution *in vivo*. Furthermore, the "CREDIBLE" system can be used to monitor bacterial or viral infections *in vivo* and, in particular, to gain a more detailed insight into the role and function of various virulence factors during infection (e.g. secreted bacterial effector proteins, outer membrane vesicles).

Presentation: Wednesday, February 21, 2018 from 8:30 – 8:45 in room Audimax Saal.

086/MPV

The secreted SplB is a novel protease from *S. aureus* that disrupts host complement attack by direct cleavage inactivation of host central complement proteins

P. Dasari*[1], M. Nordengrün[2], L. Steil[3], N. Reiher[1], C. Skerka[1], U. Völker[3], B. M. Bröker[2], P. F. Zipfel[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Infection Biology, Jena, Germany*
[2]*Institute of Immunology and Transfusion Medicine, University Medicine Greifswald, Greifswald, Germany*
[3]*Interfaculty Institute of Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany*

Staphylococcus aureus is a commensal bacterium that causes both nosocomial- and community-acquired infections. The incidence of S. aureus infections is rapidly increasing. This Gram positive pathogen causes infections ranging from superficial skin infections to life-threatening invasive infections, particularly in immune-compromised individuals. S. aureus expresses multiple virulence factors and immune evasive proteins. Most clinical S. aureus isolates express six serine protease-like proteins SplA–SplF. The exact function of each of the Spl proteins is not clear. To define the role of Spl proteins, and explore if they modulate host immune response for the benefit of the bacteria, we recombinantly expressed all six Spl proteins and investigated whether Spl proteins cleave complement proteins. Here we identify SplB as a protease that cleaved and inactivated the two central complement proteins C3 and C4. SplB specifically cleaved the α-chain of the two complement proteins and also cleaved the activation products C3b, iC3b, C3c, C3d, and C4b. This effect was specific, as SplB did not cleave the human complement inhibitors Factor H or C4BP. Furthermore, SplB cleaved the terminal complement pathways proteins C5, C6, C7, C8 and C9. Staphylococcal SplB mediates this proteolytic effect also in NHS. This inactivation of both C3 and C4 resulted in lower C3b opsonisation and reduced phagocytosis of S. aureus by human neutrophils and furthermore in less C5b-9 surface deposition. In conclusion, we identify the first function of SplB as a bacterial protease which cleaves and inactivates human complement proteins, inhibits all three complement pathways, and reduces clearance by human innate immune cells. Thus SplB is a novel virulence factor of S. aureus

Presentation: Wednesday, February 21, 2018 from 9:00 – 9:15 in room Audimax Saal.

087/MPV

The intercellular adhesin (*ica*) locus of *Staphylococcus epidermidis* harbours a non-coding RNA critically involved in the control of polysaccharide intercellular adhesin (PIA)-mediated biofilm formation

M. F. Lerch[1], S. M. K. Schoenfelder[1], M. Eckart[1], G. Marincola[1], K. U. Förstner[1], C. M. Sharma[1], K. M. Thormann[2], W. Ziebuhr*[2]
[1]*University of Wuerzburg, Molecular Infection Biology, Würzburg, Germany*
[2]*University of Gießen, Microbiology & Molecular Biology, Gießen, Germany*

Polysaccharide intercellular adhesin (PIA)-associated biofilm formation is mediated by the intercellular adhesin (*ica*) locus and represents a major pathomechanism of *Staphylococcus epidermidis.* Here we report on the detection and characterization a novel long non-coding (nc)RNA, named IcaZ, which is approximately 400 nucleotides in size. *icaZ* is located downstream of the *ica* repressor gene *icaR* and partially overlaps with the *icaR* 3′ UTR. *icaZ* exclusively exists in *ica*-positive *S. epidermidis*, but not in *S. aureus* or other staphylococci. IcaZ is transcribed as a primary transcript from its own promoter during early- and mid-exponential growth and its expression is induced by low temperature and ethanol stress. Interestingly, inactivation of *icaZ* completely abolishes PIA production. IcaZ acts as an activator of PIA production via induction of *icaADBC* transcription, through targeting the *icaR* mRNA UTRs and controlling *icaR* mRNA on the posttranscriptional level. Other than in *S. aureus*, control of *icaR* mRNA in *S. epidermidis* does not involve *icaR* mRNA 5′/ 3′ UTR base pairing. This suggests major structural and functional differences in *icaADBC* operon regulation between the two species that also comprise the recruitment of ncRNAs. Together, the IcaZ ncRNA represents an unprecedented novel species-specific player involved in the immediate control of *S. epidermidis* PIA production.

Presentation: Wednesday, February 21, 2018 from 9:15 – 9:30 in room Audimax Saal.

088/MPV

The long non-coding RNA SSR42 controls virulence of *Staphylococcus aureus* in response to external signals

J. Horn*[1], M. Klepsch[1], M. Manger[1], C. Wolz[2], B. Hüttel[3], R. Reinhardt[3], A. Otto[4], D. Becher[4], T. Rudel[1], M. Fraunholz[1]
[1]*University of Würzburg, Chair of Microbiology, Würzburg, Germany*
[2]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*
[3]*Max Planck Genome Centre, Köln, Germany*
[4]*Ernst-Moritz-Arndt Universität Greifswald, Institute for Microbiology, Greifswald, Germany*

Introductions: *Staphylococcus aureus* is a major human pathogen producing a plethora of different virulence factors and toxins to successfully kill host cells and cause severe blood and tissue infections.
Aims: Aim of this work was the functional and molecular characterization of the 1232 nt long non-coding RNA (ncRNA) SSR42 and the elaboration of its impact on *S. aureus* virulence.
Methods: Knockout of ncRNA SSR42 leads to a global transcriptomic and proteomic reprofiling revealing its role in regulation of erythrocyte lysis, cytotoxicity, and biofilm formation. Among other, promoter activity and hemolysis assays were used to decipher the role of the ncRNA in *S. aureus* virulence regulation.
Results: Here we report that *S. aureus* uses SSR42, to regulate the expression of a multiplicity of virulence factors and that SSR42 constitutes the effector of the AraC-type transcriptional regulator, Rsp. We show that expression of SSR42 is induced upon stimulation with subinhibitory concentrations of antibiotics as well as cell-damaging substances. We further demonstrate that SSR42 is acting upstream of the SaeRS two-component system and identified multiple factors influencing SSR42 transcription in the complex circuit regulating *S. aureus* virulence.
Conclusions: Our data shows that *S. aureus* uses not only the quorum sensing effector RNAIII, but also SSR42 to regulate major virulence pathways.

Presentation: Wednesday, February 21, 2018 from 9:30 – 9:45 in room Audimax Saal.

089/MPV

***Pasteurella multocida* Toxin manipulates the host cell metabolome**

S. Chakraborty[1], B. Kloos[1], A. Hafner[2], F. Uhle[2], K. Kubatzky*[1]
[1]*Zentrum für Infektiologie, Universitätsklinikum Heidelberg, Medizinische Mikrobiologie und Hygiene, Heidelberg, Germany*
[2]*Universitätsklinikum Heidelberg, Anästhesiologische Klinik, Heidelberg, Germany*

Introduction: Toxigenic *Pasteurella multocida* strains produce the *Pasteurella multocida* toxin (PMT), a classical AB toxin that causes bone destruction due to atrophic rhinitis in pigs. PMT is a deamidase that constitutively activates specific heterotrimeric G protein alpha subunits, such as Q209 of the Gq alpha subunit. Eventually, this results in the activation of downstream signalling cascades that are involved in cytoskeletal rearrangement, proliferation, differentiation and survival of cells. We showed that PMT induces the formation of functional, bone-resorbing osteoclasts from macrophages. Physiologically, the formation of osteoclasts cells is initiated by the cytokines RANKL and M-CSF. PMT, however, uses a non-canonical pathway that relies on Gq-mediated activation of NFATc1 and the NFkB-mediated production of pro-inflammatory cytokines.
Aims: It is known that PMT-generated osteoclast are smaller and have a lower number of nuclei than classical osteoclasts. Although this suggests a lower resorptive capacity, PMT-derived osteoclasts have similar activity and we investigated how PMT can compensate the defect in fusion.

Results: When we investigated the activation of Rho GTPases, the central regulators of the actin cytoskeleton, we could show that PMT persistently activates RhoA and Rac1, which likely is the reason for the absence of the classical osteoclast actin ring and the decreased fusion of osteoclast progenitors. Since the process of bone resorption is highly energy-demanding, we further investigated the effect of PMT on the cellular metabolism. Further analysis showed that PMT induces mTOR, a kinase that plays a central role in the regulation of cell growth and metabolic activity. Seahorse analysis confirmed an increased oxygen consumption rate and ATP production of toxin-treated cells. However, the mitochondrial copy number of toxin-treated cells was not elevated, suggesting that the mitochondrial activity of these cells is enhanced without the need to stimulate mitochondrial biogenesis. Indeed, we could show that the expression of OXPHOS components is elevated. Recently it was published that Gq is localised not only at the plasma membrane but also at the outer and inner membrane part of mitochondria where it regulates the balance between mitochondrial fission and fusion and the folding of christae. When we checked for the presence of Gq in purified mitochondria, we found Gq to be deamidated and thus explaining the increased metabolic capacity of toxin-treated cells.
Summary: PMT-treated osteoclasts show comparable bone resorption due to their ability to highly express OPXPHOS and to generate an excess amount of ATP which seems to increase the resorptive capacity of the cells.

Presentation: Wednesday, February 21, 2018 from 9:45 – 10:00 in room Audimax Saal.

WORKSHOP 16
Cases in clinical Microbiology and infectious Diseases (StAG KM) (TED Session)
21 Febr. 2018 • 08.30–10.00

090/KMV, 091/KMV, 092/KMV, 093/KMV

These Abstracts will not be published.

Presentation: Wednesday, February 21, 2018 from 08:30 – 10:00 in room Hörsaal 1.

WORKSHOP 17
Nosocomial Outbreaks (FG PR)
21 Febr. 2018 • 08.30–10.00

094/PRV

A large scale outbreak of multiresistant *Enterobacter aerogenes* associated with intraoperative placement of TEE probes

E. M. Klupp[1], B. Kuhls[2], J. C. Kubitz[2], B. Reiter[3], J. K. Knobloch*[1]
[1]*University Medical Center Hamburg Eppendorf, Institute for Medical Microbiology, Virology and Hygiene, Hamburg, Germany*
[2]*University Medical Center Hamburg Eppendorf, Department of Anaesthesiology, Hamburg, Germany*
[3]*University Heart Center Hamburg, Department of Cardiovascular Surgery, Hamburg, Germany*

Introduction: In April 2017 a possible cluster of cases with multiresistant *Enterobacter aerogenes* (MR-EA) was observed at a tertiary care center with five carbapenem resistant strains during a period of two month detected in patients of different wards. Analysis of patient data revealed a prior colonization with carbapenem susceptible MR-EA in four of five patients. Evaluation of MR-EA with different resistance phenotypes revealed a possible large-scale outbreak with MR-EA and an outbreak management team was established.
Methods: Clonal relationship between carbapenem resistant and susceptible MR-EA was determined by PFGE. Extended screening procedures were put into execution (including two point prevalence studies) and a case control study was performed. Patients were included if at least a rectal swab and material of the respiratory tract was send for microbiological analysis (n=123; MR-EA positive cases n=35). Early onset of MR-EA was defined as confirmation of MR-EA carriage within 48 h after surgical procedures. Modified microbiological investigation of TEE probes was performed by placement of the distal end of the probes in 1 L of sterile 0.85 % NaCl solution for at least one hour with repeated flexion of the ransducer tip in all directions.
Results: PFGE revealed carbapenem resistant and susceptible MR-EA to be clonaly relatetd, indicating a large-scale outbreak. Point prevalence studies revealed a significant ($p<0.01$) correlation between cardiac surgery procedures and the observation of MR-EA. In early onset patients MR-EA was exclusively detected in specimen of the upper airways, indicating the oral cavity as the portal of entry. The removal of all oral applicable pharmaceuticals didn´t disrupt the transmission of MR-EA. Intraoperative placement of a TEE probe didn´t reach significance in the case control studies ($p=0.1$), but all MR-EA-positive patients received intraoperative TEE probe placement by one of three TEE probes. No correlation with a specific TEE probe and cases was observed. Repeated microbiological examination of the TEE probes (swabbing with moistened swabs) were negative for MR-EA. However, in a modified analysis the TEE probes could be confirmed to be contaminated by different microorganisms (including MR-EA) even after repeated cleaning and disinfection cycles and testing for impermeableness using validated procedures. Replacement of the three TEE probes immediately stopped further transmission of ME-EA.
Conclusions: Despite a validated cleaning and disinfection process three independent TEE probes used for intraoperative placement could not be sufficiently rescued from bacterial contamination causing a large scale outbreak by MR-EA. The process of intraoperative TEE probe placement should be optimized in the future with a focus on protective measures (including sterile probe covers) and optimized cleaning and disinfection processes.

Presentation: Wednesday, February 21, 2018 from 8:45 – 9:00 in room Hörsaal 3.

095/PRV

Long-term, low-frequency cluster of a GIM-1-producing *E. hormaechei* ssp. *steigerwaltii* in a tertiary care hospital in Germany

A. F. Wendel*[1,2], S. Meyer[2], R. Deenen[3], K. Köhrer[3], S. Kolbe-Busch[2], K. Pfeffer[2], M. Willmann[4], A. J. Kaasch[2], C. R. MacKenzie[2]
[1]*City of Cologne Hospitals, Institute of Hospital Hygiene, Köln, Germany*
[2]*Heinrich-Heine Universität, Institute of Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany*
[3]*Heinrich-Heine Universität, Biological and Medical Research Center, Düsseldorf, Germany*
[4]*University of Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany*

Objectives: *Enterobacter cloacae* complex is a common cause of hospital outbreaks. A retrospective and prospective molecular analysis of multidrug-resistant clinical isolates in a tertiary care centre demonstrated an outbreak of a GIM-1 metallo-beta-lactamase-producing *E. hormaechei* ssp. *steigerwaltii* affecting 23 patients between 2009 and 2016.
Methods: Carbapenem-non-susceptible *Enterobacteriaceae* isolates from routine clinical care were analysed by PCR, targeting relevant carbapenemase genes (*bla*IMP, *bla*VIM, *bla*GIM-1, *bla*NDM, *bla*IMP, *bla*KPC and *bla*OXA-48). Genotyping was carried out using whole-genome sequencing. Core-genome MLST (cgMLST) using the SeqSphere+ software and Maximum-likelyhood phylogenetic analyses were performed. Genetic and phenotypic resistance profiles were compared. Transposon and plasmid sequences were determined by reference mapping and *de novo* assembly. Epidemiological data were collected from clinical charts and environmental sampling was performed twice on the medical ward with the highest number of patients carrying the *E. hormaechei*.
Results: All isolates were sequence type 89 by conventional MLST and displayed a maximum difference of 49 out of 3643 targets in the cgMLST scheme. One clonal complex of highly related isolates (<= 15 allele difference) contained 16 patients, but epidemiological data only suggested six transmission events. The *bla*GIM-1-gene was embedded in a class-1-integron

(In770) and the Tn*21*-subgroup transposon Tn*6216* (KC511628) on a 25-kb plasmid. Environmental screening detected one colonized sink trap in a service room. No further *bla*GIM-1-positive *E. hormaechei* has been isolated since 2016.
Conclusion: Routine molecular screening of carbapenem-non-susceptible Gram-negative isolates detected a long-term, low-frequency outbreak of a GIM-1-producing *E. hormaechei* ssp. *steigerwaltii* clone. No clear environmental reservoir was found. This highlights the necessity of molecular surveillance.

Presentation: Wednesday, February 21, 2018 from 8:30 – 8:45 in room Hörsaal 3.

096/PRV

Analysis of a carbapenemase-producing *Klebsiella pneumoniae* – outbreak in a secondary care hospital

D. F. Peter*[1], M. Krakau[2], S. Maczewski[1], B. Pehlivan[1], F. Ragalmuto[3], F. Mattner[1]
[1]*Kliniken Köln gGmbH, Institut für Hygiene, Köln, Germany*
[2]*Krankenhaus Köln-Holweide, Medizinische Klinik, Köln, Germany*
[3]*Krankenhaus Köln-Holweide, Anästhesiologie, Köln, Germany*

Background: In October 2016 an OXA-48-producing *Klebsiella pneumoniae* (KPN) strain was isolated from a clinical specimen from a patient who had been admitted to an interdisciplinary intensive care unit (ICU) four days earlier. Prospective surveillance of multidrug-resistant Gram-negative organisms (MDRO) yielded further OXA-48-positive KPN strains.
Methods: Prospective surveillance of MDRO was established as routine procedure. All OXA-48-positive KPN isolates were typed using randomly amplified polymorphic DNA (RAPD) technique. Line-listing was used to analyse epidemiological connections between patients with identical isolates. All patients on the ICU underwent a series of prevalence-screenings for MDRO. Direct and indirect contacts were traced, screened and monitored, leading to further prevalence screenings on peripheral wards. Staff was trained in hand-hygiene and isolation precautions.
Results: Between October 2016 and April 2017 prospective surveillance yielded seven additional patients with OXA-48-producing KPN with first detections on four different general wards, suggesting an outbreak. Isolates from six patients showed identical RAPD patterns, indicating transmission. 39 direct and indirect contacts were identified. The patient identified as index case had initially not been isolated upon ICU-admission despite having just arrived from Turkey, where he had been treated in a hospital for pneumonia.
Clinical departments involved were Medicine, Surgery, and Urology. Positive patients and contact patients were shifted to a total of five different general wards. Line-listing did not fully explain the distribution pattern within the hospital. Contact tracing however finally produced one patient as hypothetical missing link although no material could be obtained. Prevalence screenings did not yield further patients.
Conclusion: Prospective surveillance of MDRO helped detect a slowly evolving widespread outbreak of OXA-48-producing KPN. Prompt isolation might have prevented transmission. Clinical staff should stick to indications for immediate isolation for patients treated abroad until screening results are negative. Screening of contact patients and implementation of isolation precautions were effective for outbreak control.

Presentation: Wednesday, February 21, 2018 from 9:00 – 9:15 in room Hörsaal 3.

097/PRV

Whole genome sequencing revealed a prolonged and spatially extended outbreak of PVL-positive MRSA and improved source investigation and elimination

A. Kossow*[1], S. Kampmeier[1], S. Janne[1], F. Schaumburg[2], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene , Münster, Germany*
[2]*University Hospital Münster, Institute for Medical Microbiology , Münster, Germany*

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) remains an important pathogen to cause nosocomial infections. Therefore, specific infection control measures to prevent the spread of this pathogen are implemented. In this context, whole genome sequencing (WGS) helps to better investigate the spread and characterization of strains.
Objectives: Here, we show that WGS can reveal potential transmission events that would have been undetected otherwise due to their timely and spatially unrelated first detection.
Material and Methods: In our institution, all patients are screened for MRSA upon admission via nasal/pharyngeal swabs. All isolates are analyzed by WGS and subsequent core genome multilocus sequence typing analysis (cgMLST) to determine possible clonal relationships between isolates from different patients as part of our routine surveillance strategy. Isolates differing in ≤6 cgMLST alleles are defined as being potentially related and undergo in-depth epidemiological investigations. MLST sequence types (ST) and *spa* types were extracted from the WGS data *in silico*.
Results: In October 2016, we observed two cases of PVL positive MRSA spa type t008 (one colonization [patient P1], one infection [P2]) within three days. Both cases had been treated at clinic A even though P1 was detected upon admission to a different clinic [B]. The two isolates differed in 1 cgMLST allele only. In March 2017, two outpatients were found to be MRSA positive with cgMLST genotypes differing in 1 (P3) or 2 (P4) alleles to P2. Both patients were now treated with MRSA infections but had stayed in clinic A in the past. P3 had been tested negative for MRSA upon initial admission at clinic A. P4 had not been tested for MRSA previously.
Environmental samples taken on clinic A were negative for the outbreak strain. During on-site evaluation and discussion with the staff on clinic A it became clear that one healthcare worker (HCW) associated with A was colonized with MRSA. The HCW had been screened for MRSA as a patient before having surgery. The respective strain also belonged to the outbreak cluster. After successful decolonization of the HCW no further cases of the outbreak strain occurred.
Summary: In this case, WGS helped to reveal a spatially and temporally extended outbreak of PVL positive MRSA t008. Subsequent in-depth epidemiological investigations including on-site evaluation and communication with HCW were necessary to interpret WGS data and to ultimately determine the outbreak source.

Presentation: Wednesday, February 21, 2018 from 9:15 – 9:30 in room Hörsaal 3.

098/PRV

Destructive Testing on Flexible Scopes - Microbial Growth after Clinical Use. Results of "ReSt" Preliminary Study.

T. Fengler*[1]
[1]*Cleanical Berlin, Berlin, Germany*

Question

- What microbial hazards are caused by used, defective (leaking), unprocessed flexible endoscopes with regard to the employees in the manufacturer"s repair departments?
- Pre-study (creating a project design for further research)
- Start of data collection to improve product design (materials, compounds)

Method

- (Min.) storage time at the manufacturer"s: 4 weeks after use on the patient for comparability of the samples (max. storage time unknown, origin and application history unknown).

- Delivery of endoscope parts (working channel, severed distal end, objective lead and cold light line) individually in plastic bags: 5 sample bags of 3 gastroscopes and 4 bags of 3 cystoscopes each. Examination of a total of 27 individual samples as a directed, not normal distributed momentary image.
- Microbiological examination based on DGKH-recommendation HYGIENIC MICROBIOLOGICAL EXAMINATION OF FLEXIBLE ENDOSCOPES AFTER PROCESSING [Hyg Med 2010; 35 (3)].
- Shredding of the individual parts and penetration of the hollow lumens with disposable endoscope brush. Separation of the brush head and insertion and agitation in 100 ml of sterile physiological NaCl together with the comminuted endoscope parts.
- Membrane filtration of the fluid samples (pore size 0.2 µm). Filter plate then laid on Columbia blood agar plate.
- Spread of 1.0 ml each directly on various selective nutrients and incubated for 44 +/-4 h at 36 +/-1 °C: Cetrimide agar, Enterococcosel agar, MacConcey agar, Columbia blood agar, GVPC agar, Middlebrook agar.

Results

Apathogenic, ubiquitous microorganisms (CNS, Micrococcus species and/or Bacillus species) were detected on almost every sample.

There was no evidence for the following microorganisms:

- Pseudomonas aeruginosa
- Enterococci
- Enterobacteriaceae
- Streptococci
- Anaerobic bacteria
- Legionella
- Mycobacteria

However, some potentially infectious microorganisms were detected (see table).

Discussion

- Device history: Significance of the findings remains limited at this stage, since too much is unknown about the endoscopes. In further studies, detailed documentation (eg. integrated data chip) should be present. In this preliminary study, however, feasibility was the main focus.
- Incidence: Even with our comparatively small number of samples, microorganisms that are typical for the subject matter have been detected. We believe that this should give rise to further microbiological studies directly on the work channel surface.
- Consequence: Microorganisms are ubiquitous, but trained processing staff are more aware of the risks of infection than technical staff in the repair shop. Those must start to regard personal protective equipment (PPE) as indispensable as the processing staff do. More frequent hand disinfection is needed, too.
- Note: A four-week quarantine of the endoscope before repair is not sufficient. Waiting is also not in the interest of the customer, who requires quick replacement of his endoscope.

Figure 1

Table: Results of microbiological testing and significance of found microorganisms. Samples were taken from 4 or 5 locations working channels of 6 endoscopes.

Microbiologally Detected	Sample (out of 27)	Findings	Significance
Micrococcus Spezies	1-4, 6-15, 17-27	25	Apathogenic, ubiquitarily prevalent (on human skin and in environment)
Aerobic Spore-formers	1-6, 9, 10, 13-15, 18, 19, 21-23, 26	17	Apathogenic, ubiquitarily prevalent
Coagulase Negative Staphylococci (CNS)	1-5, 8,10-14, 18-21, 23, 24, 26,27	19	Apathogenic, ubiquitarily prevalent
Gemella Morbillorum	15	1	Facultative anaerobic gram-positive cocci, belonging to mouth and throat flora; also known to be pathogenic (sepsis, infections)
Sphingomonas paucimobilis	17,24	2	Gram-negative non-fermenting rod bacteria; widely prevalent in the (hospital) environment; cannot be excluded as a nosocomial pathogen
Acinetobacter spezies	6	1	Gram-negative rod bacteria, prevalent in nature; may cause nosocomial infections in case of imunodeficiancy (esp. Ventilation patients)
Oligella urethralis	9	1	Known to cause Urinary Tract Infection
Methylobacterium sp.	21, 22	2	able to form biofilms and withstand high temperatures and disinfection agents; nosocomial pathogen
Mould	24	1	usually not infectious, except with underlying disease/ imunodeficiancy

Presentation: Wednesday, February 21, 2018 from 9:30 – 9:45 in room Hörsaal 3.

099/PRV

A Cluster of *Listeria monocytogenes* on a Nephrological Ward Uncovered by Whole Genome Sequencing

M. H. Pillukat*[1], F. Schaumburg[2], N. Vogelsang[3], A. Kossow[1], A. Mellmann[1], S. Kampmeier[1]

[1]*Universitätsklinikum Münster, Institut für Hygiene, Münster, Germany*
[2]*Universitätsklinikum Münster, Institut für Medizinische Mikrobiologie, Münster, Germany*
[3]*Universitätsklinikum Münster, Medizinische Klinik D, Abteilung für Nephrologie, Münster, Germany*

Background: *Listeria monocytogenes* is a gram-positive rod-like bacterium normally associated with flu-like or gastroenteritic symptoms. It is considered a classical community-associated pathogen usually transmitted via food, during pregnancy or perinatal. In contrast, nosocomial smear infection is uncommon. Here we report on a cluster of *L. monocytogenes* infections on a nephrological ward.

Patients and Methods: Within a three-week time span, blood culture samples of three patients, admitted to the kidney transplant surgery or the nephrological ward, were tested positive for a *L. monocytogenes* blood stream infection. These three patients developed only unspecific signs of a systemic infection like fever, slightly elevated C-reactive protein levels and/or blood leukocyte counts, but did not develop signs of gastroenteritis at any time. To identify possible sources of infection, environmental samples were obtained from the dialysis unit, identified as one possible interface between detected patients and included water-conducting as well as filtration systems, and were analyzed using standard environmental sampling techniques. Stool samples from all patients admitted to the nephrological unit were screened for *L. monocytogenes*. We used whole genome sequencing (WGS) and subsequent core genome multilocus sequence typing (cgMLST) to infer the relationship between the three bacteremia isolates.

Results: Patient history ruled out food-borne sources of *L. monocytogenes* infection. Both environmental samples and stool samples from other patients from the same ward were tested negative for *L. monocytogenes*. WGS and subsequent cgMLST analysis of the strains isolated from the two nephrological patients exhibited a close relationship (only 4 alleles difference sharing the same cluster type [CT]4045), while the isolate from the kidney transplant surgery patient was only distantly related (CT2789) and differed in 1669 of the in total 1688 genes present in all isolates.

Conclusion: WGS suggested a single transmission of *L. monocytogenes* on a nephrological ward and ruled out a transmission from a third patient. Although *L. monocytogenes* is usually associated with food-borne infections, due to the absence

of gastrointestinal symptoms in any patient and the lack of further infections on the affected ward, transmission via food items was unlikely and other modes of transmission, e.g. smear infection, should be taken into account.

Presentation: Wednesday, February 21, 2018 from 9:45 – 10:00 in room Hörsaal 3.

WORKSHOP 18
Epidemiology & Antimicrobial Resistance of Zoonotic Pathogens (FG ZO/FG MS)
21 Febr. 2018 • 08.30–10.00

100/ZOV

Molecular basis of tetracycline resistance in *Haemophilus parasuis*

A. Pavlovi?*[1], D. Seinige[1], S. Brogden[1], C. Kehrenberg[1]
[1]*Stiftung Tierärztliche Hochschule Hannover, Institut für Lebensmittelqualität und -sicherheit, Hannover, Germany*

Introduction: *Haemophilus* (*H.*) *parasuis* is an abundant colonizer of the upper respiratory tract in swine. Infections of native populations and environmental stress factors may lead to disease, which is characterized by clinical signs of polyarthritis, polyserositis, meningitis, and pneumonia and which causes high economic losses on pig farms worldwide. To treat the disease, antibiotics such as tetracyclines are frequently used and, as a consequence, isolates decreased susceptible to tetracycline have been reported. However, only very limited data of the genetic basis of tetracycline resistance in *H. parasuis* is currently available.
Objectives: It was the aim of the present study to identify tetracycline resistance genes and their localization on mobile genetic elements in *H. parasuis*.
Matherials and Methods: In this study, 24 *H. parasuis* isolates with elevated MICs of tetracycline (≥ 8µg/ml) were investigated for the presence of tetracycline resistance genes of classes A-E, G, H, L, M and O by PCR analysis. Transformation and hybridization experiments were performed to identify a plasmid localization of the resistance genes.
Results: The three *tet* genes, *tet*(B), *tet*(H), and *tet*(O), were detected. The gene *tet*(H) was found on plasmids of six isolates, which were transformable into *E. coli* AS19. Only one isolate harbored *tet*(H) in the chromosomal DNA. Another 14 isolates were identified as carriers of *tet*(B), while *tet*(O) genes were found to be located on plasmids of two isolates. Only one isolate was negative for all *tet* genes investigated. No correlation between the *tet* gene type and the MIC value for tetracycline was observed. Sequence analysis of the 5.6 kb *tet*(H)-carrying plasmid type pHPS1 revealed the presence of the *tetR-tet*(H) region on the plasmid as well as of mobilization genes *mobA*, *mobB* and *mobC*. Sequence analysis of the *tet*(O)-carrying plasmid type, designated pHPS2, identified also *mobA*, *mobB* and *mobC* along with the *tet*(O) gene. The plasmid types pHPS1 and pHPS2 showed 99.9% identity to plasmid p9956 from *Actinobacillus pleuropneumoniae* and 99.9% to plasmid pB1006 from *Pasteurella multocida*, respectively, suggesting intergenus transfer of small plasmids between pathogens of the respiratory tract of swine.
Conclusion. The results of this study showed that tetracycline resistance in *H. parasuis* is mediated by at least three different *tet* genes. Furthermore, this is the first report of a *tet*(H)-plasmid nucleotide sequence from *H. parasuis* and the first report of *tet*(O) tetracycline resistance genes present in *H. parasuis.*

Presentation: Wednesday, February 21, 2018 from 8:45 – 9:00 in room Seminarraum 2.

101/ZOV

Wind-driven emission of *Enterococcus faecium* from agricultural soil fertilized with poultry manure

N. Thiel*[1], V. Junker[1], S. Münch[2], P. Siller[3], O. Biniasch[4], M. Faust[5], K. Schepanski[5], T. Amon[4], U. Rösler[3], R. Funk[2], U. Nübel[1]
[1]*Leibniz Institute DSMZ, Braunschweig, Germany*
[2]*Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany*
[3]*Institute of Animal and Environmental Hygiene, FU Berlin, Germany*
[4]*Leibniz Institute for Agrigultural Engineering and Bioeconomy (ATB), Potsdam, Germany*
[5]*Leibniz Insitute for Tropospheric Research (TROPOS), Leipzig, Germany*

The application of livestock manure as fertilizer on agricultural fields results in environmental pollution with fecal bacteria. We investigated the persistence of *Enterococcus faecium* from chicken manure in fertilized agricultural soil on an experimental field site, and measured wind-driven microbiological emissions during the fertilizer application process, during subsequent tillage operations incorporating the manure into soil, and in the weeks after fertilization. Enterococcal isolates from manure and the various samples were matched by genome sequencing.
Dust formation was measured and air samples collected for microbial quantification at varying distances from the dust sources. We found that the emission of *E. faecium* was highest during fertilizer application, followed by tillage operations, even though dust development was stronger during the latter process. Viable *E. faecium* strains genomically indistinguishable from those in the manure were detectable in air samples collected at least 100 meters from the dust source. Regular sampling of the field site revealed that the same *E. faecium* strains survived in the soil for up to seven weeks following fertilization. In addition, we measured the wind-driven release of Enterococci from fertilized soil in controlled wind-tunnel experiments, to determine the parameters critical for bacterial emission and long-distance transport.
In conclusion, we demonstrate the persistence of pathogenic fecal bacteria in manure-fertilized agricultural soil for several weeks and we have assessed their potential for wind-driven emission. We use our data to model bacterial emission fluxes from agricultural soil and their dispersal, to assess the risk of dust-associated transmission, and to evaluate strategies for mitigation.

Presentation: Wednesday, February 21, 2018 from 8:30 – 8:45 in room Seminarraum 2.

102/ZOV

Fecal carriage of plasmid mediated-colistin resistance *mcr-1* gene in *E. coli* isolated from zoo animals in Pakistan

M. Mohsin*[1]
[1]*Institute of Microbiology, University of Agriculture, Faisalabad, Institute of Microbiology, Faisalabad, Pakistan*

Question: Plasmid mediated colistin resistance *mcr-1* gene has rarely been reported from captive zoo animals. In this study, we report on the detection and molecular characterization of *mcr-1*-carrying *E. coli* from zoo animals in Pakistan.
Methods: A total of 128 healthy zoo animals from Gatwala Wildlife Park, Faisalabad were screened for the occurrence of plasmid mediated colistin resistance. These animals include *Ovis orientalis* (n=15), *Boselaphus tragocamelus* (n=30), *Antilope cervicapra* (n=35), crocodile, *Pavo cristatus* (n=25) and *Pavo muticus* (n=20). Fecal samples were cultivated directly on MacConkey agar supplemented with 2 mg/L colistin. MIC of colistin was determined with broth microdilution method. Antibiotic susceptibility test was performed against polymyxin B, ceftriaxone, cefotaxime, meropenem, gentamicin, ciprofloxacin. Molecular detection of *mcr-1* and other variants were performed using PCR. Plasmid incompatibility was performed with PCR based replicon typing.
Results: A total of 10/128 (7.8%) zoo animals were found to carry *mcr-1* producing *E. coli* whereas none of the isolate carried *mcr-2* and *mcr-3* gene. Antibiotic resistance profile showed that 8/10 *mcr-1* positive *E. coli* were resistant to more than 3 classes of antibiotics thus considered as multi drug resistant (MDR). Most

common Plasmid replicon typing showed that incX4 as the most common plasmid.
Conclusions: Dissemination of *mcr-1* could be linked with spreading of particular plasmid incompatibility groups among various zoo animals in Pakistan.

Presentation: Wednesday, February 21, 2018 from 9:00 – 9:15 in room Seminarraum 2.

103/ZOV
Airborne MRSA colonization of piglets - Investigation of predisposing factors
K. Rosen *[1], F. Ebner[1], A. Friese[1], U. Roesler[1]
[1]*Freie Universität Berlin, Institute of Animal Hygiene and Environmental Health, Berlin, Germany*

Introduction: More than a decade ago, Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) was first described. LA-MRSA clonal complex (CC) 398 with the predominant *spa*-type t011 is highly prevalent in pig farming and poses a risk of transmission to humans as a zoonotic pathogen. Presently, LA-MRSA can be also isolated from companion animals and is therefore not restricted to livestock anymore. The main mode of transmission is direct contact but an airborne route is presumed due to the fact that MRSA could be regularly found in barn and exhaust air of pigsty.
Objectives: After having established the necessary dose for an airborne colonization of piglets, we aimed to define predisposing factors for MRSA colonization success.
Materials and Methods: Three groups of nine MRSA-negative piglets each were exposed in an aerosol chamber to 104 cfu/m3 LA-MRSA CC398 (*spa*-type t011) in conditioned air for 24h. In previous studies, this concentration resulted in transient colonization of piglets (comparison group). Every group was exposed in addition to one possible predisposing factor: dexamethasone administration, bacterial endotoxin in air (24h) or antibiotic treatment (Tiamulin). Blood samples were collected to investigate specific immunological parameters (except for the antibiotic-treated group). After MRSA exposition, different animal swab samples were taken (nasal, skin, pharyngeal, conjunctival and rectal swabs) three times a week for a period of 21 days to monitor the MRSA colonization. Necropsy was performed at the end of the screening period, to examine the presence of MRSA in the internal organs.
Results: Compared to the comparison group, none of the groups treated with any predisposing factor showed higher MRSA colonization rates. Whereas piglets from comparison group were MRSA-positive during the entire screening period in low prevalence, MRSA was detectable in dexamethasone-treated piglets only until day 10 after exposition. There were virtually no differences in colonization dynamics within the bacterial endotoxin group, when compared to the comparison group. Piglets treated with antibiotics were MRSA-positive only during the first screening day.
Conclusion: We found that our investigated factors did not influence MRSA colonization success. Blood sample analysis confirmed a mild immune suppression induced by dexamethasone treatment. Evaluation of immunological parameters of endotoxin-exposed animals is in process. Immunomodulation via dexamethasone or bacterial endotoxin exposure did not increase MRSA colonization rates. For endotoxin, the brief exposition period of 24h should be taken into account. We determined that short-term treatment of three applications with a pleuromutiline did not promote MRSA colonization due to co-selection.

Presentation: Wednesday, February 21, 2018 from 9:15 – 9:30 in room Seminarraum 2.

104/ZOV
Diversity of virulence genes and evolution of sequence type 141 heteropathogenic *Escherichia coli*
N. S. Gati*[1], B. Middendorf-Bauchart[1], U. Dobrindt[2], H. Karch[1], A. Mellmann*[1]
[1]*Institute of Hygiene, Hospital and Environmental Hygiene, Münster, Germany*
[2]*Institut of Hygiene, Microbial Genome Plasticity - Molecular Infection Biology, Münster, Germany*

Introduction: Heteropathogenic Shiga toxin-producing *Escherichia coli* (STEC) of multilocus sequence type (MLST ST) 141 cause both urinary tract infection and diarrhea in humans. We could show previously that they possess virulence genes typical for STEC and uropathogenic *E. coli* (UPEC) and are phylogenetically positioned between these pathogroups. However the diversity of virulence genes as well as the origin and evolution of these strains with respect to other pathogroups is yet to be elucidated.
Objectives: Our aim is to investigate the diversity of virulence genes and the evolution of ST141 using phylogenomics.
Materials and Methods: A total of 78 ST141 whole genome sequence datasets of *E. coli* isolated from different parts of the world were retrieved from the NCBI SRA database and subsequently assembled using the SeqSphere+ software (Ridom GmbH, Münster, Germany). Moreover, 25 reference genome sequences comprising different extraintestinal pathogenic (ExPEC), enteroaggregative (EAEC), adherent invasive (AIEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and commensal *E. coli* were added. We performed MLST and ribosomal (r) MLST to construct a minimum spanning tree (MST). Furthermore, we extracted up to 3,180 core genome genes for an allele-based core genome (cg) MLST analysis to construct the neighbor-joining tree (NJ). Furthermore, isolates were screened for the presence of 86 virulence genes belonging to various pathogroups using the SeqSphere+ software.
Results: The MST confirmed the previous finding that all ST141 isolates were phylogenetically positioned between STEC and ExPEC and in close proximity to AIEC. The NJ based on cgMLST showed a division into two major distinct groups. Virulence gene profiles differ significantly between the two distinct ST141 groups (Ex. 60% of group 1(n=30) lack the *sfp* gene cluster while only 8.3% of group 2 (n=48) lack the same gene cluster). Detailed analyses exhibited that ST141 strains have a diverse repertoire of virulence genes ranging from isolates carrying genes typical only for ExPEC to isolates having genes belonging to ExPEC, EHEC and EAEC. None possess genes typical of ETEC and EIEC. Preliminary analyses on the sequence level showed that the virulence genes were highly conserved within ST141 but more diverse when compared to genes of prototypic strains, e. g. the sequence variation of the vacuolating autotransporter toxin gene *vat* within ST141 was 99.95% in comparison to 99.66% obtained for the same gene within prototypic UPEC and MNEC strains.
Conclusion: Phylogenetically, ST141 is divided in at least two different groups. The heterogeneous distribution of virulence genes hints to horizontal gene transfer as a mechanism for evolution. Current investigations focus on the in-depth analysis of the evolutionary history of ST141 using among others Bayesian statistics.

Presentation: Wednesday, February 21, 2018 from 9:30 – 9:45 in room Seminarraum 2.

105/ZOV

Occurrence and distribution of *Giardia* spp. in wild rodents in Germany

C. Klotz*[1], Y. Helmy[1], D. Reil[2], R. Ulrich[3], T. Aebischer[1]
[1]*Robert Koch-Institute, Mycotic and Parasitic Agents and Mycobacteria, Berlin, Germany*
[2]*Julius Kuehn-Institute, Institute for Plant Protection in Horticulture and Forests, Münster, Germany*
[3]*Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany*

Introduction: Wild rodents can harbor a variety of different *Giardia* species, including *G. muris, G. microti* and different assemblages of *G. duodenalis*. It has been speculated that wild rodents may represent a potential reservoir for zoonotic transmission. In Germany, no molecular data are currently available on *Giardia* infections in wild rodents.
Objectives: The objective of this study was to determine the occurrence of *Giardia* spp. in wild rodents from different localities in Germany.
Methods: Fecal samples of 577 animals of the three genera *Apodemus, Microtus and Myodes* from 11 different localities in Germany were investigated for the presence of *Giardia* spp. by immunofluorescence microscopy and real-time-PCR. In addition, semi-nested PCR and sequence typing was performed at different gene loci (small subunit ribosomal RNA (ssu-rDNA), beta-giardin (bg) and glutamate dehydrogenase (gdh)) to determine the *Giardia* species/assemblage types and genetic parasite variation.
Results: The occurrence of *Giardia* was high in all three rodent genera. Species determination at the ssu-rDNA gene locus revealed that Apodemus mice, depending on species, were predominantly infected with one of two distinct G. muris sequence types. G. microti was the predominant parasite species found in Microtus and Myodes. Potentially zoonotic *G. duodenalis* were only found in few animals. Subtyping at bg and gdh genes suggested a unexpectedly broad genetic diversity within G. microti.
Conclusion: This study extends the current knowledge on sequence type variation of *G. muris* and *G. microti*. The results further indicate that small rodents do not represent a significant source for zoonotic transmission of *G. duodenalis* in Germany.

Presentation: Wednesday, February 21, 2018 from 9:45 – 10:00 in room Seminarraum 2.

Hauptsymposium 05
Whole Genome Sequencing (WGS) History & Application
21 Febr. 2018 • 10.45–11.45

106/INV

Clinical Hide and Seek: usefulness of whole genome sequencing in the analysis of the problem Gram-positive pathogens *Clostridium difficile* and "stealth" MRSA

R.V. Goering
Creighton University, School of Medicine, Omaha, U.S.A

Whole genome sequencing (WGS) offers unprecedented promise as a means of informing issues related to infectious disease. However, its efficient clinical application requires an understanding of the pros and cons of different analytical options and, to the extent possible, streamlined and standardized protocols. The generation and application of WGS data is complicated by its digital nature which requires (cryptic) digital processing before it can be transformed into a visual form suitable for interpretation. In addition, the suitability of different analytical approaches can vary depending on the concerns being addressed. These issues will be explored in the context of two problem Gram-positive pathogens – MRSA and *Clostridium difficile*.
The identification and characterization of MRSA relates to the *mec*A gene encoded within the unique chromosomal region known as SCC*mec*. However, this highly recombinogenic region can exhibit sequence variations resulting in organisms which, while phenotypically susceptible (MSSA), are actually cryptic (i.e., "stealth") MRSA capable of reversion to resistance. WGS analysis of representative clinical isolates will be discussed using reference mapping and single-nucleotide variation (SNV) detection to reveal the genetic basis of the phenomenon and its clinical implication.
The movement and spread of *Clostridium difficile* in patient populations has become of increasing importance as the organism has emerged as a global health concern. However, *C. difficile* epidemiological analysis and strain typing continue to rely on older agarose-gel "band-based" approaches. In more recent years methods such as pulsed field gel electrophoresis (PFGE) and chromosomal restriction enzyme analysis (REA) have given way to PCR-ribotyping. However, the latter still suffers from its "band based" format and inability to subtype strains to track specific isolate movement and patient-to-patient transmission. Proof-of principle WGS analysis of *C. difficile* strains will be discussed using whole-genome multilocus-sequence typing (wgMLST) as a means of more specifically identifying and tracking the movement of specific problem *C. difficile* strains.
Discussion of the above examples will consider both the utility as well as the challenges that remain in establishing WGS analysis as an efficient, reliable, and useable approach to the analysis of issues related to infectious disease.

Presentation: Wednesday, February 21, 2018 from 10:45 – 11:15 in room Audimax Saal.

107/INV

The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria

C. Giske
Karolinka Institute, Department of Laboratory Medicine, Division of Clinical Microbiology, Huddinge, Sweden

Introduction: Whole genome sequencing (WGS) offers the potential to predict antimicrobial susceptibility from a single assay. The European Committee on Antimicrobial Susceptibility Testing established a subcommittee to review the current development status of WGS for bacterial antimicrobial susceptibility testing (AST). Data searches were conducted in 2016, resulting in a publication in early 2017.
Objectives: The objective was to review the conclusions from the previously published paper, as well as to conduct updated literature searches
Materials and Methods: Literature searches were conducted with the search terms antimicrobial resistance, next generation sequencing, epidemiological cut-off values and ECOFF. Searches were done from 1 January 2017-31 December 2017.
Results: Despite and updated literature search, the conclusions from the original paper remained the same. For most bacteria of clinical relevance, the available evidence for using WGS as a tool to infer antimicrobial susceptibility (i.e. to rule-in as well as to rule-out resistance) accurately is either poor or non-existent. The primary comparator should be epidemiological cut-off values, not clinical breakpoints. The reason for this is that clinical breakpoints depend on quantifying resistance levels, which is a complex issue depending on e.g. gene expression. Many of the publicly available databases still offer limited resources in terms of detecting point mutations in chromosomal genes. The most promising results have been demonstrated for S. aureus and M. tuberculosis, whereas *Enterobacteriaceae* including *Salmonella* also show some promise, although working best for acquired resistance genes. For other species the evidence is very limited that WGS can be used to rule-in or rule-out resistance, but the method can be very useful for characterization of known resistance genes in large strain collections. Another limiting factor in the clinical setting is that many phenotypic methods are now introduced for rapid AST, and the cost and availability of technology limits to which extent WGS can be of value for AST of routine samples.
Conclusion: There is still no evidence to support that WGS can replace phenotypic AST for most clinically important bacterial species. The development should be continuingly monitored, but most likely larger well-funded projects will be needed to advance the field, and to ensure sustainability of publicly available and continuously curated databases.

Presentation: Wednesday, February 21, 2018 from 11:15 – 11:45 in room Audimax Saal.

WORKSHOP 19
One Health: Rain Water, Waste Water of Farmer Products to multiresisant Pathagogens in Humans (StAG HY)
21 Febr. 2018 • 13.00–14.30

108/HYV

It`s raining pathogens? – The microbial contamination of rainwater in separate sewer systems

C. Schreiber*[1], N. Zacharias[1], S. B. Heinkel[1], F. M. Mertens[2], E. Christoffels[2], T. Kistemann[1]
[1]*Institute for Hygiene and Public Health, Faculty of Medicine, University of Bonn, GeoHealth Centre, Bonn, Germany*
[2]*Erftverband, Bergheim, Germany*

Precipitable water is generally supposed to be hygienically unproblematic. In case of heavy rainfalls, stormwater sewers discharge the rainwater run-offs from sealed surfaces directly into the nearest water course. The water from these separate sewer systems, however, shows high loads of micro-pollutants, such as pesticides, phthalates, biocides or pharmaceuticals (Mertens et al, 2017).
The aim of our study was to assess the hygienic water quality of separate sewer outflows with a focus on human pathogenic microbes. The outflows of two separate sewer systems located in the catchment area of the river Swist (North Rhine-Westphalia) were investigated. Both separate sewers collect rainwater from sealed surfaces of rural settling areas with few farms and retail houses. The sewers differ in terms of their storage volume (3,650 and 1,400 m³) as well as the size of the sealed surfaces they received water from (0.74 and 0.24 km²). During two consecutive research projects (2009-12, 2013-16), event-based rainwater samples (n=37) were taken from the stormwater sewers. The laboratory analyses comprised the detection of several hygienically relevant bacteria: *E. coli*, *C. perfringens*, *Campylobacter* spp. and *Salmonella* spp.. Additionally, the parasites *Cryptosporidium parvum* and *Giardia lamblia* which potentially may cause severe intestinal infections by low doses, were assessed. Samples were collected from the first flush out of the basin from two different stormwater sewers in cases of overflow. Bacterial and parasitological analyses were conducted in accordance to international norms and standard methods.
Our results proved a high contamination with pathogens in both separate sewer systems, e.g. concentration of *E. coli* was about 10^4 CFU/100ml. The results of both stormwater sewers differed concerning concentrations of pathogenic bacteria. Overall, the microbial loads cannot be solely explained by surface contamination or wash-out effects from the sealed surfaces of their catchment area. Presumable, the sewer systems contain many cross connections of private households and industries, causing discharge of waste water from households and farms in separate sewers which are earmarked exclusively for rainwater run-off.
Precipitable water is contaminated with hygienically relevant micro-organisms through contact with sealed surfaces as well as cross connections in the separate sewer systems. The resulting loads of pathogens pose a high risk to pollute rivers and to impact human health.

Reference

[1] Mertens FM, Brunsch AF, Wunderlich-Pfeiffer J, Christoffels E, Kistemann T & Schreiber C (2017): Mikroschadstoffe im eingeleiteten Wasser aus einem Regenwasserkanal im Einzugsgebiet der Swist. KW Korrespondenz Wasserwirtschaft 10: 145–150

Presentation: Wednesday, February 21, 2018 from 13:00 – 13:15 in room Hörsaal 1.

109/PRV

Prevalence of clinically relevant multidrug resistant bacteria and resistance genes in a rural and a clinical/urban wastewater system

E. Sib*[1], M. Gajdiss[1], U. Klanke[1], F. Lenz[1], C. Albert[1], A. Schallenberg[1], M. Parcina[1], C. Timm[2], H. Müller[2], N. Zacharias[2], C. Schreiber[2], R. Schmithausen[3], M. Exner[3], G. Bierbaum[1]
[1]*University of Bonn, University Hospital, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany*
[2]*University of Bonn, University Hospital, Institute for Hygiene and Public Health, GeoHealth Centre, Bonn, Germany*
[3]*University of Bonn, University Hospital, Institute for Hygiene and Public Health, Bonn, Germany*

Background: Multidrug resistant bacteria play a crucial role in the healthcare system, especially because antibiotic resistance is currently increasing. This situation requires not only new antibiotics but also new approaches to control the spread of multidrug resistant bacteria and resistance genes. An important source for the dissemination of multidrug resistant bacteria is wastewater, which implies that this source should be closely monitored.
The joint project "HyReKA" is part of the BMBF (Federal Ministry of Education and Research) funding measure "Risk management of new pollutants and pathogens in the water cycle (RiSKWa)" in the funding priority "Sustainable Water Management (NaWaM)", Germany (FKZ 02WRS1377). The aim of this substudy is to examine the spread of multidrug resistant bacteria in surface water of a small river system in a rural area and an urban setting including a university hospital.
Methods: Water samples were obtained from different sampling points of clinical wastewater, throughout the local sewer system ending at a local wastewater treatment plant (WWTP). Samples were also taken from surface waterbodies upstream and downstream of the WWTP. In the rural setting, water of different sampling points in a river system with multiple WWTPs was obtained and examined. Multidrug resistant bacteria were isolated on selective agar plates (ESBL, MRSA, VRE) and preselected by morphological and physiological characteristics. The isolates were identified by MALDI-TOF MS and antibiotic resistance was tested by microbroth dilution. The resistance genes were examined using real time polymerase chain reaction (RT-PCR). For the classification of the strains, different molecular typing methods were used.
Results and Conclusions: Vancomycin resistant enterococci were found at almost every sampling point of the rural wastewater and in all samples of the clinical/urban wastewater. Methicillin resistant *S. aureus* was found in untreated, but not in treated wastewater. 3MRGN/4MRGN isolates could be identified throughout the whole wastewater system of the clinical/urban setting. The bacteria were mostly identified as *Enterobacter spp*., *Klebsiella spp*., *A. baumannii* complex, *E. coli* and *P. aeruginosa*. In the rural setting, 3MRGN/4MRGN isolates were present only in a few samples. These bacteria belong to *A. baumannii* complex, *E. coli*, *Klebsiella spp*. and *Raoultella spp*.

Presentation: Wednesday, February 21, 2018 from 13:15 – 13:30 in room Hörsaal 1.

110/PRV

The presence of antibiotic resistant *E. coli* on smallholder farmer fresh produce - farmer pre-and post-harvest hygiene practices as essential pre-requisite for food safety and quality

T. Beharielal*[1], J. Thamaga-Chitja[1], S. Schmidt[1]
[1]*University of KwaZulu-Natal, Department of Microbiology, Pietermaritzburg, South Africa*

While there exists an increasing demand for "minimally processed" fresh produce as part of a healthy modern diet, microbial contaminated fresh produce has been identified as causative agent in numerous disease outbreaks. This study therefore analysed irrigation water and fresh produce for total and faecal coliforms and *E. coli*, to assess the potential contribution of South African smallholder farmer pre-and-post-harvest practices to the microbiological quality of produce. Selected produce processing

surfaces were analysed for the presence of *E. coli*, as was done for a common foodborne pathogen, *Salmonella* spp. Microbiological analysis showed that a number of irrigation water samples used by smallholder farmers failed to meet WHO recommendations (1) for safe irrigation of "minimally processed" fresh produce, with faecal coliform levels exceeding 1000MPN/100ml. According to recommendations by the South African Department of Health (2), lettuce, parsley, carrots and spinach collected over at least 3-months, were frequently of unsatisfactory quality, with total coliforms quantified in a range from 130 to 79000 MPN/g, and *E. coli* from 2.2 to 49 MPN/g. Surfaces of farming equipment and transportation vehicles within the processing chain were found to harbour *E. coli*. However, *Salmonella* spp. was not detected in fresh produce, on surfaces or in irrigation water samples. Antibiotic susceptibility patterns of 155 randomly selected *E. coli* isolates from both fresh produce and irrigation water were determined using the EUCAST disk diffusion method and appropriate breakpoint tables (3,4,5). High levels of resistance were detected for streptomycin (95%) and amoxicillin-clavulanic acid (33%). The most frequently observed resistance profiles were STR-AMC and STR-AMC-AMP. However, only 3% of all isolates displayed multidrug resistance with one isolate showing resistance to STR-AMC-AMP-CAZ-TOB. Furthermore, numerous antibiotic resistant *E. coli* isolates from fresh produce displayed biofilm formation capabilities. The presence of such *E. coli* strains - potentially including diarrheagenic *E. coli* pathotypes - on fresh produce and within the production and processing environment highlights the need for good hygienic pre-and post-harvest practices and monitoring, especially in view of food safety and if smallholder farmers intend on supplying regulated markets.

References

[1] WHO (2006). Guidelines for the safe use of wastewater, excreta and greywater; Vol. 1.World Health Organisation, Switzerland.
[2] DoH (2002). Food control. Annexure B. Pretoria, South Africa.
[3] EUCAST (2015). Antimicrobial susceptibility testing: EUCAST disk diffusion method, V.5.
[4] EUCAST (2016). Breakpoint tables for interpretation of MICs and zone diameters, V.6.
[5] Scherer et al. (2013). *Environ. Microbiol.* 15:91-304.

Presentation: Wednesday, February 21, 2018 from 13:30 – 13:45 in room Hörsaal 1.

111/PRV

High prevalence of carbapenemase genes in Schleswig-Holstein urban wastewater

J. K. Knobloch*[1], E. M. Klupp[1], S. Schlauß[2], M. Grottker[2], K. Wellbrock[2]
[1]*University Medical Center Hamburg Eppendorf, Institute for Medical Microbiology, Virology and Hygiene, Hamburg, Germany*
[2]*University of Applied Sciences, Department for urban water science, Lübeck, Germany*

Introduction: Carbapenemase producing Gram-negative organisms (CPGN) are a major threat with increasing therapeutic challenges in many regions of the world. In Germany, carbapenemases are frequently detected in endemic *Pseudomonas aeruginosa* strains; whereas in other species CPGN are still associated with sporadic outbreaks and the hospital stay of patients in countries with a high prevalence of CPGN. In this study, we investigated the prevalence of carbapenemase encoding genes in urban wastewater.
Methods: In eight different urban wastewater treatment plants in Schleswig-Holstein (Ahrensburg, Bad Segeberg, Cismar, Flensburg, Kropp, Ratzeburg, Rendsburg, and Süderbrarup) wastewater was sampled continuously for seven subsequent days using Bühler 3010 automatic water samplers with sampling every quarter of an hour. Daily composite samples were pooled for 24 to 72 hours prior investigation of carbapenemase genes. Presence of carbapenemase genes was determined by isothermal amplification using eazyplex® SuperBug complete A or B in a Genie® II instrument including KPC, NDM, OXA-23, OXA-40, OXA-48, OXA-58 (only A), OXA-181 (only B), and VIM carbapenemases.
Results: In all tested pooled samples (n=25) carbapenemase genes were detected. OXA-58 (8/8; 100%), OXA-40 (24/25; 96 %), OXA-48 (24/25; 96 %), and OXA-181 (14/17; 82 %) were the most frequently detected alleles in pooled wastewater samples. VIM, KPC, OXA-23, and NDM carbapenemases were detected in twelve (48 %), seven (28 %), six (24 %), and four (16 %) pooled samples, respectively. In one wastewater treatment plant seven different carbapenemase genes including KPC and NDM were observed simultaneously in three independent pool samples. Most carbapenemase genes were detected repeatedly during the seven day sampling period in individual wastewater treatment plants, indicating a countinious influx of bacteria carrying these genes.
Conclusions: Carbapenemase genes are frequently observed in urban wastewater in Schleswig-Holstein. OXA-type carbapenemases were the most frequent observed alleles. However, KPC and NDM carbapenemases not yet considered as endemic in Germany were observed in a significant number of samples, indicating that these carbapenemases might become endemic in the near future. The examination of the possible sources of carbapenemases in wastewater should be forced to identify appropriate measures for the reduction of the dissemination of these resistance genes.

Presentation: Wednesday, February 21, 2018 from 13:45 – 14:00 in room Hörsaal 1.

112/PRV

Characterization of OXA-143-like encoding plasmids in *Acinetobacter baumannii* clinical isolates

K. Xanthopoulou*[1,2], J. Ertel[1,2], A. Carattoli[3], H. Seifert[1,2], P. G. Higgins[1,2]
[1]*Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Köln, Germany*
[2]*German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Köln, Germany*
[3]*Istituto Superiore di Sanità, Department of Infectious, Parasitic and Immune-Mediated Diseases, Rome, Italy*

Introduction: Carbapenem resistance in *Acinetobacter baumannii* is most frequently conferred by OXA-type carbapenemases which can be both chromosomally and plasmid-encoded [1]. OXA-143 was first identified in a carbapenem-resistant *A. baumannii* isolate from Brazil in 2004 [2]. OXA-143 and variants of it have now been found in other countries [3,4].
Objectives: The present study aimed to characterize OXA-143-like encoding plasmids among three *A. baumannii* clinical isolates from different geographical regions [2,3].
Material and Methods: Total DNA was prepared using the MagAttract HMW DNA Kit from two *A. baumannii* isolates from Brazil and one from Honduras recovered in the years 2004, 2008 and 2009, respectively. Sequencing libraries were prepared using the Nextera XT library prep kit for a 250bp paired-end sequencing run performed on MiSeq. SPAdes and Velvet were used for *de novo* assembly. Plasmid assembly and predicted gaps were confirmed by PCR-based gap closure using total DNA and/or plasmid DNA prepared with the PureYield Plasmid Midiprep System. A core-genome multilocus sequence typing (cgMLST) scheme was defined using the Ridom SeqSphere+ v.3.0 software [5]. Traditional seven-loci MLST was determined using the Oxford typing scheme.
Results: The two isolates from Brazil were assigned ST1552 and did not cluster with any international clones (IC). By cgMLST these two isolates differed by 63 alleles and harbored OXA-143-like on plasmids with highly similar scaffolds. The plasmids with a size of 3.955 bp encoding *bla*$_{\text{OXA-143}}$ and the variant *bla*$_{\text{OXA-231}}$ carried a replicase of the GR19 type and harbored in addition a mobilization protein. The isolate recovered from Honduras was assigned ST1551 and clustered with IC5 control strains. This isolate was epidemiologically unrelated to the other isolates differing by cgMLST analysis in over 1900 alleles. *bla*$_{\text{OXA-253}}$ was located in a 9.280 bp plasmid with a completely different scaffold, including a replicase of the GR12 type. Furthermore, the *bla*$_{\text{OXA-253}}$ plasmid carried a Ton-dependent receptor involved in iron uptake, a septicolysin-like gene coding for a pore-forming toxin and components of a toxin-antitoxin (TA) system.
Conclusions: In the present study OXA-143-like was found on two

different plasmid types, discernable by their replicase genes. The *bla*$_{OXA-253}$-encoding plasmid harbored a more complex set of genes including a TonB-dependent receptor and components of a TA system. *bla*$_{OXA-143}$- and *bla*$_{OXA-231}$-encoding plasmids presenting a highly similar genetic composition were found in unrelated isolates recovered within a four-year time span. This evidence suggests that these plasmids may support the spread of the OXA-143-like carbapenemases.

References

[1] Poirel, L. & Nordmann, P., CMI, 2006. 12(9):826-36.
[2] Higgins, P.G., et al., AAC, 2009. 53(12):5035-8.
[3] Zander, E., et al., AAC, 2014. 58(5):2704-8.
[4] D'Souza, R., et al., AAC, 61(5):e02676-16.
[5] Higgins, P.G., et al., PloS one, 2017. 12(6):e0179228.

Presentation: Wednesday, February 21, 2018 from 14:00 – 14:15 in room Hörsaal 1.

113/HYV

Microbial colonization of health care personnel (HCP) attire: no evidence for a short sleeve dress code in the hospital setting

A. Ambrosch*[1], K. Wahrburg[1]
[1]*Krankenhaus Barmherzige Brüder, Institut für Labormedizin, Mikrobiologie und Krankenhaushygiene, Regensburg, Germany*

Some Health Care Systems recommend short sleeves for doctors white coat, because microbial colonization of health care attire may increase the risk of cross transmission to patients (1). Since there is weak evidence for such recommendations the present study investigates the microbial colonization of white coats focusing on bacterial load and microbial spectrum including potential pathogens.

Methods: White coats from medical staff (doctors and nurses) were investigated for microbial contamination post duty at the end of each shift. For this, sampling of three (nurses) to four (doctors) areas of white coats (breast pocket / side pocket / front and sleeve (only doctors)) was performed by the contact plate method using RODAC plates (25 cm2). Contact plates were analysed for bacterial load (cfu/plate), and different colonies identified by Maldi-tof. In addition, staffs were asked when personnel attire has been changed since the local hygiene management recommend to wear white coats no longer than two shifts.

Results: A total of 700 contact plates from 200 HCP attires (100 from doctors and nurses, respectively) were enclosed for analyses. Bacterial load on attire was found to be 4fold higher on white coats which were worn for more than 1 day (2 days and more). In addition, side pockets exert the highest bacterial load (after one / two shifts: 35 (± 37) / 114 (± 130) cfu/plate) - and doctors sleeves the lowest (after one / two shifts: 12 (± 12) / 45 (± 65) cfu/plate; compared to all other areas on doctors attire $p < 0.05$, respectively). Coagulase negative Staphylococci (CNS) - particularly S. epidermidis, S. haemolyticus and S. hominis - and Bacillus spp were found to be the most common non-pathogenic germs (positivity rate of all samples: up to 70 % for CNS, and up to 45 % for Bacillus spp.). S. aureus as a potential pathogen was found to be present on 21 % of all doctors white coats (cumulative positivity rate). However, the distribution of S. aureus on the personnel attire was different: only 4 % of the investigated sleeves were positive but 15 % of the side pockets. Pathogens like *E. coli*, Acinetobacter spp. or Pseudomonas spp. were very rare and particularly found on nurses white coats.

Discussion: The present study clearly shows that bacterial load on HCP attire increased dramatically within the first two shifts on duty. Therefore, the recommendation for a routine change of white coats after two shifts seems to be reasonable. However, since bacterial load and distribution of the main potential pathogen S. aureus were lowest on doctors sleeves compared to other areas of HCP attire, no evidence was found for wearing short sleeves as recommended by others. The striking high degree of spore forming Bacillus spp. on white coats may result from the increasing use of alcoholic hand desinfection instead of hand washing.

Reference

[1] SHEA Expert Guidance, Infect Control Hosp Epidemiol 35, 107, 2014

Presentation: Wednesday, February 21, 2018 from 14:15 – 14:30 in room Hörsaal 1.

WORKSHOP 20
New Therapies and Methods (StAG HY)
21 Febr. 2018 • 13.00–14.30

114/PRV

Clinical characteristics and course of infections by influenza A- and respiratory syncytial virus (RSV) in hospitalized adults

A. Ambrosch*[1], A. Klinger[1], D. Luber[1], M. Lepiorz[2], S. Schroll[2]
[1]*Krankenhaus Barmherzige Brüder, Institut für Labormedizin, Mikrobiologie und Krankenhaushygiene, Regensburg, Germany*
[2]*Krankenhaus Barmherzige Brüder, Klinik für Pneumologie und konservative Intensivmedizin, Regensburg, Germany*

There is little evidence on the clinical characteristics and the course of complicated infections with respiratory syncytial virus (RSV) compared to influenza A in adults. Therefore, the present mono center study aims to compare infections with RSV and influenza A with regard to potential predisposing factors, clinical profile, course and outcome in hospitalized patient.

Material and Methods: the study was performed between Jan 1th and March 31 this year and included all hospitalized patients with a confirmed infection of influenza A and RSV. Patients were characterized by clinical symptoms at the time of diagnosis, laboratory parameters of inflammation and potential predisposing factors like chronical diseases of heart, lung, kidney, metabolism and tumors. Data on the length of hospital stay, origin of infection (nosocomial), rate of pneumonia, antimicrobial use, need of mechanical ventilation and hospital mortality were obtained to evaluate clinical severity and outcome.

Results: A total of 191 patients with Influenza A and 98 patients with RSV were included. Both patient groups did not differ with regard to anthropometric data and clinical symptoms: it was surprising to see that only 2/3 oft all patients exert symptoms of a respiratory infection. 16 % of influenza A and 17 % RSV infections were defined as being nosocomial. The rate of RSV-patients with chronical kidney disease was nearly double as high as in the influenza group (35 % vs. 18 %, $p<0.001$). Comparing the clinical course and outcome, patients with RSV infections had an increased rate of mechanical ventilations (RSV vs. Influenza: 10 % and 3.6 %, respectively; $p=0.05$) and mortality (14 % and 7 %, respectively; $p=0.049$) (table 1).

Conclusions: The present data clearly show that RSV is a frequent pathogen in hospitalized adults with complicated infections in the winter season. RSV infections tend to be more severe compared to influenza A, but were as frequent as influenza A of nosocomial origin. In this context, an early diagnosis seems to be helpful for a successful infections prevention management under hospital conditions.

Figure 1

%*	Influenza A	RSV	P
hospital stay (days)	11.2 (12.0)	10.9 (9.1)	n.s.
infection of nosocomial origin	16	17	n.s.
antibiotic therapy	48	62	0.013
mechanical ventilation	3.6	10	0.05
mortality	7	14	0.049

*% if not indicated otherwise

Figure 1: Clinical course and outcome of hospitalized patients with Influenza A und RSV infections

Presentation: Wednesday, February 21, 2018 from 13:00 – 13:15 in room Hörsaal 3.

115/MSV

A Genome Database for *Clostridioides difficile* implemented in EnteroBase

M. Frentrup*[1], M. J. Sergeant[2], Z. Zhou[2], N. F. Alikhan[2], M. Blaschitz[3], A. Indra[3], L. von Müller[4], M. Steglich[1,5], T. Riedel[1,5], B. Bunk[1], C. Spröer[1], J. Overmann[1,5], O. Zimmermann[6], U. Groß[6], M. Achtman[2], U. Nübel[1,5]
[1]*Leibniz-Institute DSMZ, Braunschweig, Germany*
[2]*Warwick Medical School, University of Warwick, Warwick, United Kingdom*
[3]*AGES - Austrian Agency for Health and Food Safety, Wien, Austria*
[4]*Christophorus-Kliniken, Coesfeld, Germany*
[5]*German Center for Infection Research (DZIF), Braunschweig, Germany*
[6]*University Medical Center, Göttingen, Germany*

EnteroBase is an open source and publically accessible, strain-based database for bacterial genome sequences, with daily automatic retrieval of newly published short-read data and subsequent assembly and allele calling (http://enterobase.warwick.ac.uk/). Within EnteroBase, we implemented a database for genome sequences from *Clostridioides difficile*, which currently (as of October 2017) holds 6,752 assembled genomes from *C. difficile* isolates from at least 27 countries. Genomic phylogenetic relationships can be assessed on the basis of allelic profiles for ribosomal multilocus sequence typing (rMLST; synchronized with PubMLST), core genome MLST (cgMLST), and whole genome MLST (wgMLST) schemes, and graphically represented in minimum spanning and neighbor joining trees.

We used rMLST to classify *C. difficile* isolates into clonal lineages, which largely corresponded to PCR ribotypes in a prospective study. Hence, an isolate's PCR ribotype could be predicted from its genome sequence, based on phylogenetic analysis. We applied cgMLST to trace spatial spread within and between healthcare institutions, to investigate outbreaks of *C. difficile* infections, and to characterize within-patient diversity for discriminating recurrence from re-infection. As a result, we found that the discriminatory power of cgMLST and its applicability to epidemiological investigations was similar to that of core-genome SNP analysis. However, cgMLST is less computationally expensive than SNP analysis and it can be standardized more easily. A major advantage of EnteroBase is that genome sequence data can immediately be interpreted within a global context, considering all previously published *C. difficile* genome sequences, which greatly assists in identifying outbreak sources and routes of epidemic spread. The web-based user interface of EnteroBase readily allows application by non-bioinformaticians.

Presentation: Wednesday, February 21, 2018 from 13:15 – 13:30 in room Hörsaal 3.

116/PRV

Identification of highly vulnerable essential drug targets by using CRISPR interference in *Pseudomonas aeruginosa*

A. S. Stolle*[1], J. Toska[1], F. Caro[1], J. J. Mekalanos[1]
[1]*Harvard Medical School, Microbiology and Immunobiology, Boston, United States*

Pseudomonas aeruginosa is a life-threatening nosocomial Gram-negative pathogen that was recently identified by the World Health Organization as a priority for the development of new antibiotics to combat its multidrug resistance. The identification of essential gene products provides one promising path to the identification of potential new targets for antibiotic discovery. However, essentiality alone is not a sufficient criterion to describe a promising drug target. Compounds inhibiting intracellular essential proteins often fail to have whole cell activity because active efflux by 12 or more RND efflux pumps of *P. aeruginosa* provide a particularly strong barrier for passage through the inner membrane and thus effectively prevent the accumulation of drugs in the bacterial cytosol. Thus, inhibitors that target exported proteins (proteins residing in the periplasm, inner- or outer membrane) might show higher efficacy against Gram-negative bacterial cells as long as they can enter cells through outer membrane proteins (OMPs) as is the case for some beta-lactam antibiotics. Furthermore, the level of protein that needs to be inhibited to block growth of bacteria can vary among essential proteins. Hence, highly vulnerable essential targets are favorable drug targets, since lower concentrations of compound are needed to achieve growth inhibition. In this study, we established a CRISPR interference (CRISPRi) system in *P. aeruginosa*, a method that can be used to easily manipulate expression levels of essential genes in a high-throughput fashion. We integrated a catalytic inactive version of Cas9 (dCas9) of *Streptococcus pyogenes* on the genome of *P. aeruginosa* under the control of an inducible promoter, whereas the single guide RNA (sgRNA), mediating target specificity, is expressed from a plasmid with constitutively active promoter. We determined the optimal conditions for the targeted down-regulation of essential genes, by addressing strand specificity and dose dependency to the inducer. CRISPRi in *P. aeruginosa* reliably reduces the expression of essential genes which results in growth inhibition and reduced viability of *P. aeruginosa*. With these experiments, we generated a ranked list of essential, exported gene products and their relative predicted vulnerability to inhibition. This knowledge of highly vulnerable essential genes will help to prioritize targets for the development of inhibitors that could show promise as antibiotics specific for *Pseudomonas aeruginosa*.

Presentation: Wednesday, February 21, 2018 from 13:30 – 13:45 in room Hörsaal 3.

117/PRV

Rapid Parallel Multilocus Sequence Typing (RP-MLST) – A new Application of Next Generation Sequencing for the simultaneous Identification of Sequence Type and Resistance Markers in *Escherichia coli* ST131 isolates.

P. Zimmermann*[1], A. Heisig[1], P. Heisig[1]
[1]*University of Hamburg, Pharmaceutical Biology & Microbiology, Hamburg, Germany*

Introduction: The increasing prevalence of *E.coli* ST131 with varying multidrug resistant (MDR) profiles[1] requires rapid simultaneous identification of ST and resistance gene pattern. A promising approach is next-generation sequencing (NGS) expected to provide information in time.

Objectives: The study aims at rapidly identifying both sequence type (ST) and antibiotic resistance genes by an NGS-based approach (RP-MLST).

Material and Methods: Clinical *E.coli* isolates (n=84) of phylogroup B2 with resistance to fluoroquinolones (fqR) were pre-screened by PCR for the presence of specific SNPs present in ST131 strains[2]. Fourteen O25b-positive and two negative isolates were chosen for further MLST analysis.

Size-fractioned PCR fragments of MLST-associated housekeeping and antibiotic resistance genes of individual strains were first amplified and fragmented before being pooled, barcoded and subsequently used for the preparation of an NGS Library. This library was used for Ion Torrent™ NGS runs using either Ion314™ chip (16 isolates,) or Ion316™ chip (15 isolates, one whole genome).

The sequencing-data were aligned on the Ion Torrent™ server with *E.coli* JJ1886 as reference genome. The data analysis was performed using IGV software. MLSTs were determined using the reference sequences from the University of Warwick[3] and the Institute Pasteur"s database[4].

Results: Ion Torrent™ sequencing results yielded an average coverage of at least 20 up to 100fold for MLST target regions.

Using the Warwick MLST-scheme all fourteen O25b-positive isolates were identified to be of ST131. According to the Pasteur-scheme thirteen of these were identical to correlating[5] ST43, one isolate with an yet undescribed allele of *pabB* is closely related to ST43. The two O25b-negative isolates were of ST14 and ST73 (Warwick) or ST6 and related to ST30 (Pasteur), respectively.

All isolates also showed mutations in *gyrA, parC, marR*, and *acrA* presumably fqR associated. Beta-lactamases of TEM-type (n=11) and/or CTX-M-15 (n=5) enzymes were identified in all but one isolate.

Conclusion: Rapid parallel (RP) MLST-NGS is a novel and highly reliable method for simultaneous identification of MLST and antibiotic resistance gene pattern in *E.coli* ST131 isolates. It could

easily be extended to hundreds of genes of interest and adopted to identify other high-risk clones in combination with MDR genes.

References

[1] Nicolas-Chanoine *et al.* 2014
[2] Clermont *et al.* 2009
[3] Wirth *et al.* 2006
[4] bigsdb.pasteur.fr/ecoli/ecoli.html
[5] Clermont *et al.* 2015

Presentation: Wednesday, February 21, 2018 from 13:45 – 14:00 in room Hörsaal 3.

118/PRV

***Enterobacter aerogenes* on a Neonatal Intensive Care Unit – Outbreak Investigation using Fourier-transform infrared spectroscopy**

A. G. Dinkelacker[1], B. Schulte[1], S. Grashorn[1], C. Gille[2], I. B. Autenrieth[1], S. Peter[1], J. Liese*[1]
[1]*University Hospital Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany*
[2]*University Hospital Tübingen, Department of Neonatology, Tübingen, Germany*

Question: *Enterobacter (E.) aerogenes* is an infrequent cause of nosocomial outbreaks. Nonetheless, this pathogen can cause a wide range of infections in vulnerable and immunocompromised patients and is able to harbour and acquire resistance to several antibiotics. In July 2017 we noted a sudden increase in *E. aerogenes* incidence from routine microbiological screening samples from infants treated on the neonatal intensive care unit (NICU) of our university hospital. Overall, *E. aerogenes* was detected in 18 patients over the following two-month period. Two infections occurred, which included one case of bloodstream infection. *E. aerogenes* isolates exhibited changing antibiotic resistance patterns, which initially suggested that several strains were present on the NICU, thereby complicating infection control measures. The goal of this study was to investigate the outbreak and to identify transmission routes. FTIR spectroscopy was evaluated as a tool for strain typing in comparison to whole genome sequencing (WGS) as a reference.
Methods: A set of 37 *E. aerogenes* strains was examined by Fourier-transform infrared (FTIR) spectroscopy and WGS. Up to four isolates were analyzed from one patient, if different resistance patterns were detected. For FTIR spectroscopy, *E. aerogenes* isolates were cultivated for 24±1 hours on blood agar before analysis of four replicates on an IR Biotyper system (Bruker Daltonik). WGS was performed on a NextSeq platform (Illumina). The BioNumerics 7.6 software suite was subsequently used for comparing and clustering the obtained summary spectra as well as calculating a SNP-based phylogeny from assembled WGS sequences.
Results: FTIR spectroscopy grouped all 37 *E. aerogenes* outbreak isolates into two distinct clusters. This result was corroborated by WGS, which revealed the presence of two distinct clones differing by more than 30,000 SNPs. Strains with phenotypically diverse resistance pattern from the same patient were shown to be of clonal origin. All patients were colonized only by one of the two prevalent *E. aerogenes* strains, no colonization with both strains was observed. The transmission chain could be reconstructed using the phylogenetic data and revealed that almost all transmission occurred, when patients were treated in the same room on the NICU.
Conclusions: Two distinct strains were identified as the cause of a suspected polyclonal *E. aerogenes* outbreak on a NICU. Strain typing with FTIR spectroscopy showed high concordance with subsequent WGS results. Therefore, FTIR spectroscopy is a promising tool for quick and reliable outbreak investigation.

Presentation: Wednesday, February 21, 2018 from 14:00 – 14:15 in room Hörsaal 3.

119/HYV

Control of MRSA Decolonization Success: Single-day Swabbing Regimen Noninferior to Three-day Protocol

H. Frickmann*[1,2], N. G. Schwarz[3], A. Hahn[4], A. Ludyga[5], P. Warnke[2], A. Podbielski[2]
[1]*Bundeswehr Hospital Hamburg, Microbiology and Hygiene, Hamburg, Germany*
[2]*University Medicine Rostock, Institute for Medical Microbiology, Rostock, Germany*
[3]*Bernhard Nocht Institute for Tropical Medicine, Infectious Disease Epidemiology, Hamburg, Germany*
[4]*Charité – University Medicine Berlin, Institute for Microbiology, Berlin, Germany*
[5]*altran Ltd., München, Germany*

Introduction: In Germany, it is recommended that decolonization of patients carrying methicillin-resistant *Staphylococcus aureus* (MRSA) should be carried out as detailed in a recently updated guideline by the Commission for Hospital Hygiene and Infection Prevention (KRINKO) on behalf of the Robert Koch Institute (RKI). Verification of the success of decolonization is advised by swabbing of the most frequently colonized sites — i.e., nostrils, pharynx, and wounds — as well as previously colonized sites of the respective patient after the decolonization procedure on three consecutive days.
Aim: Here we assessed the recommended gold standard in comparison with a swabbing scheme comprising three swabs on a single day for noninferiority of the latter.
Methods: During a 3-year interval, MRSA patients at a German tertiary hospital were screened 2 days after decolonization approaches by the taking of three swabs. The three swabs were taken either at 3- to 4-hour intervals during screening day 1, or once daily on screening days 1, 2, and 3. Nose and throat swabs were taken by specifically trained hygiene nurses in a standardized way and subsequently analyzed using chromogenic agar and broth enrichment techniques in an accredited laboratory. The study was powered to identify at least a 15%-noninferiority margin.
Results: During the study interval, 160 patients were included, comprising 105 and 101 patients with results on all three swabs for decolonization screening of the nose and throat, respectively. Noninferiority of the single-day swabbing scheme was confirmed for both pharyngeal and nasal swabs. Fleiss"s kappa results suggested substantial agreement of both schemes regarding positive and negative test results. Compliance with the single-day scheme was better than with the 3-day scheme.
Discussion: As shown in previous studies, ending of isolation of contacts earlier during the hospital stay could result in cost savings and decreased morbidity. The better adherence to the single-day screening scheme in conjunction with demonstrated noninferiority suggests its implementation as the new gold standard. In order to do this on a broader scale in Germany, the requirements of the OPS-code 8-987 which is relevant for refunding of decolonization procedures requires adaptation.

Presentation: Wednesday, February 21, 2018 from 14:15 – 14:30 in room Hörsaal 3.

WORKSHOP 21
Recognition of and Response to Infection (FG II)
21 Febr. 2018 • 13.00–14.30

120/IIV

***Yersinia enterocolitica activates* JAK-STAT signaling and promotes nuclear localization of STAT3 in primary human macrophages**

L. Berneking*[1], M. Schnapp[1], M. Aepfelbacher[1], L. Berneking*[1], M. Schnapp[1], M. Aepfelbacher[1]
[1]*University Medical Center Hamburg Eppendorf, Hamburg, Germany*

Injection of *Yersinia* outer proteins (Yops) into macrophages via the bacterial type-III-secretion system is essential for virulence of pathogenic *Yersinia* species (*Y.pestis, Y. enterocolitica, Y.pseudotuberculosis)*. Yops effectively suppress innate immune responses in the infected macrophages to promote pathogenesis. Although the eminent role of the effector-Yops for virulence of

Yersinia in infected animals has been related to the subversion of cytokine gene expression and –production, the molecular and cellular mechanisms underlying these Yop activities are not understood.
To understand the effect of Yops on the global transcriptional response of primary human macrophages, we employed RNA-seq analysis. JAK-STAT signaling was among the most highly activated canonical pathways triggered by wild type *Yersinia* after 6 h of macrophage infection. Interestingly, we found that Stat3 and Stat3-regulated genes known to be involved in immune suppression (i.e. IL10, IL23) were upregulated by wildtype Yersinia strain compared to cells infected with a strain deficient for YopM. We further analyzed the effect of the wild type strain on pro- and anti-inflammatory cytokine expression in macrophages by qPCR and found that YopM increased the level of Interleukin-10 (IL-10) mRNA. Furthermore we could demonstrate that YopM promotes nuclear localization of STAT3 in the absence of robust STAT3 phosphorylation on tyrosine residue 705 and also disrupts interleukin-6 -induced phosphorylation of STAT3.
Thus, our study points to an unanticipated mechanism involving STAT3, IL-10 and related JAK-STAT-signalling by which Yersinia affects the function of macrophages. How Yersinia YopM hijacks the JAK-STAT module and induces nuclear translocation of Stat3 in a manner that benefits bacterial survival within the host is an intriguing area of current research.

Presentation: Wednesday, February 21, 2018 from 13:00 – 13:15 in room Seminarraum 2.

121/IIV

The expression of the deubiquitinating enzyme OTUB1 in dendritic cells is required to induce a protective immune response against intracellular parasitic infection

F. Mulas*[1], X. Wang[1], S. Song[1], M. Naumann[2], M. Deckert[3], D. Schlüter[1]
[1]*Otto von Guericke University, Institute of Medical Microbiology, Magdeburg, Germany*
[2]*Otto von Guericke University, Institute of Experimental Internal Medicine, Magdeburg, Germany*
[3]*University Hospital Cologne, Neuropathology, Köln, Germany*

Dendritic cells play a pivotal protective role in toxoplasmosis and mice deficient of DCs fail to control *Toxoplasma gondii* (*T. gondii*) and rapidly succumb to the infection. In toxoplasmosis, one of the most important function of DCs is the production of IL-12, which stimulates NK cells and T cells to produce IFN-γ. Mechanistically, *T. gondii*-induced IL-12 production by DCs is mediated by profilin, which activates NF-κB and IRF8 signaling by stimulation of TLR11 and TLR12. *In vitro* data suggest that the deubiqutinating enzyme OTUB1 regulates NF-κB signaling but *in vivo* studies on the molecular function of OTUB1 have not been reported so far.
To study the DC-specific function of OTUB1 in *T. gondii* infection, we generated CD11c-Cre OTUB1$^{fl/fl}$ mice and infected them with *T. gondii.* Compared with OTUB1$^{fl/fl}$ control mice, CD11c-Cre OTUB1$^{fl/fl}$ mice were more susceptible to *T. gondii*, with enhanced mortality due to impaired parasite control. Although the intracellular control of *T. gondii* was normal in OTUB1-deficient DCs, IL-12 production of OTUB1-deficient DCs was compromised both *in vitro* and *in vivo*. Interestingly, a 4-day administration of IL-12 enabled CD11c-Cre OTUB1$^{fl/fl}$ mice to control *T. gondii* as efficiently as control mice. We also show that the absence of OTUB1 does not affect the motility of DCs before and after *T. gondii* infection. Upon *T. gondii*-profilin (TgPFN) stimulation, OTUB1-deficient DCs showed reduced nuclear translocation of NF-κB p65, leading to a diminished production of IL-12 by OTUB1-deficient DCs.
Conclusively, DC-specific OTUB1 is required for potent IL-12 production in *T. gondii* infection, effective parasite control and survival of the infection.

Presentation: Wednesday, February 21, 2018 from 13:15 – 13:30 in room Seminarraum 2.

122/IIV

Inhibition of neutrophil necrosis controls *Mycobacterium tuberculosis* growth after removal by macrophages

T. Dallenga*[1,2], U. Repnik[3], R. Reimer[4], G. Griffiths[3], U. E. Schaible[1,2]
[1]*Research Center Borstel, Cellular Microbiology, Borstel, Germany*
[2]*German Centre for Infection Research, TTU-TB, Borstel, Germany*
[3]*University of Oslo, Department of Biosciences, Oslo, Germany*
[4]*Heinrich-Pette-Institute, Core Facility Microscopy & Image Analysis, Hamburg, Germany*

With multi drug-resistant *M. tuberculosis* variants on the rise, novel approaches to tackle the global tuberculosis epidemic are needed. Neutrophils represent the main infected cell population in lungs of patients with active tuberculosis. Here we show that *M. tuberculosis* induces necrosis of human neutrophils in an ESX-1-dependent manner. Necrosis was a prerequisite for mycobacterial growth in human macrophages after subsequent removal of infected, necrotic neutrophils. After identification of reactive oxygen species (ROS) as drivers of necrosis, we were able to prevent necrosis by pharmacological inhibition of myeloperoxidase. Thereby, we restored the capability of efferocytic macrophages to control mycobacterial growth, highlighting ROS and ROS-producing enzymes as putative targets for host-directed therapy. Taken together, host cell necrosis represents the starting point for a vicious circle leading to subsequent uptake of infected necrotic cells by other phagocytes, mycobacterial growth therein and, again, induction of host cell necrosis, a scenario that is very likely to take place in patients. Interruption of this vicious circle by inhibition of necrosis and subsequent restoration of the anti-mycobacterial functions represent an intriguing approach for host-directed therapy.

Figure 1

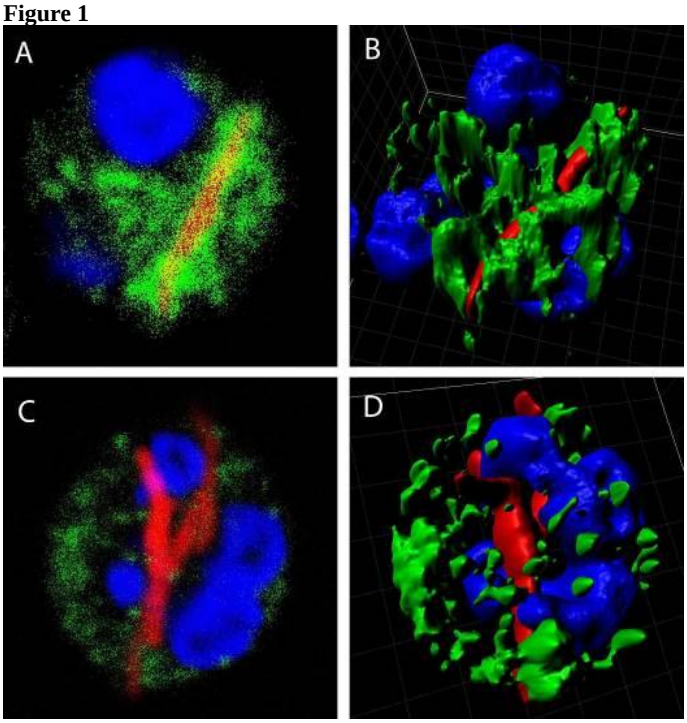
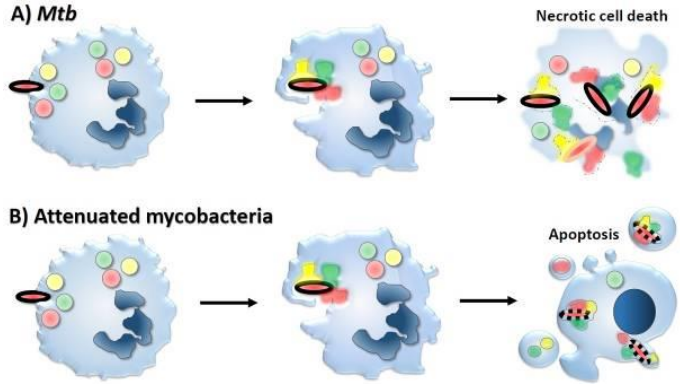


Figure 2



Presentation: Wednesday, February 21, 2018 from 13:30 – 13:45 in room Seminarraum 2.

123/IIV

The C-type lectin receptor Mincle recognizes *Group A Streptococcus* M1T1 5448

S. Mayer*[1], T. Imai[2], N. M. van Sorge[3], S. Yamasaki[2], B. Lepenies[1]
[1]*University of Veterinary Medicine Hannover, Immunology Unit & Research Center of Emerging Infections and Zoonoses (RIZ), Hannover, Germany*
[2]*Kyushu University, Division of Molecular Immunology and Medical Institute of Bioregulation, Fukuoka, Japan*
[3]*University Medical Center Utrecht, Medical Microbiology, Utrecht, Netherlands*

Group A Streptococcus (GAS) or *Streptococcus pyogenes* is a Gram-positive bacterium that commonly colonizes the human skin and nasopharynx. GAS can cause localized infections that are mainly mild and self-limiting, but it can also cause life-threatening invasive infections such as streptococcal toxic shock syndrome or necrotizing fasciitis. GAS is able to express several surface associated virulence factors that allow the entry into host cells and establishment of an infection. Host cells express pattern-recognition receptors (PRRs) to recognize distinct pathogen-associated molecular patterns (PAMPs) on the surface of pathogens. C-type lectin receptors (CLRs) are a highly diverse superfamily of PRRs that recognize carbohydrates mainly in a Ca^{2+}-dependent manner. The CLR Mincle is expressed by myeloid cells like macrophages, dendritic cells and B cells. Signalling via Mincle involves the adapter molecule Caspase-recruitment-domain family member 9 (CARD9) and initiates effector functions such as phagocytosis, cytokine and chemokine production as well as antimicrobial responses.
The role of PRRs in innate host defense in GAS infection is largely unexplored. Therefore, this work focused on the interaction of CLRs with the GAS M1T1 5448 strain.
Using a comprehensive library of recombinantly expressed CLR-human Fc fusion proteins, we identified Mincle as an innate sensor of GAS M1T1 5448, a representative of globally disseminated M1T1 clone of GAS, using flow cytometry. Furthermore, *in vitro* stimulation tests revealed that bone marrow-derived macrophages and dendritic cells from Mincle- and CARD9-deficient mice produced substantially lower levels of pro-inflammatory cytokines compared to wild-type bone marrow derived myeloid cells. However, no significant difference in survival, weight loss, bacterial burden in organs and serum cytokines was observed between Mincle- and CARD9-deficient mice compared to wild-type mice upon systemic infection with GAS M1T1 5448.
In conclusion, our results indicate that GAS M1T1 5448 is recognized by Mincle and impacts cytokine production *in vitro*, but plays a limited role during systemic murine infection with this GAS M1T1 5448 strain.

Presentation: Wednesday, February 21, 2018 from 13:45 – 14:00 in room Seminarraum 2.

124/IIV

Dissecting host-pathogen interactions using single-cell RNA-seq

A. E. Saliba*[1]
[1]*Helmholtz-Institut für RNA-basierte Infektionsforschung, Würzburg, Germany*

Intracellular bacterial pathogens can exhibit large heterogeneity in growth rate inside host cells with major consequences for the infection outcome. If and how the host responds to this heterogeneity remains poorly understood. Here, we combined a fluorescent reporter of bacterial cell division with single-cell RNA-seq analysis to study the macrophage response to different intracellular states of the model pathogen *Salmonella* enterica serovar Typhimurium. The transcriptomes of individual infected macrophages revealed a spectrum of functional host response states to growing and non-growing bacteria. Intriguingly, macrophages harboring non-growing *Salmonella* display hallmarks of the pro-inflammatory M1 polarization state and differ little from bystander cells, suggesting that non-growing bacteria evade recognition by intracellular immune receptors. By contrast, macrophages containing growing bacteria have turned into an anti-inflammatory, M2-like state, as if fast-growing intracellular *Salmonella* overcome host defense by reprogramming macrophage polarization. Additionally, our clustering approach reveals intermediate host functional states between these extremes. Altogether our data suggest that gene expression variability in infected host cells shapes different cellular environments, some of which may favor a growth arrest of *Salmonella* facilitating immune evasion and the establishment of a long-term niche; while others allow *Salmonella* to escape intracellular antimicrobial activity and proliferate.

Presentation: Wednesday, February 21, 2018 from 14:00 – 14:15 in room Seminarraum 2.

125/IIV

High salt conditions enhance the antibacterial activity of macrophages in an NO-independent manner

P. Neubert*[1], V. Schatz[1], A. Weichselbaum[1], A. Schröder[2], J. Jantsch[1]
[1]*Institute of Medical Microbiology and Hygiene, University Hospital Regensburg, Regensburg, Germany*
[2]*Institute of Orthodontics, Regensburg, Germany*

Question: Infection leads to diet-independent Na+ accumulation in the afflicted tissue. This favors pro-inflammatory activation of macrophages and facilitates pathogen removal. Na+-mediated augmentation of pro-inflammatory macrophage activation largely depends on nuclear factor of activated T cells 5 (Nfat5)-signaling. Previously, we demonstrated that increases of Na+ facilitated control of the protozoan pathogen *Leishmania major in vitro* and *in vivo*. Mechanistically, this process involved enhanced NFAT5-dependent NOS2-expression and subsequent NO production. We hypothesize that increases in extracellular Na+ favor anti-bacterial defense mechanisms in macrophages.
Methods: We use a modified gentamicin-protection assay to monitor the influence of high salt conditions on the antibacterial effects of macrophages. This experimental setup is followed by different readouts e.g. counting CFUs of remaining intracellular bacteria, confocal microscopy of infected cells and associated target molecules and western blotting.
Results: We exposed *E. coli*-infected RAW264.7- and bone-marrow derived macrophages to normal (140mM NaCl) and high (180mM NaCl) salt containing cell culture media. These experiments revealed that increased Na+-concentrations favored the antimicrobial activity. Surprisingly, increased anti-microbial activity upon HS exposure was not dependent on type 2 NO synthase or phagocyte oxidase.
Conclusions: These results imply that enhanced Na+ levels trigger macrophages to use other anti-bacterial mechanisms beyond NO production which are currently under investigation in our lab.

Presentation: Wednesday, February 21, 2018 from 14:15 – 14:30 in room Seminarraum 2.

POSTERSESSION 01
19 Febr. 2018 • 19.00-21.00

General and Hospital Hygiene (FG HY)

126/HYP

An ATP based evaluation method for the cleaning and disinfection of complex surgical devices

R. Otchwemah*[1], T. Hempen[2], S. Messler[3], S. Shafizadeh[4], T. Tjardes[4], F. Mattner[1]
[1]*Clinics Cologne, Institute for Hygiene, Köln, Germany*
[2]*Cleanpart Healthcare, Köln, Germany*
[3]*LIS Labor im Sommershof, Köln, Germany*
[4]*Clinics Cologne, Orthopedics and Trauma Surgery, Köln, Germany*

Introduction: Effective cleaning and disinfection (C&D) of surgical instruments are an important part of infection prevention in surgery. Proper preparation is particularly difficult, if cannulated tools are involved, as in anterior cruciate ligament (LCA) replacement surgery. Several outbreaks due to problems in the reprocessing of instruments have been described.
Aim: In our study we monitored the C&D of surgical instruments for LCA replacement, using an adenosine triphosphate (ATP) based detection system for organic residuals, which has not been used for this purpose before.
Materials and Methods: A LCA replacement surgeon, an infection control specialist and an employee of the central sterile service department (CSSD) chose tools and sampling points of LCA replacement surgery sets, which were frequently used and difficult to clean and disinfect. Besides the cannels of the 4.5 mm drill, the drill hand piece, the chucks for drill and K-wires and the screwdriver hand piece, the inside of the tendon fixation clamp were tested.
During a standardised, validated process, which was developed in accordance with the KRINKO and BfArM recommendations for preparation of medical devices, a member of CSSD staff performed the ATP test after mechanical C&D. The SystemSURE Plus Cleaning Verification System by Hygiena®, which uses a bioluminescence reaction for ATP detection, was applied. Following the manufacturer´s recommendations, five relative light units (RLU) were considered as threshold for proper reprocessing. Values in brackets are standard deviations.
Results: Between December 2016 and March 2017 the six selected tools of 50 LCA replacement surgery sets were analysed after C&D. In two cases the drill hand piece was not tested, which leads to a total number of 298 analysed tools. Sixty-six instruments showed values above 5 RLU (22.7%). The 4.5 mm drill accounted for 31 of 66 cases (47.0%; total mean for all 4.5 mm drills: 11.6 RLU (±9.6); median 9.0 RLU), the drill hand piece for 8 (12.1 %; mean: 6.5 RLU (±13.6); median: 2.0 RLU), the screwdriver hand piece for 4 (6.0%; mean 1.5 RLU (±3.3); median 1.0 RLU), the drill chuck for 10 (15.2%, mean: 3.8 RLU (±5.8); mean 2.0 RLU), the K-wire chuck for 9 (13.6%; mean: 43.6 RLU (±222.0); median 1.0 RLU) and the clamp for 4 (6.0%; mean 1.9 RLU (±3.9); median 1.0 RLU) cases. If RLU were above 5, instruments were reprocessed again until RLU were below 5 before their reuse.
Conclusion: Although the clinical relevance of the ATP-test and its most significant threshold are unclear, the method seems to allow identification of instruments, whose reprocessing might be problematic, even with a validated process. Thus, generally high RLU values (mean and median), as in the 4.5 mm drills, may suggest single use in future, whereas individual high RLU values, like in the K-wire chucks (high mean only), may advise special adjustments of the reprocessing workflow.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

127/HYP

Hospital-acquired Vancomycin resistant enterococci – Are surgical intensive care patients at special risk?

S. Kampmeier*[1], A. Kossow*[1], L. M. Clausen[1], D. Knaack[2], J. Wüllenweber[2], C. Ertmer[3], A. Gottschalk[3], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[2]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*
[3]*University Hospital Münster, Department of Anaesthesiology, Intensive Care and Pain Medicine, Münster, Germany*

Background: Vancomycin resistant enterococci (VRE) can cause severe nosocomial infections. In particular, immunocompromised patients are at special risk and acquisition of VRE has been associated with prolonged hospital stay and duration of previous hospitalization, neutropenia and antibiotic treatment. Frequently these risk factors are present in intensive care unit (ICU) patients.
Aim: This study aims to investigate frequency and risk factors for a VRE colonisation/infection in patients of surgical ICUs in a tertiary care centre and to correlate these factors with the occurrence of VRE.
Patients and Methods: From August-October 2017 all patients admitted to all surgical ICUs (41 beds) at the University Hospital Münster were screened for VRE. Screening was performed using rectal (5 cm *ab ano*) swabs within 48 hours after admission and directly prior to discharge of patients. Swabs were applied to chromogenic selective agar and suspected colonies confirmed by MALDI-TOF-MS (Bruker, Bremen, Germany). Susceptibility testing was performed in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. In suspected strains Vancomycin resistance genes *vanA* and *vanB* were detected using GenoType Enterococcus System (Hain Lifescience, Nehren, Germany). In parallel, the following risk factors associated with a VRE colonisation/infection were prospectively recorded: Underlying diseases, previous contact to VRE positive patients, travel history, direct transfer from hospital facilities including ICU and antibiotic treatment. VRE detections later than 48 hours after admission were defined as hospital-acquired.
Results: In total 363 patients (68% male) with a median age of 79 years (range: 14-91 years) were admitted to the surgical ICUs during the investigation period; median length of stay was 3 days (range: 1-44 days). Of all patients 339 (93.3%) were screened on admission and 256 (70.5%) on discharge. Nine patients were admitted with previously known VRE colonisation. Eleven (3.2%) further patients were VRE positive on admission. During ICU stay, seven (2.7%) patients acquired VRE (one *vanA* and six *vanB* positive strains). None of these patients developed a VRE infection.
In total all 363 admitted patients, harboured on average two of 11 recorded risk factors. Median length of stay on the ICU differed significantly (p=0.025) between patients with (11 days, range: 6-25 days) and without (2 days, range: 1-44 days) acquired VRE. All other detected risk factors did not differ significantly between both groups.
Conclusion: Although surgical ICU patients frequently host a variety of risk factors, VRE acquisition among these remained low in our study. Length of stay is the only risk factor significantly associated with hospital-acquired VRE in surgical ICU patients.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

128/HYP

Point prevalence studies in our MRE network – results of the past eight years

B. Ross*[1], M. Lorsch[2], A. E. Lauprecht [3], C. Scheytt [2], C. Tegeler [4], J. Spors[5], L. Wirmann[1], E. Heintschel von Heinegg [6], C. Weyland [7], O. Witzke [8], M. Rath[2], J. Böttcher [2], R. Kundt [2]

[1]*Universitätsklinikum Essen, Krankenhaushygiene, Essen, Germany*
[2]*Stadt Essen, Gesundheitsamt, Essen, Germany*
[3]*Kliniken Essen Mitte, Essen, Germany*
[4]*Ambulantes Operationszentrum, Essen, Germany*
[5]*Stadt Essen, Berufsfeuerwehr, Essen, Germany*
[6]*Universität Duisburg Essen, Institut für Medizinische Mikrobiologie, Essen, Germany*
[7]*Katholische Kliniken, Essen, Germany*
[8]*Universität Duisburg Essen, Klinik für Infektiologie, Essen, Germany*

Introduction: Since 2009, the city of Essen has conducted point prevalence studies in all areas of the local health care system via the Essen MRE network. So far, 11 queries have been carried out, initially twice a year, and once a year from 2011 onwards. Trans-sectoral data on MRE (multidrug-resistant pathogens) from Essen are available continuously for the past eight years.

Methods: 15 hospitals, 3 rehabilitation clinics, 423 medical practices, 58 nursing services, 72 nursing homes, three hospices and the emergency services are contacted regularly via the health authorities and asked for the number of MRE of colonized / infected patients on the reference date. A questionnaire is used to record the following pathogens: MRSA (methicillin-resistant *Staphylococcus aureus*), VRE (vancomycin-resistant *Enterococci*), 3MRGN, 4MRGN (multidrug-resistant gram-negative pathogens) and *Clostridium difficile*.

Results: The epidemiological results regarding MRSA were well reproducible and in accordance with the results of other monitoring systems. The number of MRGNs (multidrug-resistant gram-negative pathogens) in hospitals increased during the observation period. For the first time in 2017, the hospitals reported more 3MRGN (1.74%) than MRSA (1.26%) carriers. The number of VRE patients in the hospitals increased to 0.99% in 2017. Nursing homes and outpatient care services report only a few cases of VRE; rehabilitation facilities report much higher numbers. The prevalence of toxin-producing *Clostridium difficile* has been reported since 2013 at a maximum of 0.3% (nursing homes) to a maximum of 0.7% (2015) in hospitals.

Conclusion: Point prevalence studies are simple tools for MRE networks to get an idea of the current local epidemiological trends.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

129/HYP

Use of cold atmospheric air plasma sterilization and continuous monitoring of the airborne microbial challenge for in-process control of maintenance of sterility of terminally sterilized products

H. Dunkelberg*[1]

[1]*University Goettingen, Bad Sooden-Allendorf, Germany*

Question: The maintenance of sterility of terminally sterilized products at the sterility assurance level of 1:1.000.000 depends on the environmental airborne microbial concentration, the volume of the packages, the environmental air pressure and temperature changes and the filtration efficiency of the packaging material or porous components. The air flow into the packages can be calculated according to the law of Boyle-Mariotte and to the Charles law. The compatibility of the airborne microbial challenge during the storage period with the barrier performance of the packaging material should be confirmed.

Methods: An automated long-term measurement system was developed in order to continuously record the air pressure and to calculate the following end points: the cumulative air inflow into the package, the airborne microbial challenge of the package and the expiration of shelf life. Cold atmospheric air plasma sterilization was used to reduce the airborne microbial concentration in the storage cabinet and to obtain a prolonged shelf life as a result. The long-term measurement system comprised of a microcontroller, a digital air pressure sensor and a SC card for saving the data. A QT-based software was used for the desktop monitoring application in order to plot the measured and via WiFi registered air pressure values and the calculated cumulative air inflow on a graph. The settle plate method was used for monitoring the airborne microbial load inside and outside the cabinet.

Results: We obtained 66.6/300 cm² x 48 h colony forming units (CFU) as mean colony number outside and 2.8 CFU per plate inside the cabinet (reduction factor: 24). Based on 18 days recording, the program calculated the maximum periods of shelf lives using an airborne microbial concentration of 200 CFU/m³ and 10 CFU/m³ (Fig., Table). The table demonstrates that even a relatively low value of 200 CFU/m³ leads to a shelf life of several days or a few weeks even if packaging material with relatively high barrier efficiency is used. The calculated shelf lives for the different packaging material and package volumes are in the range of 21 weeks and more than 5 years when 10 CFU/m³ was used as airborne microbial concentration.

Conclusions: This study demonstrates that the storage conditions of products which should strictly meet the sterility assurance level of 10-6 can be controlled by an automated continuous recording of the relevant parameters and by a data based shelf life calculation. The data based assessment of the compatibility of the sterile barrier systems with the storage conditions leads to a prolonged shelf life if cold atmospheric air plasma sterilization is used.

Figure 1

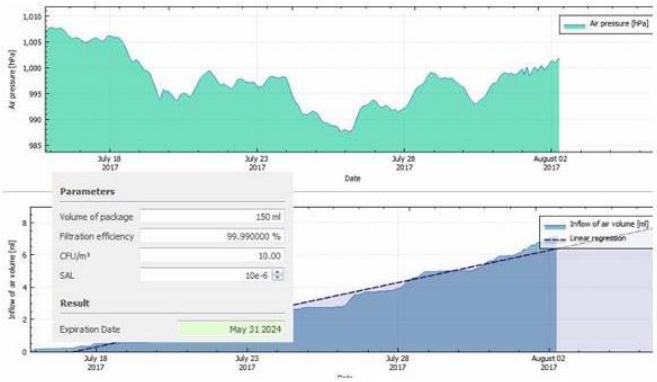


Figure 2

Data-based shelf life calculation of terminally sterilized products by continuous air pressure monitoring. Cold atmospheric air plasma sterilization was used to reduce the airborne microbial concentration

Packaging material	Volume [ml]	Filtration efficiency (%)	200 CFU/m³	10 CFU/m³
Paper/film pouches 2)	150	98,4	1 d 3)	4 w 3)
Paper/film pouches. (double wrapped) 2)	150	99.97	7 w	120 w
Non-woven/film pouches 1)	150	> 99.99	15 w	>5 y
Baskets, double wrapped paper 2)	2600	99.999	9 d	207 w
Baskets, non-woven sheets 1)	2600	> 99.99	2 d	21 w

1) Data of filtration efficiency was taken from manufacturer's IFU;
2) Filtration efficiency was determined by the exposure chamber method;
3) Calculated shelf life limitation: d = days, w =weeks, y = years

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

130/HYP

Aktuelles aus der horizontalen Standardisierung Lebensmittelmikrobiologie

B. Gerten*[1]
[1]*Merck KGaA, Life Science, Darmstadt, Germany*

Die internationale Standardisierung der Methoden zur mikrobiologischen Lebensmitteluntersuchung wird im internationalen Normungsgremium ISO/TC34/SC9 "Food Products – Microbiology" und im europäischen Gremium CEN/TC275/WG6 "Microbiology of the food chain" in enger Zusammenarbeit bearbeitet. Die Experten der beiden ISO/CEN-Normungsgremien treffen sich einmal jährlich meist direkt nacheinander, zuletzt vom 19. – 23. Juni 2017 in Tokio, Japan. Von deutscher Seite nehmen Experten aus dem DIN Arbeitsausschuss "Mikrobiologie in der Lebensmittelkette" teil, der die Arbeiten national spiegelt.
Im Rahmen eines CEN-Mandats wurden in den letzten Jahren 15 der im Anhang 1 der Verordnung über mikrobiologische Kriterien zitierte Untersuchungsverfahren neu erarbeitet bzw. überarbeitet und internationale Ringversuche zur Validierung durchgeführt.
Diese Projekte wurden in 2017 abgeschlossen und die revidierten Methoden vor kurzem einschließlich der Ringversuchsergebnisse veröffentlicht, u.a.:
Nachweis von Salmonellen (EN ISO 6579-1), Nachweis und Zählung von Listerien (EN ISO 11290), Nachweis von *Cronobacter* (EN ISO 22964), Nachweis und Zählung von *Enterobacteriaceae* (EN ISO 21528), Nachweis und Zählung von *Campylobacter* (EN ISO 10272), Nachweis von Vibrionen (EN ISO 21872), Nachweis von Yersinien (EN ISO 10273).
Aktuell beschäftigen sich die Expertengruppen mit den Themen Validierung von In-house-Methoden und alternativer Bestätigungsverfahren (EN ISO 16140 Teile 3-6), Leistungsprüfungen von Bestätigungsmedien und Reagenzien (Ergänzung zur EN ISO 11133), Überarbeitung der EN ISO 7218, PCR-Verfahren, der Thematik "Whole-genome sequencing for typing and genomic characterization" sowie:
Zählung von Hefen und Schimmelpilzen (EN ISO 21527), Zählung von *Clostridium perfringens* (EN ISO 7937), Zählung Sulfit-reduzierender Anaerobier (ISO 15213), Zählung und Bestätigung von Bakterien der *Bacillus cereus*-Gruppe (ISO 7932), Zählung von *E. coli* (EN ISO 16649), Nachweis und MPN-Verfahren *Staphylococcus aureus* (ISO 6888-3), Zählung von psychrotrophen Mikroorganismen (ISO 17410), Nachweis von *Clostridium botulinum* Toxinen.
Neue oder überarbeitete Methoden sollen möglichst einschließlich der Ergebnisse von Ringversuchen als horizontale Europäische und Internationale Normen (EN ISO) veröffentlicht werden und werden dann national als DIN EN ISO übernommen.

Presentation: February 19, 2018 from 19:00 – 21:00 in room Audimax.

131/HYP

Nomenclatural stasis or progress, the dilemma of changing taxonomic concepts and associated nomenclatural changes.

B. J. Tindall*[1]
[1]*Leibniz-Institut DSMZ, Braunschweig, Germany*

End users of nomenclatural systems are often irritated when changes are made to names of familiar entities. This problem is particularly acute when it comes to the changing the names of organisms that are encountered in the clinical environment. Recent examples include recognizing the fact that *Clostridium difficile* is not a member of the genus Clostridium and competing proposals created the names "Peptoclostridium difficile" and Clostridioides difficile. Older problems include the long established fact that members of the genera Shigella and Escherichia should be placed in a single genus or the outstanding issue of *Enterobacter aerogenes* having been placed in the genus Kelbsiella, albeit not under the name Klebsiella aerogenes. There are numerous examples of revisions in classification that will potentially alter the way existing names and taxonomic concepts are used and may affect those working in the clinical environment. In a formal context the International Code of Nomenclatural of Prokaryotes has operated a system over the past 40 years that was unique at the time of its inception and allows one to link names and taxonomic concepts of the past with those in current use. Particularly in the age of the internet, mobile phones and apps there is the potential to keep end users informed of nomenclatural changes at the touch of a button and avoid potentially life threating misunderstandings in the treatment of human pathogens that may have recently been subjected to a taxonomic revision and a change in name. Understanding the background of the rules governing the nomenclature of prokaryotes, the International Code of Nomenclature of Prokaryotes, and the fact that it seeks to set the baseline for a set of standards in classification, without restricting classification is a key element. Similarly appreciating the fact that as classifications alter the International Code of Nomenclature of Prokaryotes allows one to indirectly keep track of changing concepts (provided they are expressed clearly by authors) and the resulting links between names that are considered to be synonyms is a significant benefit that help end users to link the present with the past. In a changing taxonomic environment it is also important to realise that names have a subtle but significant influence on the way we interpret the data associated with a particular name and data set.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

132/HYP

Are large bundles more effective in preventing surgical site infections after colorectal surgery than small bundles? A supplementary analysis of 21 studies reviewed by Zywot and colleagues in the *J Gastrointest Surg* (doi: 10.1007/s11605-017-3465-3)

I. Tomsic*[1], I. F. Chaberny[2], N. R. Heinze[3], C. Krauth[3], B. Lutze[2], T. von Lengerke[1]
[1]*Hannover Medical School, Centre of Public Health and Healthcare, Medical Psychology Unit, Hannover, Germany*
[2]*Leipzig University Hospital, Department of Diagnostics, Institute of Hygiene, Hospital Epidemiology and Environmental Health, Leipzig, Germany*
[3]*Hannover Medical School, Centre of Public Health and Healthcare, Institute of Epidemiology, Social Medicine and Health Systems Research, Hannover, Germany*

Question: Successful implementation of clinical guidelines to prevent hospital-acquired infections represents a challenge for health care workers.1 To address this, the Institute for Healthcare Improvement (IHI) has developed the "bundle-"concept.2 Recently, Zywot and colleagues published a systematic review and meta-analysis on bundles to prevent surgical site infections (SSI) after colorectal surgery.3 They found an overall SSI-risk reduction of 40.2% after bundle implementation. However, they did not analyze bundle size, i.e. the number of measures included in bundles. This attribute may relate to bundle effectiveness: while originally, the IHI defined bundles to consist of 3-5 elements as compliance would be more attainable than with more comprehensive protocols, larger bundles may be more effective by including more evidence-based measures. Thus, we performed a supplemental analysis of the studies reviewed by Zywot et al.3 to test for differences in SSI-risk reduction by bundle size.
Methods: We analyzed 21 studies on SSI of all wound depths.3 Given the number and distribution of bundle sizes, bundles were categorized into 4 groups: 2-4 elements (9 studies), 5-7 elements, 8-10 elements, and 11 or more elements (4 studies each, respectively). For SSI-risks, both baseline- and cohort-sample sizes and baseline- and cohort-SSI outcomes were summed up to calculate risk differences and risk ratios for each group. OpenEpi4 was used for all analyses.
Results: Risk differences differed significantly across bundle size groups (Chi^2=15.4, p=0.002), with 11+ elements-bundles standing out with a risk reduction of 63.3% (risk difference: -12%), while results were 27.1% (-5.3%), 41.9% (-6.8%) and 29.8% (-5.8%) in the groups with 2-4, 5-7 or 8-10 elements, respectively.
Conclusions: Large bundles (11+ elements) were most effective in SSI-reduction after colorectal surgery. The reasons for this, e.g. whether bundle success "only" depends on specific components, remain undetermined, and thus also whether larger bundles should

be recommended. Possibly, larger bundles with key elements are especially effective. Further analysis should examine which bundles of which size, and which elements, are associated with highest compliance and SSI-reductions.

References

[1] Gebhardt FE, Wantia N. [Prevention of nosocomial infections by bundles. Evidence and practical implementation]. Med Klin Intensivmed Notfmed 2013;108:119-124
[2] Resar R, Griffin FA, Haraden C, Nolan TW. Using care bundles to improve health care quality. IHI Innovation Series white paper. Cambridge, MA: IHI; 2012
[3] Zywot A, Lau CSM, Fletcher HS, Paul S. Bundles prevent surgical site infections after colorectal surgery: meta-analysis and systematic review. J Gastrointest Surg 2017;21:1915-30
[4] Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 3.01. www.openepi.com, updated 2013/04/06, accessed 2017/09/22

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

133/HYP

A microbiological-hygienic view on RSFs as a fourth purification stage within waste water treatment

N. Zacharias*[1], A. Brunsch[2], F. M. Mertens[2], E. Christoffels[2], T. Kistemann[1], C. Schreiber[1]
[1]*Institute for Hygiene and Public Heath, University Hospital Bonn, GeoHealth Centre, Bonn, Germany*
[2]*Erftverband, Bergheim, Germany*

The environmental quality standards for surface water bodies have been significantly expanded through the recent amendment of the German regulation on surface water bodies. The newly introduced substances comprise mainly anthropogenic trace elements. Limit values are only established in currently applicable regulations, if the water is indicated for certain uses, e.g. abstraction of irrigation water for agricultural use, or recreation. Quite often, however, surface water bodies which are not explicitly designated for such utilization are nevertheless used for hygiene-sensitive purposes (e.g. water sports or playing children).
In the course of climate change precipitation patterns are expected to change in terms of quantity, incidence and frequency. As a result, longer periods of dry weather are possible, which may lead to extreme low-water discharges with high portions of purified waste water in the watercourses.
In order to improve the purification performance of waste water treatment plants (WWTP), the implementation of a fourth purification stage is intensively discussed. Processes such as UV disinfection, activated carbon filtration, ozonation and membrane bioreactors are in the focus of interest. The effect of these methods on chemical trace elements is substance-specific; a reduction of microbial concentrations could also be observed (KÖNIG 2002, RUDOLPH et al. 1992). Recently, retention soil filters (RSF) are also increasingly attracting attention as an alternative, extensive treatment technology for discharged combined waste water.
In order to better assess the effectiveness of RSFs as an additional purification stage for WWTPs, a RSF test facility was set up at a municipal WWTP. This facility consists of three semi-technical RSFs with a filter area of 1.5 m² each. Two of the filters contain original material from large-scale RSF systems, which already have been in operation for years. The filters were fed exclusively with treated waste water from the WWTP. For most hygienic-microbiological parameters an additional reduction of about 1-2 log steps could be observed. By means of built-in sampling tubes, it was possible to sample individual filter layers (0.1 m, 0.3 m, 0.75 m) in addition to the inlets and outlets. The results demonstrate that the first centimeters of the RSFs are responsible for the major part of reduction. Thus, the process achieves similar or even better reduction rates than the other methods discussed as fourth purification stage, which are also dose-dependent in their effect (e.g. UV irradiation and ozonation) (RUDOLPH et al. 1993).

References

König R (2002): Abwasserdesinfektion mittels UV-Licht - "Renaturierung" der Oberen Isar als Badegewässer. *Kurzfassung*. Umwelt Magazin 12:36
Rudolph, K.-U., Oberg, C. and T. H. Nelle (1992): Stand der Technik bei der Desinfektion von Abwasser und Schwerpunkte der Forschung. Wasser Abwasser Umwelt 134(1), 1 - 9.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

134/HYP

Infection Control Visits in Accordance with §23 of the German Protection Against Infection Act – The Situation of Intensive Care Units in Lower Saxony

C. Henke-Gendo*[1]
[1]*Governmental Institute of Public Health of Lower Saxony (NLGA), Hospital Epidemiology, Hannover, Germany*

Introduction: Since 2013 the "AG Krankenhaushygiene", a subgroup of the infection control committee of Lower Saxony"s public health officers, has been organizing biennial campaigns in order to enhance and structure infection control inspections in accordance with §23 of the German Protection Against Infection Act.
Methods: In 2014 and 2015 local health authorities were invited to inspect the intensive care units (ICU) of their assigned hospitals with the aid of a standardized checklist. In order to gather information about the current situation of ICU in Lower Saxony, they were asked to share their anonymized checklist data with the Governmental Institute of Public Health of Lower Saxony (NLGA).
Results: In total, 839 beds of 73 ICU and intermediate care units (IMC) inspected in 2014 - 2016 could be included in this analysis. This comprises at least a third of ICU beds in Lower Saxony. 56 of the 73 wards come from primary care hospitals. IMC (median = 15 beds) were larger than ICU (10 beds) and combined ICU/IMC (7 beds). Overall, in 25% of the ICU, the number of beds was smaller than the recommended minimum number of 8-12 beds, which is regarded as relevant to sustain medical expertise. On average, one nurse takes care of 2.6 patients during morning shifts, 2.7 patients during late shifts and 3.2 patients during night shifts. 7 out of 63 ICU were not able to present any surveillance data during inspection. Another 10 wards were not able to present data on mandatory surveillance of nosocomial infections. The most common shortcomings identified during inspection were old buildings fabric and physical defects (in 15% of cases), as well as insufficient separation of clean and dirty items (12%) and shortage of space (10%).
Conclusion: The high number of very small and/or combined ICU/IMC mirrors the structural difficulties of a territorial state like Lower Saxony, where the next maximum care hospital might be far away due to sparse settlement. This leads to the necessity of having intensive care facilities available even in small houses. The data also shows that identified shortcomings, like physical defects of the old buildings fabric are common, not only in Lower Saxony, but presumably throughout Germany. Therefore, responsibility for solving these structural issues cannot only be allocated to each single hospital, but has to be pursued in respective expert societies and with political measures.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

135/HYP

Experimental Contamination of Medical Equipment and Surfaces with highly contagious Pathogens

B. Magsig*[1], U. Roesler[1]
[1]*Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany*

In recent years, rare and highly contagious diseases have occurred more often in Germany due to factors such as climate change and increased travel activities. However, there are only few treatment centers in Germany that are specialized in these types of infections. Those usually will not make the initial or suspicious diagnosis; it is

more likely that an infected person will go to the emergency of a "normal" hospital or doctor`s office.
For this reason, a joint project investigates to which extent "normal" hospitals can isolate and treat patients with highly contagious diseases in a short term. This includes an efficient decontamination of used rooms or medical equipment with the idea to reuse them. The goal of this study is the controlled, aerogenic contamination of medical devices/materials used in an intensive care unit and the subsequent evaluation of the available decontamination possibilities.
All experiments are occurring in an aerosol chamber with an individual controllable air-conditioning system (e.g. temperature, humidity). For characterization of the generated aerosols, samples are taken at different points of the chamber using a GRIMM particle counter and impingement, so that the pathogenic concentration in the air can be determined in various areas. Used surrogate pathogens are *Staphylococcus (S.) aureus* as a representative of gram-positive, airborne bacteria and *Geobacillus (G.) stearothermophilus* as representatives of spore-forming agents. For validation of this experiment, the required amount of pathogens is set to 1E+06 colony-forming units (CFU) per cubic meter of air. For evaluation of the generated aerosols several factors were examined. These included the mortality rate of the pathogens, the particle size and the distribution of the particles depending on different atmospheric conditions (30, 50%, 70% humidity).
First results show a reduction of 3 lg from aerosolized *S. aureus* compared to *G. stearothermophilus*. Thus far, there is only a very small influence of the humidity on the death rate of aerosolized *S. aureus* (within one lg). In contrast, it was found that the sample position in the chamber had insignificant influence on the vitality of the bacteria. *G. stearothermophilus* shows that neither the humidity nor the position in the chamber had influence on the number of measured CFU.
The next step is to contaminate medical devices, such as vitality monitors, wall coverings or surfaces used in hospitals, with the above-mentioned pathogens as well as with an additional viral surrogate. These devices/materials will then be decontaminated with various kinds of disinfectants and methods in order to be able to assess their decontamination efficiency. The number of pathogens on the surfaces will be reduced to such an extent that it is comparable with the exciter concentrations found in hospitals.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

136/HYP

Airborne bacteria in hospital operating rooms during ongoing surgery

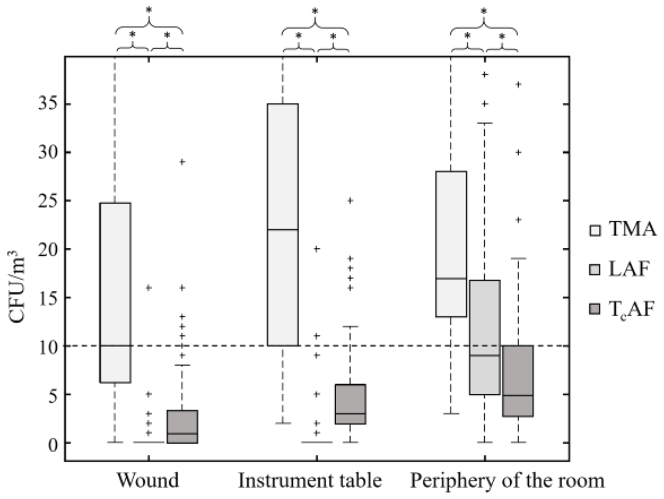
M. Alsved*[1], A. Civilis[2], P. Ekolind[3], A. Tammelin[4], J. Jakobsson[1], T. Svensson[1], M. Ramstorp[1], T. Santl-Temkiv[1], P. A. Larsson[2], M. Bohgard[1], J. Löndahl[1]
[1]*Lund University, Ergonomics and aerosol technology, Lund, Sweden*
[2]*Helsingborg's Hospital, Operating Department, Helsingborg, Sweden*
[3]*Avidicare AB, Lund, Germany*
[4]*Karolinska Institutet, Department of Medicine Solna, Unit of Infectious Diseases, Stockholm, Sweden*

Introduction: Post-operative infections obtained from open-wound surgeries constitute an unnecessary load on both healthcare and affected patients. It is well established that increased air cleanliness reduces the number of post-operative infections. Therefore, the ventilation system is important in order to reduce the number of infectious particles in the air during surgery. Ventilation with high airflow, as in operating rooms, consumes a high amount of energy, and it is thus desirable to find energy efficient solutions.
Objectives: The purpose of this work was to evaluate air quality, energy efficiency and working environment comfort for three different ventilation techniques in operating rooms.
Method: The newly developed ventilation system temperature controlled airflow (T_cAF) was compared with the conventionally used turbulent mixed airflow (TMA) and laminar airflow (LAF). In total, 750 air sample measurements were performed during 45 orthopaedic operations: 15 for each type of ventilation system [1]. The concentration of colony forming units (CFU)/m^3 was measured at three locations in the rooms: close to the wound (<0.5 m), at the instrument table and peripherally in the room. The working environment comfort of the ventilation was evaluated by the operating staff in a questionnaire.
Results: Our study shows that both LAF and T_cAF maintains CFU concentrations in the air during ongoing surgery significantly below 10 CFU/m^3 at the wound and at the instrument table, and for T_cAF also in the periphery of the room, see Figure 1. The median CFU concentration in TMA was at or above 10 CFU/m^3 at all locations. T_cAF used less than half the airflow to that of LAF, resulting in a 28% reduction in energy consumption. The working environment comfort was perceived less noisy and having less draft in the T_cAF than the LAF ventilation.
Summary: Both the LAF and T_cAF ventilation maintain high air cleanliness with low CFU concentrations throughout the operation. TMA is less efficient in removing bacteria from the air close to the patient.
Figure 1: Box plot of measured CFU/m^3 in each of the ventilation systems TMA, LAF and T_cAF at three locations in the operating room. The dotted line represent the limit for ultra-clean air at 10 CFU/m^3 [2]. * indicates significance between groups, $p<0.05$.
This work was supported by Avidicare AB, Swedish Energy Agency, FORMAS and AFA.

References

[1] Alsved, M., et al. (2017). Temperature controlled airflow ventilation in operating rooms compared with laminar airflow and turbulent mixed airflow. J Hosp Infect. Online: http://dx.doi.org/10.1016/j.jhin.2017.10.013.
[2] Lidwell, O.M., et al. Effect of ultraclean air in operating-rooms on deep sepsis in the joint after total hip or knee replacement â€" a randomized study. Brit Med J. 1982; 285:10â€"14.

Figure 1



Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

137/HYP

Abstract has been withdrawn.

138/HYP

Outbreak of coliform pathogens in the drinking water in a building of the University Hospital Leipzig?

C. Hennig*[1], P. Opitz[1], J. Kühne[1], I. F. Chaberny[1]
[1]*Universitätsklinikum Leipzig, Institut für Hygiene, Krankenhaushygiene und Umweltmedizin, Leipzig, Germany*

Question: As part of the annual microbiological examination of the drinking water in March 2016, a systemic contamination of coliform pathogens occurred in a building of the Department of Head and Dental Medicine of the University Hospital Leipzig.
Method: According to the German "Drinking Water Ordinance of 2001", coliform pathogens are classified as indicator parameters and mainly consist of a group of *Escherichia coli, Klebsiella,*

Enterobacter, *Citrobacter* and *Serratia*. Of the mentioned species only Escherichia is distinctly fecal origin [1]. Therefore coliform bacteria are indicator organisms for the sanitary quality of the drinking water system in a building with a limit value of 0 CFU (colony-forming unit) / 100 ml. The drinking water samples were determined with the DIN EN ISO 9308-1:2014-12 procedure.
Result: After analysis the associated pre-results revealed a massive detection of coliform pathogens at several sampling points. 9 of 27 (33.3%) sampling points were affected in different risers and building parts with loads of 1 CFU / 100 ml to greater than 200 CFU / 100 ml. For the direct adverting of dangers a "task force" was convened and all water points were equipped with sterile filters in a time-consuming and cost-intensive measure. In addition, a causal analysis was initiated and the responsible public health department was involved in all discussions and decision on the following actions.
Conclusion: Further follow-up examinations by an alternative accredited laboratory as well as parallel in-house examinations did not show any abnormalities at all sampling points. Due to the distribution of the affected sampling points in the house and the negative tests in the main supply the causal analysis revealed that an actual contamination was not comprehensible. Therefore, it must be assumed that the examination results were not contaminated drinking water, but rather irregularities in the sampling or laboratory examination were responsible for the crossing of the limiting values.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

139/HYP

Self-reported vaccination behaviour of medical students of the University Leipzig: Are prospective doctors a good role model?

B. Lutze*[1], B. Krüger[1], I. F. Chaberny[1]
[1]*Leipzig University Hospital, Department of Diagnostics, Institute of Hygiene, Hospital Epidemiology and Environmental Health, Leipzig, Germany*

Question: After the pre-clinical stage medical students are in regular contact with potentially infectious patients. Therefore, they are confronted with an increased number of pathogens. In the case of an infection, on the one hand, there is the risk of the disease and, on the other hand, there is the risk of transmission to the surroundings and other patients. [1] Thus, vaccinations are an effective prevention measures for medical students. Generally, the current data on the vaccination status of this individual group is low in Germany. But the scant studies on the subject have shown that the vaccination status of medical students is often incomplete. The aim is to perform a risk analysis about medical students, only to find specific reasons against vaccinations and their self-reported vaccination status. The study aimed to effectively contribute to the discussion about lapses occurring in vaccination in recent years.
Methods: A self-reported questionnaire was used as survey instrument. Medical students of the University Leipzig were interviewed in the fifth semester. The items "vaccination status", "knowledge of indicated vaccinations", "vaccination motivation" and "attitude to vaccinations" were evaluated. The participants assessed the vaccination items on a five-stage Likert scale (1 "fully correct" to 5 "not correct at all").
Results: A total of 185 questionnaires were evaluated (response rate of 60%). More than half know which vaccines are medically indicated. Over 80% of students state that they are fully vaccinated against hepatitis-A (85.4%) & hepatitis-B (93.3%), diphtheria (88.6%), mumps (91.5%), rubella (91.4%), pertussis (82.2%), polio (87.0%) and tetanus (94.4%). Almost 95% are vaccinated against measles. In contrast, only 16.8% are vaccinated against influenza. The students state that the flu is a serious disease and rate possible side effects very low. However, 43% of the participants say that they have the intention to get vaccinated against the flu. The majority are not averse to vaccinating (M=4.9) but they do not have time to get vaccinated (M=3.7) and that the probability of occurrence of the disease is too low (M=3.5).
Conclusion: The self-reported vaccination behaviour of medical students shows that they are a good example in general (positive attitude to vaccinations). In the case of measles, a higher value was found than in comparable studies [2]. They are widely vaccinated and have a moderate level of expertise which vaccines are medically indicated. Exciting is the argument against influenza vaccination: Probability of occurrence of the flu and the missing time to get vaccinated.

References

[1] Sydnor, Emily, and Trish M. Perl. "Healthcare providers as sources of vaccine-preventable diseases." *Vaccine* 32.38 (2014): 4814-4822.
[2] Petersen, S., H. Roggendorf, and S. Wicker. "Impfpräventable Erkrankungen: Wissen, Einstellung und Impfstatus von Medizinstudierenden." *Das Gesundheitswesen* 79.05 (2017): 394-398.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

140/HYP

Evaluation of the modulation of the legal obligation to notify infectious diseases in Germany for the city of Essen during the first 12 Month after implementation Author: Lorsch, Ross, Rath, Kundt, Witzke

A. M. Lorsch*[1], O. Witzke[1,2], R. Kundt[1], M. Rath[1], B. Ross[3]
[1]*Public health service Essen, Infection control, Essen, Germany*
[2]*University Hospital, Infectiology, Essen, Germany*
[3]*University Hospital, Department of Hygiene, Essen, Germany*

Introduction: In March 2016, a modulation of the legal obligation to notify distinct infectious diseases was implemented with validity due date May 2016(1). The Modulation aimed the adaption of the established obligation of notification to the actual epidemiological situation. Besides other adaptions, the modulation extended the existing laboratory reporting duty by adding the mandatory to report certain Enerobacteriacea and Acitenocter ssp. with a lack of sensitivity towards Carbapenem antibiotics (either genetically encoded Carbpenemase or phenotypical lack of sensitivity).
Laboratories report detection of either of the organisms to the local health department, which contacts the patients and the attending physicians in order to evaluate further information to meet the requirements to subsequently transfer the information to the National Reference Centre for Infectious Diseases.
Aims: The aim is to analyse the current epidemiological situation in means of prevalence of patients with Carbapenem resistant bacteria in the City of Essen. In addition, the quality of data and of submission to the reference centre is evaluated.
Materials and Methods: The data of reported Carbapenem resistant organisms between 01.05.2016 and 01.05.2017were evaluated in means of the distribution of age and sex of patients and whether the patient is reported as infected, colonized or with unknown status. Furthermore, the reports are interpreted concerning the spectrum of pathogens and distinct resistance properties. The body sites from which the samples were taken are matched to the specific spectrum of pathogens.
The report quality was determined by evaluation of discrepancies between the data, reports with missing information and by analysing the number of wrong reports.
Results: The number of reported patients with Carbapenem resistant bacteria within the determined time span was 133. The reported cases allowed a proper analysis regarding the epidemiological situation. The analysis of the distribution between the patients and the spectrum of organisms resulted in a reliable overview for the City of Essen.
Besides detailed results of the distinct distributions, a poor quality of several information required for proper submission to the National Reference Center for Infectious Diseases, was observed.
Summary: The introduction of the mandatory report of different Carbapenem resistant microorganisms allows a valid overview over the actual prevalence of patients affected by Carbapenem resistant pathogens. Yet the quality of information still requires improvements in means of documentation by the local health departments as well as transmission of information from attending physicians.

Reference

[1] IfSG-Meldepflicht-Anpassungsverordnung vom 18. März 2016 (BGBl. I S. 515)

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

141/HYP

Cleaning and disinfection procedures in Bavarian hospitals: survey of the current status

U. Kandler*[1], S. Fries[1], S. Nickel[1], C. Ertl[1], G. Valenza[1], V. Lehner-Reindl[1], C. Höller[1]
[1]*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Hygiene, Erlangen, Germany*

Introduction: Appropriate cleaning and disinfection of environmental surfaces play a crucial role in infection prevention in hospitals[1, 2]. From the choice of detergents and disinfectants to the education of staff there are numerous critical points with an opportunity for mistakes

Objectives: The purpose of this survey was to assess the organization of cleaning and disinfection procedures in Bavarian hospitals and to detect critical points which need particular attention and show room for improvement.

Methods: The department of hospital hygiene and infection prevention at the Bavarian Health and Food Safety Authority generated a standardized checklist which was sent to all local health authorities in Bavaria. They were asked to visit each hospital in their district and to collect data on the infrastructure for cleaning and disinfection procedures as well as to examine the rooms where cleaning materials are washed, dried and prepared for further application. Results from the inspection were documented in the above mentioned checklist. The completed checklists were sent back to the Bavarian Health and Food Safety Authority. The collected data were read into a data base and were analyzed by IBM SPSS Statistics 23.

Results: In the years 2016 and 2017, 385 hospitals of various sizes had to be inspected in respect of their cleaning and disinfection procedures in Bavaria. Currently, 265 hospitals passed their inspection and data have already been collected. Preliminary evaluation shows that half of the clinics employ internal cleaning services and almost all clinics provide rooms (N=250) for the reprocessing of cleaning materials but a clear separation of clean and soiled material could only be observed in 162 (= 65%) of these rooms. Other topics of concern were for example the correct dosage of disinfectant and clarification of responsibilities especially in case of housekeepers from external enterprises.

Conclusion: Preliminary results indicate that the housekeeping in Bavarian hospitals is mostly well organized. Detailed inspection of housekeeping including the assessment of educational measures, cooperation with infection prevention and control staff and admitted cleaning time per surface have been examined. The evaluation of data will be continued and more detailed information will be presented at the beginning of 2018.

References

[1] Boyce, J.M., *Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals.* Antimicrobial Resistance & Infection Control, 2016. **5**(1): p. 10.

[2] Dancer, S.J., *Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination.* Clin Microbiol Rev, 2014. **27**.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

142/HYP

Track down the problem: Includes the indication before aseptic procedure tasks which are not usually associated with the term aseptic? A comparison of the self-reported level of knowledge and hand hygiene compliance

B. Lutze*[1], I. Hartlep[1], J. Schuchardt[1], M. Vielstich[1], I. F. Chaberny[1]
[1]*Leipzig University Hospital, Department of Diagnostics, Institute of Hygiene, Hospital Epidemiology and Environmental Health, Leipzig, Germany*

Question: Results from a long-term study (the "Clean Hands" campaign in Germany) showed that the lowest hand hygiene compliance rate is found in the indication "before aseptic procedure". [1] At the same time, improving hand hygiene compliance for this indication offers the greatest potential for preventing nosocomial infections [2] and hence remains an important starting-point in the context of infection prevention. The aim is to compare the self-reported knowledge of the indication "before aseptic procedure" and the effective hand hygiene compliance of visceral surgeons. The results should generate a new approach to improve inadequate hand hygiene compliance "before aseptic procedure".

Methods: A self-reported questionnaire was used as survey instrument. The questionnaire checked the state of knowledge of aseptic procedure ("What are aseptic activities for you?"). The correct assignment of aseptic activities was examined within eleven different response options (seven correct / four not correct according to the definition of "before aseptic procedure"). For the hand hygiene compliance monitoring a standardized survey sheet was used with the focus on the indication "before aseptic procedure". The hand hygiene observations ran over three weeks on three visceral surgical stations (by external infection control hygiene specialists) during the ward rounds.

Results: The results of the questionnaires (n = 15) show that 86.7% of the physicians were unable to correctly classify the aseptic activities. "The touching patient surroundings" (80%), "listening through a stethoscope" (73.3%) and "palpation" (66.7%) were falsely described as aseptic activities. Only half of the respondents classified "intubation" as an aseptic activity correctly. The lack of knowledge is matched with the insufficient compliance of hand hygiene during the ward rounds (n = 45): Hand hygiene compliance rate of 22% (indication "before aseptic procedure").

Conclusion: The comparison of the self-reported level of knowledge and hand hygiene compliance showed that "before aseptic procedure" include many tasks which are not usually associated with the term "aseptic". It should be emphasized that the inadequate knowledge of the medical staff reflects the compliance "before aseptic procedure". In this context, further education should focus on the topic "aseptic procedure" and pay attention to practical skills. More particularly, in order to fulfill the effectiveness of tailor-made interventions, the attention must be paid to the right access of the target group.

References

[1] ASH (2017): "Aktion Saubere Hände" Compliance Beobachtungen – Referenzdaten. Berechnungszeitraum 01.01.2016 bis 31.12.2016 Erstellungsdatum 05.05.2017.

[2] WHO (2009): WHO Guidelines on Hand Hygiene in Health Care. First Global Patient Safety Challenge. Clean Care is Safer Care. Geneva: World Health Organization.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

143/HYP

Microbiological safety of human tissues – concept and requirements for quality control testing

H. Lößner*[1], R. R. Tönjes[2], D. Schilling-Leiß[2], A. Godehardt[2], I. Bekeredjian-Ding[3], O. Krut[1]

[1]*Paul-Ehrlich-Institut, Mikrobiologie, FG Mikrobiologische Sicherheit, Langen, Germany*

[2]*Paul-Ehrlich-Institut, Medizinische Biotechnologie, FG Avitale Gewebezubereitungen, Xenogene Zelltherapeutika, Langen, Germany*

[3]*Paul-Ehrlich-Institut, Mikrobiologie, Langen, Germany*

Human tissue transplants, such as corneae, cardiovascular tissues, musculoskeletal tissues and others, can be naturally colonized by microbes or might be contaminated during procurement and processing. Therefore microbiological control testing of human tissues is required in order to prevent transmission of microbial contaminants to recipients. Since August 2007 human tissues are under the scope of medicinal product legislation in Germany. Thus, compliance with European Pharmacopoeia (Ph. Eur.) is required for microbiological control testing of human tissues. However, not all specific aspects for the microbiological control testing of human tissues are covered by the compendial methods of Ph. Eur. and drafting of a new Ph. Eur. chapter in this regard has been initiated. Further standardization and improvement of microbiological test methods for the various human tissues is necessary and should be closely connected to the scientific advances in the field. At present, mostly conventional culture methods are used for microbiological control tests. These methods are relatively slow, do not detect all microorganisms and mostly cannot reveal details of virulence or resistance. These limitations will potentially be overcome by new test methods which allow a real time in depth analysis of present microorganisms. The use of such so called rapid microbiological methods (RMM) requires primary method validation according to Ph. Eur. chapter 5.1.6. Tissue establishments and clinical microbiology laboratories that are associated by contracts for microbiological testing of human tissues are advised to carefully consider current regulations or consult regulatory authorities on this matter.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

144/HYP

Detection of human pathogenic viruses in surface water via capsid integrity PCR

M. Leifels[1], L. Jurzik*[1]

[1]*Ruhr-University Bochum, Dept. of Hygiene, Social- and Environmental Medicine, Bochum, Germany*

Introduction: The study of surface waters for their enteral human pathogenic viral load is relevant as viruses pose a serious threat to public health despite their low concentration in the environment. According to the state of the art, viruses were quantified using real-time PCR (qPCR) as well as cell culture. With qPCR the quantification of infectious viruses in water is limited. In contrast, with cell culture it is possible to detect infectious viruses, but it is at the same time labor-intensive and time-consuming.

Aims: The aim of the study was to evaluate the potential applications of capsid integrity PCR (ciPCR) - qPCR with prior incubation of the sample with the intercalating dyes ethidium monoazide (EMA) and propidium monoazide (PMA) - for molecular quantification of infectious viruses in surface waters.

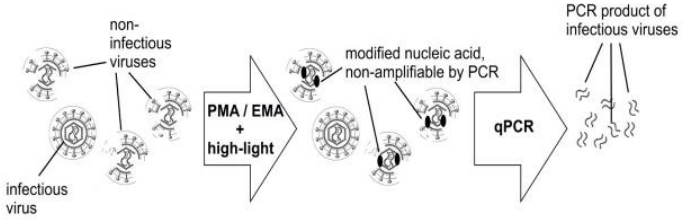
Material and Methods: EMA and PMA interacts with viral DNA/RNA and form an irreversible complex and thus prevent subsequent amplification by the polymerase of qPCR (figure 1). Both substances are unable to penetrate intact virus capsids. For evaluation the ciPCR for quantifying infectious viruses different viruses were partially or completely inactivated by heat, chlorine and UV irradiation. Subsequently, the viruses were examined for their infectivity by qPCR, ciPCR and culture-based method.

Results: Comparison of the results from qPCR with the ciPCR showed that both dyes lead to a reduction of infectivity by decreasing qPCR signals, when the disinfection method attacks the integrity of the viral capsid (temperature >55°C, 2mg/ml chlorine). Thus, heat inactivation resulted in no or minimal decrease in qPCR results for all three viruses tested, while ciPCR showed a reduction rate between log 1 and log 3.5. However, when the viruses are exposed to UV light, the two dyes used are unable to confirm the viral inactivation demonstrated by the culture methods.

Discussion: A correspondence between the decrease of the infectivity in cell culture and the nucleic acid concentration measured in the ciPCR, however, was only recognizable by a pretreatment with heat and chlorine. Both inactivation methods attack at the integrity of the viral capsid. In contrast, the UV treatment attacks less the capsid but rather the nucleic acid. Accordingly, a reduction could be detected in the cell culture, in the ciPCR and the qPCR, however, no reduction in concentration was measured.

Conclusion: In the laboratory, the use of ciPCR has helped to more realistically assess the infectious viral load of environmental samples, which allows a more accurate calculation of health consequences by using viral contaminated waters.

Figure 1



Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Antimicrobial Resistence and Drugs, Infection Prevention (FG PR)

145/PRP

Increase in Azithromycin Resistance and low Levels of Cephalosporine Resistance in Neisseria gonorrhoeae in Germany

S. Buder*[1], D. Heuer[2], S. Dudareva-Vizule[3], S. Banhart[2], T. Taminga[3], E. Guhl[1], I. Graeber[1], T. Pilz[2], P. K. Kohl[1], K. Jansen[3], V. Bremer[3]

[1]*German Reference Laboratory for Gonococci, Department of Dermatology and Venerology, Vivantes Hospital, Berlin, Germany*

[2]*Robert Koch Institute, Unit Sexually Transmitted Bacterial Infections, Berlin, Germany*

[3]*Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany*

Background: The widespread antimicrobial resistance of *Neisseria gonorrhoeae* (NG) is a serious problem for the treatment and control of gonorrhoea. Many of the previously effective therapeutic agents are no longer viable. Because NG infections are not reportable in Germany, only limited data on disease epidemiology and antimicrobial susceptibility patterns are available. The Gonococcal Resistance Network (GORENET) is a surveillance protocol to monitor trends in the antimicrobial susceptibility of NG in Germany and link this to epidemiological data and NG multiantigen sequence typing (NG-MAST) data to guide treatment algorithms and target future prevention strategies.

Methods: Between April 2014 and December 2016, data on patient-related information were collected from laboratories nationwide, and susceptibility testing was performed on 1069 N. gonorrhoeae isolates forwarded from the network laboratories to the Conciliar Laboratory for gonococci. Susceptibility results for cefixime, ceftriaxone, azithromycin, ciprofloxacin and penicillin were defined according to EUCAST 4.0 standards. Percentages, medians and interquartile ranges (IQR) were calculated. NG multiantigen sequence typing (NG-MAST) was performed for a third of these isolates.

Results: Altogether, 90% of isolates were from men. The median age was 32 (IQR 25-44) years for men and 25 (IQR 22-40) years for women (p-value<0.001). The most frequently tested materials among men were urethral (96.1%) and rectal swabs (1.7%), and among women, it was mainly endocervical and vaginal swabs

(84.3%). None of the isolates were resistant to ceftriaxone. Furthermore, 0.8-1.9% of the isolates were resistant to cefixime, 4.3-11.9% showed resistance against azithromycin, 53.4-72.0% were resistant to ciprofloxacin, and 18-29.1% were high-level resistant to penicillin**.** Selected isolates underwent NG-MAST analysis and showed a predominance of ST1407 in 2014/2015 with decreasing number during observation period and a wide range of other STs, including various new sequence types.
Conclusion: Resistance to ceftriaxone was not detected, and the percentage of isolates with resistance to cefixime was low, whereas azithromycin resistance showed a discontinuous trend with partly high levels during the observation period. The rates of ciprofloxacin resistance and penicillin resistance were very high across Germany. Continued surveillance of antimicrobial drug susceptibilities for NG remains mandatory to ensure efficient disease management.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

146/PRP
CPP-peptidoglycosidase fusion proteins targeting intracellular bacterial pathogens
T. Martins*[1], C. Rüter[1], I. Benz[1], A. Schmidt[1]
[1]*Center of Molecular biology and Inflammation - ZMBE, Institute of Infectiology, Münster, Germany*

In the last decades, the increasing rate of multidrug-resistance to classical antibiotics has driven research towards identification of other means to fight infectious diseases. Increasing evidence infers that bacteria being internalized into epithelial cells via phagocytosis cause a high incidence of recurrent infections.
Concomitantly, endolysins have attracted great interest as putative novel antimicrobials against Gram-positive bacteria, because these are not inhibited by traditional antibiotic resistance mechanisms. Fusions of CPP with endolysins or homologues to lytic transglycosidases, which maintain the high enzymatic activity, may represent novel antimicrobials.
Therefore, we propose, to use endolysins as antimicrobials combined with a CPP to kill resistant intracellular bacteria through cell lysis. For this purpose fusion proteins of recombinant variants of endolysins with CPP have been generated, expressed, isolated and purified. These fusion proteins will be assessed with respect to their cell-penetrating ability in different human primary cells as well as cell lines including immune cells.
Furthermore, we characterized their putative antibacterial activity against various Gram-positive and also Gram-negative pathogens by using confocal microscopy, flow cytometry, immunoblotting assays and by performing enzyme activity analyses to determine peptidoglycan (PG) degradation.
The results will evaluate and underline the potential of CPP-peptidoglycosidases to delivery antimicrobial agents. These novel agents – alone or in combination - might open new approaches for the treatment of infectious diseases caused by intracellular pathogens.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

147/PRP
Interleukin 26 shows immediate bactericidal effects even to multi-resistant isolates
B. Hansen*[1], G. Maschkowitz[1], R. Podschun[1], H. Fickenscher[1]
[1]*Institute for Infection Medicine, Kiel, Germany*

Introduction: Interleukin 26 (IL-26) is a highly cationic cytokine (1), which is locally produced by T lymphocytes and inflammatory cells, attaches to cell surfaces, is highly overexpressed in chronically inflamed tissues (1-3), and modulates virus replication. In addition, bacteriostatic and bactericidal effects have been reported for IL-26 (4).
Objective: This study aimed at the clarification of the previously claimed antibacterial effects of IL-26 by testing both sensitive and multi-resistant bacteria for their susceptibility to recombinant human IL-26 under standardized conditions and at the quantitation of that antimicrobial activity.
Material and Methods: Different species of Gram-positive and Gram-negative bacteria at 10E5 CFU/ml dilutions were incubated with recombinant human IL-26 (1-100 µg/ml) for 4 h. Samples were taken immediately and after 1, 2, 3 and 4 h and spread on agar plates. After incubation for 16-20 h, bacterial survival was determined by the number of colony-forming units.
Results: IL-26 was capable of killing Gram-positive bacteria, including MRSA and VRE strains with a significant reduction of the colony numbers immediately after adding IL-26 at 30-100 µg/ml concentrations. Complete killing of all Gram-positive bacteria in the respective cultures was achieved within 1 h. Regarding Gram-negative bacteria, IL-26 showed antimicrobial activity also against *Acinetobacter baumannii* with a significant reduction of the colony counts already after 1 h of incubation. However, *Escherichia coli* and *Klebsiella pneumoniae* were not significantly affected by IL-26, in contrast to the previous report.
Conclusion: The study revealed for the first time that the pro-inflammatory cytokine IL-26 functions similarly to antimicrobial peptides as a potent bactericidal agent on drug-sensitive and -resistant Gram-positive bacteria and on *Acinetobacter baumannii*. This study may provide possible therapeutic options against infections with multi-resistant bacteria.

References
[1] Knappe et al. (2000) Induction of a novel cellular homolog of interleukin-10, AK155, by transformation of T lymphocytes with herpesvirus saimiri. J Virol 74:3881-3887.
[2] Corvaisier et al. (2012) IL-26 Is overexpressed in rheumatoid arthritis and induces proinflammatory cytokine production and Th17 cell generation. PLoS Biol 10:e1001395.
[3] Miot et al. (2014) IL-26 is overexpressed in chronically HCV-infected patients and enhances TRAIL-mediated cytotoxicity and interferon production by human NK cells. Gut 64:1466-1475.
[4] Meller et al. (2015) Th17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. Nat Immunol 16:970-979.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

148/PRP
Expression of the AdeRS two-component system of *Acinetobacter baumannii* ATCC 17978 under different physiological conditions
K. Lucaßen[1], S. Gerson[1], K. Xanthopoulou[1,2], H. Seifert[1,2], P. Higgins*[1,2]
[1]*University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[2]*German Centre for Infection Research, , Bonn-Köln, Germany*

Introduction: Multidrug resistance is common in *A. baumannii* and is often mediated by efflux pumps, especially through resistance-nodulation-cell division (RND) efflux pump systems. The RND efflux pump AdeABC is regulated by the 2-component regulator AdeRS. Mutations or disruption of the gene has been shown to affect *adeABC* expression. However, regulation of *adeRS* expression has until now not been investigated.
Objectives: The objective of this study was to investigate the expression of *adeRS* under different physiological conditions.
Materials and Methods: Expression of *adeRS* was determined by β-galactosidase reporter assays. This was performed by putting the expression of the reporter gene under the control of the *adeR or adeS* promoter. Physiological conditions tested were growth on solid media (LB and Mueller Hinton agar) and motility plates supplemented with X-gal and growth in the presence of different antibiotics. Gene expression was detected by blue colonies. The influence of different antimicrobial agents was tested by disc diffusion and by gradient plates.
Results: Co-transcription of *adeRS* was demonstrated by RT-PCR. Therefore only *adeR* was investigated in further experiments. β-galactosidase reporter assays revealed constitutive expression of *adeR* in the presence of the tested antimicrobials piperacillin, ampicillin, ampicillin/sulbactam, cefotaxime,

piperacillin/tazobactam, ceftazidime, trimethroprim/sulfamethoxazole, ciprofloxacin, moxifloxacin, amikacin, gentamicin, tobramycin, cefepime, cefuroxime, meropenem, ertapenem, imipenem and kanamycin**.** However, growth on motility plates showed differences in expression of *adeR* compared to solid media. After 24 h incubation only the site of inoculation was blue while the migrating fringe was white. After 48 h incubation, the newly created migration fringe was white whereas the formerly white region turned blue. This indicates that motility inhibited expression of *adeR*. A *lacZ-adeA* construct also showed that the efflux pump was not expressed under motility conditions.

Summary: *adeR* was constitutively expressed under all tested growth conditions on solid media. However, growth on motility plates suggests that expression of *adeR* is inhibited while the cells are motile. Since the expression of *adeR* facilitates the expression of the *adeABC* efflux pump, and thereby contributes to MDR this opens up the possibility of the efflux pump regulators for novel antibiotic targets.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

149/PRP

Updated data on primary resistance of *Helicobacter pylori* in Germany

B. Bluemel[1], H. Goelz[1], B. Eisele[1], D. Hofreuter[2], E. Glocker*[2,1]
[1]*University Medical Center, Institute of Medical Microbiology, Freiburg i. Br., Germany*
[2]*Brandenburg Hospital, Brandenburg Medical School, Institute of Laboratory Medicine, Brandenburg, Germany*

Question: There are only sparse data on antimicrobial resistance of *Helicobacter pylori* in Germany. The aim of this ongoing prospective study is to keep the development of primary antimicrobial resistance of *H. pylori* under surveillance and to continuously deliver updated data on resistance.

Methods: From October 2014 until October 2017, a total of 1474 adult patients from all across Germany who had not yet received any prior eradication treatment were enrolled. Gastric tissue samples were molecular genetically tested for mutations conferring resistance to clarithromycin, levofloxacin and tetracycline. Epidemiological data were gathered and documented.

Results: About 60.6% of the enrolled patients suffered from gastritis; 14% from peptic ulcer disease; 7% did not reveal any macroscopic alterations of the gastric mucosa.

Overall, primary resistances were 10.8% for clarithromycin and 11.6% for levofloxacin; 2.8% revealed reduced susceptibility to tetracycline; 2.6% showed combined resistance to clarithromycin and levofloxacin. There was no significant difference in the resistance proportions among the different German federal states.

The underlying gastric disease did not have an impact on primary resistance.

Conclusions: Our data show that primary clarithromycin resistance is about 10% in Germany, making clarithromycin still an option for first-line eradication treatments. Genotypic susceptibility testing is a reliable method that facilitates updating resistance data in regular intervals.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

150/PRP

Reliability of Vancomycin minimum inhibitory concentration in MRSA by testing with VITEK®2 in comparison to Etest using two different culture media for primary cultivation

M. Ecker*[1], M. Thomé*[2]
[1]*Kassel School of Medicine/ University of Southampton, Kassel, Germany*
[2]*Klinikum Kassel, Institute for Laboratory Medicine, Clinical Microbiology, Kassel, Germany*

Introduction: Infections caused by *Methicillin-resistant Staphylococcus aureus* (MRSA) are a major threat for patients. Mortality is markedly increased and hospital stays are prolonged. Management and therapy are determined by strain specific antimicrobial resistance; choice and dose of the antibiotic depends on the minimum inhibitory concentration (MIC). Hence, accuracy and reliability of the measured MIC value is of huge importance for patient safety and effectiveness of antibiotic therapy. Recently, the assumption evolved, that the use of cefoxitin supplemented screening media for MRSA might result in altered MIC values, as we observed altering values between differential and selective media used for MIC measurements.

Aims: Our aim is the evaluation and comparison of the semi-automated VITEK®2 (bioMérieux, Marcy-l"Étoile, France) and the manually performed Etest (bioMérieux, Marcy-l"Étoile, France). Additionally, we aimed to evaluate the influence of different culture media on the MIC value. For this purpose, we used Columbia blood agar with 5% sheep blood (BD, Franklin Lakes, USA) and CHROMagar MRSA, which is supplemented with cefoxitin (Mast Diagnostica, Reinfeld, Germany).

Methods: 100 clinical isolates from the Klinikum Kassel have been tested for their MIC value.

All strains were proven to be MRSA in diagnostic routine and cryopreserved in the Institute of Laboratory Medicine prior to study begin.

The strains were defrosted on Columbia agar plates and after incubation, they have been plated onto Columbia and CHROMagar MRSA. On day three after defrosting, the MIC measurement occurred. Each strain from each agar plate has been tested with VITEK®2 and Etest. All tests have been performed in duplicate.

For quality control, the *mecA* positive strain ATCC® 43300™ has been tested in parallel with all other strains on each day a measurement has been performed.

In addition, we determined the colony forming units (CFU) by serial dilution technique to prove the inoculation density of each bacterial suspension used for VITEK®2 and Etest.

Statistical analysis with SPSS involved dot plots, histograms and kappa scores.

Results: Both, VITEK®2 and Etest showed high essential agreement and repeatability within each strain and culture medium. But, MIC values obtained from strains taken from CHROMagar MRSA were remarkably higher than those from Columbia.

Summary: We conclude, that the minimum inhibitory concentration of vancomycin for MRSA is more reliable, if Columbia agar has been used for cultivation. Hence, we recommend avoidance of chromogenic or nutrient deficient media, if MIC measurement is intended.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

151/PRP

Physical and material properties of *Bacillus subtilis* biofilms

S. Kesel[1], S. Grumbein[2], O. Lieleg[2], M. Opitz*[1]
[1]*LMU, Faculty of Physics, München, Germany*
[2]*IMETUM/Technische Universität München, Garching, Germany*

Biofilms are the dominant lifestyle of bacteria. In the biofilm, the single bacteria are embedded in a self-produced matrix consisting of exopolymeric substances (EPS), that protects the bacteria in the biofilm against antibiotics, chemicals and mechanical stresses, rendering the biofilm unremovable, which poses a big problem in health care and industry. In order to prevent the formation of biofilms or to cure patients from mature biofilms, we need to understand how biofilm matrix components determine the biophyscial properties of bacterial biofilms. Changing these properties may allow better penetration of the biofilm by antimicrobial agents. Using different biophysical techniques[1-4], we investigated physical properties of two *Bacillus subtilis* biofilms that differ in their biofilm matrix composition. We investigated these biofilm properties at different phases of biofilm formation, beginning with the attachment of single cells to surfaces up to micro-colony growth and fully matured biofilms. We were able to show that basal expression of EPS already affects the first step in biofilm formation[4]; the attachment of single cells to surfaces. Furthermore, by using mathematical modeling, we were able to quantify the influence of specific EPS on biofilm growth and the final dimensions of the *B. subtilis* biofilm[1]. Finally, we were able

to show that specific biopolymers determine physical properties of *B. subtilis* biofilms[2,3], such as surface stiffness, surface roughness, the biofilm wetting resistance or its bulk stiffness.

References

[1] S. Kesel, *et al.*. Matrix composition determines dimensions of *Bacillus subtilis* NCIB 3610 biofilm colonies grown on LB agar. **RSC Advances,7, 31886-31898 (2017)**

[2] M. Werb, *et al.*. Surface topology affects wetting behavior of *Bacillus subtilis* biofilms **npj Biofilms and Microbioms, doi:10.1038/s41522-017-0018-1 (2017)**

[3] S.Kesel, *et al.*. Direct comparison of physical properties of *Bacillus subtilis* NCIB 3610 and B-1 biofilms. **Applied and Environmental Microbiology, 82(8): 2424-2432 (2016)**

[4] S. Kesel, *et al.*. Carbohydrate-coating reduces adhesion of biofilm forming *Bacillus subtilis* to gold surfaces**. Applied and Environmental Microbiology, 80(19):5911 (2014)**

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

152/PRP

Evaluation of the SuperPolymyxin medium for the screening of Colistin-resistant gram-negative bacteria in stool samples

S. Przybysz*[1], C. Correa[1], K. Becker[1], R. Köck[1,2], F. Schaumburg[1]

[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

[2]*Institute of Hospital Hygiene Oldenburg, Oldenburg, Germany*

Question: Colistin is considered as one of the last resort antimicrobials for the treatment of infections with carbapenem-resistant Gram-negative bacteria. While colistin resistance conferred by chromosomal mutation is known since a long time, plasmid-encoded resistance (e.g. *mcr-1*) is now emerging particularly in South-East Asia. To improve infection control measures, there is a need for reliable, culture-based screening diagnostic approaches to detect persons carrying colistin-resistant Gram-negative bacteria (CRGN). The aim of this study was to assess the test performance and applicability of the colistin-containing SuperPolymyxin agar medium [1] when used to screen stool samples for CRGN.

Methods: This study was an ancillary study to a prospective cohort study that analysed the acquisition of antimicrobial-resistant bacteria among international travellers. Participants provided stool samples (FecalSwab) once before and once after the travel, as well as several times during their stay abroad. Samples were cultured on SuperPolymyxin medium (37Â°C, 24â€"48 h). From each agar plate, one of each phenotypically different colony was subcultured for species identification by MALDI-TOF mass spectrometry. Susceptibility to colistin was tested in all non-intrinsically resistant *Enterobacteriaceae* using Etest (bioMÃ©rieux). Colistin resistance was confirmed by broth microdilution (Sensititre, Thermo Fisher Scientific). All colistin-resistant isolates were screened for *mcr-1* (eazyplex SuperBug mcr-1, Amplex).

Results: In total, 692 stool samples from 52 participants were cultured on SuperPolymyxin medium. Bacterial growth was detected on 85% (n=588) of these plates. A median of one species (range: 1â€"3) was detected per plate (total number of isolates: 880). After species identification, all intrinsically resistant species were excluded from the final analysis including *Hafnia alvei* (n=6), *Proteus* sp. (n=23), *Providencia rettgeri* (n=72), *Serratia marcescens* (n=31) and *Morganella morganii* (n=61). *Stenotrophomonas maltophilia* (n=20) were also excluded due to missing EUCAST clinical breakpoints. In the remaining isolates (n=667), colistin resistance was detected in 19.5% (130/667) isolates by Etest. Colistin resistance was confirmed by broth microdilution in 13.6% (n=91/667) of the isolates (15.1% of the stool samples, 89/692). Of these, 15.4% (14/91) were *mcr-1* positive (all were *E. coli*). The remaining (all *mcr-1* negative) were *Enterobacter asburiae* (n=26), *Enteroacter cloacae* (n=19) and others (n=32).

Conclusions: We reliably detected non-intrinsically CRGN in 15.1% of all stool samples that were positive using the SuperPolymyxin medium. The majority of bacteria growing on the medium was colistin-susceptible, resulting in an enormous workload (species identification, susceptibility testing and confirmation).

Reference

[1] Nordman et al., A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates. J Clin Microbiol 2016;54:1395-1399.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

153/PRP

Bacterial zincophore [*S,S*]-ethylenediamine-N,N'-disuccinic acid is a potent inhibitor of metallo-β-lactamases

A. Proschak[1], J. Kramer[1], E. Proschak[1], T. A. Wichelhaus*[2]

[1]*Goethe-University, Institute of Pharmaceutical Chemistry, Frankfurt am Main, Germany*

[2]*University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt am Main, Germany*

Introduction: Carbapenemases such as metallo-β-lactamases (MBLs) are spreading among gram-negative bacterial pathogens. Infections due to these multidrug-resistant bacteria constitute a major global health challenge. Therapeutic strategies against carbapenemase producing bacteria include β-lactamase inhibitor combinations. [*S,S*]-Ethylenediamine-N,N'-disuccinic acid (EDDS) is a chelator and potential inhibitor of MBLs.

Objective: We investigated the activity of EDDS in combination with imipenem against MBL producing bacteria *in vitro* as well as *in vivo*.

Materials and Methods: Inhibitory activity of EDDS was analyzed by means of a fluorescence based assay using purified recombinant MBLs, i.e., NDM-1, VIM-1, SIM-1, and IMP-1. *In vitro* activity of imipenem ± EDDS against mutants as well as clinical isolates expressing MBL was evaluated by broth microdilution assay. *In vivo* activity of imipenem ± EDDS was analyzed in a *Galleria mellonella* infection model.

Results: EDDS revealed potent MBL inhibitory activity against purified NDM-1, weaker activity against VIM-1 and SIM-1 and marginal activity against IMP-1. EDDS did not exhibit any intrinsic antibacterial activity but enabled a dose-dependent potentiation of imipenem against mutants as well as clinical isolates expressing various MBLs. The *in vivo* model showed significant better survival rate of imipenem + EDDS treated *Galleria mellonella* larvae infected with NDM-1 producing *K. pneumoniae* compared to monotherapy with imipenem.

Conclusions: The bacterial natural zincophore EDDS is a potent MBL inhibitor and in combination with imipenem overcomes MBL mediated carbapenem-resistance *in vitro* and *in vivo*.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

154/PRP

Screening for antimicrobial resistance gene markers in *Salmonella* isolates from clinical samples, in Lagos, Nigeria

K. O. Akinyemi*[1], B. Iwalokun[2], E. Akpabio[1]

[1]*University, Microbiology, Lagos, Nigeria*

[2]*Nigerian Institute of Medical Research, Molecular Biology, Lagos, Nigeria*

Background: Salmonellosis is one of the major food borne diseases with a high incidence and severity. Antimicrobial resistance is a growing public health emergency. The emerging and re-emerging extended spectrum beta-lactamase producing *Salmonella enterica* serovars had continued to pose serious health challenge in developing countries

Aim: This study determined the prevalence and characterization of ESBL genes among multiple drug resistant-*Salmonella* species in Lagos.

Methods: A total of 127 patients with various types of medical conditions at known referral public hospitals from June 20015 to September 2015 were collected. Samples were cultured and antimicrobial susceptibility test was performed on all *Salmonella* isolates by standard methods. The isolates were further screened

for ESBL production by Double Disc Synergy test (DDST). Characterization of ESBL using three gene makers; bla CTX-M1, blaSHV, blaTEM was by standard PCR procedure.
Results: The result of the study revealed that over 60% of the 51 *Salmonella* isolates exhibited multiple drug resistance. Similarly, more than 60% of the isolates were resistant to cefotaxime and cefuroxime. A total of 12 different resistance patterns were found. About 63% of the isolates produced ESBL, of which 66.7% contained one of the three gene makers However; *bla*SHV was found in 63% of ESBL positive *Salmonella* species; followed by *bla*CTX-M1 in 36.4% and, *bla*TEM in 9.1% of the isolates. All the *Salmonella* isolates were 100% sensitive to imipenem.
Conclusion: This study revealed emergence and circulation of *bla*CTX-M-I and *bla* SHV in clinical *Salmonella* isolates in Lagos. Application of multilocus sequencing techniques and pulse field gel electrophoresis on ESBL producing *Salmonella* strains to assess the clonal relatedness is advocated. Imipenem; the first member of the carbapemem was 100% effective against *Salmonella* species. Thus, this result confers an early warning signal for the prudent use of this valuable drug.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

155/PRP
Investigation of *pmrB* mutations and potential novel colistin resistance mechanisms in clinical *Acinetobacter baumannii* isolates
S. Gerson[1], J. Ertel[2], S. Göttig[3], H. Seifert[1,4], P. Higgins*[1,4]
[1]*University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[2]*Institute for Medical Microbiology, Immunology and German Centre for Infection Research, Köln, Germany*
[3]*Johann Wolfgang Goethe-University, Institute for Medical Microbiology, Frankfurt am Main, Germany*
[4]*German Centre for Infection Research, , Bonn-Köln, Germany*
Introduction: The lack of antimicrobial agents effective against multidrug-resistant MDR *Acinetobacter baumannii* strains has led to the reintroduction of the polymyxin antibiotic colistin. However, resistance to colistin (COL) has been reported among *A. baumannii* isolates, complicating the treatment of infections significantly.
Objectives: This study aimed to investigate the mechanisms of colistin resistance in *A. baumannii* obtained from patients hospitalized in Germany, Spain and Greece between 2012 and 2016.
Materials and Methods: Resistance to COL was determined by agar dilution and microbroth dilution, and interpreted as >2 mg/l (EUCAST resistance breakpoints for *Acinetobacter* spp). *A. baumannii* isolates were investigated as isolate pairs (n=4), which were defined as two isolates obtained from the same hospital, usually but not exclusively from the same patient, that displayed a phenotype shift from low-to-high COL MICs, and were identical by rep-PCR. The isolate pairs were subjected to whole-genome sequencing (WGS) and Ridom SeqSphere+ v.3.0 software was used for *de novo* assembly and comparison. Gene expression of *pmrABC* was analysed by qRT-PCR.
Results: Isolates within an isolate pair displayed less than 10 allele differences. Isolate pairs 1-3 belonged to the international clonal lineage IC2, while the fourth isolate pair belonged to IC4. In the four isolates with high COL MICs, three amino acid substitutions (A28V, I232T and S17R) and a four amino acid deletion (ΔL9-G12) were found in PmrB (Table 1). Analysis of gene expression revealed that the amino acid substitutions A28V and S17R as well as the four amino acid deletion led to a significant increase (up to 70-fold) in *pmrABC* expression. In contrast, the amino acid substitution I232T was associated with an unchanged or decreased *pmrABC* expression, suggesting a different resistance mechanism in this isolate. Here, an additional allele difference was detected in the guanosine polyphosphate pyrophosphohydrolase/synthase (SpoT) (Table 1). SpoT synthesizes guanosine tetraphosphate and has been previously described to be involved in antibiotic resistance [1]. No mutations or disruptions were found in genes encoding porins or in genes involved in the lipid A biosynthesis.
Summary: Two novel *pmrB* mutations were associated with *pmrABC* overexpression and COL-resistance. The amino acid substitution in SpoT suggests a novel colistin resistance mechanisms in *A. baumannii* and further investigation will elucidate the impact in clinical isolates.

Reference
[1] Wu J, Long Q, Xie J (2010) (p)ppGpp and Drug Resistance. J. Cell. Physiol. 224:300-304

Isolate pair	MIC (mg/L)	Difference
1	2-256	ΔL9-G12
2	2-256	I232T and G575V (SpoT)
3	32->256	A28V
4	1-256	S17R

Table 1: Overview of isogenic A. baumannii *isolate pairs showing MIC change and PmrB/SpoT differences*

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

156/PRP
A novel antimicrobial coating inhibits biofilm formation in MRSA
A. Vaishampayan*[1], A. de Jong[1,2], J. Kok[1,2,3], C. Meyer[1,2,3], U. Landau[1,2,3,4], E. Grohmann[1,2,3,4]
[1]*Beuth Hochschule fuer Technik Berlin, Microbiology, Berlin, Germany*
[2]*University, Department of Molecular Genetics, Groningen, Netherlands*
[3]*Largentec GmbH, Berlin, Germany*
[4]*Beuth Hochschule fuer Technik Berlin, Department of Microbiology, Berlin, Germany*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known for its multiple drug resistance and its ability to form robust biofilms on medical devices. Multiple drug resistance combined with a thick biofilm makes the treatment and eradication of infections of this pathogen even more difficult. This calls for the development of novel antimicrobials, which could also be potential biofilm inhibitors. We tested the influence of the novel antimicrobial surface coating AGXX® on the transcriptome of the clinical MRSA isolate, *S. aureus* 04-02981, at different time intervals, using RNA sequencing (RNA seq). The differentially expressed genes were analyzed via the RNA seq analysis pipeline T-REx, focusing on known biofilm, and virulence-associated genes in *S. aureus*. In the biofilm screening assay, a significant reduction in biofilm formation in *S. aureus* 04-02981 was observed in presence of AGXX®. RNA seq data showed that many biofilm and virulence-associated genes of *S. aureus* were down-regulated in presence of AGXX®. The gene expression trends observed in RNA seq studies were confirmed via RT-qPCR. AGXX® also down-regulated the two-component, quorum-sensing system *agr*, surface adhesin *sdrC*, *cap5A*-a gene mediating the synthesis of capsular polysaccharide, suggesting that AGXX® might inhibit biofilm formation by interfering with quorum-sensing and by repressing genes associated with surface adhesins, and capsular polysaccharide. Our data manifest AGXX® to be an effective antibacterial and a biofilm inhibitor for *S. aureus*.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

157/PRP
***In-vitro* activity of sodium bituminosulfonate against staphylococci**
E. A. Idelevich*[1], K. Becker[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction: The sodium salt of bituminosulfonate is an active ingredient derived from sulfur-rich oil shale. It has been used since the 1930s to treat skin diseases including infectious conditions. Data on the antimicrobial activity of this agent are limited.
Objectives: This study aimed to investigate *in-vitro* activity of sodium bituminosulfonate against different staphylococcal species.

Material and Methods: The *in-vitro* activity of sodium bituminosulfonate (ICHTHYOL® light, ICHTHYOL-Gesellschaft, Hamburg) was investigated against 48 clinical staphylococcal isolates. The collection included consecutively collected 24 *S. aureus* isolates, comprising 12 methicillin-resistant *S. aureus* (MRSA) and 12 methicillin-susceptible *S. aureus* (MSSA), as well as 24 isolates of coagulase-negative staphylococci (CoNS): 12 *S. epidermidis*, 2 *S. hominis*, 2 *S. haemolyticus*, 2 *S. capitis*, 2 *S. saprophyticus*, 2 *S. auricularis*, and 2 *S. lugdunensis* isolates. Only one isolate per patient was eligible. Antimicrobial susceptibility testing (AST) was performed by broth microdilution method according to CLSI guidelines. Bacterial suspensions were prepared in cation-adjusted Mueller-Hinton broth producing final bacterial concentration of approximately 5 x 10^5 cfu/ml confirmed by colony counting of serial dilutions. Sodium bituminosulfonate was tested in double dilution concentration steps ranging from 0.03 µg/ml to 256 µg/ml. The microtiter plates were incubated at 36°C and read visually after 18±2 hours. Additionally, quality control (QC) strain *S. aureus* ATCC 29213 was tested at each testing day.
Results: Minimum inhibitory concentration $(MIC)_{50}$, MIC_{90}, and MIC range were 0.25 µg/ml, 0.25 µg/ml and 0.125-0.25 µg/ml for MRSA, and 0.25 µg/ml, 1 µg/ml and 0.06-2 µg/ml for MSSA, respectively. MIC_{50}, MIC_{90}, and MIC range for CoNS were 1 µg/ml, 16 µg/ml and 0.03-32 µg/ml, respectively. MIC of *S. aureus* ATCC 29213 was 0.06 µg/ml or 0.125 µg/ml throughout the testing, which may be used for establishing of tentative QC ranges for AST of sodium bituminosulfonate.
Conclusion: Sodium bituminosulfonate possesses high *in-vitro* activity against staphylococci, particularly *S. aureus*, including both MRSA and MSSA.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

158/PRP

Biochemical characterization of NDM-18, a novel subclass B1 metallo-β-lactamase from a clinical *Escherichia coli* isolate

J. Schauer*[1], N. Pfennigwerth[1], S. G. Gatermann[1]
[1]*Ruhr-University Bochum, Department for Medical Microbiology, Bochum, Germany*

Question: To date, bacteria of the *Enterobacteriaceae* family are the most prevalent source of hospital-acquired infections worldwide. Especially the carbapenemase producing members have emerged as a serious problem for public health. In the last years the amount of novel carbapenemase variants has increased drastically and even single amino acid substitutions in the gene can lead to a significantly altered substrate spectrum. Therefore, it is necessary to characterize these novel variants on a molecular basis. Here we report the biochemical characterization of NDM-18, a novel variant of the Ambler subclass B1 carbapenemase NDM-1. The aim of this project was to characterize the novel NDM-1 variant NDM-18 to investigate the impact of the amino acid deviations on its substrate spectrum and hydrolytic activity. Additionally, we aimed to identify the localization and genetic environment of the carbapenemase gene to gain insights into previous and future mobilization processes.
Methods: Carbapenemase detection was performed by modified Hodge test, combined disk test with boronic acid or EDTA and verification of carbapenemase genes by PCR. The NDM-18 and the NDM-1 encoding genes were cloned into the pBK-CMV vector and the thus-obtained recombinant plasmids pMB3069 and pMB3068 were transformed into *Escherichia coli* TOP10. Susceptibility studies were performed by microdilution and disk diffusion. The purification of both enzymes was performed by Fast Protein Liquid Chromatography (FPLC) and their catalytic behavior was analyzed and compared by *in vitro* hydrolysis assays through photometrical measurements.
Results: *Escherichia coli* strain NRZ-30964 was isolated in 2016 from urine of an inpatient in a German hospital and showed resistance to carbapenems. The modified Hodge test was positive and synergy with EDTA could be detected. PCR and sanger sequencing confirmed the presence of a variant of the *bla*NDM-1 gene, nominated as *bla*NDM-18 by the National Center of Biotechnology Information (NCBI). It shares 98 % amino acid identity with NDM-1 and yields a five amino acid long insertion at position 49 of its amino acid sequence. No essential domains like the signal peptide cleavage site or the characteristic zinc binding site are affected by this insertion. Biochemical characterization of NDM-18 revealed that despite the insertion of five amino acids NDM-18 forms a functional carbapenemase which mediates resistance to almost all β-lactams except monobactams. However, NDM-18 showed slightly lower hydrolysis rates for carbapenems than NDM-1.
Conclusions: The biochemical characterization of NDM-18 showed that differences in the amino acid sequence can lead to altered catalytic activities of enzymes belonging to the same family. This again may, in some cases change the therapeutic options for patients and therefore makes the characterization of novel carbapenemase variants indispensable.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

159/PRP

Viral predators against multidrug resistant *Acinetobacter baumannii*: genomic insights and *in vitro* lytic activity of newly isolated bacteriophages

M. Jansen*[1], A. Wahida[1], H. Häfner[1], K. Ritter[1], H. P. Horz[1]
[1]*RWTH Aachen University Hospital, Institute of Medical Microbiology, Aachen, Germany*

Background: *Acinetobacter baumannii* is one of the notorious "ESKAPE" pathogens, which cause inexorable nosocomial infections. Given the increasing number of multi-drug resistant clinical isolates, bacteriophages (phages) could be used as an alternative or complementation to antibiotics.
Objectives: The objective of this study was to isolate new phages from environmental samples and to test their lytic capability against multidrug resistant clinical isolates of the *A. baumannii*-complex.
Methods: Phages were isolated via the Double-Layer Plaque Assay, based on 4MRGN clinical isolates as host. For the determination of host range, the identity and distinctiveness of 36 bacterial isolates was verified by MALDI-TOF mass spectrometry and ERIC-PCR based genomic fingerprinting, along with antibiotic resistance profiles. Phage characterization included transmission electron microscopy (TEM), growth/kill curves in liquid assays combined with antibiotics, as well as genome analysis using the MiSeq-sequencing platform.
Results: In total, six phages (A1 – A6) were isolated. TEM confirmed the affiliation of phages to the *Caudovirales* order and to the *Myoviridae* family. Phages A2 – A6 could infect 50% – 69% of bacteria, whereas phage A1 could lyse only one strain. Genomic analysis revealed two phages (A1 and A5) as temperate which was apparent from the presence of attachment sites and genes encoding for integrase enzymes. Conversely, the absence of those genetic elements and the higher prevalence of phage-encoded tRNA genes identified phages A3, A4, and A6 as obligate lytic phages. Lytic activity against planktonic bacterial cells was observed for four phages using multiplicities of infection (MOIs) ranging from 1 to 10^{-6}.
The most potent phages were A3 and A6, which share a high degree of genome similarity to each other, but which show a genome wide sequence identity at nucleotide level to known phages of only 77%. Both phages demonstrated a fast adsorption to their host (> 90% after 6 min and 18 min, respectively). The temperature with the strongest lytic activity was 37 °C (phages A3 and A6) and ~24 °C (phage A6), indicating potential for therapy and also for clearance of hospital surfaces. Significant synergistic effects (i.e. complete clearance of planctonic cells) were achieved with the combination of phages and meropenem. Such synergistic effects were not seen with ciprofloxacin and colistin. Hence, the concerted activity of meropenem and phage might be an option for treatment of infections caused by 4MRGN pathogens.
Conclusion: In order to combat multidrug resistant *A. baumannii*, at least two novel phages are now available as potential cure or prevention of nosocomial infections. As a next step, *in vivo*

experiments are required to verify phage efficiency and possible shortcomings under real-life conditions.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

160/PRP

Analysis of the cell wall architecture of vancomycin resistant *S. aureus* strains

M. Hort*[1], U. Bertsche[2], G. Bierbaum[1]
[1]*University Hospital Bonn, Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany*
[2]*University of Tübingen, Microbial Genetics, Tübingen, Germany*

Staphylococcus aureus is a dominant cause of nosocomial disease around the world, leading to higher rates of treatment failure, persistent infection and mortality. Especially the elevated rates of MRSA, which comprise up to 25 % of all *S. aureus* isolates in Germany [1], are of concern for health care professionals. The drug of choice against MRSA is vancomycin. Although rare in hospital settings, the number of vancomycin intermediate resistant (VISA) strains rose significantly in recent years [2]. Here we focus on the biochemical alterations in the cell wall of a laboratory and a clinical VISA strain to elucidate mechanisms for vancomycin resistance. *S aureus* VC40 is a highly resistant VISA strain (MIC 64 µg/mL), generated by serial passage of *S. aureus* RN4220ΔmutS in the presence of vancomycin [3]. Additionally a clinical isolate *S. aureus* 137/93A (MIC 8 µg/mL) was analyzed. Both strains have a thickened cell wall, a characteristic for most VISA strains, and a significantly lower peptidoglycan crosslinking than the respective controls determined by UPLC-MS. The decreased percentage of crosslinking leads to an increase in false target sites for vancomycin. The strain VC40 also has a diminished negative cell wall charge measured by the cytochrome C assay. A less negatively charged cell surface decreases the first interaction of the positively charged vancomycin molecule with the cell envelope. Interestingly, *S. aureus* VC40 showed upregulation of multiple autolysins in qRT-PCR, because of a mutation in the regulatory gene *walK*, that hinders its autophosphorylation [4]. Autolysins degrade the cell wall and are important for cell division. Their activity is inhibited by teichoic acids and therefore the enzymes hydrolyze the cell wall septa which are teichoic acid free. In order to test the influence of teichoic acids on vancomycin resistance, a MIC assay in the presence of subinhibitory concentrations of tunicamycin was performed. So far, the inhibition of teichoic acid biosynthesis had no effect on vancomycin resistance in the strain. In conclusion, these results confirm resistance mechanisms of VISA strains towards vancomycin and show the similarities and differences between a laboratory mutant and a clinical isolate.

References

[1] RKI 2016 Epidemiologisches Bulletin 22:191-6
[2] Zhang et al. 2015 PLoS One 10(8): e0136082
[3] Schaaff et al. 2002 Antimicrob Agents Chemother. 46(11):3540-8
[4] Berscheid et al. 2014 J Antimicrob Chemother. 69(12):3190-8

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

161/PRP

Identification of new natural products with anti-microbial activity against apicomplexa and multiresistant gram-negative rods (4MRGN)

S. Shaneh Sazzadeh*[1]
[1]*Heinrich-Heine Universität, Institute of Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany*
Shabnam Shaneh Sazzadeh[1], Sarah Schmidt[1], Karin Buchholz[1], Peter Proksch[2], Klaus Pfeffer[1]
[1]*Institute of Medical Microbiology and Hospital Hygiene,* [2]*Institute of Pharmaceutical Biology and Biotechnology, Heinrich Heine University Düsseldorf, Germany*

Introduction: The launch of anti-microbial therapies has led to a decisive breakthrough in the treatment and chemo-prophylaxis of infectious diseases. Furthermore, under protection of anti-infectives, invasive and cytostatic therapies (i.e. bone marrow transplantation, tumor therapies) were enabled which, without efficient control of infections, would lead to death of immunocompromised patients. However, the recent occurrence of (multi-) resistant pathogens leads to an increase in lethality and morbidity of infected patients. Apicomplexa such as Plasmodia also develop resistance against common treatments even to artemisinine or cannot be eradicated after infection. Therefore the need for new anti-microbial drugs is urgent.
Aims: The primary aim of this project will be the identification of novel natural products (from marine sponges and endophytes) with anti-microbial activities against *Toxoplasma gondii* and multidrug resistant gram-negative rod-shaped bacteria (4MRGN), and the elucidation of their targets in pathogens in order to develop new leads for anti-microbial therapies.
Methods: To reach this aim, the first step is to identify the products, which are able to inhibit *T. gondii* proliferation without being cytotoxic against HFF (human foreskin fibroblasts). This is accomplished via Toxoplasma proliferation and MTT assays. To identify anti-4MRGN products microdilution assays are performed.
Results: Within a first round of screening, promising candidates could be detected. A total of 240 new natural products and 50 derivatives thereof were analyzed against *T.gondii* (type II, ME49 strain). Some of them have demonstrated anti-Toxoplasma activity. Furthermore, we are performing the same experiments for the virulent *T.gondii* (type I, BK strain).
Summary: This project will introduce new anti-microbial products for novel therapies of multiresistant pathogens.
Funding: GRK 2158, DFG

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

162/PRP

Fifteen years of antimicrobial susceptibility surveillance of invasive meningococcal isolates in Germany

H. Claus[1], T. Lâm[1], U. Vogel*[1]
[1]*Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction: Continuous surveillance of antimicrobial susceptibility of invasive meningococcal isolates is a major task for national reference laboratories (NRLs). From 2002 to 2016, susceptibility testing of penicillin, ciprofloxacin, and rifampicin was conducted on almost 4,500 meningococcal isolates submitted to the German NRL. Since 2010, susceptibility towards cefotaxime was tested in addition. Reduced susceptibility towards penicillin is related to mutations in the penicillin binding protein 2 (PBP2) encoded by penA.
Aims: To monitor the development of resistance towards antibiotics over time.
To correlate intermediate penicillin-susceptible phenotypes with PBP2 polymorphisms.
To correlate intermediate penicillin-susceptibility with serogroups and clonal complexes.
Material and Methods: Meningococcal strains isolated from blood and CSF were finetyped (serogroup:PorA-variable region (VR)1, PorA-VR2:FetA-VR) and analysed by Multilocus sequence typing (MLST).

Gradient diffusion antimicrobial susceptibility testing was done with E-Test® strips (BioMérieux) on Mueller-Hinton agar supplemented with sheep blood. Interpretation of minimal inhibitory concentrations (MIC) was applied according to the EUCAST breakpoint table version 7.1.
PenA alleles of strains with penicillin MICs above 0.06 µg/ml were assigned at pubmlst.org/neisseria/ sited at the University of Oxford.
Results: From 2002 to 2016, 82.2%, 99.7% and 99.7% of all isolates were susceptible to penicillin, ciprofloxacin, and rifampicin, respectively. Furthermore, all of the isolates tested with cefotaxime (n=1,560) were susceptible. Over time, the rate of penicillin-susceptible isolates varied between 61% and 89%.
An intermediate genotype based on five mutations in the transpeptidase region of PBP2 was identified in only a minor proportion of isolates with MICs of 0.094 and 0.125 µg/ml (7 and 35%, respectively). From 2002 to 2010, appr. 5% of all isolates showed an intermediate PBP2 genotype. In the following years, this value increased up to 14%.
Only 40% of the MenW strains were susceptible to penicillin. Reduced susceptibility of MenW isolates was mostly found among ST-22 complex isolates. Isolates of the predominant clonal complexes ST-11 (MenC), ST-32 (MenB) and ST-41/44 (MenB) were 73%, 83% and 91% penicillin susceptible, respectively.
We will report in addition the development of MICs over time and resistance towards rifampicin and ciprofloxacin.
Conclusions: Antimicrobial resistant isolates are rare within the German meningococcal population. The increasing trend of isolates with intermediate resistance towards penicillin needs to be monitored. Nevertheless, this trend may partially be related to expansions of certain clonal complexes with higher MICs.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

163/PRP
Prevalence of Fidaxomicin and Rifaximin Resistance in Clinical Isolates of *C. difficile*
J. Schwanbeck*[1], F. Laukien[1], I. Oehmig[1], O. Zimmermann[1], U. Groß[1], A. E. Zautner[1], W. Bohne[1]
[1]*Universitätsmedizin Göttingen, Institut für Medizinische Mikrobiologie, Göttingen, Germany*

Introduction: *Clostridioides difficile* is an increasing problem in modern healthcare environments. The two antibiotics Rifaximin (RFX) and Fidaxomicin (FDX) are recently approved drugs for *C. difficile* therapy, which both target the RNA polymerase. As stated by Babakhani *et al*.[1], resistance development to Fidaxomicin is uncommon in clinical isolates and usually associated with an impaired fitness burden.
Aims: The aim of the study was to ascertain the prevalence of RFX and FDX resistance in *C. difficile* isolates obtained from patients treated with these antibiotics before. Furthermore, the underlying resistance mechanism is supposed to be determined.
Materials and Methods: The agar dilution method was used to determine susceptibility towards RFX and FDX. The *rpoB* and/or *rpoC* locus of resistant mutants was sequenced to identify putative point mutations.
Results: Out of 138 clinical *C. difficile* isolates from 98 patients, 14 isolates (from 12 patients) were resistant towards RFX (MIC >32 µg/ml). Two isolates were resistant against FDX (MIC >64 µg/ml), which were obtained from the same patient. Susceptible isolates displayed MICs between 8-500 ng/ml. The resistance towards RFX was conferred in all cases by a R505K point mutation in *rpoB*. In addition, 10 isolates also possessed a H502N mutation and of those, 6 possessed an additional I750M mutation. The FDX resistance was conferred by a V1143D mutation in *rpoB* in both resistant isolates. The growth profile of the two FDX resistant isolates was accessed in comparison to two lab strains and to further clinical isolates and found to be in a comparable range (doubling time: *C. difficile* 630 Δ*erm:* 84 min; R20291: 74 min; FDX-res isolate A: 82 min, FDX-res isolate B: 65 min).
Conclusion: While it has been stated that a mutation conferring resistance towards FDX is very unlikely in clinical isolates, we found two resistant isolates (from a single patient) in a total of 139 tested isolates. The MICs found in these isolates are considerably higher than those previously described from other FDX-resistant clinical isolates. In contrast to previous reports, the FDX-resistant isolates do not display a growth disadvantage, suggesting that FDX resistance appears not to be associated with a considerable fitness burden.

Reference
[1] Babakhani, F., Seddon, J., & Sears, P. (2014). Comparative Microbiological Studies of Transcription Inhibitors Fidaxomicin and the Rifamycins in *Clostridium difficile*. Antimicrobial Agents and Chemotherapy, 58(5), 2934–2937.

Acknowledgment
This work was funded by the Federal State of Lower Saxony, Niedersächsisches Vorab (VWZN2889).

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

164/PRP
Resistance-plasmids and their relevance for the transfer of antimicrobial resistance determinants in *Klebsiella*
A. Irrgang*[1], G. Carl[1], A. Käsbohrer[1,2], C. Jäckel[1], M. Grobbel[1], B. A. Tenhagen[1], J. A. Hammerl[1]
[1]*German Federal Institute for Risk Asessment, Biological Safety, Berlin, Germany*
[2]*Institute for Veterinary Public Health, University of Veterinary Medicine, Wien, Austria*

Klebsiella spp. are Gram-negative opportunistic pathogens prevalent on plants, in water and soil but also colonizing a wide range of livestock/wildlife animals. Klebsiellae were recognized as an important threat to global public health due to their high level of antimicrobial resistance, mainly associated with the presence of mobile genetic elements.
In this study, resistance-plasmids of *Klebsiella* spp. isolates from livestock/wildlife were investigated to elucidate their potential for the spread of antimicrobial resistance.
Antimicrobial susceptibility testing of *Klebsiella* spp. isolates was performed using broth microdilution following CLSI guidelines and EUCAST epidemiological cut-off values. S1-PFGE, whole genome sequencing and bioinformatics were performed to reveal the genetic basis of the observed resistance and the composition of the plasmids. The transferability of the plasmids was characterized by filter-mating studies.
Molecular analysis of two multidrug-resistant *Klebsiella pneumoniae* strains revealed that the individual isolates carry one and four plasmids, respectively. These can be efficiently transferred from *Klebsiella pneumoniae* to *E. coli* and other genera of the *Enterobacteriaceae* by filter-mating studies. The plasmids differ significantly in size and genomic composition. The genetic background of the antimicrobial resistances and the transfer regions on the plasmids will be presented in detail.
Our study underlines the high mobility and transferability of resistance genes from animal derived *Klebsiella pneumoniae*, pointing at the potential transfer of these genes to human pathogens.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

165/PRP
Isolation of multi-resistant bacteria from class 1 integron hotspots in household environments
R. Lucassen*[1], D. Bockmühl[1]
[1]*Hochschule Rhein Waal, Faculty of Life Sciences, Kleve, Germany*

Introduction: Class 1 integrons have been the most commonly reported among clinical bacteria [1]. The relative abundance (RA) of class 1 integron integrase gene (intl1) was reported to be a good marker for anthropogenic pollution [2]. It was shown that detergents and biocides such as quaternary ammonium compounds (QAC) can co-select for antibiotic resistance [3, 4]. Since cleaning and personal care products are omnipresent we hypothesized

whether we find intl1 hotspots in household environments and whether bacterial isolates exhibit resistance to QACs and antibiotics.

Methods: Areas such as shower drains (SD) and toothbrushes (TB) were probed. Sewage sludge and rain water drain samples served as pos. and neg. controls. DNA was extracted and qPCR of intl1 and 16s ribosomal DNA (16srDNA) was performed, followed by determination of RA of intl1 (RA = copies/mL intl1: copies/mL 16srDNA). Bacteria were isolated and species, antibiotic profiles as well as minimal inhibiting concentration (MIC) of Benzalkoniumchloride (BKC) were determined.

Results: Intl1 was found in all sample areas (Figure1). Mean RA values of intl1 were highest in SD (32.29) and on TB (18.08). Although all sample areas exhibit a high mean RA compared to the neg. control (RA=0.77), Kruskal-Wallis test revealed that differences in mean RA values are not significant. This is caused by high CV% (coefficient of variation) values of samples of the same sample area. However, isolates of samples with high and low RA values of the same sample area show significant differences in MIC values of BKC and antibiotic resistance (Table 1). Noteworthy, only from sample areas with high RA values, biocide and antibiotic resistant bacteria could be isolated. Resistance to antibiotics corresponds well with high MIC values to BKC.

Conclusion: Household environments exposed to cleaning/disinfecting and personal care products show increased relative abundance (RA) of bacteria carrying class 1 integrons and isolates of sample areas with high RA values exhibit high MIC values to BKC and resistance to aminoglycosides and metallo-ß-lactamases. The occurrence of multi-drug resistant bacteria correlates well with MIC values of BKC ≥500 µg/mL.

References

[1] Deng et al. Resistance integrons: class 1, 2 and 3 Integrons. Ann Clin Microbiol Antimicrob (2015) 14:45.

[2] Gillings MR etal. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution The ISME Journal (2015) 9, 1269–1279.

[3] Gaze WH et al. Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment The ISME Journal (2011) 5, 1253–1261.

[4] Gaze WH et al. Incidence of Class 1 Integrons in a Quaternary Ammonium
Compound-Polluted Environment. Antimicrob Agents Chemother. 2005 May; 49(5): 1802–1807.

Figure 1

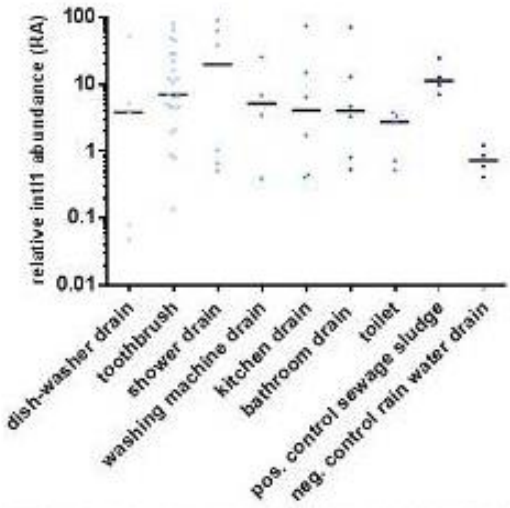


Figure1: Relative abundance of class 1 integrons in household environments

Figure 2

Strain	Sample point	Benzalkoniumchloride MIC (µg/mL)	resistance to aminoglycosides	resistance to metallo-ß-lactamases
Escherischia coli	toothbrush RA= 12.12	31,25	-	-
Pseudomonas aeruginosa	toothbrush RA= 4.40	31,25	-	-
Pseudomonas fluorescens	shower drain RA= 0.50	31,25	-	-
Staphylococcus aureus	bathroom drain RA= 4.74	62,5	-	-
Acinetobacter baumanii	toothbrush RA= 80.06	250	+	+
*Klebsiella pneumonia**	toothbrush RA= 65.79	500	+	+
Klebsiella pneumoniae	shower drain RA= 89.80	250	+	+
*Pseudomonas aeruginosa**	bathroom drain RA= 70.87	1000	+	+

*multi-drug resistant in ViTek 2 system

Table1: Comparison of bacterial isolates exhibiting high and low RA values by means of resistance to Benzalkoniumchloride and antibiotics

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

166/PRP

New method for the rapid identification and classification of multidrug-resistant *Enterobacteriaceae*

V. van Almsick*[1], B. Ghebremedhin[1], N. Pfennigwerth[2], P. Ahmad-Nejad[1]

[1]HELIOS Universitätsklinikum Wuppertal, Universität Witten/Herdecke, Institut für medizinische Labordiagnostik, Wuppertal, Germany

[2]Ruhr Universität Bochum, Department für medizinische Mikrobiologie, Bochum, Germany

Objective: Accurate detection of multidrug-resistant (MDR) *Enterobacteriaceae* constitutes a major healthcare problem and a laboratory diagnostic challenge. Aim of the present study was to develop a rapid phenotypic assay for the detection of MDR *Enterobacteriaceae*.

Method: We developed a bioluminescence-based assay to detect ATP levels which correlate proportionally with the number of bacteria. Bioluminescence was generated by BacTiter-Glo(R) microbial viability assay. ATP levels were measured after incubation of the bacteria with 1mL AST broth with and without antibiotics (ciprofloxacin, ceftazidime/cefotaxime, meropenem and piperacillin). Light emission (measured in relative light units (RLU)) was detected after 2.5 hours of incubation at 37°C by the GloMax(R) Integrated Luminescence System (Promega) and relative induction was calculated. The BD Phoenix™ was used as reference method for antibiotic susceptibility testing. For the evaluation of the ATP assay, inter alia, 60 genotyped carbapenemase-producing isolates were used. The investigated study population consisted of 185 *Enterobacteriaceae* isolates with different phenotypic resistance patterns. Results: 180 out of 185 (97%) investigated isolates were correctly identified by our ATP assay. All of the antibiotic susceptibility tests (AST) performed with ciprofloxacin and ceftazidime/cefotaxime were in accordance with the Phoenix™ AST results. In two cases the results for meropenem by the ATP assay were incorrect (2/88; 2%), once false positive and false negative. However, the incubation period for piperacillin susceptibility was extended to 4h to gain sensitivity and specificity. The results of the ATP assay for 94 out of 97 isolates were in accordance with those of PhoenixTM AST. We also performed our assay for positive blood cultures testing meropenem susceptibility and revealed 100% concordance to the PhoenixTM AST results (n=20).

Conclusion: Our bioluminescence-based ATP assay enabled accurate and rapid detection of the most common antibiotic resistances in *Enterobacteriaceae* within 2.5h and is an additional option for microbiological laboratories to identify MDR in *Enterobacteriaceae* within few hours.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

167/PRP

Diversity of *mcr-5* harbouring plasmids in German *Escherichia coli* isolates from livestock

J. A. Hammerl[1], M. Borowiak[1], S. Schmoger[1], D. Shamoun*[1], M. Grobbel[1], B. Malorny[1], B. A. Tenhagen[1], A. Irrgang*[1], A. Käsbohrer[1,2]
[1]*German Federal Institute for Risk Asessment, Biological Safety, Berlin, Germany*
[2]*Institute for Veterinary Public Health, University of Veterinary Medicine, Wien, Austria*

Colistin is considered as highest priority critically important antibiotic used to treat severe human infections caused by multidrug-resistant and carbapenemase-producing Gram-negative bacteria. Recently, Borowiak et al. reported on a novel mobilizable colistin resistance-gene, *mcr-5*, in *d*-tartrate fermenting *Salmonella enterica* serovar Paratyphi B. In this study, the diversity of *mcr-5* harbouring plasmids in *Escherichia coli* isolates of the German national monitoring programme for antimicrobial resistance in zoonotic agents from the food chain was investigated.

Antimicrobial resistance testing was performed following 2013/652/EU, using the microdilution method according to CLSI guidelines and EUCAST epidemiological cut-off values. S1-PFGE, Illumina MiSeq sequencing and bioinformatical analysis were performed to determine the genetic background of *mcr-5* harbouring plasmids.

Our results showed that the majority of the German *mcr-5* harbouring plasmids from *E. coli* are closely related to the ColE-plasmid pSE13-SA01718 of *S.* Paratyphi B (*d*Ta+). However, they often differ in size and their genetic composition but exhibit a highly conserved *mcr-5* transposon, which has been previously described in *S.* Paratyphi B (*d*Ta+) and *Cupriviadus giliardi*. Interestingly, some plasmids exhibit stronger similarities to plasmids of different incompatibility groups.

Our findings indicate that *mcr-5*-harbouring *E. coli* isolates carry different plasmid prototypes harbouring *mcr-5* on a highly conserved transposon. However, further information on the stability of *mcr-5* harbouring genetic elements, their transmission routes as well as their distribution in livestock, food products and humans were needed to assess the impact of this resistance determinant for public health.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

168/PRP

Studies on the effectiveness of antimicrobial coated surfaces in a medical practice

S. Buhl*[1], C. Bulitta[1], R. Brückner[2], S. Käs[1]
[1]*Ostbayerische Technische Hochschule Amberg-Weiden, Medizintechnik, Weiden in der Oberpfalz, Germany*
[2]*Health Complete Solutions GmbH, Bamberg, Germany*

Introduction: Efficient and effective cleaning and disinfecting (C&D) measures are an indispensable factor in the prevention of treatment-associated infections. The increase of multi-resistant pathogens (MRE) is a major risk for patients and staff. However, there are few scientific papers so far that have systematically examined and evaluated the effectiveness of cleaning and disinfection protocols in outpatient clinics. [1-2]

Goals: In this work, the infection control measures carried out by the medical personnel and the cleaning staff of an orthopedic outpatient clinic was investigated with regard to the reduction of microbiological contamination on surfaces. In a second step, the effect of an antimicrobial coating (TiTANO®, HECOSOL) on the contamination of the surfaces was assessed. The Titano® used in the experiments is applied to the surfaces by an electrospray method.

Material and Methods: The effectiveness of the current C&D-protocols was investigated by standard microbiological procedures. In order to achieve this, samples were taken at critical surfaces before and after the daily cleaning routine and culture media were incubated for 72 hours at 35°C. Colony forming units (cfu) were counted and documented. In order to compare the results subsequently, this was done with unchanged cleaning and disinfecting measures during one week before and one week after the antimicrobial coating of the medical practice.

Results: The partly high cfu-counts in the samplings indicate that there is need for action and training in cleaning and disinfection measures in the examined outpatient clinic. Although no multi-resistant gram negative pathogens (MRGN) had been detected, it should be discussed if cleaning and disinfection procedures should be tested regularly in outpatient clinics in order to prevent harm for the patient by suboptimal infection control measures. Despite the existing problems in C&D, however, a reduction of the microbial burden could be achieved after application of the antimicrobial surface technology.

Conclusion: Due to the unchanged general conditions and identical C&D procedure, this can at least partly be attributed to the antimicrobial coating. It is also clear that the TiTANO® coating alone is not sufficient and an optimal result can only be achieved through the interplay of professional cleaning and disinfection. Based on our results, the antimicrobial coating of surfaces in outpatient clinics shows the potential to provide additional safety for patients and staff. Further studies have to be done to systematically assess the potential for this technology in practice.

References

[1] Kramer A, et al.; How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis. 2006;6:130. [PMID: 16914034]

[2] Bures S, et al.; Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit. Am J Infect Control 2000;28:465–471. [PubMed: 11114617]

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

169/PRP

Prevalence of multidrug-resistant organisms in refugee patients admitted to a German University Hospital according to their reported time since arrival in Germany

C. Reinheimer*[1], P. Abdollahi[1], T. A. Wichelhaus[1], M. Hogardt[1], K. Zacharowski[2], P. Meybohm[2], H. Mutlak[2], V. A. J. Kempf[1]
[1]*University Hospital Frankfurt, Inst. for Medical Microbiology and Infection Control, Frankfurt am Main, Germany*
[2]*University Hospital Frankfurt, Department of Anesthesiology, Intensive Care Medicine, and Pain Therapy, Frankfurt am Main, Germany*

Background and Aim: Refugees have a significant risk to carry multidrug-resistant organisms (MDRO), *i.e.* multidrug-resistant gram-negative organisms (MDRGN) and methicillin-resistant *Staphylococcus aureus* (MRSA). We hypothesized that MDRO prevalence in refugees is gradually declining to MDRO prevalence in patients without refugee history over reported time since refugees" arrival in Germany. The knowledge of dynamics in MDRO prevalence in refugees might contribute to provide appropriate infection control suggestions for refugees in German hospitals.

Material and Methods: Retrospectively, MDRO prevalence of 109 refugees admitted to the University Hospital Frankfurt, Germany and of 819 control critical ill patients admitted to intensive care unit between June 2016 and May 2017 was studied. Refugees" countries of origin and reported time since arrival were obtained by using records in the digital patient data files.

Results: 41.3% (95% confidence interval = 31.9 – 51.1) refugees and 5.7% (4.2 – 7.6) controls were positive for at least one MDRGN, respectively. Highest MDRGN prevalence was found in refugees having recently arrived (≤3months) in Germany (72.4%; 52.8-87.3). Refugees" MDRGN prevalence was continuously declining over time, reaching the controls" MDRGN prevalence most likely 18 months after their arrival with 15.4% (1.9 – 45.4) vs. 5.7% (4.2 – 7.6; p=0.14), respectively. For MRSA, no comparable dynamics were observed.

Conclusion: This is the first study demonstrating that refugees" MDRGN prevalence is declining over time since their arrival in Germany. 18 months after their arrival, refugees" and locals" MDRGN prevalence does not significantly differ anymore.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

170/PRP

Towards control of health care associated hepatitis B: Epidemiology and immunization coverage in Southwest Germany, 2015 to October 2017

G. Pfaff[1], C. Wagner-Wiening*[1], N. Ouédraogo[1]
[1]*Landesgesundheitsamt Baden-Württemberg, Gesundheitsschutz und Epidemiologie, Stuttgart, Germany*

Question: The viral hepatitis pandemic is responsible for an estimated 1.4 million deaths per year from acute infection and hepatitis-related liver cancer and cirrhosis. Of those deaths, approximately 47% are attributable to hepatitis B virus (1). We reviewed incidence and immunization coverage in order to assess progress towards control of health care associated hepatitis B and the 80% three-dose immunization coverage target set by WHO for 2018 in Baden-Wuerttemberg, Southwest Germany.
Methods and Materials: We defined active hepatitis B cases as persons reported to the mandatory surveillance between January 2015 and October 2017 with detection of HBV DNA or HBs-Antigen; additional HBs-IgM as acute HBV. We described cases by age, sex, symptoms, suspected route of transmission and HDV coinfection. Data on vaccination rates were extracted from annual school entry health examinations.
Results: Since 2015, 802 cases (median age 33, range seven months to 94 years; 69% male) of active HBV were notified; 152 cases in refugees (median age: 17); six cases of HDV coinfection. HBcIgM was detected in 113 cases (14%) (median age 44, range 10 - 88; 77% male). Nine cases (8%) were travel-related. 10 cases (9%) of acute HBV infections were associated with medical interventions. Coverage with three doses hepatitis B vaccine was above 80% since 2007, and was 88-89% in 4-5-year old children examined in 2009-2016. In 2016, about 49% of children in Waldorf kindergartens and 10% in other settings remained unvaccinated for hepatitis B.
Conclusions: Health care associated cases of hepatitis B compose less than 10% of cases notified in Southwest Germany. Hepatitis B immunization of children and youth has reached the 80% three-dose coverage target set by WHO for 2018 but the 95% target set for 2021 will require further intense action. Adolescents and young adults entering health care professions require continuing attention of employers and occupational health physicians in order to close immunization gaps and ensure individual protection against hepatitis B.

Reference

[1] World Health Organization. Global health sector strategy on viral hepatitis 2016-2021. Towards ending viral hepatitis. WHO/HIV/2016.06. Geneva 2016.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

171/PRP

Temporal changes of the composition of vancomycin-resistant *Enterococcus faecium* sequence types in inpatients of a German tertiary care hospital

M. Post[1], G. Geginat[1], K. Bauer[1], I. Tammer[1], D. Schlüter[1], J. Färber*[1]
[1]*Institut für medizinische Mikrobiologie und Krankenhaushygiene, Universitätsklinikum Magdeburg, Magdeburg, Germany*

Question: We describe the molecular epidemiology of vancomycin-resistant *Enterococcus faecium* (VRE) isolated from patients of a German tertiary care hospital over a period of three years. The study was triggered by a sharp increase of the frequency of VRE compared to wild-type *E. faecium* during the 4th quarter 2013. Despite implementation of infection control measures that included strict barrier precautions, risk adjusted VRE screening and improved environmental cleaning the frequency of VRE remained elevated. Therefore extensive typing of previously collected VRE isolates was performed in order to enable a more precise analysis of the spread of VRE among hospital inpatients.
Methods: A total of 276 VRE isolates carrying either the vanA or vanB genes were typed using multilocus sequence typing (MLST). The isolates were collected from screening samples (rectal swabs, n = 133) and from clinical specimens (n = 143) over a period of three years from 2013 to 2015. The eBURST algorithm was used to identify groups of related genotypes and the founding genotype.
Results: Overall 20 different sequence types (ST) were detected that all belonged to the clonal complex CC17. Over the whole study period the dominant STs were: ST117 (59.4%, n = 164), ST203 (9.8%, n = 27) ST17 (8.0%, n = 22), ST192 (5.4%, n = 15) and ST80 (4.3%, n = 12).
The frequency of VRE in relation to *E. faecium* sharply increased during the 4th quarter 2013 from less than 5% to 19% in 2014. During this initial period ST117 was the dominant sequence type (71.9 %). After implementation of infection control measures against VRE the frequency of ST117 decreased to 48.6 %, however, at the same time other sequence types increased.
Conclusion: Typing of VRE isolates allows in many instances to exclude a possible direct transmission of VRE among inpatients. The results of the extensive sequence typing of VRE-isolates show that the implemented infection control measures successfully controlled the initial spread of ST117 although the overall rate of VRE remained elevated. The frequency of VRE remained elevated probably because ST117 as well as other VRE sequence types were repeatedly re-introduced from outside the hospital due to an overall regional increase of VRE.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Diagnostic Microbiology and Clinical Microbiology (StAG DV/FG DKM)

172/DVP

Antibacterial activity of sphingosphines, short-chain ceramides and C6-ceramide analogs against pathogenic *Neisseriae*

J. Becam*[1], S. Hebling[1], T. Walter[2], J. Schlegel[3], J. Seibel[2], M. Sauer[3], A. Schubert-Unkmeir[1]
[1]*University of Wuerzburg, Institute of Hygiene and Microbiology, Würzburg, Germany*
[2]*University of Wuerzburg, Institute for organic chemistry, Würzburg, Germany*
[3]*University of Wuerzburg, Department of biotechnology and biophysics, Würzburg, Germany*

Introduction: Certain fatty acids and sphingoid bases found at mucosal surfaces are known to have bactericidal activity and are thought to play a more direct role in innate immunity against bacterial infections. However, little is known about the exact mechanism of lipid antimicrobial activity.
Objective: Herein, we analysed the antibacterial activity of sphingolipids, including sphingosines as well as short-chain and long-chain ceramides and azido-functionalized ceramide analogs against *N. meningitidis* and *N. gonorrhoeae*.
Materials and Methods: In order to test the effect of sphingolipids on pathogenic *Neisseriae*, commercially available sphingosine, sphinganine and phytosphingosine, C_2, C_4, C_6, C_8-ceramide and C_{16}-ceramide were employed. Antimicrobial activity was determined by broth microdilution to determine minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Kinetic killing assay evaluated the rate of bactericidal effect. To visualize uptake and transport of ceramides within bacteria, azido-functionalized ceramides (α-azido-C_6-cer, ω-azido-C_6-cer, α-azido-C_{16}-cer and ω-azido-C_{16}-cer) were synthesized. Acquisition of the fluorescent click-labelled azido-functionalized ceramides was determined by flow cytometry and visualized using confocal laser scanning microscopy (CLSM) and *direct* Stochastic Optical Reconstruction Microscopy (*d*STORM). In addition, toxicity on host cells (HBMEC, HEK293T and HepG2) was evaluated by annexin V and propidium iodide staining. Diethyloxacarbocyanine iodide was used to detect bacterial membrane potential.
Results: Sphingosine, sphinganine, phytosphingosine and short-chain ceramides (C_2, C_4, C_6-cer) as well as ω-azido-C_6-cer could inhibit growth of *N. meningitidis* and *N. gonorrhoeae*. In addition,

C_8-cer and long-chain C_{16}-cer and the C_{16}-cer analogs were not active against these species. Killing kinetic assays showed bactericidal effect of ω–azido-C_6-cer occurring within 2h whereas C_6-cer had bacteriostatic effect. The killing rate of sphingosine arose within 1h. Interestingly at a bactericidal concentration, ω–azido-C_6-cer and sphingosine had no significant toxic effect on host cells. Moreover, acquisition of the fluorescent click-labelled azido-functionalized ceramides revealed a rapid uptake by bacteria within 5 min. CLSM and super-resolution fluorescence imaging by *d*STORM demonstrates homogeneous distribution of ceramide analogs in bacterial membrane. Finally, sphingosine and ω-azido-C_6-cer induced depolarization on *N. meningitidis*.
Conclusion: Our results indicate a bactericidal effect of ω-azido-C_6-cer and different sphingosines on *N. meningitidis* and *N. gonorrhoeae* at a non-toxic concentration for mammalian cells. In addition, we demonstrated that these sphingolipids induce membrane depolarization.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

173/DVP

MIC distribution and epidemiological cutoff values for biocides in *Listeria monocytogenes* isolates from food production environments

A. Rödel*[1], M. Noll[2], S. Kleta[1], S. Al Dahouk[1], R. Dieckmann[1]
[1]*German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*
[2]*University of Applied Sciences and Arts, Institute for Bioanalysis, Coburg, Germany*

Biocide usage has been implicated in the development of biocide tolerance and antibiotic cross-resistance in bacteria. Since standardized methods for the detection of reduced susceptibility to biocides do not exist and breakpoints for biocides have not been established yet, biocide resistance in bacteria remains poorly defined. In our study, we determined for the first time the epidemiological cut-off values (ECOFFs) of 93 *Listeria monocytogenes* field isolates from food production environments for selected biocides commonly used in food industries such as benzalkonium chloride (BAC), glutardialdehyde (GDA), sodium hypochlorite (NAClO), isopropanol (IPA) and peracetic acid (PAA). ECOFFs were defined on the basis of minimal inhibitory concentrations (MIC95) determined by broth microdilution. Furthermore, the minimal bactericidal concentration (MBC) was examined. Antibiotic susceptibility testing against therapeutically relevant antibiotics was performed using the commercial test system Micronaut-S Listeria (Merlin Diagnostika, Bornheim, Germany). Whole genomes of all strains were sequenced using an Illumina MiSeq platform and screened for potential factors involved in biocide tolerance. 16% of the tested *Listeria* isolates showed reduced susceptibility to BAC. Core genome (cg) MLST analysis revealed a cluster of BAC tolerant strains. These BAC tolerant isolates, like all other strains tested, were sensitive to ampicillin, penicillin, erythromycin, linezolid and trimethroprim/sulfamethoxazole and vancomycin. However, one of the biocide-tolerant isolates as well as six other field isolates and five reference strains showed resistance to meropenem. Overall, 5% of the field isolates were resistant to gentamicin, but showed no increased biocide tolerance. All isolates were resistant to daptomycin. Multiresistance against 5 antibiotics could only be detected in two field isolates and one reference strain. Spearman correlation coefficients were determined to assess the potential relationship between biocide tolerance and antibiotic resistance. Strong correlations were detected between beta-lactam antibiotics in BAC tolerant strains. Our study showed that microbicide tolerance and antibiotic resistance may occur in *L. monocytogenes* isolates from food production environments. In order to assess the extent to which the selection pressure due to the increased use of disinfectants may enhance antimicrobial resistance development, further studies are needed to elucidate the underlying tolerance and resistance mechanisms.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

174/DVP

Rapid detection of *Enterobacteriaceae* isolates phenotypically resistant against major antibiotic classes by MALDI-TOF mass spectrometry direct-on-target microdroplet growth assay

K. Sparbier*[1], E. A. Idelevich[2], K. Becker[2], M. Kostrzewa[1]
[1]*Bruker Daltonik GmbH, Bremen, Germany*
[2]*University Hospital Münster, IInstitute of Medical Microbiology, Münster, Germany*

Question: The increasing number of (multi-) drug resistant clinical microorganisms is a global threat requiring accurate and rapid detection of their resistance patterns. This is a major pre-requisite to apply a dedicated, specific therapy of affected patients and to prevent further spreading of these strains.
Here, we investigated the reliability of a novel MALDI-TOF mass spectrometry (MS) direct-on-target microdroplet growth assay technique to determine the resistance status within a few hours.
Methods: A set of 10 *Enterobacteriaceae* comprising different resistance patterns were employed to analyze the resistance phenotypes against piperacillin/tazobactam, ciprofloxacin, gentamicin, and cefotaxime. MICs were determined by broth microdilution method. For the novel approach, 6 µl microdroplets containing 5 X105 CFU/mL in cation-adjusted Mueller-Hinton broth and the respective antibiotic at breakpoint concentration (according to EUCAST) or no antibiotic, respectively, were directly spotted onto a Biotarget96 and incubated in a humidity chamber. Subsequently, the medium was removed, matrix was spotted onto the dried spots and MALDI-TOF MS was performed. Spectra were analyzed by prototype software comparing the growth in the presence of the respective antibiotic to the growth without any antibiotic agent.
Results: The MALDI-TOF MS direct-on-target microdroplet growth assay prototype results after four hours incubation were compared to the results of the microdilution method after 20 h incubation. For all strains and all antibiotics tested, complete categorical agreement was observed except one strain that was categorized primarily as falsesusceptible for ciprofloxacin by microdilution. However, repetition of this experiment revealed resistance of this strain against ciprofloxacin by both experimental methods, novel approach and microdilution. Therefore, the wrong categorization can be considered as a technical error when setting up the microdilution.
Conclusions: The study demonstrated that the novel approach possesses the potential to determine the resistance phenotype of *Enterobacteriaceae* isolates against antimicrobial agents of various antibiotic classes. The time to result could be reduced from 20 h needed for the microdilution approach down to a few hours applying the MALDI-TOF MS direct-on-target microdroplet growth assay.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

175/DVP

Rapid Identification of Methicillin-resistance in *Staphylococcus aureus* via Bioluminescence

C. Freyth*[1], P. Ahmad-Nejad[1], B. Ghebremedhin*[1]
[1]*Institut für Medizinische Labordiagnostik, Wuppertal, Germany*

Introduction: Infections caused by *Staphylococcus aureus* are life-threatening and can manifest differently: *Staphylococcus aureus* causes simple skin and mucosa colonization to wound infections, food poisoning, pneumonia and osteomyelitis and can frequently be found in blood culture vials of critically ill patients. Regarding bloodstream infections with *S. aureus*, a rapid distinction between the methicillin sensitive and the resistant type (MSSA and MRSA) is of great importance for the selection of the therapy since infections with a resistant strain are associated with significantly increased mortality.
Question: Therefore, we developed a method that enabled us to phenotypically differentiate between MSSA and MRSA. Our aim was to keep costs and time low.
Material and Methods: The assay is based on a measurement of the bacterial concentration in a nutrient solution, using

bioluminescence to determine the ATP concentration produced by the bacteria. The test can be performed from strains deriving from culture medium (n=298) as well as directly from blood cultures (n=73) within three hours (prior incubationtimes – blood cultures or culture medium - to obtain the isolates to test are excluded). As reference we used the disc diffusion method, BD PhoenixTM (Becton Dickinson, Heidelberg, Germany) and an epsilometer test (Etest).

Results: Applying this method we can achieve a sensitivity of 97% and a specificity of 98% from culture medium and the accuracy of our method is comparable to PCR or to automated detection systems like the BD Phoenix™ (Heidelberg, Germany). Concerning blood-cultures we also achieved a sensitivity of 97% and a specificity of 98%.

Conclusion: Our ATP assay may provide an easy-to-use robust low-capacity screening option. However, compared to molecular-based screening methods the duration to ATP assay result needs 3-4 hours.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

176/DVP

Antimicrobial susceptibility testing of *Brucella melitensis* - pitfalls and recommendations by an European laboratory network

A. Tscherne*[1], E. Georgi[1], D. Jacob[2], C. Hinz[1], J. Henczko[3], P. Vollmar[1], R. Grunow[2], L. Zöller[1,4], S. Zange*[1,4]
[1]*Bundeswehr Institute of Microbiology, Central Diagnostic Unit, München, Germany*
[2]*Robert Koch-Institute, Centre for Biological Threats and Special Pathogens (ZBS 2), Berlin, Germany*
[3]*National Center for Epidemiology, Budapest, Hungary*
[4]*Bundeswehr Institute of Microbiology, National Consultant Laboratory for Brucella, München, Germany*
on behalf of the EMERGE AST group

Introduction: Brucellosis is a zoonosis mainly caused by *Brucella melitensis* (Bm) which is endemic in the Mediterranean area and the Arab world. In Germany, it plays a role as an imported infectious disease, associated with high risk of chronification and relapses. Up to now, treatment failures are not associated with antimicrobial resistance and, thus, many laboratories neglect antimicrobial susceptibility testing (AST) of Bm. Nevertheless, mutations are described, e.g. in the *rpoB* leading to RIF resistance and AST guidelines for highly pathogenic bacteria are available from the Clinical and Laboratory Standards Institute (CLSI) including breakpoints for nearly all relevant substances, except rifampin (RIF).

Aim: The European Joint Action EMERGE consists of laboratories that are specialized in highly pathogenic agents in their respective countries. One working groups aims at the development of a suitable SOP for AST of Bm and to submit it as a new regulation to the European Committee on Antimicrobial Susceptibility Testing. In a further step, the SOP is intended to be used to set up epidemiological cutoff values and to complete the clinical breakpoints for relevant substances.

Material and Methods: All isolates submitted to the National Consultant Laboratory were tested according to CLSI regulations by means of user-defined commercial microdilution plates. Isolates with MIC values above the CLSI breakpoints were further investigated using the gradient strip method and whole genome sequencing in order to identify potential genetic markers of resistance. Various modifications of the CLSI method were tested, including variations of incubation conditions and culture media. Microdilution, agardilution and gradient strip methods were compared with each other. MIC values and growth curves of Bm obtained using new culture media were compared with the CLSI-recommended Brucella broth. The modified method was validated with clinical strains, reference strains and strains showing higher MIC values for relevant substances.

Results: Using a combination of all methods, no antimicrobial resistance properties were observed among the clinical Bm isolates. For RIF and trimethoprim/sulfamethoxazole (SXT) the MIC values clustered around the breakpoints and therefore, several probably false resistant MIC values were observed using the CLSI method. The *rpoB* analyzation showed no mutations associated with RIF resistance. Modifications of culture media used for microdilution yielded an improvement of the method for SXT, but could not solve the problem of RIF testing, indicating that the breakpoint used for fastidious organisms is not suitable for Bm.

Conclusions: The microdilution method is applicable for AST of Bm, but needs improvement due to false-resistant MIC values and, therefore, the SOP was modified. In the next step, the new SOP will be validated among all EMERGE partners and strain collections will be tested to get an overview of the Bm wild type population.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

177/DVP

Does pretreatment of samples with Dithiothreitol improve bacterial detection in chronic implant infections?- Review of current clinical evidence

C. Berberich*[1]
[1]*Heraeus-Medical GmbH, Training/Med. Education, Wehrheim, Germany*

Background: The low number of planktonic bacteria in clinical samples poses a challenge in the diagnosis of chronic implant infections in orthopaedics, traumatology, cardiology and other fields. With the objective to dislodge bacteria from prosthesis-associated biofilms, sonication and – more recently – the chemical pretreatment of implants and tissue biospies with dithiothreitol (DTT) has been introduced.

Method: The available clinical studies in the period 2013 to 2017 were analysed (Medline, Embase, PubMed), in order to compare the diagnostic value of DTT-pretreatment of samples with sonication & culture of native biopsies or swabs.

Results: 2 studies from orthopaedic centers with in total n=106 patients (48 with presumed aseptic condition & 58 with prosthesis infection) compared the culture of samples after either prior sonication or prior DTT-pretreatment of explanted prosthesis material. The number of true positive cultures was higher in the DTT than in the sonication group in both studies (sensitivity: +14,5% and +12,3%; specificity: comparable). In particular *S. epidermidis* was more frequently detected in the DTT group. 1 study from a septic orthopaedic unit with n=70 patients (45 non-infected & 25 prosthesis infections) randomly allocated tissue samples from the same site either to DTT or saline pretreatment before culture. The sensitivity & specificity of the diagnosis was higher when DTT-treated tissues were plated on agar plates compared to native biopsies (+16% & +6,7%). 1 study from a trauma department enrolled 30 patients with presumed septic conditions (post-traumatic complications, implant infections or osteomyelitis) and compared the number of positive and negative samples using the specific MicroDTTect™ device or swabs. The DTT-based method showed a higher sensitivity compared to swabs (+31%) and was associated with more positive results (+15%). There were also significant differences in the type of microorganisms isolated with both methods. 1 case report described the successful detection of *S. aureus* & *P. mirabilis* from the biofilm of an aortic valve as culprits of the endocarditis case using the DTT method.

Conclusions: DTT-pretreatment of samples taken from the site of a possible chronic implant infections may improve the identification of microbial pathogens. If combined with a completely closed system of sample processing, the number & considerable costs of false negative & false positive results will be reduced. Further trials and larger studies are needed to validate this diagnostic procedure.

Figure 1

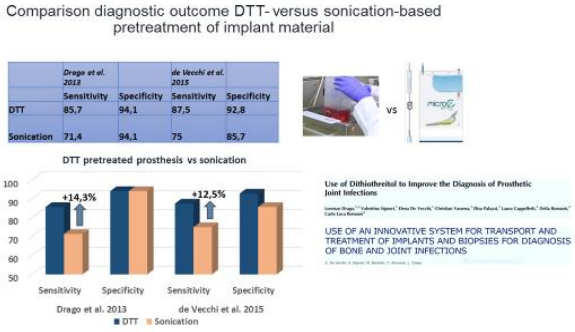
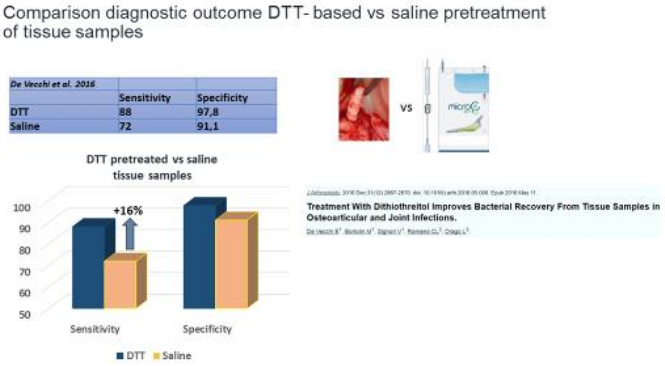


Figure 2



Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

178/DVP

16S/18S rDNA PCR and sequencing for routine diagnosis: manual and automated microbial DNA extraction

S. Keim[1], C. Baussmerth[1], M. Lustig*[1], M. Lorenz[1], C. Disqué[1]
[1]*Molzym GmbH & Co. KG, Bremen, Germany*

Broad-range rDNA PCR is widely applied in rapid clinical routine testing of microbial DNA. However, because microorganisms are present at low loads, molecular testing is challenging. Host DNA is present at a great excess and tends to reduce sensitivity and specificity by unspecific primer binding. Selective removal of human DNA (MolYsis™) significantly enhances PCR detection. Here we analyzed diverse clinical samples by two systems that are based on MolYsis™ extraction and 16S/18S rDNA Real-Time PCR with sequencing analysis.

In total, 115 routine samples from 85 patients were DNA extracted by manual SepsiTest™-UMD (UMD) and the new automated SelectNA™*plus* robot/Micro-Dx™ kit (SNP). Eluates were Real-Time PCR analyzed using the 16S and 18S rDNA assays supplied by both kits. Strains were identified by sequence analysis of amplicons (SepsiTest-BLAST/NCBI BLAST).

Sixty two samples were positive (53.9%) by UMD, SNP or both. UMD was positive with 57 (49.6%) and SNP with 50 samples (43.5%). Concordance of SNP with UMD results was 80%. Forty samples (34.8%) were identical by species with UMD and SNP. Rare bacterial (*N. menigitidis, M. morganii, Abiotrophia defectiva, Bartonella* spp., *Clostridium bifermentans*) and fungal pathogens (*C. albicans, C. glabrata, Aspergillus fumigatus, Pseudallescheria boydii, Cladosporium* spp.) were found in 11 samples (9.6%) 9 of which (7.8%) were identical by both systems. Mixed infections were detected by UMD (5.6%) and SNP (1.7%). Contamination was comparably low with UMD (3.5%) and SNP (4.3%).

The 16S/18S assays identified a great variety of bacterial and fungal pathogens in fluid and tissue biopsies. Automated sample extraction by SNP reduced time to result and hands-on time. The broad-range assays proved useful for precise and rapid culture-independent pathogen diagnosis.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

179/DVP

Detection of β-D-glucan in serum samples for diagnosis or exclusion of *Pneumocystis jirovecii* pneumonia.

K. Dichtl*[1], J. Wagener[1]
[1]*Max von Pettenkofer Institute, Faculty of Medicine, LMU Munich, München, Germany*

Pneumocystis jirovecii is an opportunistic fungal pathogen that causes a life-threatening pneumonia in immunocompromised patients, so called *P. jirovecii* pneumonia (PJP). Diagnosis of PJP in Germany currently relies on microscopic examination of bronchoalveolar lavage (BAL) fluid or quantitative real-time PCR (qPCR). However, BAL is a resource-intensive and invasive procedure that is associated with physical stress for the patient. An alternative approach for diagnosis of PJP is the detection of *P. jirovecii* antigen. β-D-glucan (BDG) is a major constituent of fungal cell walls and can be found in blood samples of patients suffering from PJP.

BDG antigen testing in Germany is currently limited to a small number of specialized laboratories, primarily because of the complex and technically challenging methodology of commercially available assays. In our retrospective study we aimed on the evaluation of a newly available turbidimetric BDG assay for detection or exclusion of PJP in patients with compatible clinical findings.

We identified and tested serum samples for BDG of 73 patients with PCR-based evidence of *P. jirovecii* in BAL fluids. The sensitivity of the BDG assay in this cohort was 86.3 %. Since it was shown that healthy individuals can be colonized with *P. jirovecii*, a qPCR cutoff is typically applied to differentiate between colonized and ill individuals. Sensitivity of the BDG assay in the sub-group with positive *P. jirovecii* PCR above the cutoff was 90.9 %. The sensitivity in the high-risk group of HIV patients was 92.3 %. All sera of a control group (n=25; comprising patients with HIV infection or hematooncologic malignancy who suffered from symptoms of atypical pneumonia but were tested negative for *P. jirovecii* DNA in BAL fluids) were tested negative for BDG.

Our results suggest that BDG detection in serum samples has a specificity which is equivalent or even higher than that of other tests for the detection of PJP. Since BDG testing in serum samples is less invasive and feasible in a timely manner using this newly available assay, testing could be routinely performed in clinical settings where PJP is suspected but BAL is not appropriate.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

180/DVP

A shortened sepsityper protocol – when every hour counts

M. Cordovana*[1], S. Ambretti[1], M. Kostrzewa[2]
[1]*Azienda Ospedaliero-Universitaria di Bologna Policlinico Sant'Orsola-Malpighi, Microbiology and Virology, Bologna, Italy*
[2]*Bruker Daltonik GmbH, Bremen, Germany*

Introduction: Sepsis is one of the leading causes of deaths in the world, with high mortality rate in critical wards (20-50%). Since the survival rate of not properly treated patients decreases by the hour, rapid identification (ID) of the causative agent is mandatory to optimize the antibiotic therapy.

The Sepsityper sample preparation kit (Bruker Daltonik, Germany) allows to perform a direct MALDI identification from the positive blood culture bottle, with a demonstrated good efficacy (75-80% from the literature).

In this study, we evaluated two shortened versions of the Sepsityper protocol, in order to further speed up the analytical and reporting times.

Methods: N=155 positive blood cultures, randomly selected after Gram staining, underwent in parallel classical Sepsityper procedure, and two shortened protocols, named "DT" (direct transfer) and "eDT" (extended direct transfer).

Both DT and eDT included the lysis and the washing steps described in the first part of the original protocol, and the direct transfer of the bacterial pellet onto the MALDI target, followed, in the eDT variant, by the addition of formic acid for *in situ* extraction of bacterial proteins.

Efficacy of the three Sepsityper protocols was evaluated comparing their ID results with the ID results of the plate subcultures.

Results: The classical protocol provided 133/155 (85.8%) correct ID, 90.2% with high confidence level (ID log score >1.8), 9.8% with low confidence level (ID log score >1.6). The failed identifications resulted mainly restricted to *S. pneumoniae, Candida* spp., *Bacteroides* spp., CoNS, and *Corynebacterium* spp..

Regarding the shortened protocols, DT provided 106/155 (68.4%) correct ID, 80.2% of them with high confidence level, while eDT provided 121/155 (78.1%), 84.3% with high confidence level. The failed identifications resulted restricted to Gram+, anaerobes, and yeast.

Both DT and eDT showed an excellent efficacy in the identification of Gram- (98% for enterobacteria, 91% including also the non-fermenting group), comparable to Ext performance (100% and 94%, respectively), while efficacy in the identification of Gram+/anaerobes/and yeast increased from 52.3% with DT to 74.4% with eDT, to 83.7% of classical protocol.

Discussion: In this study, the classical Sepsityper method showed a very good efficacy, with an overall rate of correct identifications higher than 85%, and mostly with high confidence level.

The identification results of the shortened Sepsityper protocols resulted absolutely comparable to classical method for Gram-negatives, while for Gram-positives, anaerobes, and yeast, its efficacy resulted inferior, but anyhow sufficient.

These findings suggest the possibility to include the shortened versions of Sepsityper in a combined workflow, driven by the result of Gram-staining.

Moreover, the comparison between the different protocols provided some hints for investigations to reduce the rate of non-identified samples.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

181/DVP

Rapid identification of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* using direct multiplex PCR

D. Weiß*[1,2], D. Gawlik[1,2], I. Engelmann[1,2], E. Mueller[1,2], P. Slickers[1], S. Braun[1,2], S. Monecke[1,2], R. Ehricht[1,2]
[1]*Alere Technologies /Abbott Rapid Diagnostics GmbH, R&D, Jena, Germany*
[2]*InfectoGnostics Research Campus, Jena, Germany*

A rapid identification of Gram-negative pathogens is prerequisite to diagnostic and therapeutic measures as well as to infection control. Multi-color channel real-time PCR provides accurate, sensitive and specific quantitation of target genes. The method measures exponentially amplified PCR product accumulation through dually-labeled TaqMan probes. Each probe uses a different wavelength for the determination of fluorescence signals.

A novel multiplex real-time PCR was designed, developed and validated to detect different species markers of four clinically relevant Gram-negative bacteria. This included *gad* (glutamate decarboxylase) for *E. coli*, *ecfX* (extracytoplasmic function sigma factor) for *P. aeruginosa*, *basC* (acinetobactin biosynthesis) for *A. baumannii* and *khe* (klebsolysin) for *K. pneumoniae*. Primer and TaqMan probes were designed aiming on conserved sequence regions for each target and covering all currently known alleles to ensure high accuracy of the assay.

The limit of detection was evaluated using dilution series of DNA of reference strains with a pre-determined number of genome copies. The true positive and negative rates of the assay were analyzed using colony PCR of 65 isolates of the four species considered, as well as of 62 negative controls belonging to other species. Species identifications by qPCR were compared with identifications obtained in parallel using microarray (CarbDetect AS2, Alere), VITEK-2 (BioMerieux) and MALDI-TOF (Bruker) systems.

Using direct colony PCR, all isolates of the target species were identified correctly. Controls belonging to other species were correctly tested negative. Therefore, the true positive and negative rates of this assay were 100% compared to the reference methods.

In dilution series, specific signals for all target genes were detected with templates comprising 101 genomic equivalents. Using a dilution series of DNA of the four species combined, the multiplex reaction targeting all four markers was shown to have the same limit of detection as the corresponding individual reactions.

The genes *gad*, *basC*, *khe* and *ecfX* can reliably be detected via colony PCR. The entire assay is time and cost effective. Colonies can be used directly from agar plates saving costs for DNA preparation. The assay does not require post-PCR sample handling, preventing carry-over contaminations of amplicons. This multiplex PCR could be used as rapid test to confirm or identify the target species, e.g., isolated from selective media or blood cultures or to detect them in native samples possibly combined with other PCRs targeting, e.g., carbapenemase genes (Weiss et al., 2017).

Reference

[1] D. Weiss *et al.*, *J Microbiol Methods* **142**, 20 (Nov, 2017).

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

182/DVP

A new software for automatic counting of unstained bacterial cells in brightfield microscopic images

S. Madzgalla*[1], M. Kirst[2], S. Brandes[3], D. Weiß[3,4], S. Monecke[3,4], R. Ehricht[3,4]
[1]*Jena University Hospital, Center for Infectious Diseases and Infection Control, Jena, Germany*
[2]*Blink AG, Jena, Germany*
[3]*Alere Technologies /Abbott Rapid Diagnostics GmbH, Jena, Germany*
[4]*InfectoGnostics Research Campus, Jena, Germany*

Quantification of bacterial cells is essential in microbiological labs. Currently used methods include counting of colonies on agar plates, measuring optical densities (McFarland), flow cytometry or quantitative PCR. A direct count of bacterial cells in a hemocytometer might offer advantages compared to other methods, but this approach is not commonly used. Devices are available that automatically count microorganisms based on this method, but additional hardware is not always a convenient option for a lab. Manual counting is labor-intensive and prone to human error.

We developed a tool for automatic and direct counting of unstained bacterial cells from brightfield microscopic hemocytometer images. In order to test the software, *Staphylococcus aureus* cells were loaded unstained into a counting chamber and pictures were taken at 400x magnification.

Agglomerated cells that are separated only by slight contrast are characteristic for microscopic images of bacterial cells (Fig1). Our algorithm recognizes the objects by their areal stability across a predefined gray level range. They have to be approximately circular and within a user defined size range. After one-time manual calibration with the counting chamber grid, a cell count per mm2 is returned. Results were concordant to manual counting.

The tool was compared to several freely available (open source) software solutions that also count cells or colonies on agar plates in images (*1*-*5*). With the exception of the CellProfiler pipeline by Choudhry, the approaches do not perform well in segmentation of clumped microorganisms. Another algorithm was published recently that performs well in segmentation of circular objects. However it is not yet available with a graphical user interface (*6*).

The discussed method is simple, cost-effective and independent from the experimental hardware setting. The IconoBacCounter package can be run with the freely available IconoClustÒ Software. Living as well as dead cells are detected and cellular aggregates can be separated, although motile cells have to be immobilized to be in one focusing plane. The ability of batch processing of images enhances speed and statistical significance of the analysis.

Fig1: Brightfield microscopic image of staphylococcus aureus at 400x magnification. The right image shows the overlay of valid counted objects by the software.

References

[1] P. Choudhry, *PLoS One* **11**, e0148469 (2016).

[2] I. V. Grishagin, *Anal Biochem* **473**, 63 (Mar 15, 2015).
[3] Q. Geissmann, *PLoS One* **8**, e54072 (2013).
[4] Z. Cai *et al.*, *Int J Radiat Biol* **87**, 1135 (Nov, 2011).
[5] M. Niyazi *et al.*, *Radiat Oncol* **2**, 4 (Jan 09, 2007).
[6] E. Denimal *et al.*, *Microsc Microanal* **23**, 11 (Feb, 2017).

Figure 1

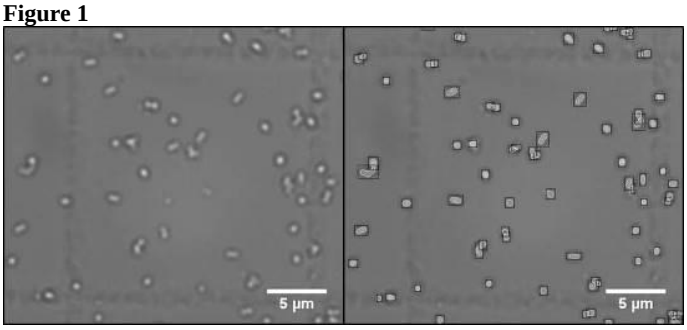


Figure 1: Brightfield microscopic image of staphylococcus aureus at 400x magnification. The right image shows the overlay of valid counted objects by the software.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

183/DVP

Assessment of the Accelerate Pheno System for direct identification of bacteria and antimicrobial susceptibility testing from positive blood cultures

B. Kohlmorgen*[1], T. Lâm[1], B. Stirzel[1], S. Mündlein[1], C. Schoen[1]
[1]*University of Wuerzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction and Objective: Rapid and reliable identification (ID) of bacteria and antimicrobial susceptibility testing (AST) are crucial for fighting mortality in septic patients. The Accelerate Pheno System (APS) (Accelerate Diagnostics, USA) is a fully automated platform performing ID and AST after red blood cell lysis and bacteria enrichment based on gel-electrofiltration and electrokinetic concentration. Identification by fluorescent in situ hybridisation and phenotypic AST is done using morphokinetic cell analysis. AST is performed if identification could be achieved and if growth controls indicated sufficient bacterial growth for AST analysis. Manual intervention is not needed except for loading the prepared specimens, reagent cartridges and test cassettes into the system. Here, we examine 30 positive blood cultures and compare ID and AST results to standard procedures.
Materials and Methods: Rapid strain identification and AST with the APS was performed according to the manufacturer"s instructions. The standard of care (SOC) for ID and AST was Vitek-MS mass spectrometry (BioMérieux, France) for identification, Vitek-2 (BioMérieux) for identification and AST as well as manual methods for MIC determination, all in line with EUCAST recommendations.
Results: Using the SOC for identification 19 Gram-positive and 14 Gram-negative bacteria species were detected, including three polymicrobial infections, each with two species. Concordant identification in a per specimen (positive blood culture bottle) analysis could be achieved in 25/30 (83.3%) specimens, including one concordant identification as off panel, which was identified as *Bacteroides* sp. with conventional methods. One ID was aborted, two IDs were misidentifications, including one polymicrobial infection and two were specimens in which APS detected a polymicrobial infection that could not be confirmed on standard media, on which we solely found one species (identified by APS). In a per bacterial strain analysis 30/33 (90.9%) strains were concordantly identified. AST was performed by APS on 10 Gram-negative and 12 Gram-positive species. Concordant AST by APS was performed in 135/146 (92.5%) analyses. Discordant susceptibilities were spotted in 4/146 (2.7%) cases (very major error), discordant resistances in 2/146 (1.4%) cases (major error) and discordant intermediate/susceptible results (if SOC showed susceptible/ intermediate results) were detected in 5/146 (3.4%) cases (minor error).
Discussion: Identification and AST by APS shows a reliable and good overall performance. APS promotes a faster way of bacterial identification and susceptibility testing, which could lead to an earlier adaption of antibiotic treatment. There is a limited panel for bacterial identification and AST, which needs to be considered in future versions of the system. Likewise, resource calculations regarding costs, laboratory workflow and capacities must be taken into account.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

184/DVP

Rapid diagnostic of infectious meningitis by implementing the BioFire FilmArray ME assay in laboratory routine: a prospective study

M. Lütgehetmann*[1], S. Pfefferle[1], M. Christner[1], M. Aepfelbacher[1], H. Rohde[1]
[1]*University Medical Center Hamburg Eppendorf, Department of Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Background: Fast and reliable diagnostic can be crucial - especially in bacterial meningitis-, since immediate antibiotic treatment is needed to ensure optimization of patients outcome. Since modern molecular diagnostic can deliver results in 1,5h the **aim** of the study was to analyses the performance of the rapid molecular diagnostics using the BioFire FilmArray meningitis (ME) assay in addition to the routine methods (culture and molecular).
Methods: Over a period of 18 months we received a total of 4623 CSF samples (from 1601 individuals). To increase pretest probability only samples in which leukocytes and/or bacteria (assessed by gram stain) were evident or urgent suspicion of infection was communicated by clinicians were subsequently analyzed.
Results: Overall, samples of n= 171 individuals matched to our risk criteria and were subjected in addition to conventional methods to rapid molecular diagnostic. Overall positivity rate was 33.3% (56/171) with bacterial pathogens detected in 54.3% (S. pneumoniae (n=18), N. meningitides (n=5), L. monozytogenes (n=3), H. influenza (n=3); S. agalactiae (n=2), E. coli (n=1)) and viral pathogens detected in 43.8% of positive samples (Enterovirus (N=11); HSV (N=7); VZV (n=5), HHV6 (n=3). We observed good concordance of results obtained using the Biofire FilmArray ME with conventional methods. However, for n= 4 samples which were designated positive (detection of *S. pneumoniae, E.coli, S. agalactiae, or HHV6*, respectively), no confirmation was obtained by reference methode. Furthermore, n=2 samples (1%) revealed false negative results in Biofire, as *S. pneumoniae* and *Parechovirus* (both with late CT values) could be detected by specific in-house PCR, respectively. In five cases, pathogens not represented by the FilmArray ME panel were detected by culture (*Bacillus sp*., *S. epidermidis, S. aureus, K. pneumoniae*) or PCR (BK virus DNA).
Conclusion: The BioFire FilmArray ME assay is a useful tool for fast and reliable diagnostic of infectious meningitis. Furthermore, risk-assessment driven selection of samples was a useful strategy to increase rate of positive samples and avoid unnecessary and cost intensive rapid diagnostics. Considering especially the additional need for antimicrobial susceptibility testing (culture), and pathogen quantification (HSV-DNA or VZV-DNA) the use of the BioFire FilmArray ME assay as stand-alone diagnostic cannot be recommended.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

185/DVP

Low Compliance with follow-up blood cultures in patients with candidemia

H. Haefner*[1], G. Haase[2], S. W. Lemmen[1], A. Kruettgen[2], I. Purr[2]
[1]*RWTH Aachen University Hospital, Infection control and hospital epidemiology, Aachen, Germany*
[2]*RWTH Aachen University Hospital, Labordiagnostisches Zentrum Bereich Mikrobiologie, Aachen, Germany*

Background: Despite of recent improvements in respect to diagnostic measures and therapy mortality of invasive candidiasis is still within the range of 15 to 50%. Persistence of candidemia has been proposed as a crucial factor for this high level of observed mortality. Therefore, we had established in our university hospital (UKA) a policy for follow-up blood cultures drawn within a time frame of 48-72h after initiation of an appropriate antimycotic therapy which were repeated until resolution of candidemia.
Objectiv: Determination of compliance of drawing blood cultures after initiation of antimycotic therapy in case of proven candidemia in the UKA. Establishing measures capable to increase the rate of follow-up blood cultures in case of candemia and thereby improving therapy and outcome of the affected patients.
Method: In the UKA notification of microscopic detection of yeast cells in case of positive blood cultures is timely performed by phone and written report. Determination of compliance to our policy concerning drawing of follow-up blood cultures was done by calculation of the time difference between this initial notification and time of drawing of a follow-up blood culture using HyBASE®. A time difference of 48-72 h was judged as "adequate".
Results: In the period from November 2016 to October 2017 candidemia episodes were observed in 51 patients. Evaluation of compliance to our policy was performed using respective data from 40 patients. In case of the remaining 11 patients evaluation could not be performed due to death within 72 h after primary notification. Compliance to our policy for drawing follow-up blood cultures was only ascertainable in 21 patients (21/40; 53 %). In case of patients in intensive care units compliance was higher (16/26; 63%) when compared to patients on standard care wards (5/14; 36%).
Conclusion: As a consequence of the determined low compliance of drawing follow-up blood cultures in case of candidemia all affected patients will be compulsorily visited by a member of the infectious disease team upon initial notification by the microbiological laboratory. Furthermore, in the respective lab report an advice for drawing follow-up blood cultures will be implemented.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Diagnostic Microbiology and Clinical Microbiology (StAG DV/FG DKM)

186/DVP

High-throughput multiplex detection of Shiga toxin-producing *Escherichia coli* from stool samples with nano-fluidics real-time PCR

M. Kiel*[1], T. Dreo[2], A. Mellmann[3], U. Dobrindt[1]
[1]*Institute of Hygiene, Microbial Genome Plasticity, Münster, Germany*
[2]*National Insitute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia*
[3]*Institute of Hygiene, Applied Hospital Hygiene, Münster, Germany*

Introduction: *Escherichia coli* (*E. coli*) is usually a mutual member of the human microbiota. Nevertheless, several pathogenic *E. coli* pathotypes exist. Especially foodborne infections with Shiga toxin-producing *E. coli* (STEC), which can lead to bloody diarrhoea and the severe haemolytic uremic syndrome (HUS), are a global health concern. To ensure the best treatment of patients and to facilitate strain typing and risk assessment a quick and reliable DNA-sequence-based detection method for STEC is important.
Aims: Our aim was to facilitate and improve DNA-based detection and typing of STEC in clinical stool samples with nano-fluidics real-time PCR.
Materials and Methods: Previously we identified a collection of biomarkers for several STEC subgroups based on comparative genomic analyses of 248 *E. coli* genomes. As a result multiplex PCR reactions were established which reliably detect the "Big Five" and additional clinically important STEC subgroups. In this work we used these new biomarker PCRs to detect STEC from stool samples with nano-fluidics real-time PCR. Crude DNA extractions from stool samples were obtained by Chelex®100 (BioRad) treatment. After a preamplification step we used the BioMark™ HD system with Fluidigm 48.48 Dynamic Array™ Integrated Fluidic Circuits (IFC) to detect 17 biomarkers in 41 stool samples as well as 9 reference strains.
Results: To facilitate the detection and typing of STEC from stool samples, we successfully employed nano-fluidics real-time PCR. Monocultures of well characterized STEC reference strains were precisely and reproducibly typed with our Fluidigm array-based approach. Furthermore, STEC biomarkers were reliably detectable in crude DNA extractions from stool samples after a preamplification PCR step. The nano-fluidics real-time PCR typing results of the stool samples were independently confirmed by standard real-time PCR.
Conclusion: With this high-throughput nano-fluidics real-time PCR system it is possible to test more samples with a reduced work load, thus facilitate DNA-based STEC typing and risk assessment in routine clinical surveillance laboratories.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Clinical Microbiology and Infectious Diseases (StAG KM)

187/KMP

Antimicrobial resistance of toxigenic and non- toxigenic *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* in Germany 2011-2017

D. Marosevic*[1], A. Berger[1], A. Sing[1]
[1]*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany*

Background: High vaccination rates led to decreasing incidence of toxigenic (DT) corynebacteria and the disease diphtheria, while non-toxigenic (NT) strains increased as infectious agents of endocarditis, septic arthritis or wound infections. Recommended first line antibiotic is penicillin or erythromycin but reliable susceptibility data are not available for potentially toxigenic species and limited information are prepared by EUCAST and CLSI for the genus. This study aims to examine antibiotic susceptibility (AS) profiles of DT and NT *C. diphtheriae* and *C. ulcerans* strains and provide data needed for the update of international guidelines.
Methods: The AS profiles of 296 NT and 135 DT strains isolated during 2011-2017 at the German consultant laboratory for Diphtheria were tested using: CLSI broth microdilution with 12 and E-test with 3 different clinical relevant antibiotics. Isolates were originating from human (n=372) invasive and non-invasive infections or animals (n=59). Majority of isolates were *C. diphtheria* (n= 299), followed by *C. ulcerans* (n=125) and a small percentage of *C. pseudotuberculosis* (n=7).
Results: Preliminary data of 220 NT and 113 DT *Corynebacterium* spp. isolates show that 44 % (n=97) and 66 % (n=75) were penicillin resistant, respectively. Erythromycin resistance was observed for 2 % (n=5) of isolates. 81 % of tested *C. ulcerans* isolates (n=86) were clindamycin resistant, while only 3 % of *C. diphtheriae* (n=7). No resistance against quinupristin-daptomicin, vancomycin or linezolid was observed.
Conclusions: The here presented data describe the largest AS testing of *C. diphtheriae* and *C. ulcerans*. High proportion of resistances and the indicative wild-type MIC distribution of the examined strains suggest that the EUCAST penicillin breakpoint is too low for this genus. Therefore, penicillin breakpoint should be updated to 0.5 mg/L. Furthermore, the MIC distribution for clindamycin urges for an update of species specific breakpoints for *C. diphtheriae* and *C. ulcerans*. Resistance against other classes of

tested antibiotics was rare, including the first line antibiotic erythromycin.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

188/KMP

Creatinine clearance may be underestimated in patients with Aerococcus sanguinicola bacteriuria (case report)

S. Kositska*[1], G. Schiffel[1], P. Weyrich[2], B. Ivandic[1]
[1]*MVZ Labor Limbach, München, Germany*
[2]*KfH-Nierenzentrum Unterhaching, München, Germany*

Creatinine clearance (CrCl) is an important parameter in the diagnostic evaluation of patients with suspected chronic kidney disease (CKD). CrCl is calculated from creatinine concentrations in serum and urine collected for 24 hours. Aerococcus *sanguinicola* (AS) is an increasing finding in urine and blood *after the introduction of* matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) and causes urinary tract infections and endocarditis in elderly or immunocompromised patients.
We report a case of a 72 years old female patient with asymptomatic AS bacteriuria. Her CrCl was very low suggesting CKD although serum creatinine was normal. Urine creatinine amounted to 1 mg/dl only and culture revealed 105 cfu of *Aerococcus sangunicola* confirmed by MALDI-TOF analysis. Creatinine concentrations were determined by Jaffé"s method. We subsequently tested this and other isolates of *Aerococcus sanguinicola in native urine and TBS broth supplemented with creatinine and found that Aerococcus sanguinicola metabolized creatinine in vitro. This* observation suggested that urine creatinine may be metabolized and underestimated in patients with AS bacteriuria. This may result in an underestimated CrCl and hence false diagnosis of CKD in these patients.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

189/KMP

Glycopeptidolipids of the *Mycobacterium abscessus* cell wall are immunodominant antigens and represent potential targets for a diagnostic assay

H. Schaefer*[1], C. Schwarz[1], A. Lewin[1]
[1]*Robert Koch-Institute, FG16, Berlin, Germany*

Introduction: Infections with non-tuberculous mycobacteria (NTM) present an increasing problem in patients with predisposing diseases such as cystic fibrosis (CF) or immunodeficiency. In pulmonary infections caused by rapid growing mycobacteria, *Mycobacterium abscessus* is the most frequent pathogen. Diagnosis of *M. abscessus* infections depends largely on symptoms of disease and confirmation by mycobacterial culture of sputum samples or bronchoalveolar lavages. Microbiological cultivation however, is time-consuming and not always feasible or reliable. A suitable assay should therefore be able to differentiate between infection with tuberculous mycobacteria and NTM, and to distinguish between ongoing infection and colonization.
Aims: We set out to identify immunodominant antigens in *M. abscessus* by ELISA and immunoblot methods. Selected antigens should not cross react with tuberculous mycobacteria, and preferably only be recognized during infection, but not upon bacterial colonization.
Materials and Methods: Heat-killed bacterial suspensions of the three different *M. abscessus* subspecies were sonicated, and alkaline-stable lipids were extracted with chloroform-methanol. The composition of lipids was analyzed by thin layer chromatography (TLC). Antigenic lipids were identified by blotting TLC-separated fractions to PVDF membranes and probed with sera from *M. abscessus* infected patients and experimentally infected animals. The immunodominant fractions were eluted and used to establish an ELISA-protocol for the detection of antibodies in blood and sputum.
Results: Sera from CF-infected patients and experimentally infected animals showed a strong reaction against the low molecular fraction in crude bacterial lysates. A more detailed analysis revealed that both lipoarabinomannan (LAM) and lipids from the cell wall were recognized. Since LAM is an essential component of all mycobacterial cell walls, and therefore not appropriate to specifically indicate NTM infection, we further analyzed the response against specific components of the lipid fraction, which are not found in tuberculous mycobacteria. Patients with culture-confirmed *M. abscessus* disease showed a strong serum antibody response against the glycopeptidolipid (GPL) fraction of TLC-separated alkali-stable lipids. Purified PGL were used to establish an ELISA protocol to detect antibodies in sera and sputum of infected patients.
Summary: We established ELISA- and Western Blot-based methods to identify immunodominant antigens in NTM infections. GPL from the cell wall were among the most dominant antigens in crude lysates and are not shared with tuberculous mycobacteria. Most patients, but none of the controls showed a strong and specific antibody response against this fraction within purified lipids in all *M. abcessus* subspecies. These preliminary results indicate that glycopeptidolipids of *M. abscessus* might provide a specific target for antibody-mediated diagnostic assays.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

190/KMP

Molecular diagnostic of integrated virus DNA in host cell genomes

C. Jurischka*[1], M. Sowa[1,2], S. Rödiger[1], J. Schiebel[1], D. Roggenbuck[1,2], P. Schierack[1]
[1]*BTU Cottbus - Senftenberg, Fakultät 2 Umwelt und Naturwissenschaften, Institut für Biotechnologie, AG Multiparameterdiagnostik, Senftenberg, Germany*
[2]*Medipan GmbH, Dahlewitz, Germany*

The integration of viral DNA into the genome of host cells bears the risk of following degenerations, where afflicted cells potentially lead to tumors. Especially anogenital cancers are strongly associated with persistent infections by high-risk human papillomaviruses (e.g., HPV 16, 18)[1]. The physical state of the virus (integrated or episomal) seems to be an important prognostic marker for cervical cancer[2]. Techniques for the risk assessment include cytological evaluations of cervicovaginal smears after Pap-test and the polymerase chain reaction (PCR)[3]. Furthermore, the fluorescence *in situ* hybridization (FISH) is a technique to determine HPV infection and integration.
For FISH analysis high-risk HPV probes should be developed for a standardized, multiplex, type-specific and quantitative detection of HPV DNA in host cells. Due to the short HPV sequence, present with just few copies, conventional FISH probes have little sensitivity. Therefore, we developed specific FISH probes targeting different regions of the HPV genome and tested them in combination with signal amplification steps.
FISH probes were produced via nick translation or PCR and biotin labeled by incorporation of biotinylated nucleotides. Biotin incorporation was checked with agarose gel electrophoresis, where biotin-labeled DNA showed a band shift in comparison to the control. Our biotinylated HPV probes were tested on HPV-positive and -negative cells and detected using fluorescence labeled streptavidin or by subsequent tyramide signal amplification (TSA). In addition to FISH the novel probes were used for chromogenic *in situ* hybridization (CISH). All analysis were performed with our fully automatized multicolor fluorescence imaging platform VideoScan[4].
The presence of HPV DNA in cell lines was confirmed by (digital) PCR. The protocol for synthesis of biotin labeled probes using HPV sequence-specific primers and gDNA as template was successful. The probes can be used for multiplex testing (HPV 16, 18, internal control). In CISH and FISH experiments, our probes showed comparable signals in CaSki cells.
Highly sensitive and specific FISH probes offer a great potential for the detection of HPV DNA in patient samples and thereby for the assessment of tumor risks. Using our probes, we got positive results in hybridization experiments using chromogenic and fluorescent detection methods with or without further amplification

steps. In future, comparative FISH experiments will be performed to check probe sensitivity in cells with low HPV copy numbers and their practicability in tissue samples.

References
[1] Zur Hausen, H. J *Natl Cancer Inst* **92**, 690–698 (2000). [2] Shukla, S. et al. *Indian J Med Res* **139**, 531–543 (2014). [3] Kroupis, C. et al. *Clin Chem Lab Med* **49**, 1783–1799 (2011). [4] Rödiger, S. et al. Adv Biochem Eng Biotechnol **133**, 35–74 (2013).

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

191/KMP

"Detection of Bacterial Pathogens in Blood by Magnetic Resonance Technology: No Culture Required?"

D. Schmidt*[1], J. Buer[1], P. M. Rath[1], J. Steinmann[2,1]
[1]*University Hospital Essen, Institute of Medical Microbiology, Essen, Germany*
[2]*Paracelsus Medical University, Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Nürnberg, Germany*

Introduction: Sepsis is the most severe complication in intensive care unit patients with a high mortality rate of up to 50%. Detection of pathogens is still based on blood cultures which lack same-day-results. The T2Dx Instrument (T2 Biosystems, USA) together with the T2 Bacteria Panel allows the detection of bacterial pathogens directly in four ml of whole blood with minimal hands-on-time. The aim of this study was to evaluate the performance of this new T2 bacterial assay in comparison with blood culture.
Materials and Methods: The fully-automated system combines amplification, hybridization and magnetic resonance technology in a single assay. The limit of detection is supposed to be about 1 CFU/ ml. The T2 Bacteria Panel can process up to seven samples and currently detects six species (*Acinetobacter baumannii*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). Time-to-result is between three to five hours. Overall, we have tested 112 blood samples from 85 patients with the T2 Bacteria Panel compared with blood culture results (BC) over a period of three months. All included patients (89.4% male, age between 15 and 89 years, median=60 years) were critically ill ICU patients.
Results: In total, data of 86 samples from 71 patients could be analysed. Single pairs of blood cultures with growth of coagulase-negative staphylococci that were considered as contaminants were excluded from the analysis. Detection rate of T2Dx regarding the six species included in the panel was three times higher than detection rate by culture (36 vs.10), in particular for gram-negative rods. Considering blood culture as gold standard sensitivity of the T2 assay was 80%, the negative predictive value was 94%. Agreement with blood culture was 66%. Time-to-result of the T2 system was between 3:36 h to 9:48 h (median= 5:53 h) depending on the number of samples that were analysed at the same time.
Conclusion: This is the first study evaluating the performance of the T2 Bacterial Panel in comparison to blood culture. Our first data indicate a good diagnostic performance with minimal hands-on-time and acceptable time-to-result. The clinical relevance of the higher positive rate for gram-negative rates in comparison to blood culture has to be further evaluated.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

192/KMP

Improving the therapeutic management of *Staphylococcus aureus* bacteraemia in a tertiary care centre via personal microbiological consultations

S. Kampmeier*[1], A. Mellmann[1], G. Peters[2], B. C. Kahl[2]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[2]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction: *Staphylococcus aureus* bacteraemia (SAB) is a common cause of morbidity and mortality, which can occur primarily or secondary from a wide range of causative sources. Optimal strategies require an early, intravenous therapy with a narrow-spectrum *S. aureus* effective antibiotic, intensified screening for the infectious focus including its possible eradication and repeated blood culture collections until clearance of bacteraemia.
Aim: The presented study aimed to optimize the therapeutic management in patients with *S. aureus* bacteraemia via personal microbiological consultations in addition to microbiological telephone advices.
Methods: For the better assessment of the quality of SAB therapy, we defined the following quality-of-care indicators (QCI): correct choice and dosage of antibiotic agent, early start of treatment, correct duration of treatment, screening for the infectious focus and collection of control blood cultures on day 3/4 after initiated targeted treatment. Each QCI received one point with an achievable maximum QCI score of 6.
During the pre-intervention period (February to July 2016), patients with SAB were identified using patients' information and microbiological laboratory systems.
During the intervention (February to July 2017), in addition to the microbiological telephone advices personal microbiological consultations were performed including specific recommendations based on the 6 QCIs listed above following a standardized protocol.
Using the QCI scoring system, therapeutic management of SAB prior and during the intervention period was compared. In addition, the microbiological eradication or persistence of *S. aureus* in blood cultures collected on day 3/4 after targeted therapy was evaluated.
Results: In the pre-intervention period, 50 patients suffering from SAB could be retrospectively identified during February and July 2016. Quantification of the therapeutic management resulted in an QCI score of 2.46. In 21 out of 50 patients (42%), blood culture samples were collected on day 3/4 after targeted treatment, resulting in 13 patients with pathogen persistence. During the intervention period, 45 patients experienced SAB. In total, the QCI score increased to 4.84. Most lacking points were control blood culture samples and the duration of therapy. In 17 of 45 cases (38%), the maximum QCI score of 6 was reached. Of the 33 collected blood culture samples on day 3/4 after targeted treatment, 91% of blood cultures revealed microbiological eradication.
Conclusions: Personal microbiological consultations can improve the therapeutic management of SAB within clinical settings. Difficulties during SAB treatment especially affect the duration of therapy and the collection of control blood cultures.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

193/KMP

Serum (1→3)-β-D-glucan and galactomannan levels in patients with cystic fibrosis

J. Träger[1], V. Melichar[1], R. Meyer[1], J. Held*[1]
[1]*Universitätsklinik Erlangen, Mikrobiologisches Institut, Erlangen, Germany*

Introduction: *Aspergillus fumigatus* is frequently encountered in sputum samples of Cystic Fibrosis (CF) patients and has traditionally been considered as saprophytic airways colonization. However, this mere bystander role is challenged by recent data and there is evidence that *Aspergillus fumigatus* is accelerating lung function decline.
(1→3)-β-D-glucan (BDG) and galactomannan (GM) are highly sensitive fungal biomarkers that are used to diagnose invasive

aspergillus infection. However, their properties in CF-patients are largely unknown.

Goals: BDG and GM levels in CF-patients are to be analyzed in order to find correlations with clinical parameters especially the *Aspergillus*-culture results and the lung function.

Methods: All patients from the University Medical Center Erlangen CF-cohort with an archived serum and a paired respiratory sample taken between September 2015 and October 2016 were enrolled. BDG and GM were measured retrospectively and clinical parameters were collected. Univariate and multivariate analyses were performed to reveal causal relationships.

Results: 104 patients were included in the study. *Aspergillus fumigatus* was persistently detected in 22 patients (21%) and serum BDG and GM levels in these patients (89 pg/ml and 0.30 ODI, respectively) were significantly higher than in those without persistent detection (40 pg/ml, p=0.022 and 0.15 ODI, p=0.013, respectively). In addition, patients with *Aspergillus* detection were significantly older, had a higher body mass index, were more often *Pseudomonas aeruginosa* colonized and treated more frequently with nebulized antibiotics. The forced expiratory volume in 1 second (FEV1) was lower in *Aspergillus*-positive patients but this difference was not significant (p=0.057).

27 patients (26%) had elevated BDG levels (median 108 pg/ml) and 7 patients (7%) elevated GM levels (median ODI 0.7). Interestingly, patients with an elevated BDG were more frequently *Aspergillus* culture-positive (40.7 versus 14.3 %, p=0.004) and had a significantly lower FEV1 than patients with a normal BDG (61.6 versus 77.1 %, p=0.007). In order to examine which parameters were independent predictors of the lung function we performed a multivariate analysis with FEV1 as dependent variable. BDG (p=0.002), WBC (p<0.001), *Staphylococcus aureus* (p=0.011) and *Pseudomonas aeruginosa* (p=0.023) detection turned out to be independent predictors of the FEV1. These parameters were able to explain 52.6% of the variation of the FEV1.

Summary: CF-patients with persistent *Aspergillus*-detection have elevated BDG and GM levels which are located between healthy and invasively infected patients. BDG but not *Aspergillus fumigatus* culture results proved to be an independent predictor of the FEV1. Thus it seems that BDG may be the superior parameter to predict lung function in CF-patients.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

194/KMP

Intrapartum detection of Group B Streptococci (GBS) by point of care (POCT) real time PCR testing

M. Bermudez-Martinez[1], L. von Müller*[1]
[1]*Christophorus Kliniken, Institut for Laboratory Medicine, Microbiology and Hygiene, Coesfeld, Germany*

Introduction: Intrapartum antibiotic prophylaxis (IAP) of pregnant women colonized with group B streptococci (GBS) was established to prevent GBS transmission followed by early onset sepsis of neonates. As colonization status varies optimized screening should be performed few hours before delivery; however, intrapartum testing cannot be achieved by standard culture-based analysis due to prolonged turn-around time. Real-time PCR with POCT is now available for intrapartum testing in < 2 hours.

Aims: In the present study, we tested if sensitivity of PCR (GenePOC) was appropriate for intranatal testing. Second, we compared GBS detection by antenatal and intranatal screening for selection of an optimized time-point for GBS analysis.

Material and Methods: Rectovaginal swabs of 300 pregnant women were tested in parallel by culture with solid media (CNA, GBS Chrome) and enrichment culture (LIM broth) on the one hand and by POCT real time PCR (GenePOC) from direct swab and LIM broth culture on the other hand. Comparison between antenatal and intranatal testing was performed for 133 patients (44%) with history of antenatal voluntary testing.

Results: Direct PCR was characterized by high sensitivity (95,8%) and specificity (95,5%) as compared to culture (Table 1). 11 (3,7%) additional patients with GBS colonization were detected by PCR only whereas 2 patients remained PCR negative with positive culture, presumably due to low number of colonies detected. Detection rates could not be increased by enrichment culture (LIM broth) which was independently confirmed by subculture and also by PCR from LIM broth.

Comparison between antenatal and intranatal screening revealed stable colonization status for most patients but for 15% of patients the colonization status changed (Table 2). 11% of patients became GBS negative and 4% GBS positive as compared to antenatal testing.

Conclusion: The European consensus conference recommended intranatal GBS screening for better selection of patients for intranatal antibiotic prophylaxis. If GBS screening was based on intranatal POCT PCR addition 4% of patients with previously undetected GBS colonization (antenatal screening) can benefit from IAP whereas unnecessary IAP could be saved for 11% patients with undetectable GBS directly before delivery.

Figure 1

		Culture		
		Positive	Negative	Total
PCR direct Swab	Positive	46	11	57
	Negative	2	238	240
	Total	48	249	300
PCR LIM Broth	Positive	43	7	50
	Negative	5	245	250
	Total	48	252	300

Figure 1: Direct comparison between culture and POCT PCR (GenePOC) from direct swabs (<2 hours) and after LIM Broth enrichment culture (12 hours).

Figure 2

Antepartum culture	Intrapartum culture	n (%)
+	+	24 (18)
+	-	14 (11)
-	-	90 (68)
-	+	5 (4)

Figure 2: Comparison between antenatal and intranatal culture-based GBS screening

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

195/KMP

Pathogen Identification and Detection of Resistance Markers from Positive Blood Cultures: Evaluation of the Unyvero BCU Cartridge

D. Schmidt*[1], J. Buer[1], P. M. Rath[1], J. Steinmann[2,1]
[1]*University Hospital Essen, Institute of Medical Microbiology, Essen, Germany*
[2]*Paracelsus Medical University, Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Nürnberg, Germany*

Introduction: Identification of pathogens from blood culture samples is still dependent on the growth of the pathogens and time-consuming. The Unyvero Application (Curetis, Germany) is a semi-automated microbiological analysis system that combines sample preparation and qualitative pathogen detection in a disposable cartridge. The blood culture unit cartridge (BCU) currently detects 34 bacterial and fungal pathogens and 16 antibiotic resistance markers within five hours.

Aim. The aim of the study was to evaluate the performance of the BCU and to assess the agreement between BCU and traditional pathogen identification.
Materials and Methods: Over a period of five months we analyzed 50 blood culture samples of 46 critically ill patients (61% male, 39% female) from the age of one month to 84 years (median= 61 years). Initially, microscopically positive blood culture samples (BACTEC FX, BD, Germany) were randomly selected. Identification and susceptibility testing were performed with MicroScan WalkAway96 plus (Beckman Coulter, Germany). Additionally, blood culture media were spiked with one isolate each of *Aspergillus fumigatus*, *Candida glabrata*, *Mycobacterium abscessus subsp. abscessus*, *Listeria monocytogenes*, *Staphylococcus aureus* (mecC) and *Enterococcus faecium* (vanA, vanB).
Results: The overall agreement between both methods was approximately 95%. The BCU cartridge missed two isolates that could be found by culture (*Enterobacter cloacae*, *Staphylococcus epidermidis*). In contrast the BCU cartridge found DNA of five bacteria that could not be found by culture (3x *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Acinetobacter baumannii*). Both methods showed identical results for the resistance markers. Furthermore, samples that had been spiked were all correctly identified by the BCU cartridge.
Conclusion: The BCU cartridge is a useful tool for the rapid identification of pathogens and resistance markers directly from microscopically positive blood cultures.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

196/KMP
Characteristics of a typhoid fever outbreak in Southwestern Tanzania, detected as part of a FUO-study
B. Flach[1,2], K. Baumann[1,2], C. Godlove[3], S. Lwitiho[3], M. Clement[3], M. Chacha[3], N. Heinrich[4], M. Hoelscher[4], G. Dobler[1,2], P. Vollmar*[1]
[1]*Bundeswehr Institute of Microbiology, Bacteriology and Toxinology, München, Germany*
[2]*German Partnership Program for Excellence in Biological and Health Security, München, Germany*
[3]*Medical Centre of the University of Munich, Division of Infectious Diseases and Tropical Medicine, München, Germany*
[4]*National Institute of Medical Research, Mbeya, Medical Research Center, Mbeya, Tanzania, United Republic Of*

In summer 2015, while conducting a clinical study to investigate the distribution and origin of febrile illnesses in Matema, Southwestern Tanzania, an increased emerge of *Salmonella Typhi* cases was observed. From March to June 2015 ten *S. Typhi* strains could be isolated via blood culture diagnostics.
Aim of this study was the characterization of the isolates including their antibiotic resistance profile and genotyping for phylogenetic clustering based on seven housekeeping genes used in Multiple Locus Sequence Typing (MLST)1.
Ten *S. Typhi* strains, isolated from positive BD BACTEC™ PLUS Aerobic/F and BD BACTEC™ PEDS PLUS/F blood cultures, underwent biochemical identification based on the Api 20 E® – system (BioMerieux) and serological identification according to Kauffmann-White-Scheme using antisera against *Salmonella* O-/H-antigens (sifin diagnostics). Antibiotic resistance testing by microdilution methods according to CLSI-guidelines was performed from all isolates. MLST was used to confirm the results of the biochemical identification and gain a deeper description of the circulating genotypes. For all patients tested positive for *S. Typhi*, potential co-infections with Malaria and/or HIV were determined using rapid test kits.
Seven of the ten *S. Typhi* cases (70%) could be appointed between March and April 2015. Two subsequent cases were identified in May 2015 and the last case was reported in June 2015. 70 % of cases were female and 30% male, both groups with a median age of 7,5 years (range 3 to 38 years). All isolates showed highly elevated minimal inhibitory concentrations (MIC) for ampicillin, cotrimoxazole and, with one exception, for chloramphenicol in terms of clinical resistance towards these antibiotics. However, MIC-results for ciprofloxacin, levofloxacin and ceftazidime revealed clinical sensitive strains concerning these drugs. Results of MLST confirmed the biochemical identification of all isolates and proofed the suspected outbreak due to high homology within the MLST-scheme based on seven housekeeping genes.
During a broad-range clinical study with respect to distribution and origin of febrile illnesses in Matema, Kyela region a *S. Typhi* outbreak could be detected. Genotyping, based on MLST, approved the suspected outbreak, whereby the outbreak origin could not be detected. Surprisingly, therapeutically relevant antibiotic resistances were found, although former antibiotic treatments have not been described for the patients. In conclusion these results unveil information on *S. Typhi* distribution and its antibiotic resistance in Matema, Kyela region in Tanzania and highlights therapeutic challenges in this region.

Reference
[1] Achtman M, Wain J, Weill F-X, Nair S, Zhou Z, et al. (2012) Multilocus Sequence Typing as a Replacement for Serotyping in Salmonella enterica. PLoS Pathog 8(6): e1002776. doi:10.1371/journal.ppat.1002776

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

197/KMP
Multi-parameter serology using protein microarrays
E. Müller*[1,2], T. Schumacher[3], D. Gawlik[1,2], S. Monecke[1,2], R. Ehricht[1,2]
[1]*Alere Technologies /Abbott Rapid Diagnostics GmbH, R&D, Jena, Germany*
[2]*InfectoGnostics Research Campus, Jena, Germany*
[3]*Virion-Serion GmbH, Würzburg, Germany*

Serological detection of human antibodies is performed using a variety of techniques including ELISA, immunoblotting, complement binding reaction, agglutination assays, lateral flow tests and direct or indirect immunofluorescence. Routinely, tests for different antigens are performed separately and consecutively, resulting in high costs and potentially delayed results. Besides, a relatively large amount of serum is needed and capillary blood samples are not sufficient. Cross reactivities of antibodies can hardly be detected.
We aimed to develop a rapid multi-parameter procedure that allows the parallel determination of antibodies against a number of pathogens within one reaction and in the same time normally needed for a single test.
Antigens were spotted and covalently immobilized onto microarrays at various dilutions and with various additives (Ehricht et al., 2009). Currently, 67 antigens were used that originated from 31 different pathogens including rubella, mumps, measles, varicella zoster, diphtheria, tetanus, toxoplasma, CMV, HSV, EBV, RSV, Brucella, Borrelia, Helicobacter, Chlamydia (C.) trachomatis, C. pneumoniae and Mycoplasma pneumoniae.
After protocol optimization, specific IgG antibodies from 1 µl of a native blood sample bound specifically to these antigens and were subsequently detected using precipitation staining with horseradish-peroxidase-conjugated anti-IgG-antibody and a TMB derivate.
For verification, 88 different reference sera with 210 known ELISA results for 30 pathogens were used. The multiplex protein array, we were able to achieve concordance in 195 of these 210 cases, i.e., in 93%.
We demonstrated that multi-parameter serology with protein microarrays is feasible. We determined the IgG status for 30 pathogens in parallel needing a sample volume of 1 µl and a total time of 2-3 hours only, translating in enormous potential for time and cost savings. The same procedure might in future be used not only for diagnostic purposes, but also for assay development (antigen and antibody screening). The panel of antigens can rapidly be adapted to other applications, as, e.g., in veterinary medicine. Multi-parameter serology at the point-of-care can be expected to be less expensive, less time consuming and to yield more information than conventional serological methods.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

198/KMP

Prevalence of *Staphylococcus aureus* with mucoid phenotype in the airways of patients with cystic fibrosis

F. Lennartz*[1], B. Schwartbeck[1], A. Dübbers[2], C. Kessler[2], J. Große-Onnebrink[2], H. Schültingkemper[3], P. Küster[3], S. Deiwick[1], H. Rengbers[1], J. Poßdorfer[1], G. Peters[1], B. C. Kahl[1]

[1]*Institute of Medical Microbiology, University Hospital Münster, Münster, Germany*

[2]*University Hospital Münster, Department of Pediatrics, Münster, Germany*

[3]*Clemenshospital, Department of Pediatrics, Münster, Germany*

Questions: The altered conditions present in the airways of patients with cystic fibrosis (CF) facilitate bacterial survival. Respiratory failure, resulting from chronic bacterial infection and inflammation, remains to be a leading cause of death for the patients. *Staphylococcus aureus* is one of the most frequently isolated pathogens in the respiratory tract of CF patients and is known to persist over many years up to decades within CF airways. Recently, Schwartbeck et al. discovered peculiar *S. aureus* isolates with a mucoid phenotype and excessive biofilm production, which have not been described previously. The aim of this prospective pilot study was to determine the prevalence of *S. aureus* with mucoid phenotype in CF airways over a period of three months.

Methods: We therefore analyzed specimens (sputa, throat swabs) from 81 CF patients that attended the two CF centers in Münster, Germany, during three months. From each specimen, 10 *S. aureus* isolates were picked randomly and analyzed regarding their mucoidy on Columbia blood agar and Kongo Red agar. Isolates with a mucoid phenotype were characterized by *spa* sequence typing and their biofilm production was evaluated via a static microtitre biofilm assay. All data were interpreted considering patients" age, BMI and lung function, co-infection with *Pseudomonas aeruginosa* and susceptibility to important antibiotics.

Results: In 7 of the 81 examined patients (8.6%) we detected mucoid *S. aureus* phenotypes. 37 of 1050 (3.5%) *S. aureus* isolates presented a mucoid phenotype. In a control group of patients with *S. aureus* infection (lung, bone or soft tissue infection) that did not suffer from CF, we did not find any mucoid isolates at all (0 out of 180). Mucoid and non-mucoid *S. aureus* isolates partly presented the same *spa* type, but *spa* types also differed between the two groups. Mucoid isolates in general produced more biofilm than non-mucoid isolates and were less susceptible to most of the tested antibiotics. No significant differences were found when assessing sex, BMI or lung function of the study population. Patients with mucoid *S. aureus* isolates were slightly older than patients without mucoid isolates.

Conclusions: In our prospective study, we could show that *S. aureus* with mucoid phenotype colonizing the airways of CF patients is not a rarity, but in fact can be found in 8.6% of the patients that carry *S. aureus* in their respiratory tract. We found mucoid isolates to be significantly less susceptible to penicillin, gentamicin, amikacin and rifampicin. Further prospective multicenter studies are necessary to determine possible correlations between patients" age, severity of the CF disease, antibiotic treatment, co-infection with other pathogens and the occurrence of mucoid *S. aureus* isolates.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

199/KMP

Use of broad-spectrum antibiotics in German acute care hospitals

B. Schweickert*[1], M. Feig[1], M. Schneider[1], N. Willrich[1], M. Behnke[2], L. A. Pena Diaz[2], P. Gastmeier[2], M. Abu Sin[1], H. P. Blank[1], D. Richter[1], E. Missa[1], T. Eckmanns[1]

[1]*Robert Koch-Institut, Abt. 3, Berlin, Germany*

[2]*Institut für Hygiene und Umweltmedizin, Berlin, Germany*

Introduction: Prudent use of broad-spectrum antibiotics is subject of antibiotic stewardship (ABS) activities in order to preserve their efficacy for treatment. Surveillance data on antibiotic consumption provide a basis for the assessment of the actual situation and consecutive planning and evaluation of interventions. In 2014, the Robert Koch-Institute, in cooperation with the National Reference Center for the Surveillance of Nosocomial Infections, built up an electronic, web-based surveillance system aiming to support hospitals in the implementation of antibiotic consumption surveillance and to provide an appropriate tool for local ABS-efforts.

Objectives: Data on the consumption of broad-spectrum (BS)-antibiotics are presented.

Methods: The calculation of antibiotic consumption values is based on the ATC/DDD (Defined Daily Dose) method of WHO with the modification that RDDs (Recommended Daily Doses) are used instead of DDDs. Target value is the antibiotic consumption density (CD) expressed in RDD in relation to 100 patient days (PD). The group of BS-antibiotics comprises the following substances: piperacillin/enzyme-inhib., quinolones, 3.-5. gen. cephalosporins, carbapenems, aminoglycosides, fosfomycin, tigecycline. Data are confined to full inpatients of 140 general acute care hospitals; ambulatory care and day-clinics, pediatric and psychiatric services have been excluded. Data from the years 2015/16 are presented as medians with interquartile range (IQR) for the hospitals as a whole and stratified by service line (medical, surgical), ward type (ICU, general ward) and hospital size (<=800,>800).

Results: CD of total antibiotic consumption and BS-antibiotics are presented in Table 1. CD of BS-antibiotics are significantly higher in ICUs than in general wards with medical services presenting with higher values than surgical services. In the hospitals as a whole and within the categories ICU, medical and surgical service, BS-antibiotics account for 38%, 54%, 43% and 27%, respectively of total antibiotic consumption. Ranking the constituting single substances we see different patterns: While in ICUs piperacillin/taz. holds the first position followed by carbapenems, in surgical general wards quinolones are the most frequently used agent. Stratification by hospital size shows that in ICUs and medical service lines CD of BS-antibiotics are 20%-30% higher in the bed category >800 beds in comparison to

Summary: Surveillance data show volume and density of antibiotic consumption in different hospital areas and assist in focusing ABS-activities.

Figure 1

Table1 Consumption densities (RDD/100PT) of total antibiotic consumption* and broad-spectrum antibiotics of 140 acute care hospitals stratified by ward type, service line and hospital size

Consumption density RDD/100PT	Hospitals, total						ICU					
	total		<=800		>800		total		<=800		>800	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Total Consumption*	44.7	39.4-49.2	44.0	36.8-48.8	48.0	46.8-57.7	87.6	76.5-104.8	85.0	72.4-101	111.4	99.6-129.2
Broad-spectrum antibiotics	16.7	13.9-20.3	16.6	13.5-20.2	19.1	14,6 - 25	50.9	41.2-60.9	50.6	39.8-58.1	65.1	49-73.5
Fluoroquinolones	5.0	3.8-6.8	4.9	3.7- 6.7	6.9	5.3-7.7	9.6	6.1-13.2	9.2	6 - 12,9	11.6	9.5-15.9
3. gen.cephalosporins	3.8	2.3-6.3	3.9	2.4-6.4	2.4	1.6-3.4	6.6	4.6-10.3	6.6	4.6-10.5	6.3	4.5- 8.0
Piperacillin/enzyme-inhib.	4.7	3.4-5.7	4.6	3.3-5.7	4.9	3.6-7.4	17.6	14.1-20.6	17.6	14.2-20.6	17.2	13.3-20.2
Carbapenems	1.8	1.1-2.5	1.6	1 - 2,4	3.7	2.0-4.9	11.7	7.3-15.9	10.9	7.2-15.6	16.6	14.4-25.4
Aminoglycosides	0.2	0-0.2	0.1	0-0.2	0.4	0.2-0.5	0.6	0.3-1.1	0.5	0.3-0.9	1.4	0.9-2.6
Fosfomycin	0.1	0.1-0.3	0.1	0.1-0.3	0.2	0.1-0.4	0.1	0-0.4	0.1	0-0.3	0.5	0.3-1.9
Tigecyclin	0.0	0-0.1	0.0	0-0.1	0.2	0.1-0.5	0.5	0-1.4	0.3	0-1.1	1.7	0.9-5.0
4./5. gen. cephalosporins	0.0	0 - 0	0.0	0 - 0	0.0	0-0.4	0.0	0 - 0	0.0	0 - 0	0.3	0 - 4.4

Consumption density RDD/100PT	Medical services						Surgical services					
	total		<=800		>800		total		<=800		>800	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Total Consumption*	43.4	36.6- 51.1	43.0	35.4-51	49.1	42-59.6	38.0	29.7-46.5	36.9	29.3-45.4	44.0	38.5-48.8
Broad-spectrum antibiotics	17.9	13.8-23.4	17.7	13.7-22.9	21.8	14.9-28.5	10.4	7.3-13.9	10.4	6.6-13.8	10.1	8.8-14.6
Fluoroquinolones	4.8	3.4-7.7	4.6	3.4-6.6	8.5	4.8-9.9	4.0	2.7-5.9	3.9	2.6-5.9	4.7	4 - 5,8
3. gen. cephalosporins	4.8	2.5-7.9	5.5	2.6-8.6	3.0	2 - 4,6	1.7	0.8-3.3	1.8	0.9- 3.7	1.1	0.6-1.7
Piperacillin/enzyme-inhib.	4.8	3.7-6.6	4.8	3.7-6.2	5.8	3.8-8.6	2.3	1.4-3.5	2.3	1.3-3.5	2.8	1.8-3.4
Carbapenems	1.5	0.8-2.1	1.3	0.7-1.9	3.5	1.9-5.1	0.7	0.3-1.1	0.7	0.3-1	1.1	0.8-1.9
Aminoglycosides	0.1	0- 0.2	0.1	0-0.1	0.3	0-0.3	0.1	0- 0.1	0.1	0- 0,1	0.2	0,1-0.5
Fosfomycin	0.1	0-0.2	0.1	0-0.2	0.1	0-0.2	0.1	0-0.3	0.1	0-0.3	0.1	0.1-0.4
Tigecyclin	0.0	0-0	0.0	0-0	0.1	0-0.2	0.0	0-0	0.0	0-0	0.1	0-0.3
4./5. gen. cephalosporins	0.0	0 - 0	0.0	0 - 0	0.0	0 - 0.3	0.0	0 - 0	0.0	0 - 0	0.0	0- 0.1

*total antibiotic consumption: A07AA, J01, J04AB02, P01AB01

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

200/KMP

***Staphylococcus aureus* bacteremia: Latest data from a Medical Microbiology Laboratory Located in the Ruhr Valley**

C. Rompf*[1], M. Bienia[1], A. Sägers[1], A. B. Pranada[1]
[1]*MVZ Dr. Eberhard & Partner Dortmund (ÜBAG), Medical Microbiology, Dortmund, Germany*

Introduction: *Staphylococcus aureus* bacteremia (SAB) is quite frequent [1] and shows high mortality rates of about 30% [2].
Recent studies have shown that evidence based clinical management of SAB can improve the patients' clinical outcome [3].
Objectives: In this study we have analysed recent microbiological as well as clinical data to assess the current situation in SAB. These could be used for antimicrobial stewardship interventions for further improvement of the clinical management of SAB.
Materials and Methods: Data about blood cultures in the nine-month period from January to September 2017 were analysed retrospectively. Additional clinical data were taken from the discharge letters or from direct communication with the clinicians.
Diagnostic was performed using the BD BACTEC blood culture system (BD, Heidelberg). For identification either MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) or VITEK 2 XL (bioMérieux, France) were used. Antimicrobial susceptibility testing was performed according to EUCAST standards.
MRSA isolates were confirmed by an in-house real-time PCR assay targeting the genes for *mecA* and *mecC*.
Results: For the period from January to September 2017, a total of 23547 blood culture bottles from N=5820 individual patients were analysed. About 17.5% of the bottles turned positive. For n=1603 patients (27.5%) at least one bottle showed growth.
A total of n=232 patients (4.0%) showed a SAB. Of these, n=23 (9.9%) could be confirmed as MRSA.
Regarding the detection time of SAB, for 81.9 % of these patients the blood culture became positive within the first 24 hours, 95.8 % within 48 hours.
Further clinical data could be obtained for n=130 patients with SAB (57% male, 43% female, mean age 70.2 years).
Of these, n=19 patients (14.6 %) died. A total of n=55 (42%) had invasive devices.
For 99 patients information about antimicrobial therapy was available. Of these, n=11 (11.1%) initially already received an adequate antimicrobial. For further n=47 patients therapy was changed, resulting in 58.6% receiving an antimicrobial directly targeting SAB.
Regarding the discharging letters, 64/80 (80%) documented the SAB.
Summary: We have evaluated recent data about SAB and its clinical management over a nine-month period.
Regarding diagnostics, detection time was fast with 81.9% of SAB patients having a positive blood culture within the first 24 hours. About 9.9% had an MRSA bacteremia.
Clinical management still can be improved with antimicrobial stewardship interventions as probably more patients could be treated with antimicrobials directly targeting SAB.

References

[1] Laupland, Clin Microbiol Infect 2013; 19:492-500
[2] Kaasch et al., J Infect 2014, 68:242-251
[3] Vogel et al., J Infect 2016, 72:19-28

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Infection Immunology (FG II)

201/IIP

Inhibition of acid sphingomyelinase enhances bacterial induced colitis

J. Meiners*[1], L. Japtok[2], R. N. Kolesnick[3], J. Zöller[1], W. Hansen[1], A. Westendorf[1]
[1]*University Hospital Essen, Institut of Medical Microbiology, Essen, Germany*
[2]*University of Potsdam, Department of Toxicology, Potsdam, Germany*
[3]*Memorial Sloan-Kettering Cancer Center, Laboratory of Signal Transduction, New York, United States*

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder, which is characterized by repeated cycles of mucosal damage, ulceration and regeneration. Although the pathogenesis is not fully understood, impairment of the epithelial barrier function plays an important physiological role. Many signaling molecules involved in the pathogenesis of IBD such as TNF-alpha or IL-1beta cause alterations of the lipid composition in the cell membrane by activation of various phospholipases, sphingomyelinases and other lipid modifying enzymes. Therefore, increasing evidence implicates a function of acid sphingomyelinase in intestinal homeostasis and the development of ulcerative colitis. In the present study, we started to determine the function of acid sphingomyelinase (ASM) during bacterial-induced colitis. *Citrobacter (C.) rodentium* is a natural mouse gram-negative mucosal pathogen that has the ability to regulate epithelial barrier integrity and induces colon pathology very similar to the pathology seen in human IBD. In first experiments we identified that sphingomyelin and ceramide concentrations are significantly decreased in the colon of *C. rodentium* infected mice in a time dependent manner. In contrast, infection of ASM-/- mice or mice which were treated for two weeks with amitriptyline for ASM inhibition, showed an increase in sphingomyelin and ceramide within 7 days post infection. This increase was accompanied by an enhanced loss of body weight and more severe inflammation with strong crypt hyperplasia in the colonic tissue compared to *C. rodentium* infected wild type mice. Interestingly, ASM deficient mice showed higher colony-forming units (CFU) of *C. rodentium* in the liver and spleen compared to infected wild type mice, suggesting an increased translocation of the bacteria from the intestine. These preliminary data support that sphingomyelin play an important role in intestinal inflammation and might therefore be a target for therapeutic intervention. Detailing the impact of this enzyme in intestinal inflammation will allow the development of new and more specific therapeutic strategies in inflammatory bowel diseases.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

202/IIP

MyD88-dependet recognition of *Anaplasma phagocytophilum* by murine granulocytes

B. Müller[1], S. Kirschnek[1], F. von Loewenich*[2,1]
[1]*Department of Medical Microbiology and Hygiene, University of Freiburg, Freiburg i. Br., Germany*
[2]*Institute of Medical Microbiology, University of Mainz, Mainz, Germany*

Question: *Anaplasma phagocytophilum* is an obligate intracellular bacterium that replicates in neutrophil granulocytes. It is transmitted by *Ixodes* spp. ticks and elicits febrile disease in humans and animals such as sheep, cattle, horses, dogs and cats. In the early phase of infection, *A. phagocytophilum* is controlled by NK-cell derived IFN-γ, whereas bacterial elimination strictly depends on $CD4^+$ T-cells. *In vivo*, $MyD88^{-/-}$ mice were unimpaired in pathogen control. Thus, it is not clear to date which pattern recognition pathway leads to immune stimulation by *A. phagocytophilum*.
Methods: To study this, we used in vitro generated murine granulocytes derived from immortalized progenitors with defects for different pattern recognition pathways. Wild-type neutrophils and $MyD88^{-/-}$, $Trif^{-/-}$, $MyD88^{-/-}$ $Trif^{-/-}$, TLR2, 3, 4, 7, $9^{-/-}$, $TLR7^{-/-}$, $TLR9^{-/-}$, $NOD1^{-/-}$, $NOD2^{-/-}$, $NALP3^{-/-}$, $DAP12^{-/-}$ and $FcR\gamma^{-/-}$

neutrophils were infected with *A. phagocytophilum*. The in vitro growth of *A. phagocytophilum* in the gene-deficient granulocytes, the induction of iNOS mRNA and the production of RANTES, MIP-1α, TNF and IL-6 by the host cells were measured.
Results: No growth differences were seen between wild-type and gene-deficient neutrophils indicating that *A. phagocytophilum* might escape the antimicrobial effector mechanisms of granulocytes. Only the TNF-production was significantly lower in MyD88$^{-/-}$, MyD88$^{-/-}$ Trif$^{-/-}$ and TLR2, 3, 4, 7, 9$^{-/-}$ *A. phagocytophilum* infected granulocytes compared to wild-type cells. Since *A. phagocytophilum* lacks the ligands of TLR2, 3, and 4, TLR7$^{-/-}$ and TLR9$^{-/-}$ granulocytes were studied. However, the TNF-production in TLR7$^{-/-}$ and TLR9$^{-/-}$ granulocytes was not significantly lower than in wild-type cells.
Conclusions: Thus, although dispensable *in vivo*, MyD88 signaling might be involved in the recognition of *A. phagocytophilum*. However, the receptor involved remains unclear.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

203/IIP
Functional analysis of lymphotoxin β receptor-regulated effector molecules during the immune response against intracellular pathogens
A. Wichert*[1], U. Sorg[1], D. Degrandi[1], K. Pfeffer[1]
[1]*Heinrich-Heine Universität, Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany*

Introduction: Studies have shown that cytokines are able to induce potent cell autonomous effector mechanisms in host cells which can inactivate pathogens that replicate intracellularly, such as *Toxoplasma gondii* (*T. gondii*). *T. gondii* is an obligate intracellular protozoan parasite that resides and replicates within a so-called parasitophorous vacuole (PV), which protects the parasite from host defence. Data from our lab demonstrate that signalling via the interferon γ receptor (IFNGR) induces a family of murine 65kDa guanylate-binding proteins (mGBPs), which play an essential role for cellular survival after infection with intracellular pathogens. mGBP2 deficient mice (mGBP2$^{-/-}$) exhibit a profound susceptibility to *T. gondii* infection (1). Compared to WT animals, Lymphotoxin β receptor deficient (LTβR$^{-/-}$) mice (2) also show significantly increased mortality: About 40 % of the mice die in the acute phase of infection and further 55 % die during the chronic phase. Additionally, LTβR$^{-/-}$ mice show delayed/decreased up-regulation of mGBP expression in the acute phase of *T. gondii* infection (3).
Objective: The aim of this project is to further elucidate the role of the LTβR in the IFNγ pathway, in the regulation of mGBPs, and in the adaptive immune response to *T. gondii* infection.
Methods: Immune responses (particularly B and T cell responses) of LTβR$^{-/-}$ compared to WT mice after *T. gondii* infection will be characterized in detail using line blots, qRT-PCR, flow cytometry and Western blot analysis. Furthermore, mGBP localization and function in *T. gondii* infected LTβR$^{-/-}$ mice will be investigated in *in vivo* and *in vitro* experiments using immunofluorescence, qRT-PCR and Western blot analysis.
Results: First results confirm that in the acute phase of infection LTβR$^{-/-}$ mice fail to up-regulate mGBP expression in an appropriate manner and preliminary data show defects in Ig-class switching. On the other hand, preliminary data suggests that LTβR$^{-/-}$ mice are able to generate *T. gondii* specific CD8^{+} T cells which could explain the survival of a percentage of LTβR$^{-/-}$ mice. Also, LTβR$^{-/-}$ bone marrow derived macrophages (BMDMϕ) are able to up-regulate mGBPs in an IFNγ dependent manner, although in unstimulated cells baseline expression of at least some mGBPs differs between WT and LTβR$^{-/-}$ BMDMϕ.
Conclusion: These first results together with the upcoming planned experiments will provide new insights about the role of the LTβR in the IFNγ pathway, in the regulation of mGBPs, and in the adaptive immune response to *T. gondii* infection.

References

[1] Degrandi D, Kravets E, Konermann C, Beuter-Gunia C, Klümpers V, Lahme S, Wischmann E, Mausberg AK, Beer-Hammer S, Pfeffer K. *PNAS* 2013;110(1):294-9.
[2] Fütterer A, Mink K, Luz A, Kosco-Volbois MH, Pfeffer K. Immunity 1998;9(1)59-70.
[3] Behnke K, Sorg UR, Gabbert HE, Pfeffer K. Mediators of Inflammation, Volume 2017, Article ID 7375818.

Funding: MOI III – Molecules of Infection III – Manchot Graduate School

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

204/IIP
mGBPs and interacting proteins in host defense
L. Legewie*[1], N. Steffens[1], E. Kravets[1], D. Degrandi[1], K. Pfeffer[1]
[1]*UKD, Med. Microbiology and Hospital Hygiene, Düsseldorf, Germany*

Introduction: *Toxoplasma gondii* (*T. gondii*) is an obligate intracellular protozoan, which is able to infect almost all warm-blooded animals. Invasion results in the establishment of a parasitophorous vacuole (PV) within the infected cell. Members of the murine guanylate binding protein (mGBP) family such as mGBP2 and mGBP7 are rapidly recruited to the PV [1]. mGBP2 has already been shown to attack the parasite directly [2].
Objectives: The first objective of this project, the purification and subsequent analysis of the GTPase activity of mGBP7, will help to provide a better understanding of the biochemistry of GBPs. The next aim of this project is to elucidate the molecular mechanisms that impair the vital functions of the *T. gondii* PV. Therefore, we aim to identify mGBP7 interaction partners originating from the host cell or the invading *T. gondii*. Moreover, we want to unravel the factors required for PV membrane association of mGBP2 and mGBP7.
Methods: The GTPase activity of mGBP7 and its oligomerization status were analysed using the malachite green phosphate assay and SEC-MALS. The interaction partners of mGBP7 will be identified performing co-immunoprecipitation and mass spectrometry experiments. For the verification of mGBP7 interaction partners we will perform FRET and MFIS analysis. The factors required for PV membrane association of mGBP2 and mGBP7 will be determined by the generation of knock-out cell lines of the identified interaction partners via CRISPR/Cas9.
Results: The first objective, the analysis of the GTPase activity of mGBP7, has already been accomplished and yields interesting insights into the biochemistry of mGBPs. The Hill coefficient *h* of mGBP7 indicates a positive cooperativity of GTP hydrolysis, yielding a maximum velocity (v_{max}) of 461.5 nmol P_i/min per mg of protein and a half maximal concentration constant $K_{0.5}$ of 174.9 µM. In accordance to the Hill coefficient the SEC-MALS results support the assumption that mGBP7 stimulates the GTPase acitivity in a cooperative manner.
Conclusion: These first results concerning the GTPase activity of mGBP7 together with the upcoming planned experiments will shed light on the biochemical and biological function of IFNγ-induced mGBP7 in host resistance against the protozoan parasite *T. gondii*.

References

[1] Kravets E, Degrandi D, Weidtkamp-Peters S, Ries B, Konermann C, Felekyan S, Dargazanil JM, Praefcke GJ, Seidel CA, Schmitt L, Smits SH, Pfeffer K. *J Biol Chem* 2012;287:27452-27466.
[2] Kravets E, Degrandi D, Ma Q, Peulen TO, Klümpers V, Felekyan S, Kühnemuth R, Weidtkamp-Peters S, Seidel CA, Pfeffer K. *eLife* 2016;5:e11479.

Funding: SFB 1208 – Identity and Dynamics of Membrane Systems – DFG

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

205/IIP

Shiga toxin 2a upregulates C3 expression in gut and renal cells and also binds to C3

Chatterjee

S. Chatterjee*[1], W. Posch[1], S. Kenno[1], D. Orth-Höller[1], R. Würzner[1]
[1]*Medical University of Innsbruck, Division of Hygiene and Medical Microbiology, Innsbruck, Austria*

Introduction: Complement is a well characterized component of the innate immunity and plays a crucial role in detection and elimination of invading pathogens. Infection with Enterohemorrhagic *Escherichia coli* (EHEC) is a major cause of EHEC-associated Haemolytic Uraemic Syndrome (eHUS) which may progress via uncontrolled activation of complement. Shiga toxin 2a (Stx2a), one of the most potent virulence factors of EHEC, activates complement via the alternative pathway and also binds to factor H (FH) (Orth *et al*., 2009). Although substantial advancements have been achieved in decoding the disease progression, the role of complement in both gut and blood has only recently begun to be explored.
Objectives: The aim of this study is to investigate the role of complement, with special emphasis on the pivotal complement protein C3 expression, in the gut epithelial cell line HCT-8 and the conditionally immortalized glomerulo-endothelial cell line CiGEnC and its further implications upon stimulation with Stx2a.
Methods: An *in vitro* cytotoxicity assay was performed to evaluate the Stx2a concentrations and time points at which the HCT-8 and CiGEnC cells were still resistant to the cytotoxic effects of Stx2a. Further, cells were stimulated for varied time points within this period with suitable concentration of Stx2a and reverse transcription-quantitative PCR (RT-qPCR) was employed to analyze the regulation of C3 in both HCT-8 and CiGEnC cells. Enzyme-linked immunosorbent assay (ELISA) was performed to evaluate the binding of Stx2a to C3b.
Results: Cytotoxicity assay revealed that HCT-8 cells were significantly more resistant towards the cytotoxic activity of Stx2a than CiGEnC cells. RT-qPCR analyses revealed the upregulation of C3 with increasing concentrations of Stx2a in both cell lines, but results being more profound in CiGEnC cells. In addition, Stx2a showed dose dependent binding to C3b when tested by ELISA.
Summary: We herein report the intracellular expression of C3 in gut as well as kidney cells upon stimulation with Stx2a. While in the extracellular space, humoral immunity can impede invading pathogens. Although this is not always sufficient to prevent infection, all cells need innate mechanisms to detect and incapacitate pathogens. We hypothesize that one method of pathogen detection is to take advantage of the intracellular upregulation of complement components. Given that C3 should otherwise be absent inside the cell, we propose this could act as an invasion signal and provide opportunities of further investigations into the pathogenesis in eHUS.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

206/IIP

C-type lectin receptor recognition of cell wall components of different corynebacteria species

C. Kersten*[1], J. Schick[1], B. Lepenies[2], R. Lang*[1]
[1]*Institute of Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany*
[2]*Tierärztliche Hochschule, Hannover, Germany*

Invasive infections caused by non-toxigenic corynebacteria are increasing in several countries. How innate immunity recognizes corynebacteria is only very incompletely understood. Lipoglycans from the coryncebacterial cell wall are recognized by toll like receptor 2 (TLR2), which seems to be vital for the macrophage activation. The members of the order Corynebacterinae, like mycobacteria and nocardia, share a glycolipid-rich cell wall, with abundant mycolic acids. Several C-type lectin receptors (CLRs) have been characterized as pathogen recognition receptor (PRR) binding to carbohydrates and glycolipids present in fungal and bacterial cell walls.

We have recently shown that the CLR Mincle, the receptor for the mycobacterial cord factor trehalose-6,6-dimycolate, directly binds to glycolipid extracts of several pathogenic and non-pathogenic corynebacteria species. Consequently, NO and G-CSF release induced by corynebacterial glycolipid extracts was strongly reduced in macrophages lacking Mincle. TLR2 was essential for activation of macrophages by whole corynebacteria, and induced strong upregulation of Mincle expression, indicating synergy of TLR and CLR pathways in innate responses to corynebacteria. Expression of several other CLR (e.g. Dectin-2, Mcl) is induced by TLR stimulation, and these may contribute to recognition of corynebacteria. Therefore, we here extend the investigation of host sensing of corynebacteria by CLRs, testing a role for Dectin-1, Dectin-2, L-SIGN and MCL. Direct interactions with glycolipid extracts from several corynebacteria strains are analysed in binding assays using CLR fusion proteins. Furthermore, the binding of intact bacteria to CLR fusion proteins is analysed by flowcytometry. These studies will provide additional insight in the cooperation of TLR and CLR pathways in innate defence against corynebacterial infection in humans and animals.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

207/IIP

Local Tissue Perfusion Affects the Outcome of Cutaneous *Leishmania major* Infection in Different Mouse Strains

V. Schatz*[1], Y. Struessmann[1], J. Wild[1], U. Ritter[2], J. Jantsch[1]
[1]*University Hospital Regensburg, Medical Microbiology, Regensburg, Germany*
[2]*Institute of Immunology, Regensburg, Germany*

Native vascularization and capillary density display genetic variability and depend on environmental factors. Moreover, inflammation of infected tissue is correlated with extensive remodelling of capillary network which affects the local perfusion, the oxygen supply, and the outcome of disease. Previously, BALB/c mouse strain was identified to share a different locus responsible for perfusion recovery compared to C57BL/6 strain yielding lower numbers of native collaterals. Therefore, we analysed the contribution of perfusion in cutaneous *Leishmania major* infection model in self-healing C57BL/6 and susceptible BALB/c mouse strains. In C57BL/6 mice, *L. major* skin lesions at maximum size displayed the lowest tissue oxygen levels, which did not affect the perfusion largely. The onset of infected tissue reoxygenation was paralleled by prominent increase in local tissue perfusion and lesion size diminishment. In susceptible BALB/c strain, *L. major* infection showed non-resolving clinical course with continuing increase in lesion size and markedly reduced tissue oxygen levels. Furthermore, infected lesions did not demonstrate any elevation in perfusion values and were not accompanied by tissue reoxygenation. Together, these data suggest that genetic variability highly influences the perfusion of *L. major* infected tissue and support the hypothesis that an increase in perfusion is critical for reoxygenation and thereby resolving the infection.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

208/IIP

iNOS induction inhibits IDO mediated antibacterial effects in human retinal pigment epithelial cells

C. Ufermann[1], K. Spekker-Bosker[1], M. Oldenburg[1], S. K. Eller[1], W. Däubener*[1]
[1]*Heinrich-Heine Universität, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Düsseldorf, Germany*

Infectious uveitis can be caused by a broad spectrum of microbes, e.g. by *Staphylococcus aureus*, human Cytomegalovirus (hCMV) and the apicomplexan parasite *Toxoplasma gondii* (*T. gondii*). Endogenous endophthalmitis is most frequently caused by infections with the gram positive bacterium *Staphylococcus aureus*. Retinal infection by Cytomegalovirus is a frequent AIDS defining disease in HIV-positive individuals. In addition, *T. gondii*-induced retinochorioiditis is a major issue of

ocular toxoplasmosis in infants and immunocompetent adults. Retinal pigment epithelium (RPE) cells form a selectively permeable monolayer between the neural retina and the highly permeable choroidal vessels. Thus, RPE cells bear important regulatory functions and are targeted by pathogens *in vivo*. Upon microbial infection, interferon-gamma (IFN-γ) is produced as one of the major cytokines of the adaptive immune response. IFN-γ induces a broad spectrum of effector molecules, which includes the expression of the tryptophan (trp) degrading enzyme indoleamine 2,3-dioxygenase (IDO). We found that IDO is active against *T. gondii*, *Staphylococcus aureus* and hCMV in hRPE.
Additionally we could identify that the inducible nitric oxide synthase (iNOS) is induced in hRPE by a co-stimulation with IFN-γ, IL-1β and TNFα. iNOS is well known as an effector molecule directed against several bacteria and parasites. Surprisingly we found that iNOS did not act synergistically with IDO and did not enhance IDO mediated antimicrobial effects. Furthermore iNOS activity in hRPE cells was low and not sufficient to mediate antimicrobial effects. But iNOS nearly completely inhibits IDO mediated tryptophan degradation and IDO mediated bacteriostasis. Therefore iNOS expression in hRPE mediates anti-inflammatory effects and blocks overwhelming tryptophan degradation and subsequent production of toxic tryptophan metabolites.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

209/IIP

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

A. Gossens*[1], M. Schneeweiß[2], M. Poceva[1], A. Schmidt[1], K. Loser[2,1], C. Rüter[1]
[1]*Institute of Infectiology, ZMBE, Münster, Germany*
[2]*Institute of Experimental Dermatology and Immunbiology of Skin, Münster, Germany*

Psoriasis is one of the most common immune-mediated chronic inflammatory skin disorders affecting approximately 2-3% of the population. A common disadvantage of all currently available drugs for treating this cutaneous disease is that they need to be applied systemically. Previously, we identified the bacterial effector protein YopM of pathogenic Yersinia as a novel cell-penetrating peptide. Furthermore, once inside the cell, YopM is able to down-regulate the transcription of several pro-inflammatory cytokines such as TNF-α. These novel findings suggest a potential immunotherapeutic application. To investigate whether YopM might be applicable as a topical therapeutic agent for the treatment of psoriasis, we utilized the murine imiquimod (IMQ)-induced psoriasis model. In this model, daily topical application of IMQ on mouse back skin induces psoriasis. Here, we applied rYopM either topically or subcutaneously for 5 consecutive days. 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.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

210/IIP

Characterization of mGBP proteins in the immune response against chlamydia and intracellular parasites

J. Lichte*[1]
[1]*Heinrich-Heine Universität, Institute of Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany*
Jens Lichte[1], Daniel Degrandi[1], Valesca Lindenberg[1], Katja Möllecken[2], Hans Hegemann[2], Klaus Pfeffer[1]
[1]*Institute of Medical Microbiology and Hospital Hygiene, Düsseldorf*
[2]*Functional Genome Research of Microorganisms, Düsseldorf*

Introduction: Guanylate-binding Proteins (GBPs) are expressed after IFNγ stimulation in most cell types and constitute important factors in cell-autonomous immunity. Via GTP-binding and two-step hydrolysis, GBPs are able to relocate to pathogen-containing vacuoles (PCV). Localization-analyses could reveal that during an infection most murine GBPs (mGBPs) are recruited to the parasitophorous vacuole (PV) of *T.gondii* as well as to inclusions of *C.trachomatis* (1). Interestingly, although mGBP-members share a high protein identity (2), strong differences in the localization-frequencies could be obtained with mGBP1 and mGBP2 showing the highest frequencies to both membranous structures (1). Additionally, mGBP9 seems to play an important role in chlamydial infections, as frequency of accumulation to chlamydial inclusions was comparable to mGBP1 and mGBP2. For mGBP9 recruitment to the *T.gondii* PV only moderate localization-frequencies could be detected.
Objectives: Aim of this project is the functional characterization of mGBP9 in different infection models. Therefore an mGBP9-deficient (*mgbp9* -/-) cell- (NIH 3T3 fibroblasts) and mouse-line will be generated. Subsequent *in vitro* and *in vivo* studies shall reveal the molecular function of mGBP9 and if mGBP9-deficiency increases the host susceptibility to various pathogens. Furthermore, co-localization analyses via GFP-tagged mGBP9 and other mCherry-tagged mGBPs will be done to investigate possible interaction partners of mGBP9 on PCV regarding clearance of infections.
Methods: For generating *mgbp9* -/- fibroblasts/mice the CRISPR/Cas9 gene editing system was/will be used. Expression of exogenous mGBP9 will be utilized for molecular function analyses (expression of point-mutated mGBP9) as well as for co-localization analyses (expression of GFP-tagged mGBP9) after IFNγ stimulation and infection with *T.gondii* and *C.trachomatis*. Loss of function effects and co-localization will be monitored by confocal microscopy and other high-performance microscopy techniques.
Results: The generation of *mgbp9* -/- fibroblasts via CRISPR/Cas9 is already accomplished and could be confirmed by PCR and sequencing. To verify the absence of the mGBP9-protein in these cells a mGBP9-specific antibody is currently generated and verified for specificity.
Summary: It was shown that mGBP9 accumulates at PCV such as *T.gondii* PVs and *C.trachomatis* inclusions. In-depth analyses of mGBP9-function and intracellular localization in infection studies could lead to a better understanding of immunity against intracellular pathogens and open new therapy-concepts.

References

[1] Lindenberg et al., PLoS One 2017;12(9):e0185273
[2] Degrandi et al., Immunol 2007, 179 (11) 7729-7740

Funding: RTG1949, DFG

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

211/IIP

Effect of the antimalarial drug pyrimethamine on the resolution of experimental cerebral malaria

R. Bhattacharjee*[1], S. Arnim[1], P. Gobrecht[1], E. Budinger[2], J. Goldschmidt[2], D. Schlüter[1,3], G. Nishanth[1]
[1]*Otto von Guericke University, Institute for Medical Microbiology, Magdeburg, Germany*
[2]*Leibniz Institute for Neurobiology, Magdeburg, Germany*
[3]*Helmholtz Centre for Infection Research, Braunschweig, Germany*

Introduction: Cerebral malaria (CM) is a severe complication of human malaria. Experimental cerebral malaria (ECM), caused by *Plasmodium berghei* ANKA (*Pb*A) is the widely used rodent disease model to study the pathogenesis of cerebral malaria. The accumulation of *Plasmodium*-infected red blood cells to the brain endothelial cells triggers the disruption of the blood-brain barrier (BBB). The drug pyrimethamine has been used successfully to treat malaria. However, the effect of the drug on the resolution of cerebral malaria associated brain pathology remains elusive.
Objective: Our aim is to understand the effect of the drug pyrimethamine on the resolution of experimental cerebral malaria.
Materials and methods: C57BL/6 mice were injected with *Pb*A-infected red blood cells. One group of mice was treated with

pyrimethamine starting at day 5 post infection (p.i.) while the control group received phosphate buffer saline (PBS). The parasite load in the peripheral blood was enumerated daily until day 14 p.i. The intracerebral accumulation of CD8+ T cells was analysed by flow cytometry. The BBB integrity was assessed by Evans blue staining. Expression of chemokines and cytokines in brain was measured by qRT-PCR. Brain pathology was studied by immunohistochemistry. Single-photon emission computed tomography (SPECT) was performed to visualize the changes in regional blood flow.

Results: All of the pyrimethamine treated mice were protected from ECM while the control mice succumbed to the infection by day 7 p.i. Pyrimethamine treatment significantly reduced the parasite burden in erythrocytes, accompanied by reduced infiltration of CD8+ T cells to the brain. Evans blue injection showed minimal damage to the BBB post treatment. The levels of proinflammatory cytokine TNF and chemokines, CXCL-9, CXCL-10 and CXCL-11 were significantly reduced in different regions of the brain post treatment. Pyrimethamine ameliorated brain pathology with reduced neuroinflammation and haemorrhage as well as reduced endothelial cell activation and apoptosis. SPECT imaging of control mice showed heterogeneous and diffused hypo-perfusion in the olfactory bulbs and cortical region, while pyrimethamine treated mice showed normal perfusion in the olfactory bulbs and cortex.

Conclusion: Our study delineates the pathophysiological alterations taking place in the brain upon pyrimethamine treatment, thereby providing a better understanding of the disease resolution. These data could be useful for the development of adjuvant therapies for CM.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

212/IIP

Regulation of innate immune response by cylindromatosis (CYLD) in murine *S. aureus* infection

M. Raptaki*[1], E. Medina[2], D. Schlüter[1,2], G. Nishanth[1]
[1]*Institute of Medical Microbiology, Otto-von-Guericke University, Magdeburg, Germany, Magdeburg, Germany*
[2]*Helmholtz Centre for Infection Research, Braunschweig, Germany*

Introduction: *Staphylococcus aureus (S. aureus)* has long been associated with nosocomial infections. The emergence of methicillin-resistant *S. aureus* has increased the mortality risk in *S. aureus* infection. Therefore the development of alternate therapies becomes all the more essential. The innate immune response against *S. aureus*, which involves mainly neutrophils and macrophages, is primarily mediated by the NF-κB pathway. CYLD negatively regulates the NF-κB pathway by removing K-63 linked polyubiquitin chains from several signaling molecules and thereby regulates the immune response.

Objective: To investigate how deficiency of CYLD regulates the outcome of murine *S. aureus* infection.

Materials and methods: Wild-type (WT) and Cyld$^{-/-}$ mice were intravenously infected with 2 x 10^7 CFU of *S. aureus* and the weight loss was monitored daily until day 49 p.i. To determine how CYLD deficiency influences the pathogen clearance, the bacterial loads in liver, kidneys and spleen were quantified on days 2, 5 and 20 p.i. Cytokine production by macrophages and neutrophils, the two main cell populations which play an important role in immune response to *S. aureus* infection was analyzed by flow cytometry. To delineate the role of CYLD in macrophages, *in vitro* bone marrow-derived macrophages (BMDM) from WT and Cyld$^{-/-}$ mice were stimulated with IFN-γ for 24 hrs followed by infection with *S. aureus*. The bacterial load in the macrophages was enumerated 24 hrs p.i. The production of anti-bacterial nitric oxide (NO) in the culture supernatant was measured by Griess assay. To identify the underlying CYLD-regulated signaling pathways, the activation of NF-κB, ERK1/2 and p38MAPK were analyzed by Western Blot in IFN-γ-stimulated *S. aureus*-infected BMDM.

Results: Our data show that CYLD-deficiency results in less reduction in body weight and improved pathogen control in liver, kidney and spleen. Furthermore, Cyld$^{-/-}$ mice harboured increased numbers of macrophages and neutrophils in the liver and spleen accompanied by increased production of proinflammatory cytokines compared to WT mice. Upon high dose infection the Cyld$^{-/-}$ mice had an increased survival rate compared to WT mice. Additional in vitro experiments showed that CYLD deficiency in BMDM resulted in enhanced activation of NF-κB, p38MAPK and ERK1/2 pathways, and increased production of anti-bacterial NO, leading to an improved pathogen control.

Summary: Our study identifies CYLD as an important inhibitor of anti-staphylococcal immune responses and a potential therapeutic target to treat *S. aureus* infections.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

213/IIP

IFN-α/β is essential for CDN-mediated CTL generation and B cell activation.

D. Lirussi*[1], A. Hamouda[1], K. Schulze[1], T. Ebensen[1], C. A. Guzmán[1]
[1]*Helmholtz Center for Infection Research, Vaccinology and Applied Microbiology, Braunschweig, Germany*

Introduction: Cyclic di-AMP (CDA) and cyclic di-GMP (CDG) are promising adjuvants and immune modulators among cyclic di-nucleotides (CDN). These adjuvants are not only able to induce a profuse antibody production, but also a predominant T helper 1 response and cytotoxic CD8 T lymphocytes (CTL) (Ebensen et al., 2011), which enables its use for vaccination against intracellular pathogens, as well as in cancer immune therapy (Woo et al., 2014;Wang and Celis, 2015). Nevertheless, a comprehensive understanding of CDN mode of action is a prerequisite for their successful translation in the clinic. Since we recently disclose the key role of IFN-α/β signaling for CDA immunogenicity (Lirussi et al., 2017), based on the biological activities of IFN-α/β, we hypothesized that IFN-α/β might be required not only for CTL induction by CDG, but also for B cell activation/maturation under general CDN action.

Objectives: To assess the importance of IFN-α/β signaling on the development of CTL and the activation of B cells post immunization using CDA or CDG as adjuvant. **Materials and Methods:** We immunized WT B6 and IFNAR1-/- (IFN receptor KO mice) with the model antigen ovalbumin (OVA) and CDN and measure intracellular cytokine production by ELISPOT and intracellular cytokine staining. IgG was measured by ELISA. The CTL response was measured after vaccination by a CTL assay (Wang and Golding, 2005). We traced antigenic fate under CDN immunization by flow cytometry and confocal microscopy. CTL proliferation was measured by passive transfers of CFSE stained CD8 OT-I T.

Results: We demonstrate that activation of B cells by these adjuvants is dependent on IFN-α/β signaling which resulted in diminished specific IgG titters. We found that IFN-α/β is essential not only for cross-presentation but also for CTL generation under CDA and CDG stimulation.

Summary: These findings are of particular interest, in view of the many attempts to use CDN in cancer immunotherapies and also for its use in prophylactic vaccines, which in most cases requires an appropriate cellular and humoral response.

References

[1] Ebensen, T., Libanova, R., Schulze, K., Yevsa, T., Morr, M., and Guzman, C.A. (2011). Vaccine 29, 5210-5220.
[2] Lirussi, D., Ebensen, T., Schulze, K., Trittel, S., Duran, V., Liebich, I., Kalinke, U., and Guzman, C.A. (2017). EBioMedicine 22, 100-111.
[3] Wang, W., and Golding, B. (2005). Immunol Lett 100, 195-201.
[4] Wang, Z., and Celis, E. (2015). Cancer Immunol Immunother 64, 1057-1066.
[5] Woo, S.R., Fuertes, M.B., Corrales, L., Spranger, S., Furdyna, M.J., Leung, M.Y., Duggan, R., Wang, Y., Barber, G.N., Fitzgerald, K.A., Alegre, M.L., and Gajewski, T.F. (2014). Immunity 41, 830-842.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

214/IIP

A p38/MK-2-dependent phosphorylation checkpoint controls cytotoxic RIP-1 signaling in bacteria-infected macrophages

J. Gropengießer[1], L. Novikova[1], H. Schimmeck[1], N. Czymmeck[1], K. Ruckdeschel*[1]
[1]*University Medical Center Eppendorf, Institute for Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Yersinia enterocolitica engages a type-III-secretion system to modify host immune responses. One of the translocated Yops, YopP, impairs pro-inflammatory NFkappaB and MAPK signaling pathways and triggers apoptosis in infected macrophages. We wondered about the cellular signals that regulate onset of macrophage apoptosis. Our data show that the host cell kinase RIP-1 and its kinase activity are required for efficient apoptosis induction by *Yersinia*. It was furthermore revealed that YopP suppresses the phosphorylation of RIP-1 conferred by the p38/MK-2 pathway. The MK-2-mediated RIP-1 phosphorylation loop was induced by TLR activation in bacteria-infected macrophages. Interestingly, the inhibition of the phosphorylation of RIP-1 by p38/MK-2 promoted bacteria-induced apoptosis when the IKK-beta kinase activity was simultaneously impaired. This indicates that MK-2 phosphorylates RIP-1 to negatively regulate the RIP-1 pro-apoptotic activity together with IKK-beta. As YopP impairs the activation of p38/MK2 as well as of IKK-beta, the cytoprotective phosphorylation of RIP-1 is lost upon *Yersinia* infection which facilitates RIP-1-dependent apoptosis. Thus, the concerted subversion of both pathways by YopP releases the pro-apoptotic activity of RIP-1 and converts RIP-1 to an inducer of macrophage apoptosis. The inhibition of p38/MK-2 alone was sufficient to promote RIP-1-mediated necroptosis when caspases were blocked. p38/MK-2-dependent phosphorylation therefore controls apoptosis as well as necroptosis induction by RIP-1.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

215/IIP

C-type lectin receptor (CLR)-Fc fusion proteins as tools to screen for novel CLR/bacteria interactions: an exemplary study on *Campylobacter jejuni* isolates

S. Mayer[1], R. Möller[1], J. Monteiro[1], B. Lepenies[1], C. Josenhans*[2,3,4,5]
[1]*Veterinary School Hannover, Hannover, Germany*
[2]*Hannover Medical School, Medical Microbiology, Hannover, Germany*
[3]*Ludwig Maximilians University Munich, Medical Microbiology, München, Germany*
[4]*DZIF, partner site Hannover/Braunschweig, Hannover, Germany*
[5]*DZIF, partner site Munich, München, Germany*

Introduction and Aims: C-type lectin receptors (CLRs) are eukaryotic carbohydrate-binding receptors that recognize their glycan ligands often in a Ca2+-dependent manner. Upon ligand binding, myeloid CLRs in innate immunity are able to trigger or inhibit a variety of signaling pathways, thus modulating effector functions such as cytokine production, phagocytosis and antigen presentation. CLRs are known to bind to various pathogens, including viruses, fungi, parasites and bacteria. The bacterium *Campylobacter jejuni* (*C. jejuni*) is a very frequent Gram-negative zoonotic pathogen of humans, causing severe acute intestinal symptoms. Interestingly, *C. jejuni* has the enzymatic capacity for both O- and N- glycosylation. It expresses multiple glycosylated surface structures, for example the capsular polysaccharide (CPS), lipooligosaccharide (LOS) and envelope proteins.
Methods and Results: The present work describes innovative methods and applications based on CLR-Fc fusion proteins to screen for yet unknown CLR/bacteria interactions using *C. jejuni* as a proof-of-principle example. ELISA-based detection of CLR/bacteria interactions offers the possibility for a first pre-screening that can be confirmed using flow-cytometric-based binding analyses and visualized using confocal microscopy. By applying these methods, we identified mDectin-1 as a novel CLR recognizing two selected *C. jejuni* isolates with different LOS and CPS genotypes.
Summary: In conclusion, the here-described methods and applications of CLR-Fc fusion proteins represent useful methods to screen for and identify novel CLR/bacteria interactions.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

216/IIP

***Candida albicans* alters the Dectin-1 and Dectin-2 host-mediated dendritic cell respons**

N. Reiher*[1,2], M. J. Reza[1], C. Skerka[1,3], P. F. Zipfel[1,2,3]
[1]*HKI Leibniz Institute for Natural Product Research and Infection Biology, Infection Biology, Jena, Germany*
[2]*CSCC Center for Sepsis Control and Care, Jena, Germany*
[3]*Friedrich-Schiller-University, Jena, Germany*

Introduction: Humans are challenged by invading microbes every day. To overcome this attack we need an intact innate immune system that kills the invaders (1). The initial step to induce an appropriate immune response is the recognition of foreign microbes by pattern-recognition-receptors (PRRs) which are expressed on the surface of immune cells, like dendritic cells, that are the linkers between the innate and the adaptive immune system. Dectin-1 and Dectin-2 are PRRs that can bind to highly conserved microbial-associated-molecular-patterns (MAMPs), which are shared by a number of microbial pathogens. Dectin-1 and Dectin-2 are mainly responsible for the recognition of fungal cells wall proteins, like β-glucan and mannan on *Candida albicans* yeast and hyphae (2). *Candida albicans* is a dimorphic fungus that colonizes the skin and mucosal surfaces in most of the humans as a harmless commensal. However, in immunocompromised humans candida can induce severe systemic infections, which often end up in sepsis. Mortality and morbidity rates of patients who suffer from disseminated candida infections are furthermore unbearable high, whereas the development of antifungal drugs is still little successful. Therefore, it is important to investigate the interaction of *C. albicans* and human innate immune cells, i.e. dendritic cells, to understand the molecular mechanisms of fungal counterstrike and immune interference.
Objectives: Aim of this project is to characterize general features how *Candida albicans* evades the recognition by Dectin-1 and Dectin-2 on human dendritic cells by secreting CRASP11 and how CRASP11 influences the dendritic cell function.
Methods: Expression and purification of recombinant CRASP11. For binding studies Biolayer Interferometry, Confocal Microscopy, ELISA and Immunoprecipitation and for functional assays Cytokine ELISAs and Flow Cytometric Analysis were used.
Results: We identified a new candida protein, i.e. CRASP11, which is secreted by the fungus and binds to both Dectin-1 and Dectin-2 with affinities in the nanomolar range. Soluble CRASP11 colocalizes with Dectin-1 and Dectin-2 on the surface of dendritic cells and by activating the cells induces the production of high levels of anti-inflammatory IL-10 in a time- and dose-dependent manner.
Conclusion: *Calbicans* modifies the dectin-mediated pattern recognition by secretion of CRASP11. The immune evasion protein CRASP11 can bind to both Dectin-1 and Dectin-2 on the surface of human dendritic cells and modulates the inflammatory cytokine response. CRASP11 induces anti-inflammatory IL-10 and simultaneously down-regulates the expression of pro-inflammatory mediators (IL-1b and TNF-α).

References

[1] Luo, S., et al., 2013
[2] Brown, G.D., et al., 2002

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

217/IIP

The differential contribution of secreted Ece1 peptides in the *Candida albicans* – macrophage interaction

A. König*[1], L. Kasper[1], S. Mogavero[1], B. Hube[2,3,1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*
[2]*Center for Sepsis Control and Care, University Hospital, Jena, Germany*
[3]*Friedrich Schiller University, Jena, Germany*

The opportunistic human pathogen *Candida albicans* uses different strategies to evade from or adapt to phagocyte antimicrobial activities and to survive phagocytosis. Filamentation of *C. albicans* is induced upon phagocytosis of yeasts by macrophages, which results in escape of the fungus from these immune cells and host cell damage. Further, hypha formation is connected with induction of pro-inflammatory cytokines in these immune cells.
We investigate the role of the hypha-associated gene *ECE1*, which is highly upregulated during filamentation and pathogenicity of *C. albicans*. The encoded protein Ece1 is a polyprotein comprised of eight peptides (I-VIII), of which peptide III (Candidalysin) is capable of producing lesions in host cell membranes[1]. Apart from Candidalysin, almost all other Ece1 peptides are secreted upon hypha formation[1].
ECE1 itself is dispensable for phagocytosis of *C. albicans*, subsequent filamentation in and hyphal outgrowth from macrophages. However, the secreted peptide toxin Candidalysin is involved in immune cell death and NLRP3-inflammasome activation.
We further investigate the role of Non-Candidalysin Ece1 peptides during the interaction of *C. albicans* with host cells. Preliminary data show that peptide V, one of the most abundant secreted Ece1 peptides[1], induces pro-inflammatory cytokine induction in macrophages without damaging the host cell, thus highlighting the importance of Non-Candidalysin peptides during the fungus-host interaction.
Single peptide knockout strains and synthetic peptides are used to address the question of fungal infectivity and the infection process. The secretion of peptides is determined using LC/MS analysis. Further, cytokine production and damage of the host cells is quantified using ELISA or LDH measurement respectively.
Concluding, we propose a dual function of Ece1: Candidalysin-mediated lesion formation is beneficial for *C. albicans* immune evasion by providing an escape route for the fungus, but might as well contribute to antifungal host defense through activation of pro-inflammatory pathways.
Non-Candidalysin Ece1 peptides might facilitate the Candidalysin action, but might as well possess an independent role during the infection process.

Reference

[1] Moyes *et al.* 2016, Candidalysin is a fungal peptide toxin critical for mucosal infection, Nature 532, pp. 64–68

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

218/IIP

***Histoplasma capsulatum* shapes host cell metabolism in alveolar macrophages**

B. Lohse*[1], D. Friedrich[1], D. Zapf[1], G. S. Deepe[2], J. Rupp[1]
[1]*University of Lübeck, Department of Infectious Diseases and Microbiology, Lübeck, Germany*
[2]*University of Cincinnati, Division of Infectious Diseases, Cincinnati, United States*

Histoplasma capsulatum is a dimorphic fungus which is spread globally. Upon inhalation, the pathogen switches from environmental mold form to pathogenic yeast phase causing severe infections in immunocompromised patients. Inside the lungs, *H. capsulatum* is phagocytosed by resident alveolar macrophages (AM). Prior to the adaptive immune response, macrophages are unable to combat the pathogen. During infection, there is granuloma formation which are areas of low oxygen. Herein, macrophages were shown to stabilize hypoxia-inducible factor (HIF)-1α, a known regulator of innate immune response and metabolism. In monocyte-derived macrophages, we could show that enhanced protein levels of HIF-1α enable fungicidal activity against *H. capsulatum*. Analysis of the host cell metabolism revealed higher respiratory and glycolytic activity during infection. HIF-1α stabilizers shut down mitochondrial respiration. In other intracellular infections the modulation of host cell metabolism has already been shown to determine the fate of the pathogen.
We aimed to analyze the influence of HIF-1α and host cell metabolism on pathogen survival in *H. capsulatum*-infected AM.
Human AM were isolated from broncho-alveolar lavage (BAL) of patients. Further, we used murine AM from wild type (*wt*) and myeloid HIF-1α knock-out C57BL/6 mice (HIF-1a-/-). Cells were infected with *H. capsulatum* yeast (MOI 5:1) and analyzed 6 h and 24 h post-infection (hpi). HIF-1α protein was enhanced by pharmacological stabilizer (IOX2) and HIF-1α protein levels were assessed by Western blot analysis. GLUT-1 and PDK1 gene expression was determined by qRT-PCR and host cell metabolism was measured by Seahorse XF96 analyzer. To inhibit glycolysis, cells were treated with 2-deoxyglucose. Intracellular *H. capsulatum* was quantified by plating recovered yeasts on agar 24 h pi and counting colony forming units (CFU) after 7 days.
Histoplasma capsulatum induced HIF-1α in human and murine AM ($p \leq 0.01$, n=3). Further, the expression of HIF-1α dependent metabolic genes GLUT-1 ($p \leq 0.01$, n=6) and PDK1 ($p \leq 0.001$, n=6) was upregulated 2.1-fold and 1.7-fold respectively during infection. In *wt* AM, mitochondrial respiration ($p \leq 0.001$, n=4) and glycolysis ($p \leq 0.001$, n=5) were both enhanced during infection. The absence of HIF-1α did not alter glycolytic activity in infected cells (n=3). Enhanced HIF-1α protein did not affect pathogen survival (n=5) while inhibition of glycolysis reduced viable *H. capsulatum* by 68% +/- 9% 24 hpi (n=5).
Our findings show a crucial role of host cell glycolysis in survival of *H. capsulatum* in AM. In contrast to HIF-1α-dependent fungal immunity in monocyte-derived macrophages, AM use a HIF-1α-independent way in fungal killing. Future investigations aim to identify intermediates of glycolysis responsible for promoting intracellular *H. capsulatum* growth and survival in AM.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

327/IIP

Pancreatic Secretory Granule Membrane Major Glycoprotein GP2 of Different Mammals

C. Bartlitz*[1], J. Noack[1], M. Jakopec[1], R. Kolenda[1], P. Schierack[1]
[1]*Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology, Faculty of Environmental and Natural Sciences, Senftenberg, Germany*

Introduction: GP2 is a protein that is expressed in the pancreas, on membranous cells (M cells) of the small intestine and on cells in various mucous glands in the digestive, respiratory and genital tracts. It can be found extra- and intracellular as well as membranous-bound and secreted. GP2 takes part in binding of type 1 fimbriae (T1F)-positive bacteria. T1F are one of the most adhesive organelles of commensal and pathogenic bacteria such as *E. coli* and *Salmonella*. On the top of T1F the FimH protein is located, which interacts with molecules. Binding to bacteria cause an immune response including autoimmunity against GP2 which is involved in inflammatory bowel diseases such as Crohn"s disease and ulcerative colitis.
Objectives: Several studies have shown that the GP2 structure differs depending on the mammal species. However, GP2 structures of different mammals have not yet been compared.
Materials and Methods: The expression of two human, two porcine and two bovine GP2-isoforms was performed with two different systems. First, the baculovirus expression system was used. For this system bacmid-DNA was generated, which contained the different GP2 isoforms. Then recombinant baculovirus was produced to infect SF9 insect cells that express the protein. GP2 was purified on self-packed gravity flow Ni-NTA resin columns and was evaluated with silver staining. Second, the lentiviral expression system was used. For this system, the GP2 sequences were cloned into plasmid pLVX to generate lentiviruses

containing the GP2 isoforms. Then human, porcine and bovine cell lines were transduced with virus. The cell lines were tested for expression of GP2 by indirect immunofluorescence assay. Proteins and cells could be used for adhesion assays.
Results: The protein expressed by the two systems represented both, secreted and membranous-bound, protein models. The GP2 isoforms of the mammal species differed significantly; moreover, there was a clear difference between the two isoforms of each mammal. This difference was also shown at the indirect immunofluorescence assay. The used antibodies in the assay with bovine isoforms were not prepared for these isoforms; nevertheless, the antibodies bound to GP2. But the signals of both bovine isoforms differed in magnitude.
Conclusion: The GP2 protein varies among mammals. Finally, there are six different isoforms of three mammals which can be used for adhesion assays. Future in vitro studies can help to determine the adhesion of bacteria to GP2 in regard to host specificity.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Eukaryotic Pathogens (FG EK)

219/EKP
Yeast cells formed from *Candida albicans* hyphae differ from primary yeast cells
M. Polke[1], C. Dunker[1], F. Wittig[1], I. D. Jacobsen*[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany*
[2]*Friedrich-Schiller-University, Institute for Microbiology, Jena, Germany*

The ability of the human pathogenic fungus *Candida albicans* to switch between the yeast form and filamentous hyphal growth is an important virulence determinant. Because hyphae are considered to be the invasive form mediating cell damage by physical force and expression of hypha-associated virulence factors, research has focused on analysis of this morphology. However, both hyphae and yeast are involved in the pathogenesis of disseminated infections. It furthermore has been shown that the properties of *C. albicans* yeasts generated in planktonic culture differ from yeast cells dispersed from filaments in biofilms, with the later showing enhanced virulence properties (Uppuluri et al., 2010, PLoS Pathog 6(3): e1000828).
Classical biofilms are not formed in tissue during systemic infection but yeast cells in tissue are likely generated from filaments by mechanisms involving quorum sensing. We aimed to determine whether such yeast cells (termed secondary yeast) also differ from yeast cells grown in non-hypha inducing conditions (primary yeast). Therefore, we grew wildtype *C. albicans* and an *eed1*Δ/Δ mutant, which we previously showed to be hypersensitive to quorum sensing, in hypha-inducing conditions at cell densities that facilitate spontaneous reversal from the hyphal to the yeast growth forms. Secondary yeast cells that budded off filaments were collected and compared to yeast grown in yeast promoting conditions in a comprehensive phenotypic screen.
Secondary yeast cells differed from primary yeast regarding growth in some – but not all – media, resistance to oxidative and cell wall stress, response to inhibitors of mitochondrial function, and the filament-inhibitory effect of farnesol. Importantly, several of these phenotypes were dependent on the temperature used and differed between liquid culture and assays on solid media. Furthermore, we observed alterations in the interaction with some types of host cells. However, and in contrast to what has been reported for biofilm disperser yeast cells, virulence of primary and secondary yeast cells was comparable in a murine model of *C. albicans* peritonitis.
These results demonstrate that *C. albicans* yeast cells display significantly different phenotypes depending on the pre-culture conditions. As the phenotypes were maintained during assays that allowed several generations of replication, it appears unlikely that they can be explained by altered nutrient storage or pre-culture-dependent differences in cell wall composition alone. Rather our observations suggest a certain level of "environmental imprinting" by as of yet unknown mechanisms that may (i) significantly affect the behavior of yeast cells *in vitro* versus *in vivo*, (ii) thereby possibly limiting the conclusions that can be drawn from in vitro phenotype assays for *in vivo* situations, and (iii) highlighting the importance of accurate reporting of pre-culture conditions for the comparison of data.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

220/EKP
tRNA modification influence virulence in pathogenic *Candida* species
B. Böttcher[1,2], P. Sarin[3], H. Drexler[4], S. Leidel[3], S. Brunke*[1]
[1]*Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*ZIK Septomics, Host Fungal Interfaces, Jena, Germany*
[3]*MPI Molecular Biomedicine, RNA Biology Laboratory, Münster, Germany*
[4]*MPI Molecular Biomedicine, Mass Spectrometry Unit, Münster, Germany*

The polymorphic yeast *Candida albicans* is a common cause of disseminated fungal infections, while its close genetic relative, *Candida dubliniensis*, is rarely associated with candidiasis. To investigate these interesting differences in virulence on a genetic level, we performed a screen of reciprocal gene transfer libraries. We found a gene which upon transfer into the *C. dubliniensis* genome phenocopied important morphological traits of *C. albicans*. Unexpectedly, this gene codes for an ortholog of *Saccharomyces cerevisiae* Tcd2, a tRNA-modifying enzyme.
tRNAs are frequently heavily modified in all domains of life to increase translational efficiency and fidelity. However, little is known about the effects of these modifications especially on fungal morphology and virulence. In our screens we found that the *C. albicans* gene, called *HMA1*, strongly affects morphology of *C. albicans* under nitrogen starvation. Even more importantly, in a *hma1*Δ mutant hyphae formation – a central virulence factor of *C. albicans* – was significantly reduced *in vitro* and in an *ex vivo* infection model of human oral epithelium. Despite their close relatedness, these effects were not observed for the *C. dubliniensis* ortholog. We found that these defects also reduced virulence, as *C. albicans hma1*Δ was significantly attenuated in an embryonated egg infection model.
We used liquid chromatography mass spectrometry to quantify ct6A37 tRNA modifications and validated the molecular function of Hma1. Our data indicate that Hma1 has a similar threonylcarbamoyladenosine dehydratase function as Tcd2 in *S. cerevisiae*, but differs in activity between *C. albicans* and its less virulent cousin, *C. dubliniensis*. In fact, different tRNA modification levels of *C. albicans* wild type and mutant, as well as *C. dubliniensis*, suggest a connection to the virulence potential of the strains. Based on these data, we propose a model how tRNA modification levels can affect morphology, and consequently virulence, in *Candida* species.
In summary, our data suggest that tRNA modification to is connected to virulence of *C. albicans* via Hma1, showing that the regulation of fungal pathogenicity mechanisms extends beyond transcriptional networks.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

221/EKP

Secreted Aspf22 from *Aspergillus fumigatus* binds to CD4 expressed by human T helper cells inducing Th2 bias

P. Dasari*[1], M. Kemmisaki[1], I. Shopova[2], D. Wartenberg[2], N. Beyersdorf[3], T. Dandekar[3,4], M. Dittrich[4,5], C. Skerka[1], A. A. Brakhage[2,6], P. F. Zipfel[1,6]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Infection Biology, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Jena, Germany*
[3]*Institute for Virology and Immunobiology, University of Würzburg, Würzburg, Germany*
[4]*Department of Bioinformatics, University of Würzburg, Würzburg, Germany*
[5]*Department of Human Genetics, University of Würzburg, Würzburg, Germany*
[6]*Friedrich Schiller University, Institute for Microbiology, Jena, Germany*

The opportunistic fungal pathogen *Aspergillus fumigatus* is a ubiquitous saprophytic filamentous fungus. *A. fumigatus* can cause a spectrum of diseases ranging from hypersensitivity pneumonitis, allergic asthma and allergic bronchopulmonary aspergillosis to life-threatening invasive aspergillosis infections in immune-compromised individuals. *A. fumigatus* has developed multiple evasion strategies to fight host innate and adaptive immune attack. Aspergillus evades host complement attack by recruiting several plasma complement regulators, like Factor H, FHL-1, C4BP, and plasminogen. In addition, CD4+ T cells regulating adaptive immunity are critical for antifungal immunity. However, the molecular mechanisms of how *A. fumigatus* modulates human CD4+ T cell responses are not clearly understood. We hypothesized that *A. fumigatus* secretes proteins that bind and modulate T cell response. To this end we used a new in-silico approach which predicted that Aspf22 of *A. fumigatus* binds to human T cells via CD4. To confirm this predicted interaction, recombinant Aspf22 from the *A. fumigatus* was expressed and binding to human CD4 was evaluated by ELISA and surface plasmon resonance analysis. Aspf22 bound to CD4 and also bound to human T cells via CD4. Upon stimulation of naive human CD4+ T cells with anti-CD3/CD28 monoclonal antibodies (mAb) binding of Aspf22 to CD4 enhanced expression of the cytokines IL-2, IL-4, IL-5, and IL-10, but not IFNγ or IL-17, as measured by intracellular staining of PMA/ionomycin-restimulated cells. However, Aspf22 alone did not induce any cytokine expression. In addition, Aspf22 induced the expression of the activation markers CD69 and CD25. Furthermore Aspf22 increased/enhanced proliferation of the anti-CD3/CD28 mAb stimulated naïve CD4+ T cells. Thus, by binding to CD4, Aspf22 assists in the activation of human T cells and in their polarization to IL-4 secreting Th2 cells.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

222/EKP

Interaction of eosinophils and *Aspergillus fumigatus* in the context of allergic bronchopulmonary aspergillosis

S. Schruefer*[1], A. Dietschmann[2], D. Voehringer[2], S. Krappmann[1]
[1]*Institute of Microbiology, Erlangen, Germany*
[2]*Department of Infection Biology, Erlangen, Germany*

Introduction: The saprophytic mold *Aspergillus fumigatus* (*Af*) is the causative agent of allergic bronchopulmonary aspergillosis (ABPA). This inflammatory lung disease is prevalent in patients suffering from cystic fibrosis or severe asthma due to impaired fungal clearance of inhaled conidia.
Objective: In allergic inflammations, like ABPA, eosinophils play a crucial role. Therefore, we aim at investigating the interaction of this immune cell type with *Af in vitro*. Further insights are gained by analyzing fungal determinants in the eosinophil activation and fungal killing by co-culture experiments employing defined mutant strains of *Af*.
Methods: To investigate the fungal survival of *Af* wild type compared to mutant strains in co-culture, we established a XTT-based viability assay in this system. The ability of the strains to activate eosinophils is quantified by measuring the release of IL-4 and IL-13 via ELISA. For transcriptome profiling of both cell types, we aim at dual RNA-sequencing with the results to be validated by qRT-PCR.
Results: A reduced fungal viability in the *in vitro* co-cultures could be observed for *Af* wild type conidia compared to *Af* conidia without eosinophils. In a next step, it is investigated to what extent this effect is evident for germ tubes or fungal hyphae. Moreover, a morphotype-dependent release of IL-4 and IL-13 could be measured. Furthermore we could establish a two-step extraction method to isolate total RNA of both cell types from distinct co-cultures as a prerequisite for the envisaged dual RNA-Seq studies to investigate significantly regulated transcripts as determinants of the eosinophil-*Af* interaction.
Conclusion: The results showed an anti-fungal effect of eosinophils and further analyses will reveal whether this effect is due to general growth inhibition or eosinophil induced killing. It could be demonstrated that the activation of eosinophils apparently depends on the fungal surface structure which changes during germination. In addition, RNA-sequencing will give an overview of transcriptional changes in eosinophils caused by *Af*.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

223/EKP

***Candida albicans* factor H binding molecule Hgt1p – a molecule also executing non-canonical functions**

S. Kenno*[1], D. Orth-Höller[1], C. Speth[1], S. Chatterjee[1], N. Gow[2], R. Würzner[1,3]
[1]*Medical University of Innsbruck, Hygiene and Medical Microbiology, Innsbruck, Austria*
[2]*University of Aberdeen, Aberdeen Fungal Group, Institute of Medical Science, Aberdeen, United Kingdom*
[3]*Innsbruck Medical University, Innsbruck, Austria*

Introduction: The opsonisation of fungal surface by C3b represents the key for the killing by immune cells. Factor H (FH) is a negative regulator of the complement alternative pathway increasing the C3b dissociation from host cells surface. For this reason FH is acquired by pathogens, conveying resistance to complement attack.
Objectives: The aim of the study was to highlight whether the blockage of FH binding molecule "high affinity glucose transporter 1" (CaHgt1p), a transmembrane protein in *Candida albicans*, increases the C3b deposition on the fungal surface due to the impairment of FH acquisition and whether this increases PMN phagocytosis. Another aim was to determine whether the *in vitro* data are corroborated *in vivo.*
Methods: FACS analyses were performed to study the blockage of CaHgt1p by an antibody anti-Hgt1p to decrease FH acquisition and thus increase C3b deposition on *C. albicans* surface. A knock-out strain (*hgt1Δ/Δ)* was used as a control. *Candida* strains were opsonized with human serum (HS) and probed by anti-FH and anti-C3b antibodies. In parallel they were stained with FITC and co-cultured with fresh human PMNs. Positive PMNs, with internalized *C. albicans*, were detected by FACS. Western analyses detected CaHgt1 on the cell wall. An *in-vivo* experiment with mice was performed to show whether the blocking of CaHgt1p influences the mice survival. The mice were injected with SN-152 wild type strain or wild type blocked by anti-Hgt1p before injection.
Results: FACS analyses showed a significantly (p<0.05) lower C3b deposition on the wild type strain compared to *hgt1Δ/Δ* knock-out strain or SN-152 blocked by anti-Hgt1p. The significantly higher (p<0.05) FH acquisition on wild type SN-152 led to an increase in C3b deposition. The lower FH acquisition on *hgt1Δ/Δ* knock-out or the blocked SN-152 strains increased significantly phagocytosis by PMNs. The western blotting of extracted cell wall proteins showed the presence of CaHgt1 in this compartment. However, the mice inoculated with SN-152 CaHgt1p blocked by antibodies unexpectedly lived longer than those inoculated with unblocked wild type.
Summary: CaHgt1p on *C. albicans* is significantly increasing FH acquisition on wild type strain, inducing a decrease of C3b opsonisation and also of phagocytosis by PMNs. The potential beneficial effect of the blocking was not seen in mice. We

speculate that CaHgt1p also exhibits non-canonical functions as FH binding molecule, potentially beneficial factor in mice and also as "moonlighting" protein in the cell wall.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

224/EKP

The peroxiredoxin Asp f3 of *Aspergillus fumigatus* protects against transient paralysis by reactive oxygen species

J. Boysen*[1,2], E. Shekhova[2,3], O. Kniemeyer[3], F. Hillmann[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Evolution of Microbial Interactions, Jena, Germany*
[2]*Friedrich Schiller University, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany*

Aspergillus fumigatus is a ubiquitous filamentous ascomycete with a generally saprophytic lifestyle. Especially in immunocompromised patients *A. fumigatus*is also known as opportunistic pathoghen, leading to severe infections. Patients with chronic granulomatous disease have a defect in their NADPH oxidase, which leads to a reduced capability to produce reactive oxygen species (ROS). Since they are also a high risk group, it is assumed that ROS are important for the protection against *A. fumigatus* infections, even so the mechanism is yet unknown. For *A. fumigatus*, the recently characterized major allergen Asp f3, a two-cysteine type peroxiredoxin with moderate thioredoxin dependant peroxidase activity *in vitro*, was shown to be of high relevance for the protection against ROS. The deletion of the gene leads to highly ROS sensitive mutants, which also showed to be avirulent in a mouse model of pulmonary aspergillosis.
Since the protective mechanism of Asp f3 during host invasive growth and in the presence of ROS is unknown, we aim to elucidate its cellular function, as well as the primary biochemical targets of ROS. To monitor the effect of ROS, we established an *in vivo* assay, which allows specific exposure to external pulses of superoxide (O2-), the primary product of the NADPH oxidase in cells of the innate immunity. Additionally we used 2D-gelelectrophoresis to analyse the redox status of the total protein content after peroxide treatment and compared the Δasp f3-strain to the wildtype. Especially proteins with a significantly different redox status were picked and identified as potential ROS-targets for further analysis.

References

[1] Henriet, S., Verweij, P. E., Holland, S. M., Warris, A. (2013). Invasive fungal infections in patients with chronic granulomatous disease. In Hot Topics in Infection and Immunity in Children IX (pp. 27-55). Springer New York.
[2] Hillmann, F., Bagramyan, K., Straßburger, M., Heinekamp, T., Hong, T. B., Bzymek, K. P., Williams, J. C., Brakhage, A. A., Kalkum, M. (2016). The Crystal Structure of Peroxiredoxin Asp f3 Provides Mechanistic Insight into Oxidative Stress Resistance and Virulence of *Aspergillus fumigatus*. Scientific Reports, 6, 33396

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

225/EKP

Knowledge of Families about Malaria in Southeast Part of Iran

H. Edalat*[1], M. Mahmoudi [1], S. Khairandish[2]
[1]*school of public health, Tehran University of Medical Sciences, Medical Entomology and vector control, Tehran, Iran*
[2]*school of dentistry, Bushehr University of medical sciences, Oral and Maxillofacial Pathology, Bushehr, Iran*

At present, Iran is in the process of eliminating malaria, in these conditions, even low number of reported cases is very important. Currently, control of malaria is focused on community-based strategies. Studies from around the world have shown that people in rural areas have a good grasp of infection by malaria while knowledge, attitudes and behavior in field of healthcare is poor. The success of this strategy relies on understanding of human behavior and social, cultural, political, economic, and environmental behavior contexts.
The study on knowledge, attitudes and behavior of people to the control of malaria is very important to find a solution in order to control the disease in this region Kerman province is located in the southeast of the Central Plateau (53°26" E, 25°55" N). Malaria as a health problem has been raised in this district to the extent that in 2006 about 1,000 cases of malaria have been reported. For this purpose a questionnaire with 40 questions was designed in 2 versions which explores various aspects of malaria (case finding, parasitology, the awareness of people living in these areas and to evaluate the economic situation and welfare). This cross-sectional descriptive study was carried out in 2012. Samples from 200 people were selected using two-stage sampling method. Villages were selected in the first stage and then householders were chosen randomly. Data were analyzed using statistical software SPSS V18 and X2 tests.
The results revealed that 45% of the audiences were male and the other 55% were female. The average age of men and women was 31±12.8 and 30±17.8 respectively. 43, 53 and 4% of them were illiterate, had elementary education and diploma respectively; Also, 22 people (11%) of respondents had a history of malaria in the last 5 years that 18 of them had Iranian nationality and the other 4 ones were citizens of Pakistan. As the results show, 198 households (96 %) of respondents had access to electridistrict that 65.6 and 28.1 % of them had access to air conditioner and ceiling fans respectively and 6.3 % of them had no cooling device.
In this study, 93 % of audience believed that mosquito bites causes malaria and 62.5 % of them believed that stagnant water is the location of malaria-carrying spawning; Also, 63.5 % of the inhabitants, believed that the highest prevalence of malaria occur in wet years and 68 % believed it is occur in warm months. The main symptoms were fever and chills and residual spraying as the best method for control the disease by vectors. Audience believed that the main way to obtain information about malaria is through the health workers and healthcare.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Infection Epidemiology and Population Genetics (FG MS)

226/MSP

Genomic and comparative phylogenetic analysis Francisella *tularensis* subsp. *holarctica,* new insights into epidemiology of tularemia in Germany

A. Busch*[1], H. Tomaso[1]
[1]*Friedrich Loeffler Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany*

Francisella (*F.*) *tularensis* is a highly virulent, Gram-negative bacterial pathogen and the causative agent of the zoonotic disease tularemia. In Germany, the European hare (*Lepus europaeus*) is considered the most common source of infection in humans. A field isolate that caused fatal tularemia in a German hare was isolated characterized and sequenced. A high quality circular genome sequence of the *F. tularensis subsp. holarctica* was generated, analyzed and characterized. Besides the genomic structure, the analysis of an oriC, unique to the *Francisella* genus and the genomic DNA and a unique methylation pattern was analyzed. Additionally whole genome sequences from *F. tularensis* isolated in the years 2008- 2015 in Germany were generated. A phylogenetic analysis allowed to determine the genetic relatedness of these and confirmed the highly conserved nature of *F. tularensis subsp. holarctica. The phylogenetic analysis* with different tools for genotyping revealed differences showed different levels of discriminatory power and allowed to established determine a genotyping strategy for *F. tularensis*. It confirmed the highly conserved nature of *F. tularensis subsp. holarctica in Germany.*

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

227/MSP

Diversity of CTX-M-15-producing *E. coli* isolates from food products in Germany

A. Irrgang*[1], L. Falgenhauer[2], J. Fischer[1], H. Ghosh[2], E. Guiral[1,3], B. Guerra[1,4], S. Schmoger[1], C. Imirzalioglu[2], T. Chakraborty[2], J. A. Hammerl[1], A. Käsbohrer[1,5]

[1]*German Federal Institute for Risk Asessment, Biological Safety, Berlin, Germany*

[2]*Justus Liebig University, Institute of Medical Microbiology, and German Center for Infection Research (DZIF), Partner site Giessen-Marburg-Langen, Giessen, Germany*

[3]*Barcelona Institute for Global Health (ISGlobal), Hospital Clínic-Universitat de Barcelona, Barcelona, Spain*

[4]*European Food Safety Authority, Parma, Italy*

[5]*Institute for Veterinary Public Health, University of Veterinary Medicine, Wien, Austria*

Extended-spectrum β-lactamase enzymes (ESBL) mediating resistance to 3rd generation cephalosporins are a major public health issue. Since food or food-producing animals are vehicles for the spread of ESBL-producing bacteria, a deeper knowledge on the occurrence and diversity of cephalosporin-resistant *E. coli* are needed.

In a large study, 404 ESBL-producing isolates derived from animal-derived food samples (e.g. poultry meat, pork, beef and raw milk) were further investigated to characterize the genetic basis of their ESBL-resistance phenotype by molecular analyses, whole genome sequencing and bioinformatical analysis. As CTX-M-15 is the most abundant enzyme in ESBL-producing *E. coli* causing human infections, this study focused on isolates harboring the *bla*CTX-M-15 gene, which were detected in 5.2 % (n=21) of the analyzed *E. coli*. PCR analyses revealed the presence of a phylogenetic group A ST167 clone that was repeatedly isolated from raw milk and beef samples over a period of six months. Sequence-based analyses of these isolates indicated that the spread of CTX-M-15-producing *E. coli* in German food samples was associated with a multi-replicon IncF (FIA; FIB; FII)-plasmid. Additionally, four ST410 isolates of the phylogenetic group A were detected of which three carried a chromosomal copy of the *bla*CTX-M-15 gene, while a single isolate harbored the gene on a 90 kb IncF-plasmid.

In conclusion, CTX-M-15-producing *E. coli* were detected in 5.2 % of the German food isolates of different sources. Among them, two prominent clonal lineages were identified that may be of importance for the dissemination of CTX-M-15-producing *E. coli* via the food chain in Germany. Transmission of CTX-M-15 harboring isolates from food-producing animals to food appears probable, as isolates obtained from livestock and human samples within the same period exhibit comparable characteristics. However, the routes and directions of transmission need further investigations.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

228/MSP

Zoonotic Pathogens in Exotic Pets – Epidemiologic Survey on VSBV-1

V. Allendorf*[1], T. Homeier-Bachmann[1], F. J. Conraths[1]

[1]*Friedrich-Loeffler-Institut, Institute of Epidemiology, Greifswald - Insel Riems, Germany*

In 2015, *Variegated Squirrel Bornavirus 1* was discovered and recognized as a new zoonotic pathogen and as the causative agent in three cases of fatal human encephalitis [1]. Subsequent investigations in the exotic squirrel holders" communities revealed more cases in the captive squirrel population in Germany. The host spectrum of the virus is not confined to the species of variegated squirrels, but is also found in squirrels of the subfamily *Callosciurinae* [2], some of which are commonly kept as pets. The squirrels themselves are asymptomatic virus shedders and can therefore be considered to serve as reservoir hosts, comparable to the white-toothed shrew in the case of *Mammalian 1 Bornavirus* [3]. Viral transmission may occur via biting or scratching lesions; viral RNA was i. a. found in the saliva, feces and epidermal tissue of squirrels [4].

Aims of this epidemiologic survey are the determination of the prevalence of VSBV-1 in the captive Sciurid population in Germany and in elucidating the entry of the virus and its distribution strategies within and among the holdings.

As a first step towards these goals, investigations regarding numbers and distribution of private holdings of all species of the family *Sciuridae* in Germany will be required for creating basic data sets. As there is no registration or control system established concerning these exotic pets, the main source will be the squirrel holder community itself. Data will primarily be gathered by an online survey promoted via social and other media. In addition, the online survey will serve as a recruiting tool for the prevalence study that will be built on the obtained data and on information from zoos where squirrels are held. For further epidemiological analysis and risk assessment, a case-control-study will be conducted.

So far, nine of 91 sampled holdings were found affected, which also included the holding where the first infected variegated squirrel was kept and a number of holdings with proven contact to it. Due to the strong selection bias, these preliminary results only allow a coarse estimation of the prevalence of VSBV-1.

References

[1] Hoffmann, B., et al., *A Variegated Squirrel Bornavirus Associated with Fatal Human Encephalitis.* N Engl J Med, 2015. **373**(2): p. 154-62.

[2] Schlottau, K., et al., *Multiple detection of zoonotic variegated squirrel bornavirus 1 RNA in different squirrel species suggests a possible unknown origin for the virus.* Arch Virol, 2017.

[3] Nobach, D., et al., *Shedding of Infectious Borna Disease Virus-1 in Living Bicolored White-Toothed Shrews.* PLoS One, 2015. **10**(8): p. e0137018.

[4] Schlottau, K., et al., *Variegated Squirrel Bornavirus 1 in Squirrels, Germany and the Netherlands.* Emerg Infect Dis, 2017. **23**(3): p. 477-481.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

229/MSP

NGS-based typing of *Clostridium difficile* using whole genome MLST and whole genome SNP

L. Hauben*[1], S. Kiekens[1], K. De Bruyne[1]

[1]*Applied Maths NV, Sales & Support, Sint-Martens-Latem, Belgium*

Clostridium difficile, is a bacterium that can cause symptoms ranging from diarrhea to life-threatening inflammation of the colon, most commonly affecting older adults in hospitals or in long-term care facilities. *C. difficile* infections typically develop during or within a few months after a course of antibiotics, as good germs that protect against infection are destroyed for several months. During this time, patients can get sick from *C. difficile* picked up from contaminated surfaces or spread through a carrier e.g. health care provider"s hand. Each year, more than a half million people get sick from *C. difficile*, and in recent years, *C. difficile* infections have become more frequent, severe and difficult to treat.

Typing methods are essential epidemiological tools in infection prevention and control. With the advent of benchtop sequencers using NGS technology, bacterial WGS became feasible even in smaller clinical laboratories. WGS has already been used to characterize several outbreaks worldwide and, is likely to replace currently used typing methodologies due to its ultimate resolution. The current bottleneck for WGS routine surveillance resides in the fact that it is laborious and time-consuming.

In this work, we assessed the use of wgMLST and wgSNP to enhance the analysis and detection of bacterial outbreaks. The method was implemented as an easy to use high-throughput data processing pipeline.

Pangenomic wgMLST can be seen as the extension of conventional MLST, incorporating many more loci and thus providing higher

resolution. The pangenomic defined scheme for *C. difficile*, includes over 8000 loci and allows for the detection of subtype- or outbreak-specific markers. To determine the locus presence and to detect the allelic variants, an assembly-free and a BLAST-based algorithm have been implemented. Once allele assignments have been calculated, different (sub-)typing schemes can be defined and used for cluster analysis. A cluster defined as such can then be further characterized by wgSNP. SNP variants are detected by mapping the WGS reads to a reference chosen from within the cluster to maximize the resolution. Analysis is performed on publicly available data.
WGS combined with automated data analysis pipelines holds great promise for epidemiological surveillance of bacterial pathogens. The BioNumerics® 7.6 software makes typing *C. difficile* isolates up to strain level using whole genome sequencing easily accessible to everyone.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

230/MSP

Epidemiology of meningococcal disease in Germany

W. Hellenbrand*[1], H. Claus[2], J. Elias[2], O. Wichmann[1], M. Frosch[2], U. Vogel*[2]
[1]*Robert Koch Institute, Immunization Unit, Berlin, Germany*
[2]*Institut für Hygiene und Mikrobiologie, Nationales Referenzzentrum für Meningokokken und h. influenzae, Würzburg, Germany*

Introduction: In Germany, meningococcal C (MenC) vaccination is recommended for one year-olds and MenACWY and B vaccination for risk groups. Invasive meningococcal disease (IMD) incidence is lower than in many European countries.1
Aims: To describe recent changes in IMD epidemiology.
Materials and Methods: We matched IMD notification data to national reference laboratory data and analysed these over time according to demographics, serogroup, finetype (Serogroup:PorAVR-1,PorAVR-2:FetAVR) and clonal complex.
Results: IMD incidence declined from 0.91 cases/100.000 inhabitants (N=754) in 2002 (MenB: 0.58, MenC: 0.28) to 0.36 in 2015, increasing to 0.41 in 2016 (N=340; Men B: 0.23; MenC: 0.09). Infants had highest incidences (MenB: 4.88; MenC: 0.44) followed by 15-19 year-olds (MenB: 0.77; MenC: 0.13). Significantly decreasing trends were observed in all age groups < 50 years for MenB and < 20 years for MenC. In 2016, MenW and MenY incidence increased to 0.038 and 0.046, respectively, from an annual mean of 0.015 and 0.026 in 2002-2015. While MenW increased in all age groups without a regional pattern, MenY cases increased mainly in teenagers in southwestern German states.
B:P1.7-2,4:F1-5 (ST-41/44cc) remained the commonest finetype and was over-represented in southwestern states. Finetype B:P1.22,14:F5-1 (ST-269) emerged in Rhineland-Palatinate in 2012, the age distribution evolving from predominantly teenagers/young adults to young children. C:P1.5,2:F3-3 and C:P1.5-1,10-8:F3-6 (associated with cases in men-who-have-sex-with-men in 2012-13,2 but not thereafter) remained the most frequent MenC finetypes (both cc11 (ST-11)). In 2016 finetype C:P1.18-1,3:F5-8 emerged predominantly in Berlin, associated with increased MenC incidence in adults. Finetype W:P1.5,2:F1-1 (ST11), associated with increased MenW incidence in several European countries, only accounted for 9/28 MenW cases in Germany in 2016. The remainder were due to finetype W:P1.18-1,3:F4-1 (ST22; N=4), the commonest finetype from 2005-2014 (49% of all MenW strains), and rarer finetypes (N=1-2 cases each). While Y:P1.5-2,10-1:F4-1 remained the commonest MenY finetype, the MenY increase in 2016 was mainly due to Y:P1.5-1,2-2:F5-8 (both ST-23cc/clusterA3).
In 2016, 149/240 tested isolates (62.1%) were sensitive to penicillin; 6.7% were resistant. All were sensitive to cefotaxime, all but one sensitive to rifampicin and 5 resistant to ciprofloxacin (2.1%).
Conclusion: IMD incidence remains low in Germany, with MenB predominant, although small absolute increases in MenC (in adults), MenW and MenY incidence occurred in 2016. Comprehensive surveillance permits early identification of clusters, emergent clones and antibiotic resistance for implementation of preventive measures.

References

[1] ECDC. Stockholm: ECDC; 2016.
[2] Hellenbrand W, et al. PLoS ONE 2016;11:e0160126.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

231/MSP

***Staphylococcus aureus* complex in the Straw Coloured Fruit Bat (*Eidolon helvum*) in Ile-Ife, Nigeria**

A. Olatimehin[1], A. Shittu[1], F. Onwugamba[2], K. Becker[2], F. Schaumburg*[2]
[1]*Obafemi Awolowo University, Department of Microbiology, Ile-Ife, Nigeria*
[2]*University Hospital Münster, Münster, Germany*

Question: Bats are important food sources in some regions in Africa but could also be a reservoir for several pathogens. Sporadic reports suggest that fruit bats could be colonized with the *Staphylococcus aureus* complex, and might disseminated bacteria though faecal shedding. However, it is unclear to what extent humans and bats share identical clones, which might point towards cross-species transmission. The aim of this study was to analyze the prevalence and clonal structure of members of the *S. aureus* complex (i.e. *S. aureus*, *S. argenteus* and *S. schweitzeri*) in Straw Coloured Fruit Bat (*Eidolon helvum*).
Methods: Faecal samples were collected from *E. helvum* in Obafemi Awolowo University, Ile-Ife, Nigeria. *S. aureus* isolates were confirmed by MALDI-TOF mass spectrometry. Antimicrobial susceptibility was tested using Vitek2 automated systems (bioMÃ©rieux). Each isolate was *spa*-typed. Multilocus sequence typing (MLST) was done exemplarily for one isolate per *spa* type. One isolate per *spa* type per sampling site and date was included in the final analysis. Isolates were screened for the presence of *lukS*/*lukF*-PV and the immune evasion cluster (*scn*, *sak*, *chp*) to assess adaptation to the human host. A Neighbor-Joining Tree was constructed using the concatenated sequence of the MLST typing scheme to ultimately distinguish the three species within the *S. aureus* complex.
Results: A total of 250 fecal samples were analyzed and 53 isolates were included in the final analysis (see above). They belonged to *S. aureus* (n=25), *S. schweitzeri* (n=15) and a divergent clade closely related to *S. argenteus* (n=13). All three species were associated with different MLST sequence types (the three most common ST in brackets): *S. aureus* (ST1726, ST3959, ST4047), *S. schweitzeri* (ST3962, ST4316, ST4326) and *S. argenteus*/divergent clade (ST3958, ST3961, ST3980). All isolates were negative for the *scn*, *sak*, *chp* genes. PVL was detected in 80% (n=20) of the *S. aureus* isolates and in 15% (n=2) of *S. argenteus*/divergent clade. No *S. schweitzeri* was PVL positive. All isolates were susceptible to oxacillin, macrolides, glycopeptides, tetracycline and contrimoxazole. Only *S. aureus* was resistant to penicillin (*bla* positive).
Conclusion: We found a high proportion of *S. argenteus*/divergent clade and *S. schweitzeri* isolates from faecal samples of *E. helvum* in Ile-Ife, Nigeria. The absence of antimicrobial resistance and immune evasion cluster suggests a limited exposure of these isolates to the human host.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

232/MSP

Hospital-acquired Methicillin-resistant *Staphylococcus aureus* (MRSA) from Pakistan: molecular characterization by microarray technology

D. Gawlik*[1,2], B. Jamil[3,4], M. A. Syed[5], A. A. Shah[6], S. Abbasi[7], E. Müller[1,2], A. Reißig[1,2], R. Ehricht[1,2], S. Monecke[1,2,8]

[1]*Alere Technologies /Abbott Rapid Diagnostics GmbH, Jena, Germany*
[2]*InfectoGnostics Research Campus, Jena, Germany*
[3]*COMSATS Institute of Information Technology, Department of Bioscience, Islamabad, Pakistan*
[4]*National University of Medical Sciences (NUMS), Rawalpindi, Pakistan*
[5]*University of Haripur, Department of Microbiology, Haripur, Pakistan*
[6]*Fauji Foundation Hospital, Department of Pathology, Rawalpindi, Pakistan*
[7]*Al-Sayed Hospital (Pvt) Ltd. 1-Hill Park, Rawalpindi, Pakistan*
[8]*Technical University of Dresden, Institute for Medical Microbiology and Hygiene, Dresden, Germany*

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a serious problem for many years in healthcare and infection control. Methicillin resistance is associated with *mec* genes (*mecA/C*) on SCC*mec* elements which are potentially mobile genetic elements in Staphylococci. They may contain other genes such as recombinase genes (*ccr*), additional resistance markers (such as *blaZ*, *fusC*) and a variety of accessory genes (including ACME). Up to date, twelve main SCC*mec* types and a wide variety of variants have been described. There is a multitude of data on the population structure of MRSA, and on its changes, from Western Europe, Australia, Japan and the US. However, from other parts of the world such data are, unfortunately, absent. This includes Pakistan. The prevalence of MRSA in Pakistan is known to be high, but very few studies have described the molecular epidemiology of the different MRSA clones circulating in the country.

The purpose of the study was to investigate nosocomial MRSA infections from dialysis/kidney transplant units of two tertiary care hospitals of Rawalpindi in Pakistan. All strains were identified by conventional phenotypic methods and then subjected to genotyping by microarray hybridization. Six clonal complexes (CC) and thirteen strains were identified. The most common strains were i) PVL-positive CC772-MRSA-V, "Bengal Bay Clone" (twelve isolates; 27.3%), ii) ST239-MRSA [III+*ccrC*] as well as a CC8-MRSA-IV strain similar to UK-EMRSA-14 (both with five isolates; 11.4% each) and iii) another CC8-MRSA-IV strain as well as CC6-MRSA-IV (both with four isolates; 9.1% each). Several of the strains detected indicated epidemiological links to the Middle Eastern/Arabian Gulf region. In addition, to the five ST239 isolates mentioned above, one isolate of a second strain of ST239-III was identified that differed in presence of *sasX* and in some SCC*mec* associated genes. Both variants have already been observed elsewhere, but in different geographic regions (Northern Eurasia and South-East Asia). This observation suggests that several, and independent, importations of ST239-III to Pakistan might have occurred.

Further studies are needed to type MRSA from countries with less known epidemiology, such as Pakistan, and to monitor the distribution and spread of strains as well as possible links to global travel, migration and commerce.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

233/MSP

Molecular Characterization of Extended-Spectrum ß-Lactamase-producing *Escherichia coli* Isolates from the University Hospital Erlangen, Germany

G. Valenza*[1], M. Werner[2], D. Eisenberger[1], S. Nickel[1], V. Lehner-Reindl[1], C. Höller[3], C. Bogdan[2]

[1]*Bavarian Health and Food Safety Authority, Hygiene, Erlangen, Germany*
[2]*Universitätsklinikum Erlangen, FAU Erlangen-Nürnberg, Mikrobiologisches Institut – Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany*
[3]*Bavarian Health and Food Safety Authority, Hygiene, Oberschleißheim, Germany*

Introduction: Extended-spectrum ß-lactamase (ESBL)-producing enterobacteria are spreading worldwide in health care settings and represent a threat to human health due to limited treatment options, prolonged hospitalizations, increased mortality, and rising costs. In particular, *Escherichia (E.) coli* multilocus sequence type 131 (ST131) is the most prevalent ESBL-producing *E. coli* in humans and is a major cause of urinary tract infections, urinary sepsis and neonatal sepsis.

Objectives: The aim of this prospective study was to investigate the molecular epidemiology of ESBL-producing *E. coli* at the University Hospital Erlangen, Germany.

Materials and Methods: Between November 2015 and February 2016, a total of 54 consecutive non-replicate clinical isolates of *E. coli* with a resistance to cefotaxime (MIC > 2 mg/l) or ceftazidime (MIC > 4 mg/l) were investigated for the presence of ESBL-genes by PCR and sequencing. Moreover, genetic diversity of all ESBL-producing isolates was investigated by multilocus sequence typing (MLST).

Results: ESBL-genes were detected in 51 isolates. The most common ESBL types were CTX-M-15 (*n* = 21; 41.2%), CTX-M-1 (*n* = 12; 23.5%), CTX-M-14 (*n* = 7; 13.7%) and CTX-M-27 (*n* = 7; 13.7%). Fifty isolates could be assigned to 23 different sequence types (STs), whereas one isolate could not be assigned. The globally dominant ST131 occurred with an overall frequency of 37.2% (*n* = 19). Major non-ST131 sequence types were ST38 (*n* = 4; 7.8%), ST10 (n = 3; 5.9%) and ST1193 (n = 3; 5.9%). Moreover, ciprofloxacin resistance occurred in 16 out of 19 of the ST131 isolates (84.2%) and in 18 out of 32 non-ST131 isolates (56.2%).

Summary: Among the ESBL-producing *E. coli* of this study, ciprofloxacin resistance occurred more frequently in ST131 than in non-ST131 isolates. Further studies need to address whether a reduction of fluoroquinolone usage in hospital settings may help to decrease the prevalence of *E. coli* ST131.

Figure 1

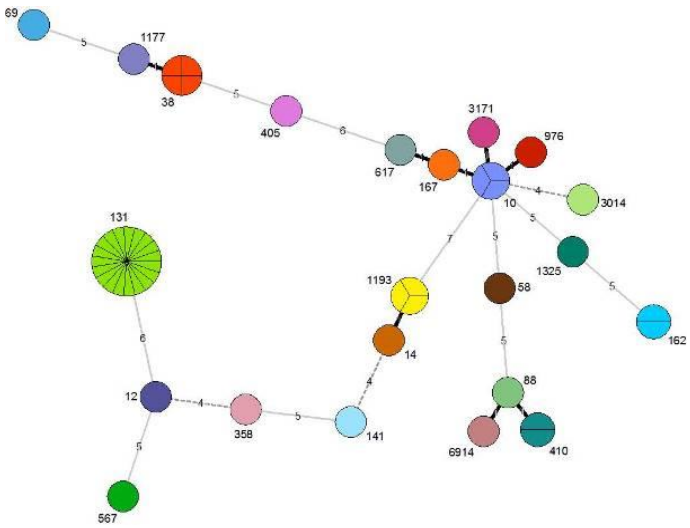


Figure 1: Minimum spanning tree (MST) generated from sequence types (STs) identified among ESBL-producing Escherichia coli isolates investigated in this study by multilocus sequence type (MLST) analysis. STs are displayed by color-shaded-grouped circles with size proportional to the numbers of strains. Branch numbers indicate allele differences between STs.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

234/MSP

Obesity as a risk factor for severe course of influenza virus infection during the 2015/2016 season at the Jena University Hospital

S. Deinhardt-Emmer*[1,2], J. Styra[3,1], R. Egerer[2], M. Baier[2], M. Hoffmann[4], M. Bauer[1,4], M. Schmidtke[3], B. Löffler[3,1]
[1]*Jena University Hospital, Center for Sepsis Control and Care, Jena, Germany*
[2]*Jena University Hospital, Institute of Medical Microbiology, Jena, Germany*
[3]*Jena University Hospital, Section of Virology, Institute of Medical Microbiology, Jena, Germany*
[4]*Jena University Hospital, Department of Anesthesiology and Intensive Care Medicine, Jena, Germany*

Introduction: The 2009 novel influenza A(H1N1) pandemic rapidly spread worldwide and shows the greatest pathogenic potential for humans (1). Influenza A infections (IAV) generate various disease symptoms; however, primary viral induced sepsis is a rare diagnosed disease entity (2). Risk factors for severe course of IAV infection were described previously and recent reports indicate a correlation between obesity and a severe outcome of the A(H1N1)pdm09. Obesity as a comorbid condition is associated with a modified pulmonary host defense against IAV.
Objectives: We report a retrospective review of patients with influenza virus infection to determine outcome of infection and risk factors for Intensive Care Unit (ICU) admission during the influenza season 2015/2016, because seasonal influenza virus-associated bronchopneumonia is one of the infectious diseases with the highest population-based mortality.
Materials and Methods: All patients who had a positive influenza virus result from respiratory specimen during November 1, 2015, and April 1, 2016 were included in this single-center retrospective study. Characteristics of IAV and IBV infected patients were collected from a total of 114 patients. A total of 10 IAV strains (H1N1) were selected sequence analysis and examination of susceptibility to neuraminidase inhibitors with chemiluminescent neuraminidase inhibition assay.
Results: Influenza A (H1N1) is the predominant strain during the 2015/2016 influenza season (56,14%). 114 patients were positively tested and 16 had to be treated at ICU. This group was more frequently infected with IBV (56,25%). A total of 49 patients (83%) have shown a BMI>25, whether 5 patients with BMI>40 were admitted at the ICU. A total of 3 died in septic shock as a consequence of primary virus-associated sepsis. Genotypical analyzation and susceptibility for NAIs revealed no higher virulence of analyzed viruses.
Discussion: Viruses becoming an increasing reason of sepsis. High virulence of influenza A virus or specific host factors could lead to severe courses of infection. The investigated viruses indicate no higher virulence than other viruses of the influenza A(H1N1)pdm09-strains. Obesity could be shown as a risk factor contributing to severity of infection for influenza A(H1N1)pdm09-strain (3), especially because younger people are affected who are not yet suffering from the comorbidities of obesity.

References

[1] **Kong, W. et al.** 2015. Microb Pathog **89:**62-72.
[2] **Singer, M. et al.** 2016. JAMA **315:**801-10.
[3] **Louie, J. K., et al.** 2011. Clin Infect Dis **52:**301-12.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

235/MSP

Trends in the clonal lineages of methicillin-resistant *Staphylococcus aureus* in bacteremia isolates from North Rhine-Westphalia, 2012-2016

J. Schmitz[1], A. Mellmann[1], A. Jurke[2], R. Köck[1], S. Bletz*[1]
[1]*Institute of Hygiene, Münster, Germany*
[2]*North Rhine-Westphalian Center for Health, Münster, Germany*

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of severe nosocomial infections. Previous studies have shown that the clonal structure of MRSA differs regionally and locally due to outbreaks, dissemination via patient transfers or distribution in the community.
Aim: In this study, we investigated the trends in clonal structure of MRSA isolated from bacteremia cases in North Rhine-Westphalia (NRW; 17.8 million inhabitants) from 2012 to 2016.
Method: Microbiological laboratories in NRW were asked to send all MRSA isolates from blood cultures obtained from patients in NRW in 2012 and 2016 to a typing facility together with information on the patient's age, sex and geographic information (first 2 numbers of postal code). All isolates were typed using *S. aureus* protein A gene (*spa*) typing. For cluster formation of *spa* types the Based Upon Repeat Pattern (BURP) algorithm of the StaphType™ software.
Results: Overall, 1,981 MRSA isolates (2012: n=1,083; 2016: n=898) from blood cultures were collected in 17 postal-code-regions (PoR) in NRW. 62.5%; (2012: 61.8%; 2016: 63.5%) of the bacteremic patients were male; mean age was 74 years (2012: range 0-95 and 2016: 0-97). Typing identified 186 (2012: 124 and 2016: 107) *spa* types. The five predominant *spa* types in 2012 were t003 (n=428 isolates; 39.5%), t032 (n=326; 30.1%), t022 (n=31; 2.9%), t264 (n=22; 12.0%) and t045 (n=21; 1.9%). In 2016, the five predominant *spa* types were t032 (n=290 isolates; 32.3%), t003 (n=283; 31.5%), t022 (n=24; 2.7%), t014 and t020 (both n=16; 1.8%).
In 2012, *spa* types belonged to the following *spa* clonal complexes (*spa*-CCs): 003 (48.9%), 032 (41.9%), 008 (1.6%), 038/740 (1.5%), 034 (1.3%), 004 (1.0%), 683/030 (0.6%), no founder (0.2%), singletons (1.4%), and excluded types (1.6%). In 2016, the distribution of the *spa*-CCs was as following: 032 (47.6%), 003 (40.8%), 127 (1.8%), 004 (0.4%), 015 (0.4%), 690/786 (0.3%), five *spa*-CCs with no founder (4.8%), singletons (1.7%), excluded types (2.2%).
The *spa* types t011/t034 characteristic for livestock-associated MRSA occurred mainly in the Muensterland region (2012: 6.0% of all regional isolates and 2016: 8.7%).
Conclusion: Although the general distribution of MRSA types did not change during the last 5 years, we detected regional differences in this representative survey. Current analyses focus on in-depth characterization of differences with respect to the spatial and temporal distribution of MRSA bacteremia cases in NRW.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

236/MSP

Molecular Typing of *Neisseria gonorrhoeae* Strains in Germany (2014 -2016) by *Neisseria gonorrhoeae* Multiantigen Sequence Typing (NG-MAST)

S. Banhart[1], S. Buder[2], T. Pilz[1], S. Dudareva-Vizule[3], T. Taminga[3], E. Guhl[2], I. Graeber[2], V. Bremer[3], P. K. Kohl[2], K. Jansen[3], D. Heuer*[1]
[1]*Robert Koch Institute, Fachgebiet, Berlin, Germany*
[2]*German Reference Laboratory for Gonococci, Hospital Berlin, Germany Department of Dermatology and Venerology, Berlin, Germany*
[3]*Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany*

Introduction: Worldwide, an increase in antimicrobial resistance (AMR) of *Neisseria gonorrhoeae* (NG) is observed threatening treatment and control of gonorrhoea. In Germany, NG infections are not reportable. Thus, data on gonococcal AMR and circulating sequence types are limited. The Gonococcal Resistance Network (GORENET) is a surveillance protocol to monitor trends in the occurrence of antimicrobial resistance of NG in Germany and link this to epidemiological data and NG multiantigen sequence typing

(NG-MAST) data to guide treatment algorithms and target future prevention strategies.
Goals: Describe prevalence and trends of gonococcal sequence types in Germany (2014 to 2016) and associations to AMR.
Materials and Methods: Between April 2014 and December 2016, 1069 NG were collected in the frame of GORENET. AMR was determined by E-test and 320 of these isolates were typed by NG-MAST (Martin et al., 2004). Briefly, DNA was extracted and fragments of *porB* and *tbpB* were amplified by PCR. Fragments were purified and sequenced by Sanger sequencing. Sequence types (ST) were evaluated using the global database www.ng-mast.net. Genogroups were assigned to STs which shared one allele and exhibited >99 % homogeneity in the other allele.
Results: 320 isolates were typed (106 in 2014; 96 in 2015 and 120 in 2016). We detected 100 different STs from 200 isolates in 2014/15 and 77 different STs including 17 new STs in 2016. The most prevalent STs in 2014/15, were ST1407 (n=16), ST2992 (n=13) and ST2400 (n=8). In 2016, ST5441 (n=9) and ST387 (n=8) were the most prevalent STs. The genogroup G1407 decreased from 27 to 5 isolates. Furthermore, numbers of isolates resistant to cefixime slightly decreased.
Summary: The NG population in Germany appears highly diverse. In 2014/15, G1407 was most predominant in Germany. These STs have been associated with cefixime resistance. In 2016, prevalence of G1407 decreased and less resistance to cefixime was detected. Interestingly, prevalence of ST5541 and ST387 increased in 2016 suggesting that clonal replacement could contribute to the observed decrease in cefixime resistance. To verify these results, increasing numbers of isolates should be tested.

Reference
[1] Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. 2004. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J. Infect. Dis. 189:1497–1505.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Gastrointestinal Infections (FG GI)

237/GIP
Comparative analysis of *H. pylori* infection in Nigeria and South Africa
P. F. Palamides*[1], T. F. Jolaiya[2], I. A. Akindele[3], E. Loell[1], M. Carranza[4], A. Clarke[3], S. Smith[2], R. Haas[1]
[1]*Max von Pettenkofer Institute, Faculty of Medicine, München, Germany*
[2]*University of Nigeria, NIMR – Nigerian Institute of Medical Research, Lagos, Nigeria*
[3]*University of Fort Hare, MPMERG - Department of Biochemistry and Microbiology, Alice, South Africa*
[4]*LMU München, Institut für Pathologie, München, Germany*

Around 50% of the world"s population is infected with *Helicobacter pylori* which is the causative agent for development of chronic gastritis, peptic ulceration and a risk factor for certain forms of gastric cancer. Antibiotic resistance is, especially in developing countries, an emerging problem resulting in frequent failure of first-line therapy. Representing one of the oldest bacterial species of the human age, *H. pylori* is also a commonly used organisms to study coevolution and microbe-host interactions. So far the evolutionary most ancient strains have been found in South Africa, making Africa one of the most interesting places for comparison to well-studied European or East Asian strains. Here we want to present the comparison of two study cohorts, from Nigeria and South Africa focusing on the epidemiological situation, common virulence factors and their antibiotic resistance patterns. Therefore we established a cooperation with a study center at the University of Nigeria in Lagos, Nigeria and the University of Fort Hare in Alice, South Africa. For evaluation of the epidemiological situation questionnaires, gastroenterologic and histopathologic findings were evaluated as well as *H. pylori* reisolated from gastric biopsies. Isolates were characterized for multiple virulence factors e.g. CagA-, VacA, DupA expression and correlated with patients gastroenterologic and pathologic findings. Antibiotic resistance to amoxicillin, clarithromycin, metronidazole and tetracycline was examined using E-tests and *in-vitro* growth assays. Phylogenetic classification was performed using MLST analysis, Sanger sequencing and Next Generation Sequencing. The results will be discussed.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

238/GIP
BaiCD gene abundance is negatively correlated with *Clostridium difficile* infection
P. Solbach*[1,2,3], S. Woltemate[2,3], P. Chhatwal[2,3], E. Tacconelli[4,5], M. Buhl[5,6], M. Gerhard[7,8], M. Vehreschild[9,10], N. Jazmati[10,11], J. Rupp[12,13], M. Manns[1,2], O. Bachmann[1,2], S. Suerbaum[2,3,14]
[1]*Hannover Medical School, Dept. of Gastroenterology, Hepatology and Endocrinology, Hannover, Germany*
[2]*German Center for Infection Research (DZIF), Hannover-Braunschweig, Germany*
[3]*Hannover Medical School, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[4]*Tübingen University Hospital, Division of Infectious Diseases, Department of Internal Medicine 1, Tübingen, Germany*
[5]*German Center for Infection Research (DZIF), Tübingen, Germany*
[6]*Tübingen University Hospital, Institute of Medical Microbiology and Hygiene, Tübingen, Germany*
[7]*Technische Universität München, Institute for Medical Microbiology, Immunology and Hygiene, München, Germany*
[8]*German Center for Infection Research (DZIF), München, Germany*
[9]*University Hospital of Cologne, Department of Internal Medicine, Köln, Germany*
[10]*German Center for Infection Research (DZIF), , Bonn, Germany*
[11]*University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Köln, Germany*
[12]*University Hospital Schleswig-Holstein, Department of Infectious Diseases and Microbiology, Lübeck, Germany*
[13]*German Center for Infection Research (DZIF), Hamburg, Germany*
[14]*Max von Pettenkofer Institute, Chair of Medical Microbiology and Hospital Epidemiology, München, Germany*

Background: *Clostridium difficile* infection (CDI) is a major cause of hospital-acquired diarrhea. Preconditions are colonization with *C. difficile*, but also a breakdown of the colonization resistance, of which secondary bile acids are believed to constitute a decisive component. 7α-dehydroxylation is one of the key steps in primary to secondary bile acid transformation, and required genes have been located in a single *bile acid inducible* (bai) operon in *C. scindens* as well as in *C. hiranonis*. The prevalence of these species in human fecal samples – particularly with regard to samples tested positive for *C. difficile* – and in the setting of fecal microbiota transfer (FMT) in CDI patients has not been studied.
Aim: To analyze *baiCD* gene abundance in *C. difficile* positive and negative fecal samples.
Material and Methods: A species-specific qPCR for detection of *baiCD* in *C. scindens* and *C. hiranonis* was established. Fecal samples of patients with CDI, toxigenic *C. difficile* colonization (TCD), non-toxigenic *C. difficile* colonization (NTCD), of *C. difficile* negative (NC) patients, and of two patients before and after fecal microbiota transfer (FMT) for recurrent CDI (rCDI) were tested for the presence of the *baiCD* gene cluster.
Results: In NC samples, the prevalence of the *baiCD* gene cluster was significantly higher than in CDI samples, with 72.5% (100/138) vs. 35.9% (23/64; $p<0.0001$), respectively. No differences were seen between NC compared with NTCD samples or TCD samples. Both rCDI patients were *baiCD* negative at baseline, but one patient turned positive after successful FMT from a *baiCD* positive donor. B*aiCD*/16S rDNA ratio calculation showed no significant difference between the relative abundances in both groups (NC vs. CDI; $p=0.3244$). The median of the NC group was 0.006% (IQR 0.0005%-0.376%) and for the CDI group 0.009% (IQR 0.0002%-7.8%). In addition, stool consistency did not measurably influence the relative abundance of bacterial species in this context of complex microbial communities.
Conclusion: *BaiCD* gene positive species are reduced in fecal samples of patients with *C. difficile* infection as compared to asymptomatic carriers or *C. difficile* negative fecal samples.

Furthermore, we present a case of *baiCD* gene positivity observed after successful fecal microbiota transfer for recurrent CDI.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

239/GIP

Analysis of the interaction between the human pathogen *Helicobacter pylori* and neutrophils

I. K. Behrens*[1], B. Busch[1], R. Haas[1]
[1]*Max von Pettenkofer Institut, Medizinische Mikrobiologie und Krankenhaushygiene, München, Germany*

Helicobacter pylori (H. pylori) is a Gram negative, spiral shaped, motile human pathogen colonizing the stomach of half of the worlds population. An infection with these bacteria can cause mucosa associated lymphoid tissue, ulcer disease, and gastric cancer. In the human body neutrophils act as a first line of defence. They produce reactive oxygen species and phagocytose bacteria to protect against pathogen attacks. The first step of the infection process is adherence of the bacteria to host cells involving adhesins. *H. pylori* interacts via its outer membrane proteins like HopQ on cell receptors, such as carcinoembryonic antigen related cell-adhesion molecules (CEACAMs). Afterwards the bacterium injects its oncoprotein CagA via its type IV secretion system (T4SS) into the host cells, where it gets phosphorylated. The aim of this project is to study the interaction between *H. pylori* and neutrophils and their consequences for signalling and migration. Therefore CagA translocation was examined via a ß-lactam dependent reporter system and CagA phosphorylation via Western Blot analysis. Furthermore the production of reactive oxygen species, phagocytosis and migration were studied. *H. pylori* translocate CagA via its T4SS into neutrophils, whereas neutrophils phagocytose *H. pylori*. How is *H. pylori* able to survive in the human stomach and persist there for a long time? These studies might lead to a better understanding of the host pathogen interaction.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

240/GIP

Minimal SPI1-T3SS effector requirement for *Salmonella* enterocyte invasion and intracellular proliferation *in vivo*

K. Zhang[1], A. Riba[1], M. Nietschke[2], N. Torow[1], U. Repnik[3], A. Pütz[1], M. Fulde[4], A. Dupont[1], M. Hensel[2], M. Hornef*[1]
[1]*RWTH Aachen, Institute of Medical Microbiology, Aachen, Germany*
[2]*University of Osnabrück, Division of Microbiology, Osnabrück, Germany*
[3]*University of Oslo, Dept. of Biosciences, Oslo, Norway*
[4]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*

Non-typhoidal *Salmonella* represent a major causative agent of gastroenteritis worldwide. Hallmark of the pathogenesis is their ability to actively invade the intestinal epithelium by virtue of their type III secretion system (T3SS) that delivers bacterial virulence factors directly into the host cell cytosol. The role of these virulence factors during enterocyte entry and intraepithelial growth has only been investigated *in vitro.* However, immortalized cell lines lack the overlaying mucus layer, final cell lineage differentiation, apical-basolateral polarization as well as continuous migration along the crypt villus axis. Here we took advantage of our new neonatal infection model that allows visualization of bacterial entry and intraepithelial growth and employed it in combination with *Salmonella* Typhimurium strains carry various single and multiple mutant on major *Salmonella pathogenicity island* (SPI)1 encoded virulence factors as well as wildtype and gene-deficient mice. Our results demonstrate the essential but redundant role of SipA and SopE2 for enterocyte invasion and mucosal translocation. In contrast, the formation of intraepithelial microcolonies, the recruitment of Lamp1 to *Salmonella* positive endosomes and SPI2 reporter activity, require expression of either SipA and SopB or SipA and SopE2 but are independent of SopA as well as host MyD88 signaling. Our results thus provide the first characterization of the minimal set of SPI1 T3SS factors required for enterocyte invasion and intraepithelial proliferation *in vivo*.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

241/GIP

The influence of postnatal infectious challenge on enteric tissue maturation

S. Schlößer*[1], N. Fazel Modares[1], K. Zhang[1], U. Repnik[2], A. Dupont[1], M. Hornef[1]
[1]*University Hospital Aachen, Institute of Medical Microbiology, Aachen, Germany*
[2]*Oslo University, Section for Physiology and Cell Biology, Oslo, Norway*

Enteric pathogens remain a major health problem worldwide, especially for young children. A better understanding of the mechanisms that drive disease development and progression in neonates and young infants is crucial to improve therapeutic options.
Here, we used the neonatal *Salmonella enterica* subsp. enterica serovar Typhimurium infection mouse model established by Zhang *et al.* (2014, PLoS Pathog 10(9): e1004385) to investigate the influence of early infection on small intestinal cell differentiation and tissue development in mice. Gene expression and immunofluorescence analyses reveal alterations of the antimicrobial peptide repertoire as well as changes in cell differentiation and metabolic enzyme activity. Together, our results indicate a possible impact of early *Salmonella* infection on intestinal tissue development with a shift of both small intestinal innate immunity and metabolism towards a more adult phenotype.
Our work is expected to give new insights on the short and long term consequences of neonatal enteric infection on intestinal homeostasis and disease susceptibility.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

Microbiota, Probiota and Host (FG PW)

242/PWP

Getting closer to *in vivo*: A 3D cell culture model to study the molecular impact of probiotic *E.coli* Nissle 1917 on the host

J. Gonzalez*[1], L. Pund[1], M. A. Schmidt[1], C. Cichon[1]
[1]*Institute of Infectiology, Microbiology, Münster, Germany*

Infectious diseases contribute to a wide range of deaths worldwide. With regard to the increasing problems of antibiotic resistance, there is an urgent need for the development of novel therapeutics. New *in vitro* cell culture models are necessary that bridge the gap between cell culture based research and more complex *in vivo* animal models. There has been a recent shift from the original "flat biology" approach (2D) to three-dimensional models (3D) of human intestinal epithelia which more precisely mimic the *in vivo*-like phenotypic characteristics like cell polarity, barrier function, and mucin production. This offers the possibility of a meaningful dissection of host-microbe interactions.
Employing the mucus-producing human colonic cell line HT-29 MTX E12, we compare the cellular responses after co-incubation with the probiotic *E. coli* strain Nissle 1917 under different cell culture conditions. HT29 MTX-E12 cells growing on transwell filters result in polarization and the formation of functional tight junctions with a three dimensional architecture resembling colonic crypts [1]. Nevertheless, gastrointestinal epithelial cells grown in standard transwell cultures still do not mimic a proper mucosal surface. A wide range of 3D cell culture systems exist by now. We employ the NASA-designed rotating wall vessel (RWV) bioreactor, which has been used for studying the cellular and molecular responses of hosts and microbes [2]. The RVW allows dynamic culture conditions of epithelial cells under low physiological fluid shear stress which influences cellular differentiation and development. Furthermore cells growing on Cytodex-3 micro-carrier beads (collagen type-I-coated) lead to a 3D tissue-like matrix.

Our preliminary results derived from the gene expression profiles of HT-29 MTX E12 cells (RT2 Profiler Arrays, Qiagen), revealed distinct differences between our cell culture models. The 3D cell culture model generates meaningful host responses to microbes, more closely comparable to an *in vivo* infection. These might help to provide a better understanding of the fundamental mechanisms governing host-microbe interactions as well as a potential faster translation of basic science to the clinics.

References

[1] Navabi N, McGuckin MA, Lindén SK. (2013) Gastrointestinal cell lines from polarized epithelia with an adherent mucus layer when cultured in semi-wet interfaces with mechanical stimulation. PloS One. 8:e68761.
[2] Barrila J, Radtke AL, Crabbé A, Sarker SF, Herbst-Kralovetz MM, Ott CM, Nickerson CA. (2010) Organotypic 3D cell culture models: using the rotating wall vessel to study host-pathogen interactions. Nat Rev Microbiol. 8(11)

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

243/PWP

Identification and characterization of immuno-active factors of *Enterococcus faecium*

F. Ghazisaeedi*[1], L. H. Wieler[1,2], M. Fulde [1], K. Tedin[1]
[1]*Freie Universität Berlin, Institut für Mikrobiologie und Tierseuchen, Berlin, Germany*
[2]*Robert Koch-Institut, Berlin, Germany*

Introduction: *Enterococcus faecium* SF68 (NCIMB 10415) is a bacterial probiotic strain shown to improve symptoms of intestinal inflammation and infection in human and animal studies. In a prior animal feeding trial in our lab, significant reductions were detected in immune-associated gene expression of intestinal tissues and associated lymphoid organs in post-weaning piglets supplemented with the probiotic strain[1].
Aim: To Identify and characterize possible factors involved in observed immunomodulatory effects of *Enterococcus faecium* SF68 using *In vitro* models.
Materials and Methods: Cell-free, whole bacterial lysates of commensal and pathogenic *Enterococcus* strains were screened using an NF-κB reporter derivative of the porcine intestinal epithelial cell line IPEC-J2 and the human intestinal epithelial cell line Caco-2 to determine effects on host cell NF-kB activtion. The kinetics of NF-κB activity of treated cells were determined over a 24h period. Cytotoxic effects and host cell recovery of NF-κB activity after removal of bacterial lysates was also determiend, as well as responses to different TLR-ligands and NOD protein-ligands
Result: All tested *E. faecium* isolates showed inhibitory effects on NF-κB activation, compared to untreated cells. In contrast, lysates prepared in the same manner and concentrations from *E. avium*, *E. gallinarum* and *E. casseliflavus* isolates did not show similar effects. No measurable host cell cytotoxicity was detected using different cell viability and cytotoxicity assays. Treated cells no longer responded to TLR2, TLR1/TLR2, or TLR4-ligands, although the cells remained capable of NF-κB activation in response to TLR5- and TLR3-ligands. Assays using proteinase K or heat treatment indicate the factor(s) present in *E. faecium* SF68 are proteinaceous in nature.
Summary: The results indicate *E. faecium* strains produce an immuno-suppressive factor with inhibitory effects on NF-κB activation in porcine and human intestinal epithelial cell lines.
Keywords: *Enterococcus faecium* SF68, NF-κB, intestinal epithelial cells

Reference

[1] Siepert. et al (2014), *Enterococcus faecium* NCIMB 10415 supplementation affects intestinal immune-associated gene expression in post-weaning piglets, *Vet Immunol Immunopathol*, 157:65-77.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

244/PWP

Intestinal inflammation and *salmonella* typhimurium colonization are influenced by epithelial fucosylation status

A. Galeev*[1], A. Suwandi[1], T. Sterzenbach[2], P. Rausch[3,4], J. Baines[3,4], G. Grassl[1]
[1]*Medizinische Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Hannover, Germany*
[2]*University of Osnabrück, Division of Microbiology, Osnabrück, Germany*
[3]*Max Planck Institute for Evolutionary Biology, Evolutionary Genomics, Plön, Germany*
[4]*Christian-Albrechts-University of Kiel, Kiel, Germany*

Introduction: The *FUT2* gene encodes the α-1,2-fucosyltransferase – an enzyme responsible for the expression of ABO and Lewis histo-blood group antigens on the gastrointestinal epithelium and in bodily secretions. It is estimated that approximately 20% of Caucasians ("non-secretors") have nonsense mutations in *FUT2*, and thus, lack certain glycan structures in mucus and on epithelial cells. *Salmonella* utilizes multiple fimbriae for adhesion to and subsequent invasion of host tissues. The *std* fimbrial operon of *S*. Typhimurium encodes the π-class Std fimbriae, which binds terminal α-1,2 fucose residues.
Objectives: To determine the role of FUT2-glycans for *S*. Typhimurium infections.
Materials and Methods: *S*. Typhimurium WT and *stdA*-deficient strains were evaluated for their ability to colonize *Fut2*$^{+/+}$ and *Fut2*$^{-/-}$ mice. Effect of the *stdA* overexpression in *E. coli* was tested *in vitro* in human epithelial cell lines expressing α-1,2 fucose on the surface. StdA fimbriae expression was assessed by flow cytometry, western blotting and immunohistochemical staining. Fucosylation status was confirmed by lectin staining.
Results: The intestine of *Fut2*$^{-/-}$ mice was significantly less colonized with *Salmonella* compared to *Fut2*$^{+/+}$ controls. Furthermore, decreased histopathological changes and less inflammatory cell infiltration were observed in the colon tissue of *Fut2*-knockout mice compared to the *Fut2*$^{+/+}$ controls. Interestingly, the increased bacterial colonization and inflammation in *Fut2*$^{+/+}$ mice were abrogated upon infection with a *S*. Typhimurium *stdA*-deficient strain. Adhesion assays revealed that *E. coli* expressing Std fimbriae adhered significantly better to differentiated, *FUT2*-expressing Caco2 and HT29-MTX-E12 cell lines than control *E. coli*. Adhesion was sensitive to competition with UEA-1 lectin which has affinity to α-1,2 fucose.
Summary. Overall, these results indicate that Std-fucose interaction is important for colonization and *Salmonella*-triggered intestinal inflammation.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

245/PWP

Action of Probiotic Bacteria against Skin Pathogens

G. Khalfallah*[1], E. Heine[1], M. Möller[1], R. Lütticken[1]
[1]*DWI Leibniz-Institute for Interactive Materials, Aachen, Germany*

Probiotic bacteria ("probiotics"), mostly from the order *Lactobacillales*, are ascribed to exert beneficial effects on their host. Usually they are applied by the oral route thereby inhibiting oral or intestinal pathogens or acting favorably on the intestinal or on the entire immune system.
Question: Few data exist on the application of probiotics by other than the oral route or on other surfaces of the human body, especially onto the skin[1]. Our approach uses dried living probiotics enclosed into devices allowing the diffusion of their antimicrobial products acting against skin pathogens. Such devices could be used to treat skin diseases like acne, venous leg ulcers, or burn wound infections.
Methods: Four probiotic strains known to produce antimicrobial substances were selected: 2 *Streptococcus salivarius* (K12 and M18)[2], 1 *Lactobacillus plantarum* (8P-A3)[3], and 1 *Bacillus coagulans* (DSM-1). These strains were tested for their antimicrobial action on selected strains of the skin pathogens

Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Cutibacterium acnes*. The antagonism was investigated by four plate methods: (1) The Line test, where a streak of the pathogen was applied on the agar, and then the probiotic was streaked perpendicularly to the pathogen line; (2) the Membrane test where the pathogens were incorporated into the agar medium, a membrane placed on the surface, and the probiotic was applied onto the membrane; (3) the Double-Layer-Agar test where the probiotics were inoculated into the lower medium layer and the target bacteria were spotted onto the surface of the upper layer[3]; (4) the Deferred Antagonism test[2].

Results: The *L. plantarum* and the two *S. salivarius* showed an antagonistic activity against all the target bacteria in the Line test. *B. coagulans* also inhibited *C. acnes* and *S. aureus*. The inhibitory effect of the two *S. salivarius* and *B. coagulans* against *S. aureus* and *C. acnes* was confirmed by the Membrane test. In the Double-Layer test *P. aeruginosa* and *S. aureus* were also inhibited by all the incorporated probiotic bacteria. The *S. salivarius* tested by the Deferred Antagonism method displayed a clear antagonistic activity against the target bacteria. The *L. plantarum* proved also its efficacy against *C. acnes* in this test. The highest antimicrobial activities of the lactic acid bacteria were seen, when they were cultivated anaerobically.

Conclusions: We could show that certain probiotic lactic acid bacteria produce antimicrobial substances that diffuse through semipermeable membranes. Our intention is to apply this principle to treat skin diseases to which the pathogens contribute.

References

[1] F. H. Al-Ghazzewi and R. F. Tester, Beneficial microbes 5 (2), 99-107 (2014).
[2] J. Manning, E. M. Dunne, P. A. Wescombe, J. D. Hale, E. K. Mulholland, J. R. Tagg, R. M. Robins-Browne and C. Satzke, BMC Microbiol 16 (1), 225 (2016).
[3] A. Tsapieva, N. Duplik and A. Suvorov, Beneficial microbes 2 (4), 255-261 (2011).

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

National Reference Laboratories and Consiliary Laboratories (StAG RK)

246/RKP

Evaluation of *in silico* tools for sequence-based serovar prediction of *Salmonella spp.* at the National Reference Center

S. Banerji*[1], S. Simon[1], A. Tille[1], A. Flieger[1]
[1]*Robert Koch-Institute, Wernigerode, Germany*

Introduction: The genus *Salmonella* comprises more than 2.600 different serotypes. Serotype determination is crucial for comprehensive pathogen surveillance. The German National Reference Center (NRC) for *Salmonella* and other bacterial enteric pathogens at the Robert Koch-Institute receives around 3,000 *Salmonella* isolates from human infections per year for further characterization. Classical subtyping methods like serotyping are still prominent at NRC, however Next Generation Sequencing-based techniques are gaining more and more importance.

Aim: The seroformula of *Salmonella* spp. consists of the lipopolysaccharide (O-) antigen and two alternatively expressed flagellar (H-) antigens. Classical serotyping utilizes specific antisera for agglutination of the respective antigens. With the availability of whole genome data, we wanted to assess the suitability of two selected *in silico* subtyping methods for routine serotype prediction. The first tool named SeqSero (by Zhang and colleagues) determines the serotype based on the loci for the relevant O- and H-antigens in the bacterial genome. The outcome of this method is thus analogous to classical serotyping. The second method developed by Achtman and colleagues is based on a 7 locus MLST scheme, which looks for allelic differences in 7 conserved housekeeping genes and reveals distinct sequence types (STs). Closely related STs can be arranged into e-burst groups. Since e-burst groups strictly correlate with the serotype, this method can be used for indirect serovar prediction.

Methods: We analyzed 562 *Salmonella* genomes from the NRC strain collection. Whole genome sequencing was performed on a MiSeq benchtop sequencer using Illumina"s v3 chemistry. Raw reads were submitted to the SeqSero software (www.denglab.info/SeqSero) that was adapted for batch analysis. The 7 gene MLST was performed with the Ridom™ SeqSphere+ software where the scheme described above (available from www.enterobase.warwick.ac.uk) was pre-installed.

Results: The analysis of 562 *Salmonella* genomes with the two *in silico* typing tools yielded a high correlation with our laboratory serotyping results, i.e. 93.7% for SeqSero and 95.2% for MLST. SeqSero assigned the wrong serotype to one isolate and failed in yielding a serovar for 20 isolates. MLST failed assigning a ST to 18 isolates, with 8 isolates being non-typeable by both methods. In nine cases of divergent results between laboratory and *in silico* methods, both *in silico* tools predicted an identical serovar, indicating that the respective laboratory results were incorrect.

Summary: The evaluation of two different *in silico* methods for *Salmonella* serotype prediction based on whole genome sequencing data showed that both methods highly correlated with laboratory serotyping results. Since both methods complement each other with respect to the provided information, we conclude that a combination of both tools is best suited for routine *in silico* serotyping at the NRC.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

247/RKP

Report of the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2017

N. Pfennigwerth*[1], M. Korte-Berwanger[1], A. Anders[1], F. Lange[1], J. Schauer[1], S. G. Gatermann[1]
[1]*Ruhr-Universität Bochum, Medizinische Mikrobiologie, Bochum, Germany*

Question: Multidrug-resistance in *Enterobacteriacea*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drug 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 still challenging for the microbiological laboratory.

Material and 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 and WGS methods allow the detection of still unknown β-lactamases.

Results: A total of 5233 isolates were investigated for carbapenemases in the National Reference Laboratory in 2017 until October 11th. Specimen sources were mostly rectal swabs (23.3 %), urinary (15.7 %) and respiratory samples (12.5 %). Carbapenemases were found in 1408 *Enterobacteriaceae* strains (47,3 %), 364 *P. aeruginosa* (27.9 %) and 391 *A. baumannii* (97.3 %). The most frequent carbapenemases in *Enterobacteriaceae* were OXA-48 (n = 515), VIM-1 (n = 265), KPC-2 (n = 147), NDM-1 (n = 213), KPC-3 (n = 62), OXA-181 (n = 58), NDM-5 (n = 57), OXA-232 (n = 47) and OXA-244 (n = 24). NDM-4, NDM-7, VIM-2, VIM-4, VIM-12, VIM-52, IMP-8, GIM-1, IMI-2, IMI-9, IMI-14, IMI-16, GES-5, OXA-58 and others were found in less 10 isolates each. In *P. aeruginosa*, VIM-2 was the most frequent carbapenemase (n = 245), followed by VIM-1 (n = 24), IMP-7 (n = 18) and GIM-1 (n = 15). NDM-1, GES-5, VIM-1, VIM-4, VIM-28, FIM-1, IMP-1, IMP-2, IMP-13 and IMP-28 were found in less than 10 isolates each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (n = 297), followed by OXA-72 (n = 51) and NDM-1 (n = 10). OXA-58, GES-11 and GIM-1 were found in less than 10 isolates each.

Conclusions: A variety of different carbapenemases has established in Germany. However, the molecular epidemiology in

Germany with a predominance of OXA-48 differs significantly from observations made in other countries like Greece, Italy or the USA. Compared to previous years, variants of OXA-48 are again on the rise, together with variants of NDM and VIM.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

248/RKP

***Bartonella* spp. in Domestic and Wild Animals and their Ticks in Hesse, Germany – Serology, PCR and Microbiome analysis**

Y. Regier*[1], T. Hain[2], K. Komma[2], A. Pulliainen[3], A. Laisi[3], C. Sonntag[1], R. Kaufmann[1], K. Strauch[1], Y. Wiegand[1], H. Podlich[1], C. Jung[1], A. Hillebrecht[1], W. Ballhorn[1], V. A. J. Kempf[1]
[1]*University Hospital, Goethe-University, Institute for Medical Microbiology and Infection Control, Frankfurt am Main, Germany*
[2]*Justus-Liebig-University, Institute of Medical Microbiology, Giessen, Germany*
[3]*University of Turku, Institute of Biomedicine, Turku, Finland*

Introduction: Zoonotic agents pertaining to the genus *Bartonella* are usually transmitted via blood-sucking arthropod vectors and cause a constantly increasing number of human and animal diseases.
Aims: In this "*one health*" approach, we screen wild and domestic animals and their ticks from Hesse, Germany for the presence of *Bartonella* spp. or related infections.
Materials and Methods: To date, 81 ticks from 52 dogs, one tick from one cat, 82 ticks from 34 roe deer, one tick from one raccoon and four ticks of four boars from Hesse were screened via PCR. Serum of the cat and dogs was screened for anti-*Bartonella*-antibodies via an indirect immunofluorescence assay and EDTA-blood of wild animals was examined via PCR. Screening for the presence of *Bartonella* spp. in ticks and EDTA-blood was conducted via 16S-23S-ITS- and 16S-rDNA-PCRs. To distinguish the closely related *B. schoenbuchensis* and *B. capreoli,* rpoB-PCR and subsequent detailed sequence analysis was performed.
Results: Three different *Bartonella* spp. were found in total. *B. henselae* was detected in three ticks from two roe deer. *B. capreoli* was detected in the blood of two roe deer and *B. schoenbuchensis* was detected in the blood of seven deer and in three ticks of two deer. Furthermore, a co-infection with *B. schoenbuchensis* and *B. capreoli* was detected in the blood of two deer. One dog was seropositive for anti-*Bartonella*-antibodies. Microbiome analysis of ticks is ongoing and will reveal the prevalence of pathogenic bacteria in those ticks to assess the infection risk for both humans and animals.
Conclusion: The results indicate low *B. henselae* prevalence in dogs and their ticks in Hesse, Germany whereas the ruminant associated *B. capreoli* and *B. schoenbuchensis* (which is suspected to cause deer ked dermatitis) can be found regularly in roe deer and their ticks.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

249/RKP

Food-borne botulism due to a commercial hummus product in Slovakia involving the unusual botulinum neurotoxin subtype A3

L. Mad'arová[1], B. Dorner[2], L. Schaade[3], V. Donáth[4], M. Avdi?ová[1], M. Fatkulinová[1], J. Strhársky[1], I. Sedlia?iková[1], C. Klement[1], M. Dorner*[2]
[1]*Regional Authority of Public Health Banská Bystrica, Banská Bystrica, Slovakia*
[2]*Robert Koch-Institut, ZBS3 - Biologische Toxine, Berlin, Germany*
[3]*Robert Koch-Institut, Berlin, Germany*
[4]*F. D. Roosevelt Teaching Hospital, Banská Bystrica, Slovakia*

Introduction: Food-borne botulism is a life-threatening illness resulting from the uptake of botulinum neurotoxin contained in contaminated food or beverages. The botulinum neurotoxins (BoNTs) are a heterogeneous family divided into seven serotypes (A–G) with more than 40 subtypes. Serotypes BoNT/A, B, E, and F cause the disease in humans. The BoNTs are produced by at least six different *Clostridium* species (*C. botulinum* Groups I–IV, *C. baratii* and *C. butyricum*). When food is contaminated with spores these spores can germinate under favourable anaerobic conditions and secrete the BoNTs. Nowadays almost exclusively ill preserved home-made foods are involved, while cases due to commercial product are extremely rare. After reaching the neuronal synapses the BoNT is taken upon interaction with specific receptors and gangliosides and translocate its light chain into the neuronal cytosol. Here it cleaves the so-called SNARE proteins at a serotype-specific position preventing the further release of neurotransmitter.
Aim: A case of food-borne botulism occurred in Slovakia 2015. Epidemiological and laboratory investigations were conducted to identify the inducing agents.
Materials and Methods: Suspected food items were analysed by anaerobic enrichment culture. Presence of BoNT genes was done by conventional PCR and qPCR. BoNT-production of isolates was confirmed by mouse bioassay. 16S rDNA and BoNT genes were amplified, Sanger sequenced and sequences were compared to GenBank entries.
Results: Out of the suspected food items tested a commercial hummus product was found positive for the presence of *C. botulinum* type A. The product was immediately recalled from Slovakian and Czech market. From the leftover packaging three isolates resembling *C. botulinum/C. sporogenes* were obtained out of which two were positive for the BoNT/A gene. Sequencing of the *bont* gene and comparison with known BoNT/A subtypes revealed the very rare subtype BoNT/A3 produced by *C. botulinum* Group I. BoNT/A3 is the most divergent subtype with nearly 16% difference at amino acid level when compared to the prototype BoNT/A1. BoNT/A3 has only been once involved in food-borne botulism before during the tragic outbreak at Loch Maree in Scotland in 1922 killing 8 people.
Conclusion: Here we report a case of food-borne botulism due to a commercial hummus product sold in Slovakia and the Czech Republic. Laboratory investigation showed that the outbreak was caused by a very rare subtype of the botulinum neurotoxin serotype A (BoNT/A3). A3 is not only a very rare subtype but, with up to nearly 16% difference at amino acid level, it is most distant to all other BoNT/A subtypes, making it more prone to evade immunological and PCR-based detection. Thus, our report should raise awareness among testing laboratories that this subtype has recently occurred in Europe again and should encourage them to validate their immunological or PCR-based methods against this divergent subtype.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

250/RKP

An unusual case of human bite infection and discovery of the suggestive suspect via Next Generation Sequencing

A. Berger*[1], L. Stalling[2], A. Dangel[3], R. Konrad[3], A. Sing[1]
[1]*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Infektiologie, Konsiliarlabor für Diphtherie, Oberschleißheim, Germany*
[2]*MEDILYS Laborgesellschaft mbH, Hamburg, Germany*
[3]*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany*

Question: In Germany 30 000 to 50 000 injuries are caused by bites every year. Dog and cat bites are very common, while human bites are relatively rare (up to 20% of all bite injuries in some urban areas). About 30–60% of the infections are casued by a mixture of aerobic and anaerobic bacteria, including unusual pathogens transmitted via the saliva. We report the transmission of a non-toxigenic *Corynebacterium diphtheriae gravis* strain by a human bite in a 57 year old homeless alcohol-addicted male person resulting in an ear abscess. NGS data of analysed *C. diphtheriae* strains allow the suggestion of a potential victim-offender relationship. Multilocus sequence typing (MLST) to characterize the patients isolate and NGS was performed in addition to 75 tox-*C. diphtheriae* strains collected in the CLOD in order to analyse epidemiological and regional clusters in Germany.
Methods: Strain identification was performed by biochemical differentiation (API Coryne; bioMèrieux, Germany) and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Susceptibility testing was performed according to the CLSI and EUCAST guidelines. Toxigenicity was verified by real-time PCR.

MLST based on seven housekeeping loci was performed and analysed using the respective database (http://pubmlst.org/cdiphtheriae). Next generation sequencing analysis was carried out with the isolate using Illumina Nextera XT libraries and an Illumina MiSeq.
Results: A non-toxigenic *C. diphtheriae* biovar *mitis* strain sensitive to penicillin, erythromycin and clindamycin was identified in a mixed wound infection with *Streptococcus dysgalactiae, Prevotella buccae* and other *Prevotella s*pp. After abscess opening the patient was initially treated with clindamycin but was interrupted by the patients preterm discharge. MLST data extracted from the NGS data yielded sequence type (ST) 8 which was the most frequently found sequence type (41/76 analysed strains). Interestingly, our NGS data support the theory of the suggestive biting person: two days before our patient was hospitalized a 42 year old female patient was taken to the same hospital suffering from a hand wound infection growing an identical ST8 strain with no allele or SNP difference compared to the patient strain. She had reported problems with domestic violence.
Conclusions: In the last years the number of non-toxigenic *C. diphtheriae* wound and blood culture isolates sent to the German Consiliary Laboratory on Diphtheria are increasing. Especially homelessness and drug/alcohol abuse are predominant risk factors for invasive and noninvasive *tox*-negative *C. diphtheriae* infections. MLST showed 20 different sequence types with ST8 as the most predominant sequence type. NGS analysis enabled the further analysis of the ST8 regional and epidemiological clusters. NGS data enable the suggestion of a potential victim-offender relationship with regional and temporal correlation.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

251/RKP
Development of an *Bartonella henselae* specific Human IgG ELISA
M. Jost*[1], A. Latz[2], V. A. J. Kempf[1]
[1]*Universitätsklinikum Frankfurt, Institut für medizinische Mikrobiologie und Krankenhaushygiene, Frankfurt am Main, Germany*
[2]*NovaTec Immundiagnostica GmbH, Dietzenbach, Germany*

Introduction: *Bartonella henselae* causes cat scratch disease (CSD), an often self-limiting lymphadenitis in immunocompetent patients, and several other clinical entities. While cats are the natural reservoir for *B. henselae*, the pathogen is transmitted by cats, cat fleas and eventually by other arthropods. The clinical symptoms underlying CSD might be similar to those being suspicious for malignant tumors. Thus, an easy and reliable method like serological analysis for *B. henselae* is desirable.
Objective: The aim of this study is to design an ELISA for detection of anti *B. henselae* to overcome the shortcomings of the currently used immunofluorescent test (IFT) like subjective and limited reproducible results and high working time of technicians.
Material and Methods: Test development is based on different *B. henselae* strains and quality assured patient sera. These sera were positively tested for anti *B. henselae* antibodies via conventional anti-*B. henselae*-IgG-IFT and, moreover, these patients showed typical symptoms or were PCR-positive for *B. henselae*. Antigens were separated by ion exchange chromatography and fractions examined in lineblots. Potential fractions were further tested and optimized for ELISA coating.
Results: Patients with *B. henselae* infections show different patterns of antibody expression in western blots. Thus, there is obviously no universally usable antigen for diagnosis detectable. Crude antigen preparations (both liquid grown and with cell culture) are not working reliably as they do not react with numerous patient sera. However, our tests show that there are certain protein fractions from liquid-grown *B. henselae* which react reliably and results from lineblots were successfully transferred to an ELISA-format with sufficient sensitivity.
Conclusion: We show a strategy for antigen testing and selection from *B. henselae* protein preparations for ELISA-based serology. Further processing of antigens is under investigation so that in future an ELISA for B. henselae is possible.

Funding: This study is financed by the state Hesse within the LOEWE III project (Landes-Offensive zur Entwicklung wissenschaftlich-ökonomischer Exzellenz).

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

252/RKP
Laboratory surveillance report: invasive *H. influenzae* isolates from infants in Germany 2009-2016
T. Lâm*[1], H. Claus[1], U. Vogel[1]
[1]*Würzburg University, Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction: The role of non-typeable *H. influenzae* (NTHi) for the rising incidence of invasive *Haemophilus influenzae* (Hi) infections especially in elderly patients is well documented. Recent reports point at NTHi as a major cause of invasive infections in neonates and a risk factor for abortion (Collins S et al., JAMA. 2014;311:1125-1132; Collins S et al., Clin Infect Dis. 2015).
Objectives: The objectives of this study were to provide an update on the laboratory surveillance for invasive Hi infections 2009-2016; to provide prevalence data of invasive Hi infections in children, especially neonates; and to analyse the role of NTHi in invasive Hi infection in neonates.
Materials and Methods: A retrospective data base analysis of isolates from blood and CSF submitted to the German National Reference Laboratory for Meningococci and *H. influenzae* (NRZMHi) was carried out for Hi from 2009 to 2016. Statistical analysis of serotype data from neonates and women aged 15-45 years was done using Fisher"s exact test.
Results: A total of 82 Hi from children aged <1 year out of 2175 isolates from blood or cerebrospinal fluid were identified. Hif (n=17; 21%) and Hib (n=13; 16%) were the most frequent capsular types. However, most isolates were NTHi (n=46; 56%). Neonates, defined as children aged <=1 month (n=29), were almost exclusively infected by NTHi (n=27; 93%). The subgroup of patients aged 1-11 months (n=52) was mainly infected by capsulated isolates (n=34; 65%) with Hif (n=16; 31%) and Hib (n=12; 23%) as the most frequent serotypes. The difference in serotype distribution was significant between these two age groups (Fisher"s exact test; p<0.1). The number of isolates analysed from neonates is small in comparison to the number of about 700,000 births per year in Germany. Incidence of NTHi in the age group of < 1 year was 2.87 / 100,000 according to statutory notification data (Survstat@RKI). The serotype distribution of women aged 15 to 45 years was not significantly different from the one found in men.
Summary: Our data confirm the significance of NTHi infections in neonates. The data presented may be below the true incidence due to probably high underestimation. No difference was found between invasive *H. influenzae* infections of women in childbearing age and of men in the same age cohort. However, our data do not include the serotype distribution of carriage isolates. Further studies are necessary on the role of maternal carriage for neonatal infections in Germany.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

253/RKP
Typing and resistance characteristics of *Campylobacter jejuni* and *Campylobacter coli* isolates from patients in Germany Fruth A1, Tietze E1, Garn T1 and Flieger A1 1 Robert Koch Institute, Wernigerode, Germany
F. Angelika*[1]
[1]*Robert Koch Institut, Infektionskrankheiten, Wernigerode, Germany*

Introduction: *Campylobacter* is a fastidious gram-negative bacterium considered to be a common cause of acute, self-limiting gastroenteritis. The number of *Campylobacter* infections reported in Germany has been increasing since 2007 (https://survstat.rki.de). Reporting of cases is based on different diagnostic tools, such as

Ag-ELISA from stool samples (*C. jejuni* and *C. coli*), RT-PCR and strain cultivation with selective culture methods. Conventional methods detect approx. 80% of causative agents and commercial PCR kits do not distinguish between species. Therefore the notified increase in reported cases observed since 2014 cannot be ascribed to an increase of *C. jejuni* or *C. coli.*

Aims: The aim of the study was to analyze the distribution of *Campylobacter* resistance properties and different capsule) types of *C. jejuni.*

Methods: Isolates were identified by PCR according to Linton et al. (1996). MIC determination by broth microdilution tests in 96-well-MTP according to CLSI directions with epidemiological cut-off (ECOFF) values of EUCAST (http://mic.eucast.org/Eucast2/) as well as based on DIN values for *Enterobacteriaceae* were performed. *C. jejuni* were analysed for typing by multiplex PCR of cps-gene loci according to Poly et al. (2011).

Results: The collected data of resistance behaviour and results for subtyping of major capsular types of Campylobacter may reflect the epidemiological situation of this zoonotic pathogen in Germany. Regional differences, for example between rural regions with large-scale livestock farming and big cities, might be expected. More than 50% of investigated *C. jejuni* and *C. coli* isolates were resistant to ciprofloxacin. Multidrug resistance was increasingly observed in both species from 2005 to 2014 in particular co-resistance to the therapeutically relevant fluoroquinolones, macrolides and aminoglycosides. A slight reduction of this trend was observed since 2015 and is still under observation. Among the most frequently detected capsule types some seemed to be linked to kind of consumed food in Germany (HS2 to raw milk, HS4/CG8486 to poultry), as it had seen on different small outbreaks.

Frequency of reported cases with *C. jejuni* infections within distinct age groups is correlated to frequency of detectable capsule types with only slight differences in variants.

Conclusion: Analyses of capsule types were applied for further epidemiological investigation. It allowed a preselection of cases to be processed by NGS based typing (MLST, *fla*A/B and *por*A sequencing) or PFGE. This approach may be a time and cost consuming benefit. Geographical differences in prevalence of certain capsule types may be explained by different food delivery channels and regional producers.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

254/RKP

Molecular analysis of *Enterococcus faecium* strains from bloodstream infections isolated between 2011 and 2017 in Germany revealed a preferred occurrence of ST117 and an increasing number of *vanB*-type VRE

G. Werner*[1], J. Bender[1], C. Fleige[1], U. Geringer[1], A. Weller[1], D. Lange[1], S. Fuchs[1], I. Klare[1]

[1]*Robert Koch Institute, Infectious Diseases, Wernigerode, Germany*

Objectives: We determined the strain type of VRE from bloodstream infections as a surrogate marker for invasive *E. faecium* strain types prevalent in German healthcare institutions.

Methods: *E. faecium* isolates were received from blood cultures of German patients with bacteraemia/sepsis between 2011 and June 2017 on a voluntarily basis. Until 2015 we have determined the MLST type by a combination of 7 PCRs and subsequent Sanger sequencing. The *van* genotype was determined by a multiplex PCR for *vanA* and *vanB.* From 2016 onwards we performed NGS and extracted corresponding MLST types (ST) and core genome MLST types (CT) (based on 1423 core genome genes; Ridom SeqSphere+; Ridom GmbH, Muenster).

Results: We have analysed and compared the MLST types of 388 invasive *E. faecium* isolates, mainly VRE. Isolates of 5 MLST types summed up to more than 80% of all isolates. ST117 is by far the most frequent MLST type occurring in 41% of cases during the study period followed by ST203, ST80, ST17, and ST192. Numbers of isolates with ST117 increased over the years and constituted ca. 50% of all isolates from 2015 onwards. ST80 is a new ST type occurring more than twice per year only since 2015. NGS data allow allocation of CT types with a much higher discriminatory power than ST. Among the 91 *E. faecium* blood culture isolates in 2016, only 4 CTs appeared more than three times and all of them were ST117: CT36 (n= 14; 15%), CT71 (n= 12; 13%) und CT469 (n= 8; 9%). Up to 2014 more *vanA*-type VRE were identified than *vanB*-type strains annually; whereas from 2015 onwards this was reversed. We have determined NGS data for 16 ST80 isolates in 2016/2017 and only 2 isolates revealed an identical coregenome cluster type (CT1017). This diversity in CT types among ST80 suggests that the increased appearance of ST80 strains does not reflect a recent clonal expansion.

Conclusion: NGS data combined with a high resolution typing scheme based on a core genome MLST allocate a distinct and highly discriminatory strain/cluster type (CT). By using the same typing language in different laboratories, successful VRE strain types spreading across regions and neighbouring countries in Europe could be identified. For instance, ST117/CT71 is a widely disseminated strain type also prevalent in The Netherlands and Denmark. Supposed spread of recent ST types such as ST80 could be refused based on a more discriminatory typing scheme used for CT types. The increased recognition of VanB type VRE in general in Germany is also recognized by analysing the collection of blood culture isolates sent to the NRC as a surrogate marker recognizing specific trends.

Figure 1

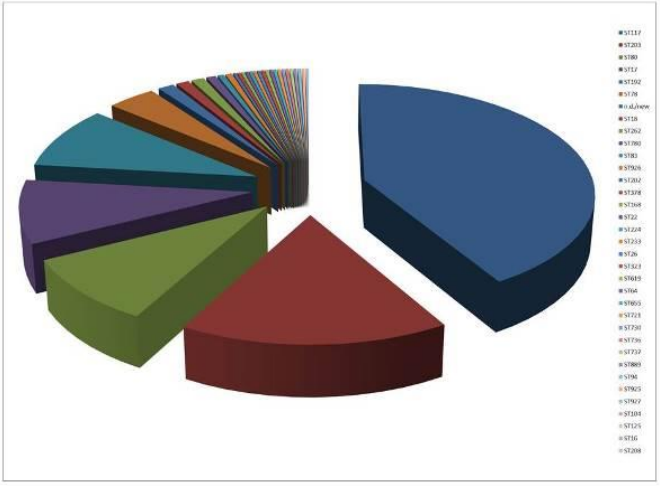


Figure 1: ***Distribution of MLST types among E. faecium isolates from bloodstream infections of German hospital patients, 2011 to June 2017.*** *(MLST types are presented clockwise, beginning on the top with descending frequency)*

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

255/RKP

Some like it hot – temperature resistance of Borrelia burgdorferi s.l.

V. Fingerle*[1], S. Stockmeier[1], C. Hizo-Teufel[1], C. Hartberger[1], A. Sing[1], G. Margos[1]

[1]*Bayerisches Landesamt f. Gesundheit und Lebensmittelsicherheit, National Reference Center for Borrelia, Oberschleißheim, Germany*

Introduction: In recent years hyperthermia therapy for so called "chronic Lyme borreliosis" was established. The supporters of this method claim, that all B. burgdorferi sensu lato species will be killed in vitro at 41.6°C, a temperature that, according to the supporters, can be reached in the human body for two hours by the hyperthermia method. However, these claims are based on a study that investigated survival of Borrelia strains when cultured for seven days at higher temperature, while in vitro data regarding survival of Borrelia when confronted with higher temperature for short term are poor.

Aim: The aim of the study was to validate or falsify the claim that Borrelia burgdorferi strains are reliably killed when confronted with temperatures up to 42°C for 2h and 6h.

Material and Methods: Five strains belonging to B. burgdorferi s.s., B. afzelii, B. bavariensis, B. garinii and B. spielmanii were incubated in parallel for 2h at 33°C, 41°C and 42°C or for 6h at at 33°C and 42°C. All strains then were cultivated at 33°C for at least 6 days. In regular intervals motility and growth of the Borrelia was monitored by dark field microscopy.
Results: Incubation experiments for 2h revealed that there was no significant effect on any of the strains at 41°C, while at 42°C the B. bavariensis was killed. Incubation for 6h at 42°C resulted in killing of the B. bavariensis and the B. spielmanii strain while the others all survived.
Conclusions: With respect to killing of human pathogenic B. burgdorferi, hyperthermia at 41.6°C for 2h is not a reliable method since most Borrelia obviously are not relevant affected by this temperature. This study underlines the importance of doing proper validation before new or modified therapies are introduced.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

256/RKP

Published data do not provide proof of human pathogenicity of *Borrelia valaisiana*

G. Margos[1], A. Sing [1], V. Fingerle*[1]
[1]*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, GE2, Oberschleißheim, Germany*

Introduction: The genospecies *B. valaisiana* was described in 1997 (1) and is found frequently in *Ixodes ricinus* ticks in Europe. It was noticed that more and more publications and reviews consider *Borrelia valaisiana* as human pathogenic.
Aim: The aim of our study was to scrutinize published data for evidence of human pathogenicity of *B. valaisiana*.
Materials and Methods: A Pubmed search was performed using the words "Borrelia valaisiana" and "human" to retrieve publications reporting on the presence of *B. valaisiana* in human samples. Such publications were screened and the evidence for human pathogenicity of *B. valaisiana* was scrutinized.
Results: Six publications from Europe and Asia reported the presence of *B. valaisiana* DNA but – to the best of our knowledge – no single cultured isolate from a human patient was reported. Several of the samples positive for *B. valaisiana* DNA were also positive for human pathogenic *Borrelia* genospecies such as *B. garinii, B. afzelii* or *B. burgdorferi* sensu stricto making it difficult to judge which of these was the symptom causing *Borrelia* species. In addition, data reported from Asia suggest misidentification of *B. valaisiana* instead of *B. yangtzensis*.
Conclusion: The data provided and discussed in this poster, i.e. the scarcity of *B. valaisiana* DNA in human patient samples (n = 12 in 20 years), methodological ambiguities and the absence of a single cultured isolate combined with the relatively high prevalence of *B. valaisiana* in questing *I. ricinus* ticks, led us to propose that *B. valaisiana* is not human pathogenic.

Reference

[1] Wang G, van Dam AP, Le Fleche A, Postic D, Peter O, Baranton G, de Boer R, Spanjaard L, Dankert J: **Genetic and phenotypic analysis of *Borrelia valaisiana* sp. nov. (*Borrelia* genomic groups VS116 and M19)**. *Int J Syst Bacteriol* 1997, **47**(4):926-932.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

257/RKP

The Consultant Laboratory on Tularemia in Germany

K. Heuner*[1], D. Jacob[1], S. Klee[1], R. Grunow[1]
[1]*Robert Koch Institut, Berlin, Germany*

Tularemia is a rare but re-emerging zoonotic disease in Germany. The causative agent of tularemia is Francisella tularensis. In Europe only F. tularensis ssp. holarctica was found in humans to cause tularemia so far. The infection source is quite often due to direct contact to infected wild animals, furthermore insect vectors especially ticks and contaminated environmental sources like dust play a role in transmission of the pathogen to humans. According to the infection protection act tularemia is a notifiable disease and an increase of the number of reported human cases over the last years can be observed. Seroprevalence studies showed an unexpected high positive rate in humans and animals. It can be assumed that tularemia is probably substantially underestimated in Germany.
Since 2015 the Division of Highly Pathogenic Microorganisms (ZBS 2) at the Centre for Biological Threats and Special Pathogens of the Robert Koch Institute is the appointed consultant laboratory for tularemia for the Public Health sector in Germany. The consultant laboratory offers diagnostic and scientific support by applying accredited microbiological, molecular and serological methods, typing of strains, and antimicrobial susceptibility testing (AST) according to the Clinical and Laboratory Standards Institute (CSLI, USA) guidelines. In addition, the laboratory is active in research projects like development of growth media, molecular epidemiology, the virulence mechanisms of F. tularensis and the pathogen-host interaction.

Furthermore, the laboratory supports the establishing of quality assurance schemes, including tularemia diagnostics at national and international level, fulfilling the role of main coordinator of the European Joint Action "Efficient response to highly dangerous and emerging pathogens at EU level" (EMERGE - http://www.emerge.rki.eu/Emerge/). One working group of EMERGE is engaged in the elaborateness of suitable standard operation procedures for AST of F. tularensis (beside SOPs for all other highly pathogenic bacteria) with the plan to provide this SOP to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for recommendations in Europe.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

258/RKP

The Consultant Laboratory on *Bacillus anthracis* in Germany

C. Cox*[1], S. Klee[1], D. Jacob[1], R. Grunow[1]
[1]*Robert Koch-Institut, ZBS2, Berlin, Germany*

Anthrax is a worldwide occurring but, at least in Germany, rare zoonotic disease. However, an outbreak of anthrax in drug users has been emerging in Germany and other European countries in the years 2009-2012. Livestock also became affected during the past years. The last anthrax outbreak among cattle in Germany occurred in April 2014 in Saxony-Anhalt. The causative bacterium *Bacillus anthracis* is also considered as a biological agent which could potentially be released deliberately. The spores of the agent are extremely resistant to environmental conditions and chemical disinfectants.
Single cases and outbreaks of anthrax as well as samples of suspected intentional release are raising questions on diagnostics as well as on laboratory and clinical management. Consequently, the first consultant laboratory for anthrax in support of the Public Health sector in Germany has been appointed in May 2014. It is located at the Division of Highly Pathogenic Microorganisms (ZBS2) at the Centre for Biological Threats and Special Pathogens of the Robert Koch Institute. The consultant laboratory offers diagnostic and scientific support by applying accredited microbiological, molecular and serological methods, antimicrobial susceptibility testing, typing of strains (SNP analysis and MLVA), and providing recommendations and advice, e. g. on disinfection and decontamination.
The consultant laboratory is also active in research projects on anthrax, including the investigation of *B. anthracis* strains having caused the outbreaks among heroin users, as well as the ones of *Bacillus cereus* biovar *anthracis* representing a new variation in the *Bacillus cereus* group found so far in different regions of Africa. Furthermore, the laboratory supports the establishing of quality assurance schemes, including anthrax diagnostics at national and international level, fulfilling the role of main coordinator of the European Joint Action "Efficient response to highly dangerous and emerging pathogens at EU level" (EMERGE - http://www.emerge.rki.eu/Emerge/). In the framework of the

EMERGE strain repository, NGS based characterization of around 30 *B. anthracis* isolates originating worldwide is planned.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

259/RKP

Quality assurance for characterization and resistance testing of *Clostridium difficile* isolates

F. Berger*[1,2], A. Mellmann[2,3], A. Nimmesgern[1,2], M. Bischoff[1,2], L. von Müller[2,4], B. Gärtner[1,2]
[1]*Institute of Medical Microbiology and Hygiene, Homburg, Germany*
[2]*National Reference Laboratory for Clostridium difficile, Homburg, Germany*
[3]*Institute of Hygiene, Münster, Germany*
[4]*Christophoros Kliniken, Coesfeld, Germany*

Introduction: *Clostridium difficile* (new taxonomic name *Clostridioides difficile*) is the predominant cause of hospital acquired diarrhea and may result also in severe disease and hospital outbreaks with mandatory reporting in most European Countries. Molecular typing and also resistance testing are required for characterization of isolates which is of special interest for outbreak analysis. However, a variety of methods are used and a information about comparability between laboratories are still scarce.
Material and Methods: In 2016 the German National Reference Laboratory for *Clostridium difficile* initiated a ring trial for Germany speaking countries with six different clinical isolates. Isolates were characterized before by PCR ribotyping (RT) and whole genome sequence typing in independent specialized laboratories. Five isolates were toxigenic (RT001, RT002, RT014, RT027, RT078) while RT010 was non-toxigenic. The 21 participating laboratories from Germany, Austria, Switzerland and Luxembourg used a broad variety of methods for molecular characterization and resistance testing.
Results: For identified ribotypes a high concordance could be shown for epidemic outbreak strains of RT027 and RT078. Lower concordance was found for identification of the endemic ribotypes as RT010, RT014, and RT001. Flourochinolone (moxifloxacin) resistance was reliably detect by all except one laboratory. However, in respect to quantitative analysis of MIC the difference between participating laboratories was high for standard antibiotics (metronidazole, vancomycin); however, despite several log differences of MIC the isolates were correctly classification as sensitive or resistant.
Discussion: Molecular typing methods showed high concordance only for epidemic RTs. Differences between attributed ribotypes were predominantly associated with different typing methods uses. The quantitative differences detected for MIC testing also underlines the necessity of harmonization which is also supported by harmonization protocols of European study groups (ESCMID: ESGCD, ECDC: ECDIS).

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

260/RKP

Genomics of two sister species to understand host association in Lyme Borreliosis: *Borrelia bavariensis* and *B. garinii*

N. S. Becker[1], A. Paulus[1], G. Margos*[2], A. Sing[2], V. Fingerle[2]
[1]*Ludwig-Maximilian University, Division of Evolutionary Biology, Planegg-Martinsried, Germany*
[2]*Bavarian Health and Food Safety Authority, National Reference Centre for Borrelia, Oberschleißheim, Germany*

Introduction: According to our current knowledge, *Borrelia bavariensis* and *B. garinii* are the taxonomically closest species in the *Borrelia burgdorferi* sensu lato species complex in Eurasia that do not share the same reservoir hosts. Indeed, both species are human pathogens and are widespread in Eurasia, but *B. bavariensis* uses rodents as reservoir hosts whereas *B. garinii* is adapted to birds. These two species thus form an ideal system to look for genetic factors involved in host association and to understand how *Borrelia* can switch to a new host type. It has been suggested that acquisition or loss of, or recombination on, coding sequences located on the numerous plasmids (potentially > 20) of *Borrelia* can lead to changes in host invasiveness and probably host adaptation.
Aim: The initial aims of our study were to provide completed reference genomes for *B. garinii* and *B. bavariensis* and to conduct comparative genomics with particular emphasis on plasmid content and structure to estimate intra-specific plasmid variability.
Materials and Methods: We combined data from Pacific Bioscience long-read sequencing with Illumina MiSeq data to describe the full genomes of about 30 strains for each species and reconstruct their evolutionary history.
Results: Whereas the main chromosome of both species show low divergence, their plasmid contents differ and we could identify plasmids that were specific to one species and are thus good candidates for playing a role in host association. We identified several recombination events and plasmid rearrangements within and between species and hypothesize that host switches may be facilitated by recombination events of genes/plasmids conferring adaptation to a new host type. Finally we analyzed sequences of the so-called CRASP (for Complement Resistance Acquiring Surface Proteins) known to be associated with human complement resistance and identified non-synonymous divergences between the two species that could be involved in evading the innate immune system of different hosts.
Conclusion: We conclude that these two sister species represent an ideal system for the study of host association in *Borrelia* and propose further studies including *Borrelia* transformation and functional genetics to unravel the evolution of host association in these two, and potentially other, *Borrelia* species.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

261/RKP

The National Reference Center for *Helicobacter pylori*: consulting, diagnostics and research

K. Dichtl*[1], R. C. Bader[1], S. Suerbaum[1]
[1]*NRZ für Helicobacter pylori am Max von Pettenkofer-Institut, LMU München, München, Germany*

Helicobacter pylori infection is the second most common infectious disease of mankind. Complications include duodenal and gastric ulcers, gastric cancer, and gastric MALT lymphoma. Emerging resistance against eradication therapy challenges health care providers: in 2017, clarithromycin-resistant *H. pylori* was graded a global high priority pathogen by the World Health Organization in order to guide discovery and development of new antibiotics.
Since 01.01.2017, the National Reference Center (NRZ) for *H. pylori*, appointed by the Federal Ministry of Health and the Robert Koch Institute, is located at the Max von Pettenkofer Institute in Munich (Faculty of Medicine, LMU Munich), and directed by Prof. Sebastian Suerbaum. The NRZ provides advice to laboratories, practitioners, and patients, on a wide range of questions ranging from epidemiology, diagnostics to therapy of *H. pylori* infection. Diagnostic methods offered at the NRZ include cultivation, molecular detection, genotypic and phenotypic susceptibility testing, detection and typing of virulence factors (*cagA*, *vacA*), multilocus sequence typing and assignment to phylogeographic populations, and whole genome sequence analysis. In addition to this direct contribution to health care, the NRZ aims to enable research projects by providing expertise, strains, and reagents, and will initiate studies related to epidemiology, antimicrobial resistance and approaches to improve diagnostic procedures in collaboration with other scientists. The NRZ promotes fundamental research aiming to extend our knowledge about *H. pylori* infection. In particular, the NRZ and the affiliated research groups at the Max von Pettenkofer Institute investigate in the fields of genetic variability, evolution and molecular epidemiology. The website of the NRZ provides a wide range of information on *H. pylori* and the activities of the NRZ, as well as a collection of relevant links.

Presentation: Monday, February 19, 2018 from 19:00 – 21:00 in room Audimax.

POSTERSESSION 02
20 Febr. 2018 • 17.00-18.30

Microbial Pathogenesis (FG MP)

262/MPP

The bacterial host determines the life-cycle of Sa3-bacteriophages

C. Rohmer*[1], C. Wolz[1]
[1]*University of Tübingen, Interfaculty institute of microbiology and infection medicine, Tübingen, Germany*

Staphylococcus aureus possesses a set of virulence factors necessary for infection of the human host some of which are encoded on bacteriophages. Sa3-phages integrate specifically into the *hlb*-gene therefore leading to loss of β-hemolysin production. However, these *hlb*-converting phages provide additional virulence genes to their host bacterium representing human specific immune modulatory factors. About 90% of all *S. aureus* strains of human origin carry these *hlb*-converting phages, whereas animal strains usually are devoid of them. We aimed to analyze the interference of the bacterial host background with the biology of these Sa3-phages, especially Φ13. Phage cured *S. aureus* strains 8325-4, USA300-c, Newman-c (clonal complex 8) and MW2-c (clonal complex 1) were lysogenized with Sa3-bacteriophage Φ13Kana carrying a kanamycin resistance cassette. Phage integration into the *hlb*-gene was proven by Multipex-PCR and pulsed-field gel electrophoresis (PFGE). PFGE analysis followed by Southern hybridization revealed multiple phage copies integrating into the *hlb*-gene during first step of lysogenization. After subcultivation stable single phage integrants were obtained. Quantitative PCR was established to measure integrated and excised phage genome copies after induction with subinhibitory concentrations of mitomycin C and reconstitution of the *hlb*-gene. In vitro phage transfer assays were performed by co-culturing lysogens with phage free recipients without mitomycin C. Phage transfer and phage induction was significantly higher in MW2c compared to 8325-4. In summary, the bacterial background has a severe impact on phage mobilization. The molecular mechanism of bacterial host-interaction is under investigation.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

263/MPP

Bacterial LPX motif-harboring virulence factors constitute a species-spanning family of cell-penetrating effectors

S. Norkowski[1], B. Körner[1], L. Greune[1], A. S. Stolle[2], M. L. Lubos[1], P. R. Hardwigde[3], M. A. Schmidt[1], C. Rüter*[1]
[1]*Universitätsklinikum Münster, Institut für Infektiologie, ZMBE, Münster, Germany*
[2]*Harvard Medical School, Dept. of Microbiology and Immunobiology, Boston, Germany*
[3]*Kansas State University, Manhattan, United States*

Effector proteins are key virulence factors of pathogenic bacteria that target and subvert the functions of essential host defense mechanisms. Typically, these proteins are delivered into infected host cells via the type III secretion system (T3SS). Recently, however, several effector proteins have been found to enter host cells in a T3SS-independent manner thereby widening the potential range of these virulence factors. Prototypes of such bacteria-derived cell-penetrating effectors (CPEs) are the *Yersinia enterocolitica*-derived YopM as well as the *Salmonella typhimurium* effector SspH1.
Here, we investigated specifically the group of bacterial LPX effector proteins comprising the *Shigella* IpaH proteins, which constitute a subtype of the leucine-rich repeat (LRR) protein family and share significant homologies in sequence and structure. With particular emphasis on the *Shigella* effector IpaH9.8, uptake into eukaryotic cell lines was shown. Recombinant IpaH9.8 (rIpaH9.8) is internalized via endocytic mechanisms and follows the endo-lysosomal pathway before escaping into the cytosol. The N-terminal alpha-helical domain of IpaH9.8 was identified as the

protein transduction domain (PTD) required for its CPE ability as well as for being able to deliver other proteinaceous cargo. rIpaH9.8 is functional as a ubiquitin E3 ligase and targets NEMO for poly-ubiquitination upon cell penetration.
Strikingly, we could also detect other recombinant LPX effector proteins from *Shigella* and *Salmonella* intracellularly when applied to eukaryotic cells. In this study, we provide further evidence for the general concept of T3SS-independent translocation by identifying novel cell-penetrating features of these LPX effectors revealing an abundant species-spanning family of CPE.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

264/MPP

Intracellular phenotypes of *Salmonella enterica* sv. Typhi and Paratyphi A

T. Reuter*[1], M. Hensel[1]
[1]*Universität Osnabrück, Microbiology, Osnabrück, Germany*

Introduction: *Salmonella enterica* is a frequent facultative intracellular pathogen. The serotypes *Salmonella* Typhi (STY) and Paratyphi A (SPA) are highly human adapted and able to cause systemic diseases (Dougan and Baker, 2014). Only few studies report on the cellular microbiology of STY, and studies on interactions of SPA with host cells are almost absent. *Salmonella enterica* serovar Typhimurium (STM) is commonly used as model system for intracellular pathogenesis of highly host adapted STY and SPA. After invasion of the host cell, STM resides inside a membrane-bound compartment, known as *Salmonella*-containing vacuole (SCV). By expressing the SPI2-encoded (*Salmonella* pathogenicity island 2) T3SS (type 3 secretion system) STM is able to translocate effector proteins across the SCV membrane into the host cell. These effectors are necessary for the maturation of the SCV and formation of *Salmonella*-induced filaments (SIFs). Since it was reported that the function of the SPI2-T3SS is not required for STY intracellular survival and replication in human macrophages, the question raises whether observations made for STM are suitable to explain STY and SPA pathogenesis (Forest *et al*., 2010).
Aims: We want to establish the cellular microbiology of intracellular STY and SPA. The focus will be on the role of the SPI2-T3SS for intracellular lifestyle. Using gentamicin protection assays the intracellular replication of WT and mutant strains in permissive and non-permissive cell lines should be determined. Furthermore the SCV morphology, formation of SIFs and the role of various effector proteins for requirement of SIF formation should be investigated. In addition we want to use reporter strains with dual-colour sensors to distinguish between living and dead/not metabolic active bacteria and to determine release into the cytosol for STY and SPA.
Material and Methods: To analyze the intracellular lifestyle of STY and SPA, we infected RAW264.7 macrophages, HeLa cells and THP1 cells with STM and STY WT and mutant strains and determined x-fold replication after 24 h p.i. To investigate SCV morphology and SIF formation we infected HeLa cells with STY and SPA and performed fluorescence microscopy.
Results: Compared to STM, the typhoidal *Salmonella* strains show distinct phenotypes regarding their intracellular replication. Furthermore the presence of an SCV, SIFs and translocated SPI2-T3SS effector proteins required for SIF formation in HeLa cells infected with SPA were observed.
Summary: The preliminary results suggest that the SPI2-T3SS plays a role for intracellular pathogenesis of STY and SPA in human host cells. It is necessary to get a more detailed view at the exact functions of the SPI2-T3SS in STY and SPA now. To achieve that further analysis of the intracellular survival of STY an SPA in various permissive and non-permissive cell types is necessary.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

265/MPP

The role of *Salmonella* stress response systems for the intracellular lifestyle of *Salmonella* Typhimurium

M. Schulte*[1], M. Meisel[1], M. Hensel[1]
[1]*University of Osnabrueck, Microbiology, Osnabrück, Germany*

Introduction: *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S.* Typhimurium) is a Gram-negative facultative intracellular pathogen able to invade epithelial cells of the intestine and to survive and propagate within host cells. Intracellularly, *Salmonella* is enclosed by a special membrane-bound compartment termed *Salmonella*-containing vacuole (SCV). Also subpopulations were found that escape the SCV into the host cell cytosol. The offensive virulence factors controlling these processes are very important, however, also defensive virulence traits are essential for the intracellular lifestyle. Stress response systems (SRS) of *S.* are able to sense assaults by host cell defense mechanisms, such as antimicrobial peptides, reactive oxygen and nitrogen species, ubiquitination, autophagy or acidification. Since intracellular pathogens have evolved its ability to sense such assaults, SRS enable the pathogen to survive and replicate within the host cell and are responding in a temporal and spatial controlled manner. (Guest and Raivio, 2016; Runkel et al., 2013).
Aims: We want to clarify the contribution of SRS to the intracellular lifestyle of *S.* Typhimurium within mammalian host cells using a competitive-index assay. By characterization of the cell-individual stress response of intracellular *Salmonella* by using different CLEM and TEM techniques as well as reporter strains we aim to characterize the local nano-environment of bacteria of distinct intracellular subpopulations. In addition, a dual-colour vitality sensor should be used to distinguish between living and dead/not metabolic active bacteria.
Material and Methods: *S.* Typhimurium SRS mutant strains were generated using Red mutagenesis. By using a competitive index (CI) assay we calculated the relative fitness of a mutant strain to WT. RAW264.7 macrophages were coinfected with a SRS mutant that harbours an antibiotic resistance cassette and *Salmonella* WT. X-fold replication after 16 h post infection was determined and the CI of WT compared to the mutant strain was calculated. The dual-colour vitality sensor is a plasmid that constitutively expresses e*gfp* and is able to express *dsred* under control of a *tetA* promotor.
Results: The SRS mutants show different phenotypes in relation to their relative attenuation. Flow cytometry analysis revealed that the vitality sensor shows fast induction kinetics of approximately 30-60 min and turned out be very efficient for the detection of the metabolic state of *Salmonella in vitro* as well as *in vivo*.
Summary: The preliminary results suggest that various SRS of *Salmonella* have a crucial role for the intracellular survival and pathogenicity of *S.*Typhimurium within mammalian host cells. Now it is important to get a more detailed view at the contribution of SRS of *Salmonella* for its intracellular pathogenesis. We want to uncover various stress factors sensed by intracellular *Salmonella* and get to know how the pathogen respond to these stresses.

Figure 1

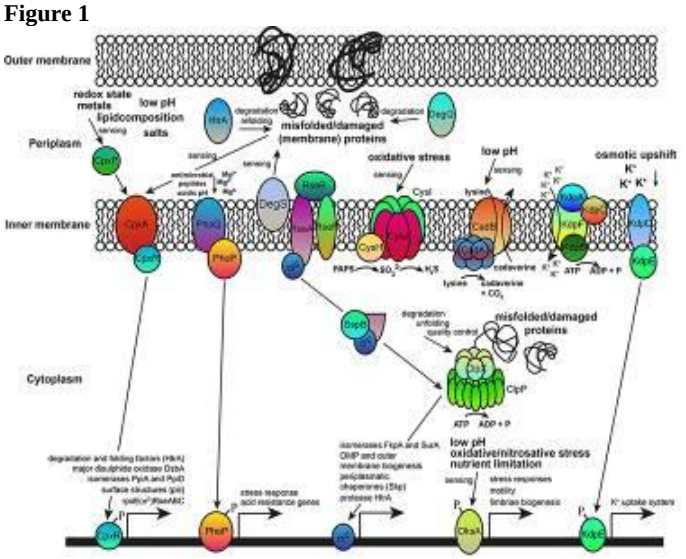


Figure 1: Subset of SRS of *S. Typhimurium*. An abundance of SRS is available that are able to sense the external but also internal environment. They enable the pathogen to survive and replicate within host cells and respond in a temporal and spatial controlled manner.

Figure 2

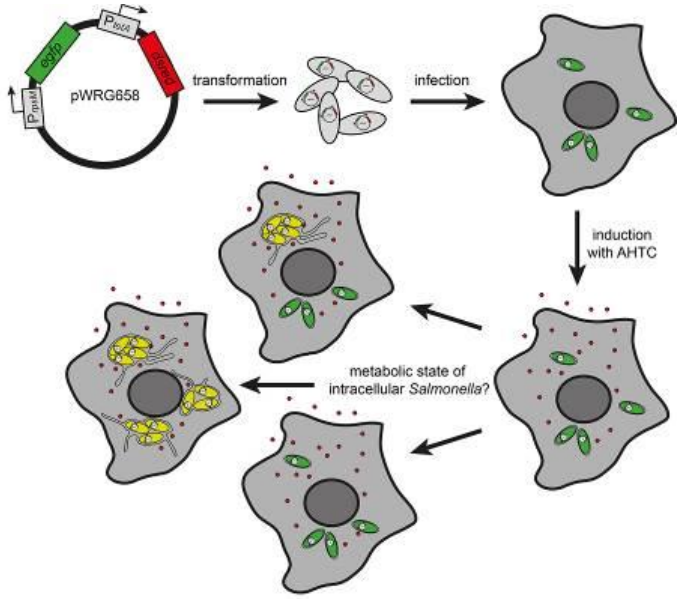


Figure 2: Overview of function of dual-colour reporter. After infection of host cells with constitutive green fluorescent bacteria and induction by 100 ng/ml AHTC, the metabolic state of intracellular *Salmonella* can be detected.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

266/MPP

S. aureus invasion into host cells: functions of fibronectin

S. Niemann*[1], J. Eble[2], B. Löffler[3], H. Van de Vyver[1], G. Peters[1]
[1]*University Hospital of Münster, Institute of Medical Microbiology, Münster, Germany*
[2]*University of Münster, Institute of Physiological Chemistry and Pathobiochemistry, Münster, Germany*
[3]*Jena University Hospital, Institute of Medical Microbiology, Jena, Germany*

Introduction: *Staphylococcus aureus* is not only an extracellular but also an intracellular pathogen. The host cell invasion of non-professional phagocytes essentially contributes to infection development. The fibronectin bridging between *S. aureus* FnBPs and α5β1 integrins on the host cell side that facilitates the uptake of staphylococci is well investigated. In a recent *in vitro* study we showed that typical barrier cells such as endothelial and epithelial cells were highly invaded by bacteria, whereas primary human osteoblasts (phOB) and fibroblasts took up *S. aureus* to a much lesser extent [1].

Objectives: In this study we wanted to find an explanation for the differences in the uptake ability of the cells by analyzing α5β1 integrin expression and fibronectin distribution on the cells.

Materials and Methods: We analyzed the α5β1 integrin expression of different host cells by antibodies using flow cytometry. Fibronectin distribution as well as bacterial binding and uptake was analyzed by fluorescence microscopy. The number of internalized viable bacteria was determined using the lysostaphin protection assay.

Results: We detected high amounts of α5β1 integrins on the surface of phOB and fibroblast cell line cells, whereas endothelial and epithelial cell line cells had only a low integrin expression. Analysis of fibronectin distribution revealed low amounts of fibronectin on endothelial and epithelial cell line cells, whereas primary osteoblasts and fibroblasts were covered with thick fibronectin fibrils. *S. aureus* bound to these fibrils and proliferated there, but was not taken up into the host cells. When the fibronectin matrix around phOB was degraded by trypsin the uptake of *S. aureus* was increased significantly. The incubation of phOB with the supernatant of destroyed neutrophils also degraded the fibronectin fibrils, leading to an increased bacterial uptake.

Conclusions: The fibronectin on the analyzed endothelial and epithelial cells acts as the well investigated bridge builder between *S. aureus* and host cells, whereas the fibronectin matrix fibrils around phOB and fibroblasts form a mechanistic barrier against *S. aureus.* The "fibronectin shield" may function as an archaic mechanism of innate immunity. Degradation of the fibronectin matrix led to increased bacterial uptake. In acute infection processes such as in acute osteomyelitis this process is conceivable as well. Our new results extend our knowledge on the role of fibronectin in the process of *S. aureus* invasion into host cells.

Reference

[1] Strobel, M., Pförtner, H., Tuchscherr, L., Völker, U., Schmidt, F., Kramko, N., Schnittler, H. J., Fraunholz, M. J., Löffler, B., Peters, G., and Niemann, S. (2016) Post-invasion events after infection with Staphylococcus aureus are strongly dependent on both the host cell type and the infecting S. aureus strain. *Clin Microbiol Infect* 22, 799-809

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

267/MPP

Influence of neutrophil serine proteases on killing of _S. aureus_ by neutrophils

D. Kretschmer*[1], R. Stock[1], D. Pfaff[1], S. Rooijakkers[2], A. Peschel[1]
[1]*University of Tuebingen, Infectionbiology, Tübingen, Germany*
[2]*University of Utrecht, Bacterial Infections and Immunity, Utrecht, Netherlands*

Highly pathogenic *Staphylococcus aureus* secrete phenol-soluble modulin (PSM) peptides in the phagosome and extracellularly. It has been shown that in low concentrations PSMs activate formyl-peptide receptor (FPR) 2 but induce leukocyte lysis at high concentrations. Although neutrophils exhibit various kinds of weapons, only the oxygen dependent weapons play an essential role for killing of *S. aureus*. Neutrophils produce serine proteases, which have been shown to destroy various bacteria or virulence factors. Interestingly, *S. aureus* secretes three neutrophil serine protease (NSP) inhibitors: Eap, EapH1 and EapH2. We wanted to investigate if NSPs can degrade PSMs, which influence this might have on the elimination of *S. aureus* by neutrophils, and which role NSP inhibitors play in the context of *S. aureus* killing by neutrophils.

We found that treatment of PSMa3, either with low amounts of one of the three NSPs neutrophile elastase, cathepsin G or proteinase 3, led to abrogation of the cytotoxicity of PSMa3 and of FPR2 activation. Instead, we noticed an increase of FPR1 activation. To analyse the effect of the NSP inhibitors, we created a mutant, named *S. aureus* USA300 DeapDH1DH2, defect in secretion of neutrophil serine protease inhibitors.

Interestingly, elastase activity can be strongly inhibited via culture filtrates of the *S. aureus* wild type strain, whereas culture filtrates

of the serine protease inhibitor mutant does not inhibit elastase activity. Furthermore, we observed that killing of the mutant strain *S. aureus* USA300 DeapDH1DH2 by neutrophils is significantly reduced compared to the wild type.
Thus, we hypothesize that PSMs in the phagosome are protected from degradation by NSPs via the NSP inhibitors of *S. aureus* which is thereby able to circumvent killing by neutrophils

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

268/MPP

Dissecting EHEC Toxin Delivery by Creating Synthetic OMVs

A. Kehl*[1], D. Steil[1], J. Müthing[1], U. Dobrindt[1], A. Mellmann[1]
[1]*University of Münster, Institute of Hygiene, Münster, Germany*

Enterohaemorrhagic *Escherichia coli* (EHEC) are the major cause of the life-threatening haemolytic-uraemic syndrome (HUS). Major virulence factors include a panel of different toxins, the composition of which can differ significantly between strains, with Shiga toxin (Stx) being the most important one in molecular pathogenesis. Apart from free, soluble toxins the delivery via outer membrane vesicles (OMVs) is being increasingly recognized as a possible route.
However, due to OMVs carrying a toxin cocktail the contribution of each individual toxin to EHEC pathogenesis remains enigmatic. To dissect the influence of single toxins in pathogenesis, we aimed at establishing a method to create defined molecular structures mimicking OMVs.
To this end, first, the production of homogenous liposomes resembling the *E. coli* outer membrane lipid composition by sonication/extrusion was established. The quality of the liposomes was controlled via dynamic light scattering. Second, with bovine serum album (BSA) serving as a surrogate the production of protein-filled liposomes was established via dehydration/rehydration. In this process different parameters were optimized. These include a higher lipid to protein ratio, the preparation at room temperature, a lack of freeze-thawing stability, and the determination of long-term storage stability for at least half a year. Preliminary results with toxins, including Stx, instead of BSA show similar results and allow us to test these artificial OMVs either with single toxins or in combinations of desire in cellular settings.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

269/MPP

Insights into capsular biosynthesis gene regulation in *Staphylococcus aureus* by redefining the promoter architecture

D. Keinhörster*[1], S. E. George[1], C. Weidenmaier[1], C. Wolz[1]
[1]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Tübingen, Germany*

Staphylococcus aureus produces a capsular polysaccharide (CP) which has been shown to possess anti-phagocytic properties, allowing the bacterium to persist in the infected host. However, CP synthesis is highly heterogeneous within a population and mainly detectable in non-growing cells (late growth phase) (1). Capsular biosynthesis genes are encoded by a *capA-P* operon, which is regulated and transcribed by one principal promoter (P_{cap}) in front of *capA*. As P_{cap} promoter activity correlates with CP synthesis, this indicates that regulation occurs predominantly on the transcriptional level. The interplay of different regulators influencing the early-Off/late-Heterogeneous *cap* transcription at the molecular level is poorly understood. Here, we aim to decipher the molecular architecture of the P_{cap} promoter region.
Expression and regulation of *cap* was investigated by creating various deletions of the ~ 400 bp P_{cap} promoter region fused to *yfp*. Promoter-activity of the truncated fusions in different genetic backgrounds was assessed by microscopy and quantification of fluorescence during growth. Molecular analysis of P_{cap} revealed a new transcriptional start site and that a minimal promoter of ~ 50 bp is sufficient for *cap* expression. This main promoter shows a prototypic Sigma factor B consensus sequence. Sigma factor B is most active towards late growth phase, indicating that it is one of the main drivers for the temporal *cap* expression. Furthermore, binding sites of some major regulators (e.g. Rot and CodY) were localized to a region upstream of the minimal 50 bp element.
The late heterogeneous expression pattern of CP is likely determined by the interaction of transcriptional factors with Sigma factor B activity. Redefining the promoter architecture is a powerful first step to elucidate complex processes leading to temporal and bistable expression of cell surface polymers.

Reference

[1] George SE, Nguyen T, Geiger T, Weidenmaier C, Lee JC, Liese J, Wolz C, *Molecular microbiology* **2015;** *98*:1073-1088.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

270/MPP

Presence of SopE results in increased SCV damage and cytosolic release during host cell infection by *Salmonella enterica*

J. Röder*[1], M. Hensel[1]
[1]*Universität Osnabrück, Mikrobiologie, Osnabrück, Germany*

Background: The food-borne pathogen *Salmonella enterica* Typhimurium (STM) actively invades non-phagocytic cells by translocation of effector proteins by the SPI1-encoded (*Salmonella* pathogenicity island 1) T3SS (type 3 secretion system). Inside host cells STM exhibits a bimodal lifestyle, residing inside a membrane-bound compartment, known as *Salmonella*-containing vacuole (SCV), or in the host cell cytosol after escape from the SCV. STM is able to enter the host cell cytosol in various ways and at various time points. SPI1-T3SS mediates host cell invasion but also destabilizes the SCV membrane shortly after host cell entry, leading to a subpopulation of cytosolic bacteria. Vacuolar bacteria express the SPI2-T3SS for translocate effector proteins across the SCV membrane into the host cell. These effectors contribute to the vacuolar lifestyle by maturation of the SCV and formation of *Salmonella*-induced filaments (SIFs). Some of these SPI2-T3SS effectors promote the SCV integrity. The membrane of a SCV harboring the *sifA* strain is unstable, therefore bacteria are released into the cell cytosol at late time points post infection (Knodler, 2015).
Aims: We want to establish a reporter system for monitoring cytosolic presence of STM. The system will be used to identify further factors influencing the rupture of the SCV and cytosolic release.
Material and Methods: We use a reporter system based on the sensing of Glucose-6-phosphate (G6P) by the UhpABC system, which induces the expression of *uhpT*, a hexose phosphate transporter. As G6P is only present in the host cytosol and not in vacuolar compartments, we generated a reporter plasmid to monitor the cytosolic presence with constitutive expression of dsRed under control of P*uhpT*. Subsequent investigations with flow cytometry and fluorescence microscopy allows to quantify the proportion of cytosolic and vacuolar STM in infected host cells based on the induction of fluorescent reporters.
Results: Utilizing this method we could show that the trigger invasion by SPI1-T3SS causes increased SCV damage resulting in cytosolic replication. STM expressing the outer membrane protein Invasin of *Yersinia* spp. are able to invade by zipper invasion and showed only few cytosolic bacteria in contrast to bacteria invade by trigger invasion. The SPI1-T3SS effector SopE has a crucial role on SCV rupture. We could demonstrated that Hela cells infected with strains secreting SopE exhibit a larger fraction of cytosolic STM at early time points post infection.
Summary: By employing a G6P reporter system we identify that the mode of internalization affects SCV **i**ntegrity and the degree of release into host cell cytosol. Particularly the effector SopE is an important factor modulating the intracellular lifestyle in context of SCV rupture and cytosolic replication due to the level of invasion and the bacterial load.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

271/MPP

In *Staphylococcus lugdunensis* heterogeneity of fibrinogen binding is due to capsular polysaccharides and a frame shift mutation in the *fbl* gene.

L. Marlinghaus*[1], C. Lemke[1], M. Mucke[1], J. A. Ameln[1], S. G. Gatermann[1]
[1]*Ruhr-Universität Bochum, Medizinische Mikrobiologie, Bochum, Germany*

Introduction: *Staphylococcus lugdunensis* is a commensal and integral part of the normal skin flora but also an important pathogen that causes several mild to serious infections similar to those caused by *Staphylococcus aureus*, like endocarditis, sepsis, skin and soft tissue infections. The fibrinogen binding protein clumping factor A (ClfA) is one of the key virulence factors in *S. aureus* and a criteria used to distinguish *S. aureus* from other coagulase-negative staphylococci. *S. lugdunensis* is also able to bind fibrinogen by means of a ClfA homolog, the Fbl. All *S. lugdunensis* strains possess the *fbl* gene analyzed by PCR but only about 55% of the strains bind fibrinogen.
Objectives: Analyze the influence of capsular polysaccharides and *fbl* repeat length on fibrinogen binding capacity of *S. lugdunensis* and the distribution of a truncated *fbl* gene.
Materials and Methods: The fibrinogen binding capacity of capsular mutant strains was analyzed. The *fbl* repeat lengths was determined by PCR. The *fbl* genes were sequenced.
Results: The repeat region of *fbl* has a length of 300-1000 bp. In most of the strains (77.5%) the repeats were longer than 750 bp. In capsule mutants a strong increase of fibrinogen binding was observed when the repeat region had a length of 300 bp and 750 bp but not in strains with a repeat region of 850 bp and 900 bp. We partially sequenced the *fbl* of 77 non-fibrinogen binding strains and observed a frame shift mutation in 50.6% of the cases. The deletion of 4 base pair in these 39 strains is always located at position 12-15 which is a direct repeat of position 16-19. One may think this might be an on/off switch for fibrinogen binding controlled by a slipped strand mispairing mechanism. However, the distribution of a gene encoding for a beta-lactamase *blaZ* and 5 nucleotide dimorphisms upstream of *fbl* indicate that the strains carrying the truncated *fbl* may belong to one lineage. Among these strains 94% were *blaZ*-positive whereas among the strains possessing the functional *fbl* only 23.8% were *blaZ*-positive.
Conclusion: Fibrinogen binding capacity is influenced by capsular polysaccharides in strains of *S. lugdunensis* harboring an *fbl* gene with a short repeat region. In about 50% of the non-fibrinogen binding strains the *fbl* gene was truncated. These strains may be a lineage and have a very high incidence for beta-lactamase carriage.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

272/MPP

Mitochondrial apoptosis is a pathway of innate immunity

D. Brokatzky*[1]
[1]*Uniklinik Freiburg, IMMH, Freiburg i. Br., Germany*

Introduction: Mitochondrial apoptosis is induced by a core signaling pathway and can be activated by numerous signals. Recent research indicates that mitochondrial apoptosis can also operate at a "sub-lethal" level, where the system is activated but the cell stays alive.
Question and Methods: We hypothesized that low-level-pro-apoptotic signals are often generated during infection and that the signal generated can be used not to kill the cell but to activate it. We therefore tested a number of pathogens for their ability to induce sub-lethal apoptosis and tested for the activation of the infected cell. We found that DNA-damage and a DNA-damage-response was the most sensitive parameter for the detection of low-level activity of the apoptosis apparatus.
Results: During infection with five different infectious agents (bacteria and viruses) we detected DNA-damage and a DNA-damage response that depended on the activity of the apoptotic pathway but that occurred in the absence of cell death. Further, cells with inactivating mutations in mitochondrial apoptosis showed reduced secretion of cytokines upon infection with microbial pathogens.
Conclusions: These findings are evidence that bacterial and viral infections can frequently trigger "sub-lethal" apoptosis. The infected cell can react to this low-level-pro-apoptotic activity, and mitochondrial apoptosis can in this way be used as a cell-intrinsic pathogen recognition system.

Presentation: on Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

273/MPP

P2X-Receptor Antagonists Inhibit the Interaction of pore forming S. aureus Hemolysin A with Membranes

M. Schwiering[1], M. Husmann*[2], N. Hellmann[1]
[1]*Johannes Gutenberg-Universität, Molekulare Biophysik, Mainz, Germany*
[2]*Universitätsmedizin der Johannes Gutenberg-Universität, Mainz, Germany*

The pore forming alpha-toxin, or hemolysin A (Hla), is an important virulence factor of *Staphylococcus aureus*. It has been estimated that 1-2 pore(s) per cell are sufficient to kill it. To explain this remarkable efficiency, a major role of ATP-gated purinergic receptors (P2XR) has been invoked, which is now widely believed to enhance the effect of Hla. Moreover, the mechanism is thought to be of general importance for the lytic activity of pore forming proteins, including other toxins and complement. Many of the data that have been previously published in support of this concept were obtained with small MW inhibitors of P2XR. Therefore, and because we could not confirm that Hla-dependent hemolysis was enhanced by extracellular ATP, and because, in our hands, oxidized adenosinetriphosphate (oxATP) had only a minor inhibitory effect, we decided to re-investigate the effect of P2XR-inhibitors on Hla-dependent lysis. Hemolysis experiments were performed with highly sensitive rabbit erythrocytes. Fluorescence microscopy and gel-electrophoresis were employed to study binding to, and subsequent oligomerisation of Hla on membranes. Liposomes were used to investigate a potential effect of P2XR-inhibitors on the binding of Hla to pure lipid membranes, and calorimetry was used to probe for direct interaction of P2XR-inhibitor pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) with toxin. Internally S35-Met-labeled Hla was utilized to study binding and oligomerization of Hla in HaCaT cells; and the loss of ATP in Hla-treated HaCaT cells was measured with a Luciferase-based assay. First, using rabbit red cell hemolysis, we found that the inhibitory potency of P2XR-antagonists was indeed in a range previously reported for erythrocytes of other species and other toxins. However, we also found that Hla-dependent hemolysis was not enhanced by extracellular ATP. Second, oxidized adenosinetriphosphate (oxATP) had only a minor inhibitory effect. Therefore, we re-investigated the effect of P2XR-inhibitors that have been frequently used to study the role of P2XR for lysis induced by pore forming proteins. Fluorescence microscopy and gel-electrophoresis clearly showed that P2XR-inhibitors interfere with binding and subsequent oligomerisation of Hla with membranes. Also, binding and oligomerization of Hla on nucleated cells (keratinocyte cell line HaCaT) was markedly reduced by pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS). In keeping with this, Hla-dependent loss of ATP was inhibited. Unexpectedly, P2XR-inhibitors prevented Hla-induced lysis of pure lipid membranes, revealing that inhibition did not even depend on the presence of P2XR. Finally, calorimetric data and hemolysis experiments with Hla pre-treated with PPADS showed that this compound binds to Hla. Our results call for a critical re-assessment of the concept that P2XR are general amplifiers of damage by pore-forming proteins.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

274/MPP

The adherence factors HcpA and Iha of enterohemorrhagic *Escherichia coli* O157:H7 strain Sakai influence its internalization into the roots of *Valerianella locusta* grown in soil under greenhouse conditions

K. Eissenberger*[1], D. Drissner[2], A. Weiss[1], H. Schmidt[1]
[1]*University of Hohenheim, Institute of Food Science and Biotechnology, Department of Food Microbiology and Hygiene, Stuttgart, Germany*
[2]*Agroscope, Microbiology of Plant Foods, Wädenswil, Switzerland*

Within the last years an increasing number of food-associated outbreaks caused by enterohemorrhagic *Escherichia coli* (EHEC) could be traced back to the consumption of contaminated fresh produce such as salads, spinach and sprouts. As bacterial contamination may occur directly on the field *via* manure, fecal contamination, irrigation or surface water it is an important issue to evaluate whether EHEC are able to colonize plant roots and to understand the underlying mechanisms. The capability of EHEC to grow on plant leaves and to colonize these as well as its ability to internalize into the roots of leafy greens lettuce, spinach and parsley was demonstrated by several studies. However, it remains still unknown which intrinsic factors play a role during this process.

In this study we analyzed the internalization of *E. coli* O157:H7 strain Sakai and its isogenic mutants that lack one or both of the adherence related genes *hcpA* and *iha* into the roots of lamb"s lettuce.

Lamb"s lettuce was grown in non-sterile diluvial sand for three weeks. Subsequently the plants were contaminated with fluorescently labelled EHEC O157:H7 strain Sakai or its derivatives *via* irrigation water. After four days of incubation under greenhouse conditions at 21°C and 12 h day/night-cycle, plants were excavated and surveyed regarding the ability of EHEC to adhere to and internalize into the roots of lamb"s lettuce. For these purposes the plants were either washed and analyzed afterwards, or they were washed and surface disinfected prior to analysis. The roots were investigated by fluorescence microscopy for qualitative analysis as well as by homogenization and plating in order to determine the number of adherent and internalized bacteria, respectively.

By qualitative analysis *via* fluorescence microscopy *E. coli* cells were detected at/in the roots in a genotype independent manner after washing and also after surface disinfection. Homogenization and plating of washed roots showed that neither deletion of either *iha* or *hcpA* nor the deletion of both genes led to altered adherence behavior of EHEC O157:H7 strain Sakai. In contrast, the deletion of one or both genes resulted in significantly decreased levels of internalization. Surprisingly, no difference between the single deletions mutants and the double deletion mutant was detected. These results showed that HcpA and Iha play a role during infections of plant roots but not during attachment to them. Furthermore, the findings indicated that these proteins do not act in concert and that additional factors are required for root internalization.

As shown by this *in vivo* study HcpA and Iha are rather internalization factors of EHEC O157:H7 strain Sakai than adherence factors under the tested conditions. Moreover, the results suggest that these two factors are not the only virulence factors involved in internalization. Therefore, further research is needed to gain a better understanding of the underlying mechanisms.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

275/MPP

Organization of multi-binding to host factors: the glyceraldehyde-3-phosphate dehydrogenase of *Mycoplasma pneumoniae*

J. Grimmer[1], R. Dumke*[1]
[1]*TU Dresden, Dresden, Germany*

Question: Different glycolytic enzymes of many bacterial species have been confirmed as surface-displayed and interacting with host proteins. Especially in the cell wall-less mycoplasmas, multi-functioning proteins are suggested to compensate the strongly limited genome resources of these micro-organisms. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of the human respiratory pathogen *Mycoplasma pneumoniae* (M.p.) is a typical member of the class of moonlighting proteins. In previous studies, the enzyme was characterized as localized on the surface of M.p. cells and able to bind to many human extracellular matrix proteins (ECM), like fibrinogen, fibronectin, lactoferrin, laminin, vitronectin, and plasminogen. However, the molecular organization of interactions with different host factors remains unclear.

Methods: To further characterize surface-displayed and binding parts, we divided full-length GAPDH of M.p. into four recombinant proteins (GAPDH-1 to 4) and produced polyclonal antibodies. ELISA, ligand immunoblot assays and immunofluorescence experiments were used to investigate surface localization of protein parts and interactions with host factors.

Results: All investigated protein parts of GAPDH bind to human A549 cells and concentration-dependently to selected human proteins (plasminogen, fibrinogen, fibronectin and vitronectin). Furthermore, in the presence of recombinant GAPDH-1 to 4 and activators uPA and tPA, plasminogen was converted to proteolytically active plasmin. In contrast, surface localization could be confirmed for GAPDH-4 only. The following order of binding affinities (K_D values) of host factors to immobilized recombinant GAPDH-4 was measured: plasminogen > fibronectin > vitronectin > fibrinogen, respectively.

Conclusions: The results of the study confirm the surface localization of the C-terminal part of GAPDH of M.p. and the interaction of this region with different host factors. Binding in the presence of several human ECM proteins seems be regulated by differences in binding affinities.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

276/MPP

***Staphylococcus lugdunensis* cell surface-displayed enolase is a plasminogen-binding virulence factor**

M. Hussain*[1], G. Peters[1], K. Becker[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction: Many cell surface receptors expressed by staphylococci and other bacterial pathogens capture plasminogen (Plg). The acquired proteolytic activity facilitates pathogens for invasion and dissemination in the infected host tissues. The main Plg receptors include the cytoplasmic proteins enolase and glyceraldehyde-3-phosphate dehydrogenase.

Intention: Our aim is to identify and characterize virulence factors of *S. lugdunensis* in order to clarify the pathogenicity of this species that ranges between *S. aureus* and "classical" coagulase-negative staphylococci.

Material and Methods: Mass spectrometric peptide mapping was used to identify proteins. Ion-exchange chromatography (IEX) was applied to purify native *S. lugdunensis* enolase (SlEno). Recombinant *S. lugdunensis* (rSlEno) and human enolases (rHEno1) were expressed using six-His-tagged pQE30 vector.

Results: A protein band close to 52-kDa marker in LiCl extract was identified as *S. lugdunensis* enolase. The SlEno contains neither a signal peptide nor an LPXTG motif. Appling IEX from LiCl-extract, 99% pure SlEno was eluted and His-tagged recombinant proteins were purified in a single step on Ni-NTA resin. The rSlEno was found to bind specifically immobilized laminin, fibronectin, collagen type IV, Plg and rHEno1 in a dose-dependent manner. The rSlEno activity was similar to that of rabbit muscle enolase purchased from a commercial source. In the presence of rSlEno, the rate of tissue-type plasminogen activator (tPA)-dependent Plg activation significantly increased. Applying jellified matrices containing fibrinogen (Fg) to check plasmin activity, SlEno readily promoted the lysis of Fg. The assays indicated a specific interaction between Plg and enolase from *S. lugdunensis* cells, which leads to the activation of plasmin and subsequent degradation of Fg. The fibrinolytic activity was enhanced in a dose-dependent manner in the presence of enolase. A fibrin matrix was generated on membranes of Transwell cell

culture inserts to study transmigration. While the number of transmigrated bacteria was up to 512 cfu/ml in 3 min in the presence of Plg and tPA, transmigration was significantly increased when rSlEno added and the number of transmigrated bacteria reached to 2,023 cfu/ml in the same time.
Conclusion: *S. lugdunensis* displayed an enolase that functions in plasmin(ogen) activation and adhesiveness, thus, being a part of the pathogen´s virulence factor armamentarium.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

277/MPP

Establishment of a cellular *in vitro* infection model for the analysis of *Bartonella bacilliformis* pathogenicity

B. Leins*[1], M. Meister*[1], C. Sittmann*[1], K. Hipp[2], J. Berger[2], W. Ballhorn[1], V. A. J. Kempf[1]
[1]*Goethe-University, Institute for Medical Microbiology and Infection Control, Frankfurt am Main, Germany*
[2]*Max Planck Institute, Development Biology, Tübingen, Germany*

Introduction: *Bartonella bacilliformis* is a neglected bacterial pathogen and the causative agent of the biphasic Carrion"s disease, leading to hemorrhagic fever (Oroya fever) and Peruvian warts. The disease is restricted to the Peruvian Andes. Transmission is mediated by the sand fly *Lutzomyia verrucarum*.
Aims: Little is known about the infection mechanisms and the underlying pathogenicity factors of *B. bacilliformis*. Therefore, infection models need to be established to analyze the pathogenicity mechanisms. Cellular microbiology methods should help to understand the complex interactions of *B. bacilliformis* and its host (e.g. host cell tropism, cell adhesion, invasion and host cell response).
Materials and Methods: HUVECs, HeLa-229 cells and erythrocytes were co-cultured with *B. bacilliformis* (strains: ATCC35686, JB584, friendly gift of Prof. M. Minnick, Montana, USA). Bacteria and infected cells were analyzed via immunofluorescence microscopy and electron microscopy. Host cell response upon *B. bacilliformis* infection was determined on transcriptional (qPCR) and translational level (ELISA) for vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8).
Results: Due to the fact that the growth conditions of *B. bacilliformis* and their human host cells differ in terms of culture media and cultivation temperature (temperature optimum of *B. bacilliformis*: 27C°) experimental infection conditions were adapted. *B. bacilliformis* was able to invade HUVECs more efficient than HeLa-229 cells. Likewise, the gene induction of IL-8 and VEGF was significantly more increased in endothelial cells than in epithelial cells. In contrast, significant quantifiable secretion of VEGF was only detected in infected HeLa-229 cells. Furthermore, infection of human erythrocytes resulted in considerable movement and agglomeration of erythrocytes compared to uninfected controls.
Conclusion: Although cell tropism to endothelial cells is described extensively in literature our investigations show no considerable effect of *B. bacilliformis* infection on epithelial and endothelial cells. These results may point to the fact, that *B. bacilliformis* might engage unknown mechanisms or other cells e.g. erythrocytes to interact with these cells. The role of flagellin and the trimeric autotransporter adhesion BrpA need to be elucidated in detail to understand *B. bacilliformis* pathogenicity.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

278/MPP

Unraveling antibiotic resistance mechanisms and dynamics of resistant *Staphylococcus aureus* isolates during chronic airway infection in CF patients.

C. Neumann*[1], L. Ueberhorst[1], T. Tißen[1], B. Husmann[1], B. Grünastel[1], J. Sobocinski[1], S. Deiwick[1], H. Rengbers[1], B. C. Kahl[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction: *Staphylococcus aureus* is one of the first and most frequent pathogens recovered from the airways of cystic fibrosis (CF) patients with increasing prevalence rates due to early eradication strategies of *Pseudomonas aeruginosa*. A rising number of multidrug resistant bacteria has been observed and antimicrobial resistance pattern of the bacteria involved in respiratory infections of CF patients is of increasing concern. In CF lungs, the bacteria can cause long-term infections despite appropriate antibiotic therapy through adaption to this specific niche by various mechanisms including phage insertions and/or exclusions, genome rearrangements, loss of *agr* activity, emergence of small colony variants (SCVs) and mucoid isolates .
Aims: We want to analyze the dynamics and mechanisms of resistance and the association with distinct clinical manifestations. We are interested in the acquisition of resistance by tracking down the first resistant isolate during the history of chronic *S. aureus* airway infection of the CF patients in our study. We plan to investigate and compare resistance mechanisms by PCR and sequencing/genome sequencing.
Material and Methods: We conducted a prospective one-year observational study, including 14 CF patients with chronic *S. aureus* airway infection with the aim to investigate the diversity and resistance pattern of *S. aureus* in this special habitat by randomly picking 40 *S. aureus* isolates, which were characterised by phenotypic evaluation (size, hemolysis, mucoidy), genotyping (*spa*-sequence typing) and susceptibility testing.
Results: We were able to analyze 2320 *S. aureus* isolates from 58 visits of 14 CF patients. We observed a high diversity of phenotypes and resistotypes of *S. aureus* within single sputa which changed during the observation period partly dependent on clinical status and antibiotic treatment. We noticed that in patients, which were infected only by susceptible *S. aureus* strains up to 4 different clonal lineages co-existed and all these different clonal lineages persisted. In contrast, during the observation period in some patients resistant *S. aureus* isolates outcompeted isolates[B1] with other *spa*-types and these more resistant *S. aureus* strains persisted over the study period.
Summary: A high diversity of *S. aureus* phenotypes and resistotypes could be observed during our prospective one-year observational study. In the future, we plan to analyze the dynamics and mechanisms of resistance and the association of different *S. aureus* strains with distinct clinical manifestations.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

279/MPP

The virulence potency of LA-MRSA does not differ from HA-MRSA or MSSA during long term persistence in the airways of cystic fibrosis patients

S. van Alen*[1], S. Berg[1], J. Treffon[1], K. Becker[1], B. C. Kahl[1]
[1]*University Clinic Münster, Institute of Medical Microbiology, Münster, Germany*

Question: The airways of patients with cystic fibrosis are often colonized and infected with *Staphylococcus aureus* for extended periods. Both, hospital-acquired (HA) and community-acquired (CA) methicillin-resistant *S. aureus* (MRSA) have been reported to be cultured from the airways of CF-patients with a negative impact on lung function and disease severity. Recently, livestock-associated (LA)-MRSA emerged in regions with high density of pig farming. As Münster and the region of the Münsterland belong to the pig farming belt, we were able to show that LA-MRSA can be isolated and survive within CF airways for several months and years. However there is no information about the pathogenic capacity and adaptation of LA-MRSA during long term persistence

in CF patients. Therefore, this study aimed to assess the virulence properties of LA-MRSA isolated from CF patients in comparison to HA-MRSA and methicillin-susceptible *S. aureus* (MSSA).
Methods: *S. aureus* strains (MSSA, HA- and LA-MRSA) were isolated from lung specimens of CF patients treated in two certified CF centers in Münster and were genotyped using s*pa*-typing. The virulence properties of LA-MRSA, HA-MRSA and MSSA were compared based on three strain pairs consisting each of an early and late isolate of each group (n = 6 for each group). To determine the virulence between the different groups, host cell invasion, cytotoxicity, α-toxin activity and biofilm formation were compared.
Results: Overall, the pathogenic potential of LA-MRSA did not differ from MSSA and HA-MRSA. No group specific differences regarding invasion of and persistence in A549 lung epithelial cells were observed between the different groups and an overall high invasiveness was determined. Nevertheless, a high heterogeneity was observed within the groups leading to no significant differences between the early and late isolates. Furthermore, our investigations demonstrated a higher hemolysis capacity of LA-MRSA compared to HA-MRSA and MSSA. This could be linked to the absence of bacteriophage *phi3* within the *hlb*-gene of all LA-MRSA, while the bacteriophage was present in all HA-MRSA and MSSA thereby disrupting the ß-toxin gene. Moreover, biofilm formation was only detected in 2 of 19 isolates (one late LA-MRSA and one late MSSA) belonging to the *spa*-types t034 and t080.
Conclusions: The virulence properties regarding invasion, cytotoxicity and biofilm formation of LA-MRSA, which were isolated from and persisted in CF airways, were comparable with those of HA-MRSA and MSSA. However, LA-MRSA demonstrated a higher hemolytic potency in regard to rabbit erythrocytes, which needs further investigation concerning its biological *in vivo* function.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

280/MPP

Replication of *Francisella* in primary human lung tissue

K. Köppen*[1], K. Rydzewski[1], S. Hippenstiel[2], D. Fatykhova[2], K. Heuner[1]
[1]*Robert Koch-Institut, , Berlin, Germany*
[2]*Charité - Universitätsmedizin Berlin, Dept. of Internal Medicine, Infectious Diseases and Respiratory Medicine - Critical Care Medicine, Berlin, Germany*

Introduction and Aim: *Francisella tularensis* (*Ft.*) is an intracellular pathogen causing tularemia in a variety of hosts including humans and rodents. The highly virulent subspecies *Ft. tularensis* is only found in North America, whereas the subspecies *Ft. holarcitca* is moderate virulent for humans and found in the whole northern hemisphere. It has been shown that an inoculum of 10 *Ft. tularensis* bacteria in an aerosol form is sufficient to induce pneumonia in humans. So far, mainly cell cultures and mice models are used to investigate the virulence of *Francisella*. Since some *Francisella* strains are nonvirulent for humans but highly virulent for mice, the existing virulence tests are unsatisfying and insufficient. Generally, *Francisella* strains differ in host specificity and virulence, and less is known for humans. Therefore, we aimed to develop an *ex vivo* infection model using primary human lung organ tissue which provides reliable results regarding the virulence potential of *Ft.* strains for humans.
Material and Methods: Here, we established the first *ex vivo* replication model for *Ft.* in primary human lung organ cultures. All assays were performed with three *Ft. holarctica* strains including the human-derived wild-type isolate A660, the commonly laboratory-used *LVS and a non-intracellular-replicating* LVS Δ*iglC* mutant, and the newly discovered environmental *Francisella* sp. isolate W12-1067.
Results: The human lung *ex vivo* model was successfully established. The wild-type isolate A660 and LVS replicated efficiently in primary human lung tissue during an incubation period of 72 h. In contrast, the LVS Δ*iglC* mutant and *Francisella* sp. strain W12-1067 did not replicate but persist in primary lung tissue. To define the *Ft.*-infected cell types within the human lung tissue, immunohistochemical analysis are planned but still pending.
Conclusion: Here, we developed the first human *ex vivo* infection model for *Francisella* using primary human lung tissue. This infection model could be used for virulence tests of new *Francisella* strains and isolates.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

281/MPP

The role of SodM in long persisting *Staphylococcus aureus* clones from cystic fibrosis airway

J. Treffon*[1], D. Block[1], S. Reiss[2], S. Fuchs[2], L. Langhanki[3], A. Mellmann[3], S. Engelmann[4,5], B. C. Kahl[1]
[1]*University Hospital Münster, Institute for Medical Microbiology, Münster, Germany*
[2]*Ernst-Moritz-Arndt Universität Greifswald, Institute for Microbiology, Greifswald, Germany*
[3]*University Hospital Münster, Institute for Hygiene, Münster, Germany*
[4]*Technical University Braunschweig, Institute for Microbiology, Braunschweig, Germany*
[5]*Helmoltz Center for Infection Research, Microbial Proteomics, Braunschweig, Germany*

Introduction: Cystic fibrosis (CF) patients experience mucus dehydration leading to decreased mucociliary clearance, chronic bacterial infections, lung insufficiency and early death. *Staphylococcus aureus* is one of the earliest pathogens that colonizes and persists in the airways of CF patients for extended periods.
Objectives: This project aims to detect adaptation mechanisms that facilitate staphylococcal long-term persistence in the CF airways.
Methods: From a longitudinal collection of *S. aureus* strains cultured from respiratory specimens of CF patients clonal short and long persisting *S. aureus* isolates of one patient (P20) were chosen for analysis of the cytoplasmic proteome using 2D gel electrophoresis. Five additional strain pairs from other CF patients were used to verify proteomic data by qRT-PCR, functional assays and cell culture experiments.
Results: The proteomic analysis revealed multiple changes in protein abundance. Interestingly, one of the two staphylococcal superoxide dismutases, SodM, was more abundant in the late compared to the first isolate of P20. Functional assays identified higher SodM activity under oxidative stress conditions for the long persisting clone. In addition, qRT-PCR analysis confirmed the up-regulation of *sodM* in the late isolate. Interestingly, the high expression of *sodM* could also be detected for long persisting clones of four other CF patients. *In vitro* infection assays using A549 airway epithelial cells, the bronchial epithelial CF cell line CFBE41o- and the complemented cell line indicated that clinical isolates with high *sodM* expression seem to be more able to invade or to survive in these cells compared to isolates with lower *sodM* transcription. Furthermore, we detected that *sodM* is highly expressed in these internalized bacteria. Preliminary data show that upon internalization by epithelial cells the clinical isolates seem to stay in the phagosome. By invasion assays and qRT-PCR experiments we are currently analyzing, if the high expression of *sodM* in internalized bacteria can be linked to the bacterial residence in the phagosome of the epithelial cell. In addition, we plan to investigate the gene expression of different host factors in infected epithelial cells to see which of these probably provokes the up-regulation of *sodM* in internalized bacteria.
Conclusion: Our results indicate that SodM might be a protein that facilitates the long-term persistence of *S. aureus* in the CF airways by protecting the bacterium against oxidative stress and probably supporting bacterial residence in the host cells.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

282/MPP

The *Staphylococcus aureus* extracellular matrix protein (Emp) has a fibrous structure and binds to different extracellular matrices

J. Geraci[1], S. Neubauer[2,3], C. Pöllath[1,2], U. Hansen[4], F. Rizzo[5,6], C. Krafft[7], M. Westermann[8], M. Hussain[9], G. Peters[9], M. W. Pletz[2,3], B. Löffler[1,10], O. Makarewicz[2,3], L. Tuchscherr*[1,11]

[1]*Jena University Hospital, Institute of Medical Microbiology, Jena, Germany*

[2]*Jena University Hospital, Center for Infectious Diseases and Infection Control, Jena, Germany*

[3]*InfectoGnostics, Jena, Germany*

[4]*University Hospital, Institute of Experimental Musculoskeletal Medicine, Münster, Germany*

[5]*Institute of Molecular Science and Technologies, Milano, Italy*

[6]*Westfälische Wilhelms-Universität Münster, Organic Chemistry Institute and CeNTech, Münster, Germany*

[7]*University of Jena, Leibnitz Institute for photon technologies, Jena, Germany*

[8]*Jena University Hospital, Center for electron microscopy, Jena, Germany*

[9]*University Hospital, Institute of Medical Microbiology, Münster, Germany*

[10]*Center for Sepsis Care (CSCC), Jena, Germany*

[11]*Jena University Hospital, Center for Sepsis and care, Jena, Germany*

Introduction: *Staphylococcus aureus* is an important human pathogen that can cause serious invasive and persistent diseases. Adhesion to the extracellular matrix of the host tissue is a critical step in S. aureus pathogenesis. This process is essentially mediated by adhesins, which are proteins that interact with the extracellular matrix. The extracellular matrix protein Emp is a secreted adhesin that mediates interactions between the bacterial surface and extracellular host structures. However, its structure and role in staphylococcal pathogenesis remain unknown. Aim/Question: The aim of this work was to characterize the Emp structure Methods / **Results:** Using multidisciplinary approaches, including circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy, transmission electron (TEM) and immunogold transmission electron microscopy, functional ELISA assays and in silico techniques, we characterized the Emp protein. We demonstrate that Emp and its truncated forms bind to suprastructures in human skin, cartilage or bone, among which binding activity seems to be higher for skin compounds. The binding domain is located in the C-terminal part of the protein. CD spectroscopy revealed high contents of β-sheets (39.58%) and natively disordered structures (41.2%), and TEM suggested a fibrous structure consisting of Emp polymers. The N-terminus seems to be essential for polymerization. Due to the uncommonly high histidine content, we suggest that Emp represents a novel type of histidine-rich protein sharing structural similarities to leucine-rich repeats proteins as predicted by the I-TASSER algorithm. **Conclusion:** According our new findings, we suggest a role of Emp in infections of deeper tissue and open new possibilities for the development of novel therapeutic strategies.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

283/MPP

Changes in nuclease activity of *Staphylococcus aureus* as a potential mechanism to escape neutrophil extracellular trap-(NET)-mediated killing during persistence in the airways of cystic fibrosis patients

S. Herzog*[1], F. Dach[1], M. Strake[1], J. Treffon[1], B. C. Kahl[1]

[1]*Institute of Medical Microbiology, Münster, Germany*

The disease of cystic fibrosis (CF) is characterized by frequent and chronic airway infections caused by *S. aureus*. One of the hallmarks of CF is the increased neutrophil presence in the lungs, which cause tissue destruction. In context of pathogen eradication, neutrophils are capable to form so-called "neutrophil extracellular traps" (NETs) consisting of antimicrobial peptides bound to extracellular DNA structures. *S. aureus* is able to escape NETs by the secretion of nucleases, which digest NET-derived DNA fibers. This study aims to elucidate mechanisms of *S. aureus* adaptation to the hostile environment of CF lungs in relation to NET-dependent bacterial killing by human neutrophils. We hypothesize that *S. aureus* escapes NETs by increasing its nuclease activity during persistent infection.

Expectorated sputum was analyzed via fluorescence microscopy. Nuclease activity of *S. aureus* isolates was assessed on DNA agar and by DNA-degradation assays. A FRET-based fluorescence assay was established to quantify nuclease activity. *S. aureus* isolates, which were recovered from airway specimens of CF-patients during a longitudinal study, were analyzed for their nuclease activity. The transcription of *nuc1* and *nuc2* expression was determined by qRT-PCR. In addition, *S. aureus* nuclease activity was tested in the presence of sputum supernatants.

In CF sputa, *S. aureus* was found tightly enclosed by NET-related DNA fibers. Comparing clonal sequential *S. aureus* isolates (n=112) from an individual CF patient, enhanced DNA degradation ability of long-term persisting isolates was observed. Also, nuclease activity of *S. aureus* strains with normal, mucoid or small colony variant phenotypes revealed to be phenotype-dependent. In particular, SCV"s showed less DNAse activity than normal strains on DNA agar plates, unlike mucoid strains, which revealed enhanced DNA degradation. In contrast, a more inhomogeneous picture was shown by strain pairs (early, late) from different CF patients with late isolates being more active (n=6), less active (n=11) or not changed (n=12) in nuclease activity. The vast majority of DNAse activity was demonstrated to be a result of *nuc1* expression prior to *nuc2* even though *nuc2* is the first nuclease being expressed by *S. aureus* under cultivation conditions. The results of *nuc1*-transcription were in line with the results of nuclease activity experiments. Interestingly, supernatants of several CF sputa showed an inhibitory effect on bacterial DNAse activity.

Preliminary results indicate time-dependent adaptation of *S. aureus* nuclease activity in CF lungs, which also depends on the phenotype. Inhibition of bacterial nuclease activity in the presence of sputum supernatants point to the interference with host specific factors such as antibodies and/or proteases. Further experiments are ongoing to determine the impact of differential nuclease activity of clinical *S. aureus* isolates on NET-dependent killing.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

284/MPP

Critical role of HIF-1α in transmigration of *S. pneumoniae* across the blood-brain barrier

G. Devraj*[1], S. Guerit[2], J. Seele[3], J. Macas[2], A. Braczynski[2], W. Ballhorn[1], S. Günther[4], M. Mittelbronn[2], L. Ogunshola[5], R. Nau[3], S. Hammerschmidt[6], U. Ködel[7], K. Devraj[2,8], V. A. J. Kempf[1]

[1]*Klinikum der Goethe-Universität, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Frankfurt am Main, Germany*

[2]*Klinikum der Goethe-Universität, Edinger Institut/Neurologisches Institut, Frankfurt am Main, Germany*

[3]*Universitätsmedizin Göttingen, Institut für Neuropathologie, Göttingen, Germany*

[4]*Max-Planck-Institut für Herz- und Lungenforschung, Bad Nauheim, Germany*

[5]*University of Zurich, Institute of Veterinary Physiology, Zürich, Switzerland*

[6]*Ernst-Moritz-Arndt Universität Greifswald, Genetik der Mikroorganismen, Greifswald, Germany*

[7]*Klinikum der Universität, Neurologische Klinik und Poliklinik, München, Germany*

[8]*Klinikum der Goethe-Universität, Allgemeine Pharmakologie, Frankfurt am Main, Germany*

Introduction: *Streptococcus pneumoniae* causes bacterial meningitis, a severe disease leading to the infection of brain parenchyma. Mechanisms underlying the transmission of the pathogen across the blood-brain barrier (BBB) into the brain are still poorly understood. The BBB consist of endothelial cells along with pericytes and astroglia forming the neurovascular unit (NVU) which maintains the homeostasis of central nervous system (CNS). The barrier function is a result of the tight intercellular junctions and efflux transporters of endothelial cells (ECs).

Objectives: We have previously shown that hypoxia inducible factor (HIF) -1α is generally activated in bacterial infections. As hypoxia is known to cause disruption of the BBB, we hypothesize that HIF-1α activation that ultimately induces secretion of the

vascular endothelial growth factor (VEGF) is critically involved in the transmigration process of pathogens across the BBB.
Materials and Methods: We performed pneumococcal infections of brain ECs *in vitro* and performed permeability assays of EC monolayers using dextrans of various molecular sizes. Localization of the *S. pneumoniae* on ECs was visualized using confocal immunofluorescence microscopy. Expression of several components of EC cell junctions (claudins, VE-cadherin) was analyzed by qRT-PCR and Western blotting. *In vivo* analyses was performed by IHC for human brain specimen and by IHC, Eelectron microscopy (EM) for mouse samples. Finally, unbiased RNAseq anaylsis was performed on brain microvessels isolated from healthy and infected mice to investigate the genes involved in BBB permeability.
Results: Results indicate an increase in paracellular permeability of EC monolayers to dextrans of various sizes and a localization of pneumococci predominantly at the cell-cell junctions. Expression analysis by qRT-PCR and Western blotting revealed an upregulation of HIF-1α and VEGF and a downregulation of VE-cadherin upon infection *in vitro*. Loss-of-function experiments performed using the HIF-1α inhibitor echinomycin and HIF-1α *si*RNA indicated a protective effect of HIF-1α inhibition on endothelial permeability upon infection in human and murine brain ECs. *In vivo* analysis of human and murine brain tissue samples by IHC demonstrated an upregulation of HIF-1α in the brain vessels in meningeal infections. EM analysis of infected mice showed a predominant endothelial junctional localization of bacteria. RNAseq analysis of brain microvessels revealed a significant upregulation HIF-1α in the infected mice that was associated with dysregulation of several genes involved in cancer, angiogenesis, and HIF-1 signaling.
Conclusions: Results demonstrate a critical role for HIF-1α in inducing local hypoxia and ultimately leading to the transmigration of bacteria across the BBB. Targeting of HIF-1α to prevent the bacterial transmigration across the blood-brain barrier and spreading of the infection might be a potential therapeutic strategy.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

285/MPP

Characterising the assembly of the outer membrane secretin of the *Salmonella* type III secretion system

N. Singh*[1], S. Wagner[1]
[1]*Institute of Med. Microbiology and Hygiene, Cellular and Molecular Microbiology, Tübingen, Germany*
Nidhi Singh[1], Samuel Wagner[1,2]
[1]*Interfaculty Institute of Microbiology and Infection Medicine Tübingen, nidhi.singh@med.uni-tuebingen.de, samuel.wagner@med.uni-tuebingen.de*
[2]*German Center for Infection Research (DZIF)*

Many pathogenic Gram-negative bacteria use type III secretion systems (T3SS) to inject effector proteins into target host cells. T3SS are specialized nanomachines composed of over 20 different proteins that build a membrane spanning multi-megadalton so called needle complex of about 200 subunits critical for bacterial pathogenicity(1). T3SS include an outer membrane protein of the secretin family (InvG in *Salmonella* T3SS-1) and most secretins require a lipoprotein (pilotin, InvH) for the formation of a functional translocation complex in the outer membrane. Formation of the secretin complex nucleates further the assembly of the entire T3SS. This hierarchy in assembly ensures penetration of the outer membrane by the growing needle of the system(2).
The recent publication of the structure of InvG(3) now facilitates a detailed investigation of the biogenesis and function of the secretin complex in the context of T3SS. We here present data that aim at elucidating the biogenesis of the secretin from translation at the ribosome to assembly of the complete T3SS needle complex using bacterial genetics, cell fractionation, *in-vivo* photocrosslinking and functional assays.

References

[1] Diepold A & Wagner S, FEMS Microbiol Rev (**2014**), Vol 38, Issue 4, Pages 802-822
[2] Wagner S et al., PNAS (**2010**), Vol 107, No 41, Pages 17745–17750
[3] L.J. Worrall et al., Nature (2016), Vol 540, Issue 7634, Pages 597-601

Figure 1

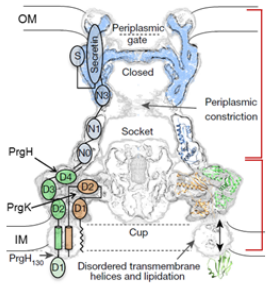


Fig. Cryo-EM central slice view of basal body reconstruction (in grey) & secretin InvG with respectively labelled domains (in blue). The domains of the inner membrane ring proteins PrgH and PrgK are shown in green and orange respectively.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

286/MPP

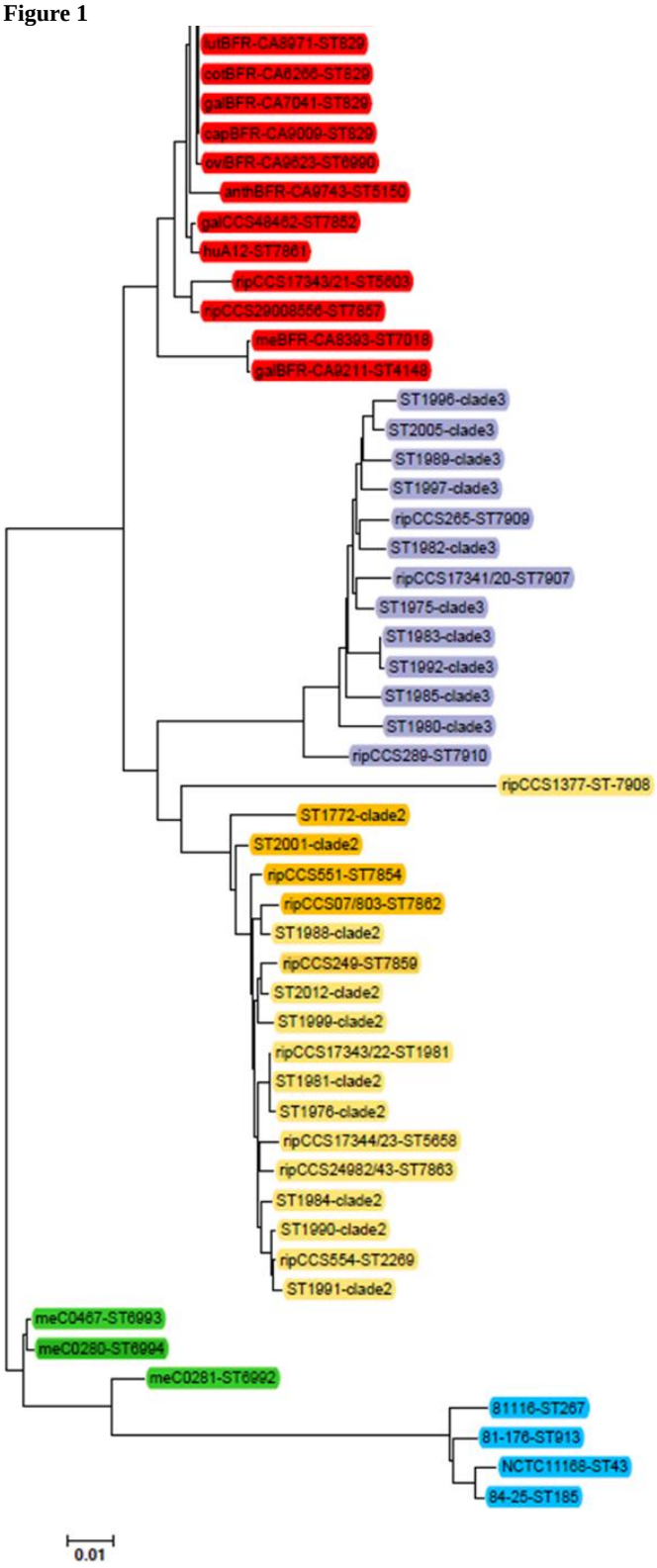
Phenotypic/Genotypic Characterisation of a putative novel *Campylobacter coli/jejuni* cluster

A. Lübke*[1], U. Groß[1], B. Bunk[2], T. Riedel[2], C. Spröer[2], J. Overmann[2], W. Bohne[1], A. E. Zautner[1]
[1]*Institut für Medizinische Mikrobiologie, Bakteriologie, Göttingen, Germany*
[2]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*

Introduction: Two of the most common causes of food-borne enteritis worldwide are *Campylobacter jejuni* and *C. coli*. With 94% identity in their 16S rRNA and 86.5% identity in their seven, MLST housekeeping genes (Dingle *et al*., 2005), *C. jejuni* and *C. coli* are thought to have evolved from a common ancestor, mainly by horizontal gene transfer. Following their speciation, ecological separation has predominantly served as the barrier to gene flow between these distinct species (Sheppard *et al*., 2008). However, recent observations of *C. coli* and *C. jejuni* isolates that contain alleles from the other species and discovery of *C. coli*/*C. jejuni* mosaic alleles in isolates of the species suggest a breakdown in this important gene flow barrier (Sheppard *et al*., 2013). This indicates a putative remerging of the two species in a process described as *despeciation*, an event that may lead to the emerging of one or several novel, medically-relevant *C. coli/C. jejuni* hybrid species (Sheppard *et al*., 2008).
Objectives and Methods: MeC0280, meC0281 and meC0467 are three *Campylobacter* isolates that were isolated from turkey (*Meleagris gallopavo*) meat slaughtered in Berlin-Brandenburg. Despite getting classified as *C. coli* by MALDI-TOF mass spectrometry analysis, MLST-based analysis indicates closer relation to *C. jejuni*. In light of the suspected, ongoing genetic introgression between the *C. jejuni* and *C. coli* species, this cluster of *Campylobacter* isolates may present one of the first *C. jejuni/C. coli* hybrid clonal complexes in the despeciation process of the genus.
In this project, a better understanding of the biology of this putative, novel *Campylobacter* despeciation clade was aimed to be gained. This was done by genome sequencing of the strains using a combination of single-molecule real-time and Illumina sequencing technology and by an in-depth phenotypic characterization of the strains based on water survival, eukaryotic cell invasion and adhesion, motility, autoagglutination and biofilm formation. In alignment of their genomic and phenotypic data, three *C. jejuni* reference strains - 11168, 81-176 and 81116 - and two *C. coli* reference strains - BfR-CA-9557 and RM2228 – were included in this study.
Results and Conclusion: The three tested isolates show a sequence identity to *C. jejuni* 11168 of 90% (meC0280) and 88% (meC0281 & meC0467). Most virulence-associated genes like *ciaB*, *htrA* or *mapA* are present in the strains and show a significant sequence coverage and identity to their *C. jejuni* 11168 counterparts. The *cdt* genes, *cdtA* and *cdtC*, however, are missing while *cdtB* is present.

Significant phenotypic differences were observed between the tested isolates and the known *C. coli* and *C. jejuni* strains in all assays. Further comparative genome analyses of these strains may help in clarifying the underlying genotypic differences between them.

Figure 1



MLST-based neigbour-jouning tree of *C. coli* and *C. jejuni* isolates: blue – *C. jejuni* isolates, red – *C. coli* clade 1 isolates, violet – clade 3, yellow – calde 2, green – *despeciation clade*

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

287/MPP

Analysis of wall teichoic acid glycosylation and its implication for horizontal gene transfer in the facultative pathogen Staphylococcus lugdunensis

D. Gerlach*[1], F. Kisters[1], M. Popp[1], S. Heilbronner[1], G. Xia[2], A. Peschel[1]
[1]*Universität Tübingen, Infektionsbiologie, Tübingen, Germany*
[2]*University of Manchester, Division of Infection, Immunity & Respiratory Medicine, Manchester, United Kingdom*

Staphylococci are an abundant bacterial species that encompasses human commensals as well as pathogens. *Staphylococcus lugdunensis* is a coagulase negative bacterial pathogen. It is mostly connected to severe cases of infectious endocarditis [1]. The cell wall of Staphylococci is functionalized with wall teichoic acids (WTA). WTA consist of poly-alditol-phosphate that is further derivatized by sugar moieties. In the recent years the biosynthetic pathways of WTA synthesis and their physiological implications have been studied comprehensively in the major human pathogen *Staphylococcus aureus*. It was demmonstrated that WTA and WTA glycosylation contribute to host colonization and antibiotic resistance [2]. Furthermore WTA serves as receptor for staphylococcal phages. Thereby WTA acts as "glycocode" guiding horizontal gene transfer (HGT) and the spreading of virulence and antibiotic resistance genes among staphylococci or other gram-positive pathogens [3]. However the biosynthesis of WTA in *S. lugdunensis* and its implication for virulence are poorly understood.

Here we present our ongoing study to identify the genes involved in WTA glycosylation in *S. lugdunensis*. By using specific biochemical and microbiological assays we tried to assess the heterogeneity of WTA modification among clinical *S. lugdunensis* isolates. Additionally we sought to identify putative WTA glycosyltransferases in *S. lugdunensis* by ectopic expression and gene deletion. We could show that *S. lugdunensis* isolates strongly differ in their uptake of S. aureus derived pathogenicity islands which implies variation in the WTA composition. Furthermore we were able to identify tagNSlug as an active WTA glycosyltranferase upon ectopic expression. However activity of TagNSlug could not be directly demonstrated in *S. lugdunensis* indicating an unknown role or a more complex WTA biosynthesis pathway that remains to be investigated.

By further investigating the WTA biosynthesis of *S. lugdunesis* we try to understand the complex network of horizontal gene transfer between staphylococci and other gram positive. Upon this knowledge we hope to develop countermeasures to combat the spread of antibiotic resistance among staphylococci.

References

[1] Liu P.Y., et al., Staphylococcus lugdunensis infective endocarditis: a literature review and analysis of risk factors. J Microbiol Immunol Infect, 2010. 43(6): p. 478–84. pmid:21195974
[2] Winstel V., et al. Pathways and roles of wall teichoic acid glycosylation in Staphylococcus aureus. International journal of medical microbiology : IJMM, 2014. 3-4: p.215-211
[3] Winstel V., et al. Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens. Nat. Comm, 2013. 4

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

288/MPP

Use of synthetic membranes for probing pathogen-host interaction

T. Eierhoff*[1,2,3], J. Galle[4], W. Römer[2,3], J. H. Hegemann[4]
[1]*Heinrich-Heine Universität, Institute of Molecular Evolution, Düsseldorf, Germany*
[2]*Albert-Ludwigs-University Freiburg, BIOSS – Centre for Biological Signalling Studies, Freiburg i. Br., Germany*
[3]*Albert-Ludwigs-University Freiburg, Faculty of Biology, Freiburg i. Br., Germany*
[4]*Heinrich-Heine Universität, Institute for Functional Microbial Genomics, Düsseldorf, Germany*

Synthetic membranes (i.e. giant unilamellar vesicles, GUVs) have long and widely been used in cross-disciplinary, bottom-up approaches to analyze lipid-lipid, protein-lipid and membrane-associated protein-protein interactions. However, in the methodology of infection research, GUVs are still underrepresented. I will present different GUV techniques which we applied in recent and ongoing work with Gram negative, human pathogens (*Pseudomonas aeruginosa* and *Chlamydia pneumoniae*) for probing pathogen-host membrane interaction to demonstrate the benefit of these complemental techniques for elucidating infection processes. In case of *P. aeruginosa*, GUV techniques helped to reveal a lectin-glycolipid (i.e. LecA-globotriaosylceramide) interaction to be sufficient for wrapping the bacterium by the host cell membrane to trigger cellular invasion (Eierhoff *et al.*, Proc Natl Acad Sci U S A. (2014) 111(35):12895-900). We will present data about lipid phase separated and non-phase separated GUVs which demonstrate membrane tension, glycolipid concentration and membrane cholesterol to be critical parameters for the membrane wrapping process. In a second project GUVs with a lipid asymmetric bilayer and incorporated fluorescent dyes were used to analyze the binding capacity of a *C. pneumoniae* protein to the phospholipid phosphatidylserine (PS), regarding binding preferences, membrane permeability and lipid distribution. We measured membrane leakage and - strikingly - a translocation of PS from the inner to the outer leaflet of the GUV membrane. These examples illustrate the versatility of GUVs as attractive membrane models for studying the molecular interaction of pathogens with the lipid scaffold of host cell membranes.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

289/MPP

High resolution imaging of the Yersinia type III secretion system components and pore complex during host cell infection

F. Huschka[1], T. Nauth[1], M. Wolters*[1], M. Aepfelbacher[1]
[1]*University Medical Center Hamburg Eppendorf, Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Pathogenic species of the genus *Yersina* employ a type III secretion system (T3SS) to inject effector proteins into host cells. The hydrophobic translocators YopB and YopD are thought to integrate into the host cell membrane and form an entry pore (named translocon) for the late T3SS substrates, i.e. the effector Yops. We aimed at visualizing YopB and YopD in relation to other components of the T3SS (LcrV, YscD) in *Yersinia enterocolitica* infected cells employing immunofluorescence microscopy techniques. Furthermore, we aimed at definining the cellular compartment of translocon expression. Upon cell contact, YopB and YopD could be visualized in close proximity of approximately 4 % of HeLa cell associated bacteria, forming irregular distributed spot like structures. By confocal immunofluorescence microscopy around 10 YopB/YopD clusters were detected per bacterial cell, which could be further resolved by super resolution microscopy (STED) revealing around 30 spots per bacterial cell. Co-staining of YopB and YopD revealed perfect co-localization within the STED spots. Furthermore, co-staining of YopB with the tip protein LcrV also showed a high degree of co-localization. Structured illumination microscopy (SIM) of *Yersinia* expressing GFP-labeled YscD (part of the inner membrane ring at the base of the injectisome) revealed a median distance of about 100 nm between neighbouring YopB and YscD

spots. These data indicate that the observed YopB/D/LcrV structures most likely represent functional translocons at the tip of T3SS needle complexes during cell infection. Translocons were almost exclusively visible in a host cell membrane enclosed compartment inaccessible to external antibodies. However, about half of the translocon positive bacteria were still accessible by external staining with smaller molecules (streptavidin), indicating that membrane integration of the translocon is induced in a specific intracellular compartment still connected to the extracellular space. In summary, the *Yersinia* translocon could be visualized at the bacteria cell contact site by high resolution fluorescence microscopy.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

290/MPP

***Staphylococcus aureus* pathogenesis: from sepsis to hematogenous chronic bone infections.**

C. Pöllath*[1,2], B. Löffler[1,2], L. Tuchscherr[1,2]
[1]*University Hospital of Jena, Institute of Medical Microbiology, Jena, Germany*
[2]*University Hospital of Jena, Center for Sepsis Control and Care (CSCC), Jena, Germany*

Introduction: Staphylococcus aureus is a gram positive bacterium, which is able to colonize epithelial surfaces of healthy individuals, as well as to cause infections, ranging from local to life threatening systemic infections, like sepsis. Moreover S. aureus can escape from the blood stream and establish infections in surrounding tissues, such as bones. However, the bacterial virulence factors that contribute to develop a blood or bone infection are still unknown.
Aim and Question: The aim of this work was to identify characteristic features of isolates that determinate the development of osteomyelitis (haematogenous or prosthesis) and sepsis in comparison to colonizing strains.
Methods and Results: To elucidate bacteria virulence factors that are associated with the development of different pathologies, as sepsis and chronic bone infections, a strain collection was established. The isolates were assigned to four different clinical categories: nasal colonization of healthy persons, sepsis without metastasis, osteomyelitis with and without haematogenous origin (prosthesis). The groups consisted of 10–14 isolates which were collected at the Jena University Hospital. These groups were genotyped for their clonal complexes and it could be shown that the distribution of clonal complexes was comparable other large German studies [1]. The strains from each group were characterized by genotypic analysis (Alere, Jena). Additionally, parameters as biofilm formation, host cells invasiveness, cytotoxicity and secretion of haemolysins were investigated. However, the groups did not show significant differences. Even that minor differences between the groups could be observed, the variation within the single groups was high. Over all, significant correlations were found between different strain characteristics independently of clinical categories.
Conclusion: Taken together, our results show that S. aureus strains causing a defined disease are not characterized by a certain set of virulence factors. Our phenotypic analysis indicates that S. aureus strains are in general equipped to induce different types of infections, including metastatic infections.

Reference

[1] Holtfreter, S., et al., Molecular epidemiology of Staphylococcus aureus in the general population in Northeast Germany: results of the study of health in Pomerania (SHIP-TREND-0). Journal of clinical microbiology, 2016. 54(11): p. 2774-2785.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

291/MPP

Reduced persister cell formation in an ATP-Synthase deficient mutant

S. Braetz*[1]
[1]*FU Berlin / IMT, Berlin, Germany*

Bacterial persister cells are slow- or non-growing bacteria with a low metabolic activity, which are able to withstand antibiotic treatment over long periods. Persistence against bactericidal agents is non-inheritable, rather it is believed to be a random process involving the activation of Toxin/Antitoxin modules [1], accumulation of toxic metabolites [2], or the activation of bacterial stress responses [3].
We investigated the role of the ATP pools in persister cell formation for *Salmonella* wild type and an *atp* operon deletion strain during the exponential phase of growth to examine the role of intracellular ATP concentration on persister cell formation.
Persister assays with exponentially growing wild type and mutant strains were performed at four-fold the MIC with Ciprofloxacin to compare bacterial survival. Additional assays with different classes of antibiotics were also conducted to check the specific susceptibility of the mutant to fluoroquinolone. Furthermore, we determine the effects of glucose to increase the intracellular ATP concentration, reduced formation of hydroxyl radicals with glutathione addition and performed persister assays under anaerobic conditions to reduce oxygen stress. In addition, we also measured the efflux activity of both *Salmonella* strains exploiting the natural fluorescence of Ciprofloxacin to compare the intracellular accumulation. Finally, we quantified endogenous superoxide production using CellROX Deep Red.
Despite reduced ATP pools, the *atp* operon mutant was found to be more susceptible to Ciprofloxacin killing, in contrast to previous studies, where poisoning the ATP-synthase with arsenate lead to an increase in persister cells [4]. The increased susceptibility was observed for fluoroquinolone but not for beta-lactam antibiotics or aminoglycosides. Increasing the intracellular ATP concentration with glucose did not restore the wild type phenotype. No effects on the efflux activity on the intracellular Ciprofloxacin concentrations were observed. However, oxygen deprivation and hydroxyl quencher increased the survival comparable to the wild type.
We hypothesize that the lack of the ATP-synthase results in increased respiratory activity resulting in an increased formation of superoxide, which may further react via the Fenton reaction to generate hydroxyl radicals. Increased oxidative stress could lead to a more rapid killing of the mutant.

References

[1] Germain, E., et al., *Molecular mechanism of bacterial persistence by HipA.* Mol Cell, 2013. **52**(2): p. 248-54.
[2] Girgis, H.S., K. Harris, and S. Tavazoie, *Large mutational target size for rapid emergence of bacterial persistence.* Proc Natl Acad Sci U S A, 2012. **109**(31): p. 12740-5.
[3] Wu, Y., et al., *Role of oxidative stress in persister tolerance.* Antimicrob Agents Chemother, 2012. **56**(9): p. 4922-6.
[4] Conlon, B.P., et al., *Persister formation in Staphylococcus aureus is associated with ATP depletion. Nat Microbiol, 2016. **1**: p. 16051.*

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

292/MPP

A neonatal CNS infection model following nasal challenge with *Listeria monocytogenes*

D. Pägelow*[1], C. Chhatbar[2], X. Liu[3], A. Beineke[4], M. Rohde[5], K. van Vorst[1], A. Nerlich[6], U. Kalinke[2], R. Förster[3], P. Valentin-Weigand[7], S. Halle[3], M. Hornef[8], M. Fulde[1]
[1]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[2]*TWINCORE, Centre for Experimental and Clinical Infection Research, a joint venture between the Hannover Medical School and the Helmholtz Centre for Infection Research, Institute for Experimental Infection Research, Hannover, Germany*
[3]*Hannover Medical School, Institute of Immunology, Hannover, Germany*
[4]*University of Veterinary Medicine Hannover, Institute for Pathology, Hannover, Germany*
[5]*Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany*
[6]*Charité Universitätsmedizin Berlin, Department of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Berlin, Germany*
[7]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*
[8]*University Hospital RWTH Aachen, Institute for Medical Microbiology, Aachen, Germany*

Bacterial central nervous system (CNS) infections remain a major cause of morbidity and mortality in the pediatric population. Commonly used parenteral infections models, however, do not reflect the early course of the disease, leaving initial mechanisms of host susceptibility, route of infection and underlying mechanisms of inflammation in the CNS largely unexplored.
The aim of this study was to establish a model of neonatal CNS infection with *Listeria monocytogenes* following mucosal challenge in order to investigate the cellular and molecular mechanisms of bacterial tissue tropism and innate immune responses.
One-day-old C57BL/6 mice were infected intranasally with *L. monocytogenes*. To determine bacterial dissemination, neonatal mice were sacrificed at various time points and organs were obtained for replica plating. Tissue tropism and immune responses were analyzed by immunohistochemistry, electron microscopy, flow cytometry and qRT-PCR.
In contrast to the well characterized gastrointestinal tropism of *L. monocytogenes* in mice, bacteria were mainly reisolated from the brain, particularly from the olfactory bulb and the cerebrum. *L. monocyogenes* was found in the cerebellum or the brain stem as well as in the blood only occasionally, indicating a non-hematogenous dissemination from the nasal cavity to the CNS. Once inside the CNS, *Listeria* induced a multifocal meningo-encephalitis as evaluated by histo-pathological examination. Interestingly, mucosal invasion was restricted to the olfactory epithelium and completely independent of the two major listerial invasins InlA and InlB. Nevertheless, electron microscopic examination clearly showed that during early time points, *Listeria* resided in olfactory sensory neurons. Later, wild-type bacteria were found to be associated with axon bundles projecting from the olfactory epithelium to the CNS. In contrast, an isogenic *Listeria* mutant lacking ActA, which facilitates intracellular motility and cell-to-cell spread, was still able to induce internalization into the olfactory epithelium but was entirely restricted to the olfactory mucosa and could not overcome the cribriform plate. Once inside the brain, wild-type *Listeria* were targeted by various immune cells. Flow cytometric and immune-histochemical analyses showed an accumulation of bacteria with a concomitant recruitment of CD45+CD11b+ microglia/macrophages as well as a significant increase of infiltrating Ly6Chi monocytes/macrophages and Ly6C+ neutrophils. As expected, mRNA of key cytokines mediating intracranial inflammation and monocyte as well as neutrophil attraction, such as *Tnfα*, *Cxcl2*, *Ccl2* and *Ccl7*, was highly upregulated.
Taken together, we propose an alternative portal of entry and route of infection for neonatal cerebral listeriosis and present a novel *in vivo* infection model to mimic the clinical features of late onset disease in human neonates.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

293/MPP

Growth-phase dependant ^{13}C-incorporation of *Legionella pneumophila* during intracellular replication in *Acanthamoeba castellanii*

M. Kunze*[1], F. Chen[2], K. Heuner[1], W. Eisenreich[2]
[1]*Robert-Koch Institut, , Berlin, Germany*
[2]*TU München, Lehrstuhl für Biochemie, München, Germany*

Introduction and Aim: *Legionella pneumophila (Lp)* is an opportunistic intracellular pathogen in humans causing Legionnaires" disease, a pulmonary infection. In the environment it is found in many protozoa, where it replicates within a so called *Legionella* containing vacuole (LCV) and restricts fusion with lysosomes. *Lp* exhibits a biphasic lifestyle, indicated in an intracellular replicative non-motile and an extracellular transmissive phase including expression of a flagellum. Accordingly it has been shown recently that *Lp* also exhibits a biphasic metabolism at least *in vitro*, where serine and glucose are used in a growth phase-dependant manner. To confirm these observations *in vivo* we want to perform isotopologue assays during infection of *Acanthamoeba castellanii*, a natural host of *Legionella*. Therefore we like to establish three time points (*exponential* E, *late exponential* LE, *postexponential* PE) for adding ^{13}C-labeled glucose or serine to generate isotopologue profiles of intracellular replicating *Lp* and appropriate mutants.
Material and Methods: Here, we generated isotopologue profiles of intracellular replicating *Lp* at three different growth phases with ^{13}C-labeled glucose and serine. All assays were performed using *L. pneumophila* Paris Wt and a *Δzwf* mutant strain, the first enzyme of the Entner-Doudoroff pathway. As an *in vivo* model we used *Acanthamoeba castellanii* (ATCC30010). Isotopologue analysis was performed using GC/MS.
Results and Conclusion: We established three different time points (E, LE, PE) in the intracellular growth of *Lp* in *A. castellanii* for isotopologue analysis. First time point indicated the replicative non-motile phase E. In the second time point LE *Legionella* starts to switch into the transmissive form, but is still within the LCV. The third point PE indicates the transmissive phase and the start of lysis of *A. castellanii*. Through an established protocol by isolating intracellular *Lp* from amoeba we can exclusively analyse the isotopologue profiles of the bacteria and appropriate mutants in a growth-phase-dependant manner.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

294/MPP

Helicobacter pylori induces DNA-damage in human cells through the apoptosis apparatus in the absence of cell death

B. Dörflinger*[1], A. Haimovici[1], G. Häcker[1]
[1]*Medical Center–University of Freiburg, Institute of Microbiology and Hygiene, Freiburg i. Br., Germany*

Around 5,000 people die in Germany every year of stomach cancer. *Helicobacter pylori* colonization is the major risk factor for development of stomach adenocarcinoma. To date, pathophysiologic mechanisms remain mostly unclear. Previous studies have shown DNA-damage-induction by *H. pylori* in vitro but the mechanism has not been clarified. Recent research indicates that low-level activation of the apoptosis apparatus can occur in human cells in the absence of actual cell death, leading to DNA-damage and oncogenic mutations.
We hypothesized that *H. pylori* induces such low-level activation of the apoptosis apparatus and that this mechanism could contribute to DNA-damage and genomic mutations by *H. pylori*.
To test this hypothesis we generated human epithelial cell lines with specific defects in components of the apoptosis-system. We analyzed these cells for activation of the apoptosis apparatus by probing for caspase-activation and DNA-damage response in the presence or absence of these signaling molecules, upon *H. pylori* infection.
We found that *H. pylori* induced DNA-damage and a DNA-damage response in cell culture as reported previously. Although this occurred in the absence of full-blown apoptosis, it did depend

on the activation of the mitochondrial apoptosis system and the caspase-dependent DNAse (CAD). Intriguingly, cytokine-secretion by infected cells partly depended on the activity of the apoptosis apparatus.
Our data demonstrate low-level activation of the apoptosis apparatus in human epithelial cells infected by *H. pylori*. We hypothesize that this activation serves the purpose of bacterial innate immune recognition. As a side effect low-level activation of the apoptosis apparatus may contribute to oncogenesis.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

295/MPP

Structural determinants of *Staphylococcus epidermidis* Extracellular matrix binding protein Embp and its interaction with fibronectin

H. Büttner*[1], M. Perbandt[2], H. Rohde[1]
[1]*Universtity Medical Center Hamburg-Eppendorf, Clinical Microbiology, Hamburg, Germany*
[2]*UHH, Structural Biology/DESY, Hamburg, Germany*

Introduction: *Staphylococcus epidermidis* is the leading cause of foreign body associated infections. The great success of this pathogen is attributed to colonize artificial surfaces via biofilm formation. Biofilm formation crucially depends on interactions between *S. epidermidis* and surface organized fibronectin. Here, we investigated she structural basis of *S. epidermidis* – Fn interactions, focusing on the role of 1MDa extracellular matrix binding protein Embp. Bioinformatics analysis showed that Embp is mainly organized by stretches of x 126 and y 170 amino acid long repeats, respectively. Structural analysis showed that both of these repeats mainly display an a-helical secondary structure. Small angle X-ray scattering analysis of recombinant Embp fragments suggest that repetitive elements are organized as elongated rods rather than being globularly folded.
Methodes: These experiments focus clinical isolate *S.epidermidis* 1585. We investigated the bacterial attachment to immobilized fibronectin in ELISA experiments. In order to map the potential site of interaction Far-Western Blot analysis and peptide library screening revealed type III repeat FN12 to be the major site of interaction. To further elucidate the interaction of Embp and fibronectin investigation of the Embp structure was begun. Bioinformatics analysis of the Embp primary sequence revealed F- and FG-repeats as most presumably the minimal structural units. By means of X-ray crystallography the structure of these repeats was determined. Small angle X-ray scattering (SAXS) was employed to get first insights in the global Embp architecture.
Results: Embp is a fibronectin binding protein of *S. epidermidis*. We identified the fibronectin type III repeat FN12 as the potential site of Embp – fibronectin interaction. On the bioinformatics level F-repeats (170 amino acids) in the FIVAR region as well as FG-repeats (126 amino acids) in the FIVAR/GA-region were identified as the minimal structural units of the Embp protein. Both of these repeats mainly display a-helical secondary structure. In SAXS studies of multiple repeats Embp presumably shows an elongated rather than globular architecture.
Conclusion: We showed Embp to be sufficient and of great importance for fibronectin binding. By means of bioinformatics analysis confirmed by X-ray crystallography minimal Embp structural units, F- and FG-repeat, were identified. Results from SAXS gave the impression that Embp posses an elongated shape. Ongoing work will investigate at the molecular level the interaction between Embp and fibronectin.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

296/MPP

Strain-specific SaeS sensing mechanism in *Staphylococcus aureus*

L. Bleul*[1], J. Ziemann[1], J. Götz[1], A. Lamsfus Calle[1], C. Wolz[1]
[1]*Medizinische Mikrobiologie und Hygiene, Universität Tübingen, Tübingen, Germany*

Staphylococcus aureus is a not only an important human pathogen but it can also colonize mucosa without causing symptoms. Due to its dual lifestyle, the expression of virulence factors requires a precise control. One of the major regulatory systems of *S. aureus* is the SaeRS two component system which has been shown to be induced in response to phagocytosis-related signals, specifically by human neutrophil peptides 1-3 (HNP1-3). The Sensorkinase SaeS consists of two transmembrane regions and a small 9-amino acid extracellular loop which makes a direct binding of HNP1-3 very unlikely. The structure and composition of both, the transmembrane regions and the extracellular loop have been shown to be important for the ability to sense HNP1-3 but the exact mechanism of sensing remains to be identified. Interestingly, SaeS of some *S. aureus* strains is unable to sense HNP1-3 despite an identical SaePQRS sequence (non-responder). We aim to analyse the role of cell-envelope components in the strain-specific sensing mechanism of SaeS. Therefore, we performed transcriptional analyses of well-defined mutants and performed protein interaction studies using different *S. aureus* strains. Cell-wall proteins, Lipoproteins, or cell-membrane modifications did not influence the SaeS sensing mechanism. However, Wall-teichoic acid (WTA) and more specifically D-alanylation of WTA were found to be involved in HNP1-3 sensing. To analyse whether SaeS dimerization is involved in the sensing mechanism, we used a bacterial two hybrid system based on adenylate-cyclase activation in *E. coli*. SaeS was shown to form dimer which requires the N-terminal membrane anchored domain. Interestingly, SaeS of strain Newman which has a constitutive Sae activity due to an amino acid substitution in the N-terminal transmembrane part of SaeS is deficient in dimerization. In addition, we examined SaeS protein stability and localization in different *S. aureus* strains using Western Blot. We could show that activation of SaeS either by HNP1-3 or by using SaeS from strain Newman (constitutively active) results in a specific shift of the SaeS band. This shift can be not detected in HG001 which is a non-responder strain. This indicates that HNP1-3 alters SaeS confirmation within the membrane which is in turn influenced by D-alanylation of the WTA in a strain-specific manner.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

297/MPP

The stringent response is involved in oxidative stress response in *Staphylococcus aureus*.

P. Horvatek*[1], A. Hanna[1], C. Wolz[1]
[1]*Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*

The stringent response is a global regulatory system activated under nutrient stress leading to growth arrest due to inhibition of replication, translation and re-programming of the transcriptome. The response is coordinated by (p)ppGpp (guanosin-tetraphosphate and guanosin-pentaphosphate) which is synthesized by activated RelA/SpoT homoloques (RSH). In *Stapyhlococcus aureus* there are additional small (p)ppGpp synthetases, RelP and RelQ, which are induced under cell wall stress conditions on the transcriptional level and mainly produce ppGpp. The bifunctional RSH senses amino acid starvation presumably through recognition of unloaded tRNA at the A-site of the ribosome and synthesizes preferentially pppGpp.
It is not known what the functional difference is between guanosin-tetra- and pentaphosphate in *S. aureus*. However down-stream consequences of the stringent response in *S. aureus* is only partially understood.
We addressed this question and performed RNA-Seq from (p)ppGpp^0 strains containing plasmids which synthesize either ppGpp or pppGpp after induction. This data revealed that pppGpp influences the oxidative stress response e.g. *perR* (Peroxide

Sensing Protein Regulator), *ahpC* (Alkyl hydroperoxide reductase), *mrgA/dps* (DNA-binding Protein from starved cells) as well as genes involved in iron storage. Interestingly, pppGpp synthesized by RSH leads to the activation of AgrA target gene *psmα*. Furthermore pppGpp induction could be linked to the activation of the sigma factor B regulon indicated by activation of *asp* and the *sarA* promoter. The RNA-seq data were confirmed by Northern blot analysis including *codY* mutant strains. Most of the observed effects detected could be verified in *codY* positive and *codY* negative strain background. This indicated that pppGpp leads to profound deregulation of oxidative stress response independent of CodY. The link between stringent and oxidative stress response could be verified by growth analysis of defined mutant strains incubated with and without diamide.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

298/MPP

Complement 5a receptor polymorphisms are associated with colonizing PVL-positive *Staphylococcus aureus* in African Pygmies

F. Schaumburg*[1], A. Witten[2], A. Flamen[3], A. Alabi[4,5], P. Kremsner[4,5], B. Löffler[6], P. F. Zipfel[7], T. Velavan[5], G. Peters[1]
[1]*University Hospital Münster, Münster, Germany*
[2]*University of Münster, Core Facility Genomics, Münster, Germany*
[3]*Hôpital Albert Schweitzer, Lambaréné, Gabon*
[4]*Centre de Recherches Médicales (CERMEL), Lambaréné, Gabon*
[5]*Eberhard Karls Universität Tübingen, Institut für Tropenmedizin, Tübingen, Germany*
[6]*University Hospital Jena, Institute of Medical Microbiology, Jena, Germany*
[7]*Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany*

Question: The Panton-Valentine leukocidin (PVL) of *Staphylococcus aureus* is a pore-forming protein-toxin that can be associated with severe necrotizing infections. The prevalence of PVL is low in isolates from Europe (3%), but exceptionally high in sub-Saharan Africa (e.g. 56% in Babongo Pygmies). PVL interacts with complement 5a receptor (C5aR) of neutrophils eventually causing cytolysis. The objective of this study was to assess if colonization with PVL-positive *S. aureus* is associated with polymorphisms of the C5aRI/II in an African Babongo Pygmy population.
Methods: Nasal and pharyngeal swabs were collected from remotely living Babongo Pygmies in Gabon to assess the colonization with *S. aureus* using selective agar (SAIDE, bioMÃ©rieux). *S. aureus* isolates were screened for the presence of PVL-encoding genes (*lukF*-PV/*lukS*-PV). Buccal swabs were taken to obtain mucosal epithelial cells for DNA extraction (Forensic Swab, Sarstedt). [F1] The C5aRI/II loci were genotyped by Sanger sequencing and sequences were screened for SNPs using the UCSC Genome Browser and the Human Genome 38 assembly (hg38). Binary logistic regression adjusted for age and gender was employed to analyze possible association of C5aRI/II genetic variants with colonization of PVL-positive *S. aureus* (Table 1).
Results: A total of 107 Pygmies were included; 69% (n=74) were colonized with *S. aureus*; 45% (n=33) were colonized with PVL-positive *S. aureus*. Twelve and seven SNPs were detected in the C5aRI and II locus, respectively. No significant association was observed between any of these SNPs and the colonization with *S. aureus*. Â In contrast, the allele frequencies of *CR5a I* SNPs rs11880097, *CR5a II* rs150649665 and *CR5a II* rs187635721 differed significantly between individuals being colonized with PVL-positive and PVL-negative *S. aureus* (Table 1). These SNPs carry a missense mutation at position 279 of the 3rd extracellular domain (rs11880097), synonymous mutation (rs11880097) or an intron variant (rs187635721).
Conclusion: African Babongo Pygmies have several *C5aR* SNPs associated with the colonization of PVL-positive *S. aureus*. Since PVL most likely acts through binding to the three extracellular domains of C5aR, the SNP *C5aR I* rs11880097 is a promising candidate to further study why PVL is widespread Africa but less common in Europe. Further functional assays are warranted to analyse the impact of this SNP on the cytotoxicity of PVL.
Table: Genotypes and allele frequencies of the three C5aRI/II variants associated with colonization of PVL-positive *S. aureus* in African Babongo Pygmies, Gabon

Figure 1

SNP loci	Genotypes and alleles	Colonization with PVL-positive S. aureus, n (%)		Odds Ratio	*P* value
		Yes	No		
C5aR *I* rs11880097 T/G	TT	30 (91)	26 (63)	Reference	
	GT	3 (9)	12 (30)	0.2 (0.06-0.9)	0.009
	GG	0	3 (7)	NA	NA
	T	63 (95)	64 (78)	Reference	
	G	3 (5)	18 (22)	0.16 (0.03-0.62)	0.003
C5aR *II* rs150649665 C/A	CC	31 (97)	30 (79)	Reference	
	CA	1 (3)	7 (18)	0.13 (0.02-1.2)	NS
	AA	0	1 (3)	0.3 (0.01-8.2)	
	C	63 (98)	67 (88)	Reference	
	A	1 (2)	9 (12)	0.12 (0.01-0.9)	0.015
C5aR *II* rs187635721 G/T	GG	27 (93)	23 (72)	Reference	
	GT	2 (7)	6 (19)	0.3 (0.06-1.7)	NS
	TT	0	3 (9)	0.1 (0.006-2.5)	NS
	G	56 (90)	52 (81)	Reference	
	T	2 (10)	12 (19)	0.15 (0.03-0.7)	0.0096

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

299/MPP

Deficiency of lysyl-transferase-lysyl-tRNA synthetase (LysX) gene in *Mycobacterium avium hominissuis* enhances intracellular growth

G. Kirubakar[1], J. Murugaiyan[2], C. Schaudinn[3], F. Dematheis[4a], G. Holland[3], M. Eravci[5], C. Weise[5], U. Roesler[2], A. Lewin[1]*

[1]*Division 16, Mycotic and Parasitic Agents and Mycobacteria, Robert Koch Institute, Berlin, Germany*
[2]*Centre for Infectious Medicine, Institute of Animal Hygiene and Environmental Health, Veterinary Faculty, Freie Universitaet, Berlin, Germany*
[3]*Division ZBS 4, Advanced Light and Electron Microscopy, Robert Koch Institute, Berlin, Germany*
[4]*Institute of Microbiology and Epizootics, Freie Universitaet, Berlin, Germany*
[5]*Institute for Chemistry and Biochemistry, Freie Universitaet, Berlin, Germany*
[a]*Current address: Flavia Dematheis, Institut für Mikrobiologie der Bundeswehr, München, Germany.*

Introduction: *Mycobacterium avium* is the most common clinically encountered species among nontuberculous mycobacteria worldwide and frequently causes lung infection in adults and lymphadenitis in children. The present study is focused on the characterization of a lysX-deficient mutant of *Mycobacterium avium hominissuis* strain 104, which encodes a lysyl-tRNA synthetase.
Objectives: This project aims to investigate the effect of the lack of LysX function on the phenotype of M. *avium* with emphasis on metabolism, resistance and intracellular survival.
Materials and Methods: Proteome analysis was performed by label free quantitative proteomics analysis. The online tools DAVID and STRING-10.5 were applied to analyze the proteome data. The metabolization capacity of the strains was tested employing BIOLOG Phenotype Microarray. Transmission electron microscopy was carried out for studying the structural features of the strains. Fluorescence microscopy of combined Nile Red- and Ziehl-Neelsen-stained bacteria were performed for visualization of the lipid content. Intracellular growth of the strains in Human blood-derived monocytes was quantified by CFU determination. Antibiotic susceptibility was examined using the Sensititre.
Results: Antibiotic sensitivity tests revealed that the lysX mutant was hypersensitive towards cationic antibiotics. Proteome analysis indicated that the lysX mutant strain undergoes a transition in phenotype by switching the carbon metabolism to β-oxidation of fatty acids along with accumulation of lipid inclusions. The lysX mutant exhibited an enhanced intracellular growth in human blood-derived monocytes, which was contradictory to the behaviour of lysX mutant from M. tuberculosis which had been reported to show defective growth in THP-1 macrophages. Thus, our study suggests an important functional role of *lysX* in the virulence of *M. avium hominissuis.*

Summary: The virulence strategies of *M. avium* are still not fully clear including the mechanisms allowing this environmental bacterium to cause chronic infections in humans. Here we demonstrated that the gene lysX, encoding a lysyl-transferase-lysyl-tRNA synthetase which is involved in the synthesis of lysinylated phosphatidylglycerol, not only impacts resistance of MAH towards cationic antimicrobials but also has an immense impact on the carbohydrate and lipid metabolism. Absence of LysX in MAH provokes a shift towards the metabolic pattern known from M. tuberculosis when present inside human macrophages and improves the ability of MAH to multiply in monocyte-derived cells. Our finding provides new insights into the regulatory network enabling this environmental Mycobacterium to act as important pathogen.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

300/MPP

Characterization of genes involved in invasion and adherence of *Campylobacter jejuni*

S. Minatelli*[1], A. Lübke[1], L. Gebauer[1], R. Lugert[1], U. Groß[1], A. E. Zautner[1], W. Bohne[1]
[1]*Institute for Medical Microbiology, Dept. of Medical Microbiology, Göttingen, Germany*

Introduction: *Campylobacter jejuni* is known as the most common cause of food-borne bacterial gastroenteritis worldwide. *C. jejuni* is able to adhere to and invade epithelial cells of the human GI tract, leading to disruption of the GI epithelial layer and producing damaging pro-inflammatory host responses.
Objectives: Novel pathogenicity factors involved in invasion and adhesion are aimed to be identified by the generation of gene deletion mutants. Candidate genes were chosen by combining proteomics data from our lab and transposon sequencing data from the literature.
Material and Methods: The mutants were generated by homologous recombination, involving the insertion of a kanamycin resistance cassette into the target gene. Caco-2 cells were used to assess the invasion and adhesion ability of these mutants. Furthermore, motility and autoagglutination of the mutants was tested.
Results and conclusion: We generated deletion mutants for 11 *C. jejuni* genes. Reductions in invasion and adhesion ability were detected in mutants for a lipoprotein and a sodium-alanine symporter. Losses in motility were observed in 7 of the 11 mutants, including the *Δlipo* mutant. The *lipo* gene may therefore be an important player in 81-176 adhesion, invasion and motility. Surprisingly, several mutants displayed increased adhesion and invasion rates, although their motility is lost. The molecular basis for this unexpected finding will be further investigated.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

301/MPP

Influence of Agr and SigB on regulation of virulence factors of *Staphylococcus saprophyticus*

J. Busse*[1], M. Korte-Berwanger[1], S. G. Gatermann[1]
[1]*Ruhr-Universität Bochum, Medizinische Mikrobiologie, Bochum, Germany*

Introduction: *Staphylococcus saprophyticus* is an opportunistic pathogen and a common cause of urinary tract infections. Several virulence factors have been described, e.g., a urease, the surface proteins Aas, SdrI and UafA, a surface-associated lipase Ssp and a D-serine-deaminase DsdA. Since D-serine is prevalent in urine and has a toxic or bacteriostatic effect on many bacteria, possession of the D-serine-deaminase seems to be an important adaption for colonization of the urinary tract. It was shown that the lipase Ssp is upregulated in presence of D-serine. However, we do not know anything about the mechanisms of regulation of the D-serine-deaminase or any other virulence factor. Bacteria often use regulation systems to modulate expression of virulence factors as a reaction to certain environmental influences. From the data on Ssp it may be announced that D-serine has an influence on the expression of a regulation system. For *Staphylococcus aureus* it is known that the two-component regulatatory system Agr and the alternative sigma-factor SigB play a major role in regulation of virulence factors. Genome analysis showed that *agr* and *sigB* also exist in *S. saprophyticus*. The physiological significance of Agr and SigB and the influence of D-serine on these systems should be investigated by construction of an *agr* and a *sigB* knock-out mutant, followed by comparative physiological tests and transcriptome analysis.
Methods: Cloning of an *agr* and a *sigB* knock-out construct was accomplished by use of a yeast recombination system. To this end, the erythromycin cassette and a 500 bp fragment upstream and downstream of *sigB* was amplified and transformed with the linearized shuttle vector pRS426 into yeast strain PJ69-4a. The assembled mutagenesis construct was cloned into the temperature sensitive replacement shuttle vector pBT2. Plasmids were purified from *E. coli* DH5α and transformed into *S. saprophyticus* strain 7108 by protoplast transformation, followed by a plasmid curing step.
Results and Conclusion: Knock-out mutants were successfully constructed. Expression of virulence factors was investigated by different physiological assays, such as urease activity testing, D-serine-deaminase activity testing, lipase activity testing or binding and biofilm assays. The *agr* and *sigB* knock-out mutants showed a diminished D-serine deaminase activity. The *sigB* knock-out mutant showed lower urease activity, when grown under the influence of 20 mM D-serine. This indicates that both systems are involved in virulence factor regulation. SigB seems to be influenced by the presence of D-serine as an environmental signal.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

302/MPP

The RNA chaperone ProQ as a global gene regulator in *Neisseria meningitidis*

S. Bauriedl*[1], L. Barquist[2], N. Heidrich[2], C. Schoen[1], J. Vogel[2]
[1]*Institut für Hygiene und Mikrobiologie, Forschung, Würzburg, Germany*
[2]*Institute for Molecular Infection Biology (IMIB), RNA Biology group, Würzburg, Germany*

Although ProQ was found to function as a RNA-chaperone and global regulator in *Escherichia coli*, its function in other proteobacteria has not been established so far. Here, we assessed the binding-sites of ProQ and is contribution to cellular homeostasis in the ß-proteobacterium *Neisseria meningitidis* [1] which is an improtant cause of bacterial meningitis and sepsis on a genome-wide level.
By using *in vivo* UV crosslinking with RNA deep sequencing (CLIP-seq) [2] we identified 1155 ProQ-binding sites in the model strain 8013 mainly situated in the 3`UTR of genes. CLIP-seq further revealed a ProQ-centered network comprising 104 potential mRNA targets and 12 small RNAs (sRNAs). The COG pathways overrepresented in the group of ProQ-associated genes include `Energy production and conversion` and the `translation`. For six of these sRNAs, the association with ProQ was also confirmed by northern-blot analysis. Rifampicin treatment experiments demonstrated that ProQ binding confers enhanced stability on these sRNAs. While deletion of *proQ* does not influence growth in rich or minimal media, the proQ protein is required for growth in a knock-out strain of the well-described RNA-chaperone Hfq [3]. The biological role of ProQ gets evident as ProQ is required for survival after DNA damage as survival rates of *proQ* deletion strains are reduced after exposure to different doses of UV light.
In conclusion, this large expression compendium allows a deeper understanding of meningococcal riboregulation thus helping to understand the biological role of ProQ in the meningococcus and providing a valuable resource for the scientific community.

References
[1] Chaulk, S., et al. RNA Biol. 2010. **7**(6): p. 812-9.
[2] Holmqvist, E., et al. EMBO J. 2016. **35**(9): p. 991-1011.
[3] Del Tordello E, et al. *J Bacteriol* 2012. **194:**6217-6232.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

303/MPP

The Small basic protein (Sbp): structure and function in *Staphylococcus epidermidis* biofilm formation

S. Weißelberg[1], C. Krambeck[1], M. Fayyaz[1], C. Jefferies[2], M. Perbandt[3], C. Betzel[3], H. Rohde*[1]
[1]*UK Hamburg-Eppendorf, Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany*
[2]*EMBL c/o DESY, Hamburg, Germany*
[3]*Institut für Biochemie und Molekularbiologie, Laboratorium für Strukturbiologie von Infektion und Entzündung, Hamburg, Germany*

Staphylococcus epidermidis is ranked under the most important pathogens causing hospital-acquired infections. Typically, these infections occur after implantation of medical devices, and association of *S. epidermidis* infections with implanted foreign material relates to the species biofilm forming ability. *S. epidermidis* biofilm formation depends on the production of an extracellular matrix which stabilizes cell-cell contacts and thus, the biofilm architecture as a whole. Apart from polysaccharides (i.e. PIA) and eDNA, proteins (e.g. Aap, Embp) play a pivotal role during biofilm assembly. Analysis of biofilm matrix associated proteins identified an 18 kDa protein with a high pI of 9.8 referred to as small basic protein (Sbp). After recombinant expression Sbp exhibits marked, concentration depended self-aggregative properties. As inferred from CD spectroscopy, the protein mainly consists of beta-sheets and some alpha helices, and small angle X-ray scattering (SAXS)-derived ab initio model revealed an elongated ellipsoidal shape with a considerably thick globular head and a small tail. Expression analysis using qRT-PCR and Western blot showed that *sbp* is expressed throughout all growth phases. Of notice, sessile growth augmented *sbp* expression during the mid-exponential growth phase. Spatial analysis of Sbp localizations demonstrated its presence in all levels of a living *S. epidermidis* biofilm. However, there was a marked accumulation of Sbp at the interface between the substratum and the basal layers of the biofilm. In fact, investigation of defined *sbp* knock out mutants and corresponding complemented strains showed that *S. epidermidis* uses Sbp to prime plastic surfaces, consequently allowing for stable bacterial adherence. In addition to its involvement in surface attachment, Sbp also contributes to PIA- and Aap-dependent biofilm formation. The impact of Sbp on the *S. epidermidis* cell wall composition is currently under investigation.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

304/MPP

Subcytolytic concentration of suilysin mediates calcium transients in human brain microvascular endothelial cells

M. T. Asmat*[1], T. Tenenbaum[2], R. Baums[3], C. Schwerk[2], H. Schroten[2]
[1]*University Of Balochistan, CASVAB, Quetta, Pakistan*
[2]*Heidelberg University, Department of Pediatrics, Pediatric Infectious Diseases, Medical Faculty, Mannheim, Germany*
[3]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*

The pore-forming suilysin, a cholesterol-dependent cytolysin secreted by *Streptococcus suis* (*S. suis*), is known as a critical virulence factor in *S. suis* meningitis. Stimulation of human brain microvascular endothelial cells (HBMEC) with *S. suis* serotype 2 strain 10, its isogenic capsule mutant or recombinant suilysin caused a substantial increase in cytoplasmic calcium concentration in contrast to stimulation with a suilysin mutant. Recombinant suilysin with reduced or no cytolytic activity hardly stimulated calcium flux in HBMEC. Importantly, the increase in cytoplasmic calcium could be abolished by chelation of cholesterol with methyl-β-cyclodextrin. In conclusion, suilysin mediates calcium influx in HBMEC.

Figure 1

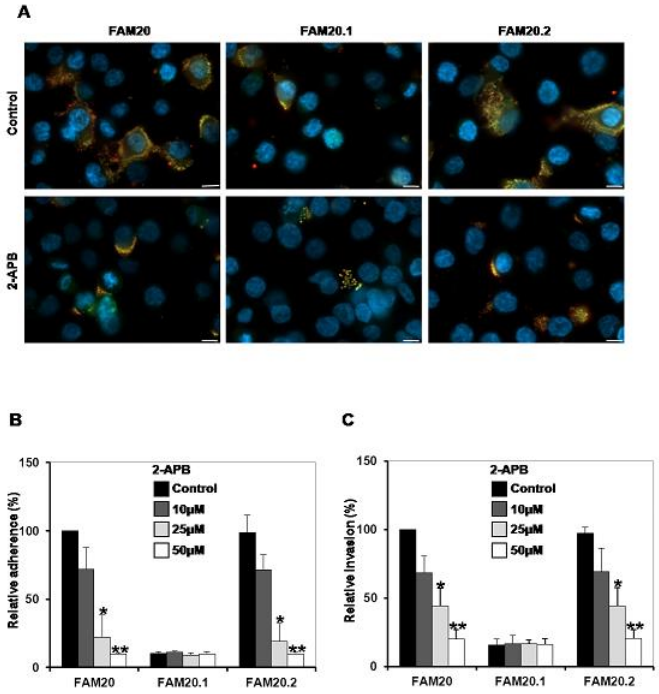
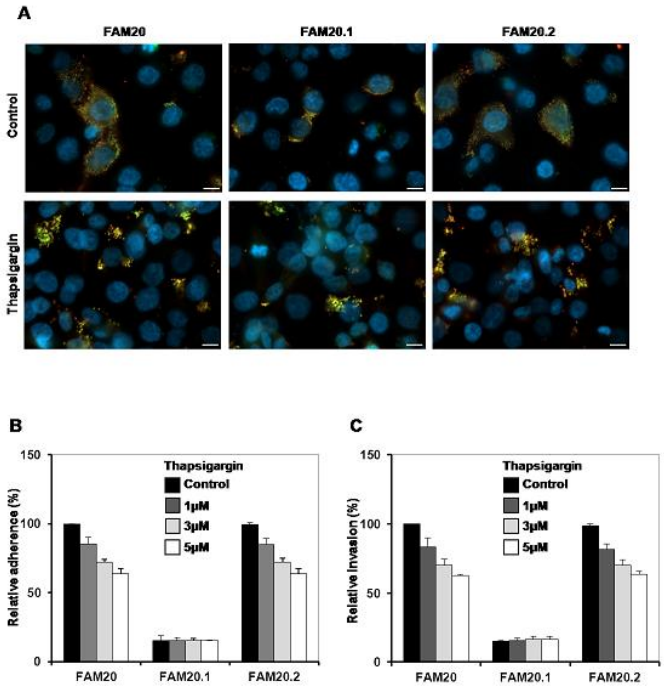


Figure 2



Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

305/MPP

Cre-recombinase based genetic engineering in *Streptococcus anginosus*

R. Bauer*[1], A. Grempels[1], S. Mauerer[1], B. Spellerberg[1]
[1]*University Hospital Ulm, Institute for Medical Microbiology and Hygiene, Ulm, Germany*

Streptococcus anginosus as one of three members of the Streptococcus anginosus group (SAG) is considered a human commensal of mucosal membranes (Whiley et al., 1992; Ruoff, 1988). However, improvements in species identification in recent years highlighted its role as an emerging pathogen. Despite the increasing number of reports about the epidemiology of SAG infections, our knowledge about the pathogenicity mechanisms is rare (Asam et al., 2014). One reason is the lack of published

genetic manipulations techniques in SAG which would facilitate the genetic investigation of virulence traits of these species.
The aim of this work was to establish a fast and reliable markerless genetic manipulation technique in *S. anginosus* based on the Cre-recombinase system.
To establish the mutation technique we performed an *in silico* investigation of the competence system of *S. anginosus* demonstrating that *S. anginosus* encodes homologues for the majority of genes that were shown to be essential for the transformation of *Streptococcus pneumoniae*. We analysed the transformation kinetic of *S. anginosus* ATCC33397 confirming that this strain possesses a *S. pneumoniae*-like competence development with a rapid increase of competence after treatment with the Competence stimulating peptide (CSP). The use of the CSP resulted in a high transformation efficiency of 0.24% ± 0.08%. To construct deletion mutants in *S. anginosus* a linear DNA fragment was generated by overlap-extension PCR consisting of two 500 bp fragments identical to the up- and downstream regions of the target gene surrounding a spectinomycin resistance gene (*spc*). In addition the fragment contains the two Cre-recombinase recognition sequences *lox66* and *lox71* flanking the *spc*. The linear deletion construct was transformed into *S. anginosus* leading to the integration of the *spc* and the loss of the target gene. In a second step the Cre-recombinase was introduced into the deletion strain leading to the excision of the *spc* and the formation of *lox72*.
The combination of the Cre-recombinase system and the CSP induced transformation allows the efficient creation of markerless gene deletions and will facilitate the investigation of the pathogenicity of *S. anginosus* at the molecular level.

References
[1] Whiley RA, Beighton D, Winstanley TG, Fraser HY, Hardie JM. *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. *J Clin Microbiol*. 1992;30(1):243-244.
[2] Ruoff KL. *Streptococcus anginosus* ("*Streptococcus milleri*"): the unrecognized pathogen. *Clin Microbiol Rev.* 1988;1(1):102-108.
[3] Asam D, Spellerberg B. Molecular pathogenicity of *Streptococcus anginosus*. *Mol Oral Microbiol*. 2014;29(4):145-155.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

306/MPP
A novel phage phiE72: an alternative therapeutic against *Staphylococcus epidermidis* infection and a potential research tool
X. Du*[1], V. Winstel[2], P. Kühner[1], Y. D. Stierhof[3], A. Peschel [1]
[1]*Tübingen University, Infection Biology, Tübingen, Germany*
[2]*University of Chicago, Cummings Life Science Center, Chicago, United States*
[3]*Tübingen University, The Center for Plant Molecular Biology (ZMBP), Tübingen, Germany*

Staphylococcus epidermidis is one of the most common pathogens causing various types of nosocomial infections in hospitals, mainly by forming biofilms on medical devices. Nowadays, the situation of increasing number of *S.epidermidis* developed resistance to antibiotics is calling for alternative therapeutics. Besides, a novel research tool is also expected since study of the pathogenicity of *S.epidermidis* is limited due to genetic manipulation failure caused by strong genetic barrier mechanisms, especially the clinical ones. Recently, we isolated a new bacteriophage named phiE72 from a *S.epidermidis* strain in an infected tooth of a clinical patient. Electron microscopy revealed characteristics as bacteriophages of the *Siphoviridae* family. Phage infection assay using different bacterial species showed that phiE72 has a narrow host range and is specific to *S.epidermidis*. It showed a more drastical decrease of turbidity of bacterial host cell culture even compared to the widely studied antibiotic reagent member lytic polyvalent phage phiK. PhiE72 remained stable at pH values between 5.0 and 8.0 and up to the temperature of 60 °C . PhiE72 also showed tolerance to chloroform. The fast and strong lyse property, and specificity for *S.epidermidis* indicates the novel phage phiE72 an attractive candidate for phage therapy or as a biofilm eradication agent against *S.epidermidis*. Moreover, phiE72 can transduce plasmid DNA efficiently even to strains refractory to electroporation. Therefore, phiE72 might also become a valuable research tool for plasmid transduction for *S.epidermidis* strains, which are often difficult to transform.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

307/MPP
One transcript, distinct stabilities: The methionine biosynthesis operon in *Staphylococcus aureus*
F. D. R. Wencker*[1], S. M. K. Schoenfelder[1], W. Ziebuhr[1]
[1]*Julius-Maximilians Universität Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*

N-formyl methionine is the universal N-terminal amino acid of prokaryotic proteins making methionine indispensable for bacterial growth. The common human pathogen *Staphylococcus aureus* is capable of synthesising methionine *de novo* and therefore to sustain in niches where the amino acid is lacking. Recently, we identified a unique hierarchical control pathway regulating *de novo* methionine biosynthesis in *S. aureus* involving stringent-response mediated CodY control in combination with a T-box riboswitch and RNA decay (1). Riboswitches are *cis*-acting RNA regulatory elements, located in 5"-untranslated regions (5"-UTRs) of genes. They typically undergo a conformational change upon interaction with a specific effector molecule, resulting in downstream gene expression control. The T-box family of riboswitches represents transcription termination control systems which bind uncharged cognate tRNAs as effector molecules. The T-box riboswitch residing in the 5"-UTR of the *S. aureus metICFE-mdh* methionine biosynthesis operon specifically interacts with uncharged methionyl-tRNAs $tRNA^{Met}$. In addition to T-box riboswitch-mediated transcription control, the *met* leader/*metICFE-mdh* mRNA was shown to undergo processing and rapid degradation involving various RNases (1). Here we demonstrate that stability of the *metICFE-mdh* mRNA varies over the length of the transcript with a longer lifespan towards the 3'-end of the transcript. Furthermore, we were able to determine the exact processing sites of the *met* leader and *metICFE-mdh* RNA, respectively by cRACE (rapid amplification of cDNA ends from circularised RNA). RNase III showed to be central to *met* leader RNA processing and degradation. We solved the secondary structure of this exceptionally long (440 nt) T-box riboswitch RNA using in-line probing to confirm the presence of structures required for RNase III cleavage.
From the combined data we hypothesise that targeted RNA decay might represent another level in the hierarchical methionine biosynthesis control network influencing translational efficiency and adjusting the protein amounts of the distinct enzymes of the pathway to current requirements.

Reference
[1] Schoenfelder, S. M.K. et al. 2013. Methionine Biosynthesis in *Staphylococcus aureus* Is Tightly Controlled by a Hierarchical Network Involving an Initiator tRNA-Specific T-box Riboswitch. Plos Pathogens 9(9).

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

Food Microbiology and Food Hygiene (FG LM)

308/LMP

Chemical speciation of selenium in selenized yeasts (dietary supplements), food contaminating molds and button mushrooms (*Agaricus bisporus*)

A. Prange*[1,2], M. Sari[1], S. von Ameln[1], S. Ellinger[1], R. Hambitzer[1], L. Bovenkamp-Langlois[2], J. Hormes[2]
[1]*Hochschule Niederrhein, Kompetenzzentrum für Angewandte Mykologie und Umweltstudien (KAMU), Mönchengladbach, Germany*
[2]*Louisiana State University, Center for Advanced Microstructures and Devices (CAMD), Baton Rouge, United States*

Question: Which chemical speciation of selenium is present in selenized yeasts, molds and mushrooms?
Method: X-ray absorption near-edge structure (XANES) spectroscopy using synchrotron radiation was used as an *in situ* approach to investigate chemical speciation in different (micro-)biological and environmental systems. The method is non-destructive and sensitive and yields information about the local geometric and electronic environment and the effective charge of a chosen atom within a molecule and, therefore, also of the formal oxidation state. Furthermore, it is possible to analyze the XANES spectra quantitatively to obtain relative percentages of the selenium species which contribute to the speciation [1].
Results and Conclusion: XANES spectroscopy was applied successfully to characterize the chemical speciation selenium *in situ* in different "selenized" (grown on selenite agar/broth) microorganisms and in mushrooms. The results show that molds can accumulate selenite and (some species) oxidize it to selenate, whereas yeasts (e.g. selenized *Saccharomyces cerevisiae* as dietary supplements) and *Agaricus bisporus* (button mushroom) also accumulate selenite, however, they incorporate selenium and form seleno-amino acids in different ratios (selenomethionine, selenocysteine). The form(s) in which selenium is present strongly affects the intake of selenium in human nutrition when using selenized yeasts or mushrooms as dietary supplement.

Reference

[1] Prange A., Modrow H. (2002). X-ray absorption spectroscopy and its application in biological, agricultural and environmental research. *Re/Views Environ. Sci. Bio/Technol.* **1**: 259-276

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

309/LMP

Antifungal activity of selected natural preservatives against the foodborne molds *Penicillium verrucosum* and *Aspergillus westerdijkiae* under different pH and a_w values

I. Schlösser[1,2], A. Prange*[1,2]
[1]*Hochschule Niederrhein, Mikrobiologie und Lebensmittelhygiene, Mönchengladbach, Germany*
[2]*Universität Witten/Herdecke, Institute für Virologie und Mikrobiologie, Witten, Germany*

Introduction: Food spoilage caused by molds may represent a considerable economic loss to the food industry in addition to being a health risk for consumers with respect to mycotoxin producing mold species. Despite the desire for safe food, there is an increasing consumer demand to avoid or diminish chemical food additives. From the ancient times until now numerous plant extracts with antimicrobial activity have already been known, including essential oils (EOs) and their active components.
Aims: The present study examines the antifungal effect of the EO *Origanum vulgare* its active components carvacrol and thymol and active components of other EOs, namely eugenol and *trans*-cinnamaldehyde against *Penicillium verrucosum* and *Aspergillus westerdijkiae* (former known as *A. ochraceus*). In order to classify the antifungal effect, the natural preservatives are further compared with conventional synthetic preservatives. Furthermore the dependence of the natural preservatives on different pH and aw values was investigated.
Methods: The minimum inhibitory concentration (MIC) was determined by broth macrodilution for *Penicillium verrucosum* CBS 302.48 and *Aspergillus westerdijkiae* CBS 112803. To determine the influence of pH and a_w values on the antifungal effect of plant derived extracts, MICs and MFCs were determined at pH (7.0, 5.6, 4.5 and 3.5) and a_w (1.00, 0.92, 0.90 and 0.87).
Results: Regarding their antifungal inhibitory effect, the following ranking in order of decreasing antibacterial activity can be made: *trans*-cinnamaldehyde > carvacrol = thymol > eugenol.
Compared with the conventional synthetic preservatives, the natural inhibitors can very well compete with respect to their antifungal effects, and even exceed them.
All tested natural agents are tolerant to the different examined milieu conditions, with the lowest MICs recorded at pH 3.5 and 0.87 aw.
Conclusion: Natural preservatives represent a possible alternative for the preservation of food. They are tolerant to different pH and a_w conditions, although they show a marginal better inhibitory effect in the slightly acid environment as well as at lower a_w values.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

310/LMP

Anti-bacterial effect of asparagusic acid isolated from asparagus sprouts against pathogens associated with cystitis

N. Juenemann[1], A. Goepel[1], A. Maxones[1], M. Birringer[1], C. Beermann*[1]
[1]*University of Applied Sciences Fulda, Oeotrophology, Fulda, Germany*

Urinary tract infections are considered to be the most common bacterial infection worldwide microbiologically indicated by Escherichia coli and *Proteus mirabilis*. The consumption of asparagus in this respect is traditionally remedy. This approach aimed at characterizing possible anti-bacterial properties of the sulfur-containing asparagusic acid derived from asparagus sprouts. Asparagusic acid was extracted from freeze-dried and pulverized white asparagus with 5 % formic acid in methanol followed by a liquid-liquid extraction with 5 % formic acid in petrolether. The evaporated petrolether extract was purified by two silica-gel 60 chromatography steps, first eluted with 2.7 % formic acid, 39.6 % ethyl acetate and 57.7 % petrolether and second eluted with 2 % formic acid, 21,8 % ethyl acetate and 76,2 % petrolether. The purity of the asparagusic acid was estimated by LC-ESI mass-spectrometry in negative mode. The anti-bacterial potential of asparagusic acid was determined by tubidometry at 600 nm wave-length light within a serial dilution of asparagusic acid in 7.3 pH buffered nutrient medium in the range of 0.02 to 2.5 mg/ mL. The results were correlated with oxytetracycline as antibiotic reference. The amount of E. coli and *P. mirabilis* in the testing always was 1*107 CFU/ mL. The measuring was performed as independent triple-tests evaluated by GraphPad 5 Prism software. The isolated asparagusic acid was >97 % pure. The concentration of 2.5 mg/ mL asparagusic acid inhibited 85% of the growth of E. coli and 76% of P. mirabilis. Acid concentrations below 0.039 mg/ mL for E. coli and 0.078 mg/ mL for *P. mirabilis* no effectiveness remains. The anti-bacterial effect of asparagusic acid compared to oxytetracycline is 103 times lower.
In this study asparagusic acid from white asparagus sprouts revealed a relevant growth inhibiting potential against gram negative bacteria associated to urinary tract infections in a slightly alkaline milieu. Therefore, asparagusic acid consumption might be one supportive element in cystitis treatment.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

311/LMP

Revised EN ISO 22964: Evaluation of Granucult® and Chromocult® Culture Media for Pre-Enrichment, Selective Enrichment and Detection of *Cronobacter* spp.

B. Gerten*[1], M. Gampe[1], A. Bubert[1], L. John[2]
[1]*Merck KGaA, Life Science, Darmstadt, Germany*
[2]*MilliporeSigma, Life Science, Bellevue, United States*

Introduction: EN ISO 22964 has been revised into a full EN ISO standard with an extended scope to *Cronobacter* spp. detection in food products for humans and feeding animals and environmental samples. A non-selective pre-enrichment step in BPW (Buffered Peptone Water) is followed by enrichment in selective medium CSB (Cronobacter Selective Broth) and plating out and identification on chromogenc CCI (Chromogenic Cronobacter Isolation) agar.
Purpose: Growth promotion and isolation of *Cronobacter* spp. and *Cronobacter*-related species were tested and confirmed with BPW, CSB and CCI agar, as described by EN ISO 22964:2017.
Methods: For growth promotion >15 *Cronobacter* spp. strains, >10 *Franconibacter* spp. and 3 *Siccibacter* spp. strains, including type strains and wild isolates from food, environmental samples and *C. sakazakii* reference material spiked into the food matrix, were used for evaluating the media from the whole workflow. Performance testing for the quality assuarance of the culture media for productivity selectivity and specificity were tested, as given by the EN ISO standard.
Results: All *Cronobacter* spp. and non-*Cronobacter* strains from pure strains resp. spiked reference material were able to be detected, following the method given by the revised standard: non-selective pre-enrichment in BPW, incubated between 34-38 °C for 18 h ± 2h, followed by selective enrichment in CSB, incubated at 41,5 °C ± 1 °C for 24 ± 2h and plated out and identified on CCI agar, incubated at 41,5 °C ± 1 °C for 24 ± 2h. Productivity, selectivity and specificity of the media affected the performance, as specified by the standard.
Significance: For the tested Granucult® BPW, CSB and Chromocult® CCI agar, the results of this study indicate the applicability of the methods and criteria as given in the revised International Standard EN ISO 22964:2017 "Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp."

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

312/LMP

Results of a study on hand hygiene in a biscuit bakery

J. Kloska[1], S. Meyer[1], B. Becker*[1]
[1]*Hochschule Ostwestfalen-Lippe, Mikrobiologie, Lemgo, Germany*

The aim of the study was to identify the reason of high number of microorganisms on the hands of employees after washing and disinfecting.
One hundred employees of a medium-sized biscuit bakery annually produce approximately 20,000 tons of biscuits. These are mainly durable products with a low water and a high sugar content where microbiological growth is not possible. Prior to the baking process the employees have main contact with raw materials, biscuit dough and fillings. When the baking process is finished (200 °C, 13 min) the products are partly individually packed. During this process there is main contact with the products, packaging material (plastics and carton) as well as with equipment and machines. Gloves are only worn when handling chocolate products to avoid fingerprints. Requirements concerning hand hygiene in food industry are defined for example in the Infection Protection Act (2000) or Biological Agents Ordinance (2013).
The company has explained in regular employee training measures hygiene plans for cleaning and disinfection of hands. The plans are posted in the hygiene sluices. The staff must pass through a hygienic sluice with stainless steel wash basins, soap dispensers, disinfectants (contact-free), disposable towels, paper bins (contact-free) and hygiene plans before entering the production rooms. The applied hand disinfectant is listed at VAH (Association for Applied Hygiene). The hand hygiene of the employees is regularly checked. For hand check the agar contact procedure according to DIN EN ISO 10113-3 is applied. After cleaning and disinfecting three fingertips (index, middle and ring finger) are pressed onto the agar surface. Rodac plates with CASO Agar and neutralizer (Tween 80, Lecithin, Histidine, Sodium thiosulfate) are used. The plates are incubated according to DIN EN ISO 10113-3 at 30 °C and the colonies are counted after 72 hours. There are two sample methods. "Visible sampling" where the sampler is in the hygienic sluices so the staff can see him. In case of "discreet sampling" the sampler is at the entry of production, however, not visible to the staff when washing and disinfecting the hands.
In case of "discreet sampling" the results show that a germ content of > 60 cfu was detected for 43 % of the employees. Washing the hands too shortly, applying not enough soap and disinfectant as well as using the same disinfectant for more than 6 years are reasons for the high germ content on the hands.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

313/LMP

Application of cold nebulized hydrogen peroxide for inactivation of murine norovirus, bacteria and bacteria spores on surfaces in food production

M. Dabisch-Ruthe*[1], M. Weinstock[1], J. Pfannebecker[1], S. Meyer[1], B. Becker[1]
[1]*Hochschule Ostwestfalen-Lippe, Mikrobiologie, Lemgo, Germany*

Food production and processing are highly technical with devices and equipment which have to meet high hygienic design demands. Hydrogen peroxide (H_2O_2) as disinfectant has a broad spectrum of activity. For many bacteria the decomposition by cell own catalase is the determining factor for the activity of H_2O_2. Cold nebulized H_2O_2 is a superfine dry fog which prevents harmful effects of humidity and is used until now especially for environment disinfection in hospitals and pharma industries.
As norovirus and bacteria contamination of food products are a persistent problem, the current study examines, if cold nebulized H_2O_2 is an effective agent for decontamination of surfaces in food production to increase the food safety.
In this study, the norovirus surrogate murine norovirus (MNV S99 P19), Gram positive (*Staphylococcus aureus* ATCC 6538) and Gram negative bacteria (*Escherichia coli* ATCC 8739) and bacterial spores (*Geobacillus stearothermophilus* ATCC 7953) were used to determine whether a treatment with cold nebulized H_2O_2 inactivates MNV and bacteria on stainless steel carriers. Two different application systems (DCXpert, DCX Technologies GmbH and DiosolGenerator MF, DIOP GmbH & Co. KG) were used for a cold fogging decontamination with H_2O_2. After H_2O_2 treatment (60 min, max. 260 ppm H_2O_2) MNV was recovered from surfaces of untreated and treated carrier discs with swabs according to DIN EN ISO 15216-2. Infective MNV was detectable in cell culture (RAW 264.7) with plaque assay. After RNase treatment for elimination of free RNA, real-time RT-PCR was used for relative quantification of intact MNV capsids. Bacteria were treated with H_2O_2 with and without drying on stainless steel carriers according to VAH method 14 (disinfectant commission of the Association for Applied Hygiene).
Reduction of intact MNV capsids was detectable by real-time RT-PCR after H_2O_2 treatment. With cold nebulized H_2O_2 infective MNV can be reduced on stainless steel by approximately 4 $\log_{10}$ below the limit of detection (50 PFU/mL). Lower reduction rates were observed for *S. aureus* and *E. coli.* Bacteria spores *G. stearothermophilus* resulted also in reduction below the limit of detection.
The good efficacy of cold nebulized H_2O_2 against the infective norovirus surrogate MNV and bacteria spores was demonstrated. The results indicate the possibility of using cold sprayed H_2O_2 to inactivate microorganisms on equipment and devices used in food production.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

314/LMP

Standing committee "DGHM microbiological guidelines"

B. Becker*[1]
[1]*Hochschule Ostwestfalen-Lippe, Miikrobiologie, Lemgo, Germany*

The DGHM working group "Microbiological Guidelines" was founded in 1986. For more than 25 years the working group was part of the DGHM scientific expert group "Food Microbiology and Hygiene." In 2015 the working group became the DGHM "Standing Committee Microbiological Guidelines." Currently, the Standing Committee consists of 16 members from food control authorities, universities, research facilities, service laboratories and the "German Federation of Food Law and Food Science (BLL)," representing industry. When specific subjects are discussed, experts of the respective industrial sectors as well as scientists are invited as guests. The members of the committee are elected by majority voting.

The DGHM Standing Committee "Microbiological Guidelines" still pursues its aim to establish guidelines with special attention to consumer health protection. It provides values for the assessment of microbiological results for food control authorities as well as for food producers and trade. When elaborating the values, the latest scientific standards concerning the different microorganisms, aspects of good hygiene, possible production procedures (e.g. industrial, handicraft), raw material parameters, packaging, logistics, storage, trade and consumer habits are considered and discussed, among others. The values are in the sense of consumer health protection and valid until the end of the best before date.

The preamble states: The values are understood as objectified basis for the evaluation of the microbiological hygienic status. The values are determined considering national and European legislation. "They are not legally binding." Until now the directive and warning values for 33 products have been published.

Since July 2013 the values have been published exclusively through the publisher BEUTH, Hamburg. This ensures that, starting from an authorized point, the complete values including all footnotes reach the user! Currently, more than 600 users are registered and 132 company licenses granted. 200 foreign companies are registered.

It has been noted that for the assessment of products for which there are no directive and warning values, the values of similar products are often used. From the point of view of the working group this procedure is not indicated. To create the values extensive microbiological data of specific products are collected. Based on this data, the directive and warning values are created. "Similar products" are not included in the data collection! For current value publications the products are described in detail in the headlines. Also, the use of data for raw materials and intermediate controls is not provided. There are significant differences in technology and microbiology between raw materials and finished products.

The working group acknowledges all objections and opinions of the users. The group creates the values with microbiological expertise, neutral and independently, responsible with a sense of proportion and foresight in terms of consumer health protection.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

315/LMP

Antibiotic resistant bacteria and their resistance genes in biofilm samples isolated from water distribution systems of hospitality homes in Benin City, Nigeria.

F. N. Olisaka[1], F. O. Ekhaise*[1]
[1]*University of Bnein, Faculty of Life Sciences, Department of Microbiology, Benin, Nigeria*

Water distributed in residential facilities such as Hotels, Inns and Guest houses are generally intended for several purposes like drinking, bathing and washing. Microbial presence in pipe-borne water results in the colonization of the distribution systems infrastructure and biofilm formation. Biofilms are structured multicellular communities, buried in a self-produced extra-polymeric substance that functions as an obstacle to antibiotic diffusion. The aim of this study was to investigate the antibiotic resistance and their resistance genes in biofilms isolated from faucets distributing water to end users in hospitality homes. Thirty six biofilm samples were collected from 6 hospitality homes. A total of 108 biofilms bacteria were isolated using standard microbiological techniques. The bacterial isolates were characterized using the 16S rRNA sequencing and three resistance genes; tetA, tetM and ermB were detected by Polymerase chain reaction. *Alcaligenes faecalis*, *Bacillus cereus*, *Enterobacter* sp, *Lysinibacillus fusiformis*, *Methylobacterium fujisawaense*, *Pseudomonas aeruginosa*, *Providencia vermicola* and *Serratia liquefaciens*, were isolated. *Alcaligenes* spp had the highest percentage frequency (26.9 %), while the least percentage frequency was recorded for *Providencia vermicola* and *Enterobacter* sp. in all Hospitality homes. *tet*A, resistance gene was more prevalent, it was detected in 49% of biofilm isolates, *tet*M, 45%, and *erm*B, 46% in all biofilm samples. Bacterial isolates from Biofilm were highly resistant to Chloramphenicol (100%) and the lowest resistance was recorded for Imipenem (1%). In view of the above, there is therefore an urgent need for the Hotel Managements to work out modalities to checkmate the menace of biofilm bacteria associated with hospitality homes, which could pose a great public health risk due to the presence of multidrug resistant bacteria harboring antibiotic resistant genes.

Keywords: Hospitality home, biofilms, resistance genes, antibiotic resistance.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

Free Topics (FT)

316/FTP

de.NBI - German Network for Bioinformatics Infrastructure

D. Wibberg*[1,2]
[1]*Center for Biotechnology (CeBiTec), Bielefeld University, Bielefeld, Germany*
[2]*Center for Biotechnology (CeBiTec) - Bielefeld University, GRIM, Bielefeld, Germany*

In recent years, the modern life sciences research underwent a rapid development that was driven mainly by the technical improvements in analytical areas in terms of miniaturization, parallelization and high throughput of biological samples and thus the generation of huge amounts of experimental data. Prominent examples of this ongoing development are the "omics" techniques featuring the analysis of the various levels of information storage and processes in living cells, and the numerous new imaging techniques providing insights into biological systems to a hitherto unprecedented depth. The ever growing application of these novel techniques and the exploitation of the resulting data have revolutionized many fields of science and are furthermore opening new areas of basic and applied research with considerable opportunities for life sciences. The bottleneck that prevents realization of the full potential of the different "omics" technologies is not the data generation itself, but the subsequent data analysis.

The "German Network for Bioinformatics Infrastructure (de.NBI)" takes care of this challenge in many areas of life sciences with its mission to provide, expand and improve a repertoire of specialized bioinformatics tools, appropriate computing and storage capacities and high-quality data resources. These efforts are supplemented by a training program providing courses on the supplied tools.

de.NBI is an academic funding initiatve of the German Ministry of Research and Education (BMBF) and started in March 2015. The consortium currently consists of 39 project partners organised in eight service centers and one central administration and coordination unit. The service centers offer a variety of training courses and bioinformatics services, online databases, software libraries, and tools as webservices and/or for download. Furthermore, consulting on individual issues is available. Services are aimed at application users in life sciences as well as bioinformaticians and developers. The de.NBI services will be

unified with regard to standards, interchangeability and reproducibility.
Further reading at: http://www.denbi.de

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

Zoonoses (FG ZO)

317/ZOP
The role of "filth flies" in the spread of antimicrobial resistance

F. Onwugamba*[1], J. R. Fitzgerald[2], K. Rochon[3], L. Guardabassi[4], A. Alabi[5], S. Kühne[6], M. P. Grobusch[7], F. Schaumburg[1]
[1]*Institute of Medical Microbiology, Münster, Germany*
[2]*Roslin Institute, Edinburg, United Kingdom*
[3]*University of Manitoba, Winnipeg, Canada*
[4]*University of Copenhagen, Copenhagen, Denmark*
[5]*Centre de Recherches Medicales de Lambarene, Lambaréné, Gabon*
[6]*Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Kleinmachnow, Germany*
[7]*University of Amsterdam, Amsterdam, Netherlands*

Question: "Filth flies" are universal, coprophagic and synanthropic insects of the order *Diptera*, that breed on garbage, decaying matters and excrements. They are usually resident, but can cover flight distances of about 5-8 km. Flies can transmit bacteria through translocation from the exoskeleton, regurgitation, defecation. Since "filth flies" live in the proximity of livestock and humans, they might be suitable vectors for pathogens to be considered as relevant in the One Health concept (e.g. Methicillin resistant *Staphylococcus aureus* or ESBL-producing *Enterobacteriacaea*).
The aim of this work is (1) to define the roles of flies in the spread of antimicrobial resistant bacteria, (2) to highlight existing knowledge gaps and (3) to propose future research.
Methods: We performed a narrative literature review (original articles and reviews indexed for PubMed), restricted to the English language. References of identified studies were also screened for potential sources.
Results: "Filth flies" can be colonized with antimicrobial resistant bacteria of clinical relevance (mainly gram-negative bacteria, e.g. ESBL or carbapenemase-producing *Escherichia coli*). The proportions can reach 17% for ESBL-producing *E. coli* or 33% for colistin-resistant (mcr-1 positive) *E. coli* in areas surrounding poultry farms.
Considerable amounts of data of limited quality suggest a capacity for transmission. However, insufficient data are at hand to prove whether flies can transmit resistant bacteria, eventually leading to infections in animals and humans. Many knowledge gaps should be filled to achieve a better understanding of the role of flies in the spread of antimicrobial resistance.
Conclusion: Interdisciplinary, cross-border concerted action is warranted to contain the spread of antimicrobial resistance. Assuming that resistant bacteria are transmitted between animals and humans in a similar way as enteric pathogens, one can conclude that effective vector control of "filth flies" might reduce the risk of transmission of antimicrobial resistance.

Figure 1

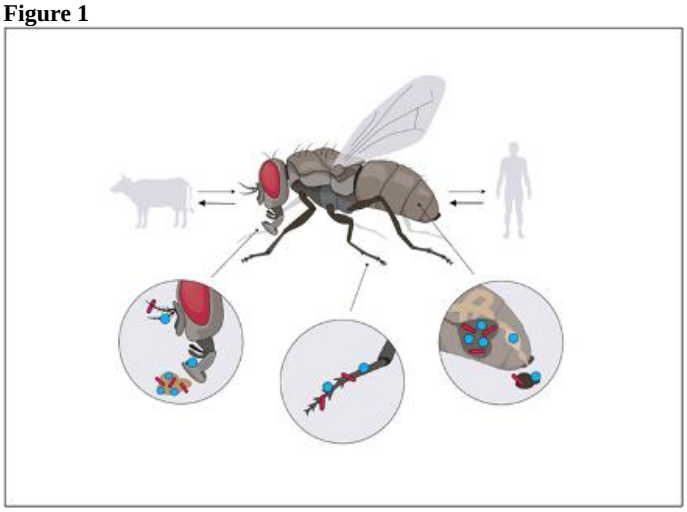


Figure 1: Showing a depiction of fly, livestock and man relationship justifying the need for a "One health" preventive effort (Picture courtesy of Christina Helmer).

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

318/ZOP
Rare, but divers - *cfr*-mediated linezolid resistance in *Staphylococcus aureus*

B. Strommenger*[1], F. Layer[1], C. Kehrenberg[2], J. Bender[1], G. Werner[1]
[1]*Robert Koch Institute, Department of Infectious Diseases, Wernigerode, Germany*
[2]*University of Veterinary Medicine, Institute for Food Quality and Food Safety, Hannover, Germany*

Objectives: Between 2006 and 2017, submissions of human staphylococcal isolates to the NRC for the verification of linezolid non-susceptibility increased continuously. In total, the NRC received 475 linezolid resistant staphylococci (371 *Staphylococcus (S.) epidermidis*, 75 *S. hominis* and 29 *S. aureus)* during this period. While the plasmid-borne resistance determinant *cfr*, which mediates the so-called PHLOPS-resistance phenotype, was detected in more than 30% of linezolid resistant *S. epidermidis* isolates, we could not detect *cfr* in any *S. hominis* isolate and in only six *S. aureus* isolates so far. The reason for the species-specific occurrence of linezolid resistance and the differential distribution of the *cfr* gene is not yet clear. Most probably, difference in plasmid content and resulting incompatibilities play an important role. Therefore the aim of the study presented herein was the characterization of *cfr*-carrying plasmids from linezolid-resistant, *cfr*-positive *S. aureus* isolates sent to the NRC.
Material and Methods: All isolates were subjected to phenotypic resistance testing. In addition, linezolid-resistant isolates were screened for the presence of *cfr* by PCR. NGS data for all isolates was generated by Illumina sequencing on a MiSeq instrument. Libraries were prepared with the Nextera XT DNA kit in paired-end-mode using v3 chemistry. A theoretical 100-fold coverage was sought. Subsequent to genome assembly using a5-miseq, initial data analysis was done with Ridom SeqSphere+. For all isolates, *spa*-type and MLST was derived from NGS data; additionally, all genomes were checked for the presence of relevant resistance gens. For further analysis, chromosomal genome data was "subtracted" from the datasets by mapping the read data to reference genomes of related clonal lineages; remaining putative plasmid sequences were assembled with a5-miseq and checked for their plasmid origin by means of BLAST. Results of in silico analyses were confirmed by plasmid digestion and Southern blot hybridization.
Results: *S. aureus* isolates were assigned to the clonal lineages CC398 (n=3) and CC30 (n=3). While CC398 isolates were broadly resistant MRSA, all CC30 isolates were sensitive to methicillin and had only a limited resistance spectrum (beyond the PHLOPS phenotype). Analysis of the plasmid content revealed a great diversity in plasmid number, size and plasmid-bound resistance determinants. In five isolates *cfr* was located on plasmids sized from app. 35 to 48 kb, while in the sixth isolate the determinant

was chromosomally located. At least three isolates carried *cfr* on plasmids harboring gene clusters associated with conjugational transfer indicating the potential of spreading the *cfr* gene across the population.
Outlook: Further work will be devoted to intra- and interspecies transfer of the identified *cfr* plasmids to understand additional mechanisms of their conditional species specificity.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

319/ZOP

Age-dependent gender differences and susceptibility rates in human isolates of *Campylobacter jejuni* and *Campylobacter coli* to important antimicrobials used in veterinary medicine

L. Hartmann*[1,2], F. Szabados[1,2], S. G. Gatermann[2]
[1]*Laborarztpraxis Osnabrück, Microbiology, Georgsmarienhütte, Germany*
[2]*Ruhr-University Bochum, Institute of Hygiene and Microbiology Department of Microbiology, Bochum, Germany*

Introduction: There is growing evidence that veterinary use of antimicrobials in food-producing animals is an important cause for emergence of resistant *Campylobacter spp.* Antimicrobial susceptibility testing using a reference method for determination of minimum inhibitory concentrations is not commonly performed and available susceptibility data from national reference laboratories may underreport antimicrobial resistance in areas of high veterinary consumption of antimicrobials. A standardized disc diffusion technique has been recently introduced for antimicrobial susceptibility testing of human isolates but broad-scale data on susceptibility results of *Campylobacter spp.* applying this method are still scarce. Furthermore, a strong relationship between Campylobacteriosis and patients age and male sex has been highlighted by nationwide surveys from several countries, while there is little information concerning *C. coli.*
Question: This study aimed to provide data on age-dependant gender differences and resistance rates of human *C. jejuni* and *C. coli*. to four important antimicrobials using a standardized disc diffusion technique in a high-incidence area of campylobacteriosis characterised by large-scale animal farming and high veterinary consumption of critical antimicrobials.
Methods: 1135 *C. jejuni* and 156 *C. coli* isolates were analysed regarding gender distribution in different age categories. Isolates were tested for susceptibility to ampicillin, tetracycline, ciprofloxacin and erythromycin using the EUCAST disc diffusion method.
Results: *C. jejuni* was more prevalent in male than female patients across most age groups with the exception of patients aged between 30-39 years. In contrast, *C. coli* was generally more prevalent in female patients with a female surplus among patients between 5-29 years, 40-49 years and 60-69 years. Resistance rates of *C. jejuni* versus *C. coli* were 48.4% vs. 46.2% for ampicillin, 64.4% vs. 46.2% for ciprofloxacin 35.1% vs. 68.6% for tetracycline and 0.06% versus 14.7% for erythromycin, respectively. Resistance to tetracycline and erythromycin was significantly more prevalent in *C. coli* compared to *C. jejuni*. Multi-drug resistance to three and all tested antimicrobials was also significantly more observed in *C. coli* than *C. jejuni*.
Conclusion: A male bias was observed for *C. jejuni* only whereas *C. coli* seems to be more prevalent in female patients. High prevalence of antimicrobial resistance was observed in *C jejuni* and *C. coli* necessitating routine antimicrobial susceptibility testing of isolated species. A mindful use antimicrobials in veterinary medicine is needed to prevent further emergence of resistant human isolates.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

320/ZOP

In-Depth Analysis of the Diversity of the Porcine Nasal Culturome

A. Schlattmann*[1], K. von Lützau[1], U. Kaspar[1], G. Peters[1], K. Becker[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*

Introduction: In recent years, research into the composition of microbial communities of various habitats has drastically increased. The underlying motif for this is the elucidation of effects that members of these microbiotas have on each other. In order to understand how these members interact with each other, however, a clear identification of and knowledge about the relationships of all constituents of the microbial communities has to be given.
Objectives: The cultivable part of the porcine nasal microbiota has been examined in this work.
Materials and Methods: Swab samples of 24 nasal cavities and 24 snout surfaces of 24 pigs (one habitat each) were taken, transported in Amies medium and processed within 24 hours. Solutions with dilution factors of 10^1, 10^2, and 10^3 were prepared and blood, chocolate, CAP, and MacConkey agar plates were inoculated with 100 µL of each solution and incubated under aerobic conditions (chocolate agar:+ 5 % CO_2). Anaerobic incubation was applied to Schaedler, Schaedler + K/V, chocolate and CAP agar plates inoculated in the same fashion. Incubation parameters for both incubation conditions were 35 °C for 48 hours. Identification of isolates was done by MALDI-TOF MS. Isolates were frozen at -80 °C. For a subset of six nasal cavity and snout surface samples each, 16S rRNA gene sequencing was performed for isolates which could not be identified by MALDI-TOF MS. Sequences were compared to the NCBI database using the BLAST algorithm.
Results: Using MALDI-TOF MS on all 48 samples and 16S rRNA gene sequencing on a subset of 12 samples, 238 different microbial species were identified. These belong to the orders of *Lactobacillales* (56), *Actinomycetales* (47), *Bacillales* (37), *Pseudomonadales* (25), *Enterobacteriales* (24), *Bacteroidetes*, *Aeromonadales* (9 each), *Burkholderiales* (6), *Neisseriales* (5), *Clostridiales*, *Saccharomycetales* (4 each), *Rhizobiales*, *Pasteurellales* (3 each), *Veillonellales* (2), *Fusobacteriales*, *Caulobacterales*, *Xanthomonadales*, and *Bifidobacteriales* (1 each). Preliminary sequence analysis of bacteria which could not be identified by either MALDI-TOF MS or 16S rRNA gene sequencing yielded six new genera and 10 new species candidates based on sequence identity to the closest match.
Conclusion: Our results suggest a vast diversity of the porcine nasal microbiota and its microbial members, some of which may not yet be described. Ongoing investigations into these unidentified isolates could further extend our knowledge of the complexity of this habitat. Cataloging and biobanking of the porcine nasal culturome will allow subsequent studies, e.g. for antagonistic interactions applying natural isolates instead of laboratory strains.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

321/ZOP

Specific differentiation of *Campylobacter fetus* spp. from other *Campylobacter* species using Fluorescence *in situ* hybridization (FISH)

M. Karg*[1], S. Poppert[2], H. Frickmann[3], H. Hotzel[4], U. Groß[1], A. E. Zautner[1]

[1]*Universitätsmedizin Göttingen, Medizinische Mikrobiologie, Göttingen, Germany*

[2]*Justus-Liebig-Universität Gießen, Institut für medizinische Mikrobiologie, Gießen, Germany*

[3]*Bernhardt-Nocht Institut, Fachbereich Tropenmedizin, Hamburg, Germany*

[4]*Friedrich-Loeffler-Institut, Institut für bakterielle Infektionen und Zoonosen, Jena, Germany*

Introduction: *Campylobacter fetus* can be further differentiated into three subspecies. *C. fetus* ssp. *fetus (Cff)* is well known to cause abortion in sheep whereas *C. fetus* ssp. *Veneralis* (*Cfv*) causes abortion mainly in cattle. Less is known about the recently in reptile intestines discovered *C. fetus* ssp. *Testudinum (Cft)*.But these subspecies have a medical issue in common in their ability to cause *C. fetus*-based bacteremia/sepsis.

Objective: It was the objective of this study to develop a method to detect specifically all three known *C. fetus* subspecies and to differentiate them from other *Campylobacter* species like *C. jejuni* ssp. *jejuni*, *C. jejuni* ssp. *doylei*, *C. coli*, *C. lari* and *C. upsaliensis* by using fluorescence *in situ* hybridization (FISH). This was done in order to be able to perform rapid diagnosis e.g. from bovine or ovine abortion samples as well as human blood samples.

Methods: Two different *C. fetus*-specific probes (a 23s-derived and a 16s-derived) were developed and used during the FISH-Analysis along with one *Campylobacter*-specific, one *Campylobacter/Arcobacter*-specific and a Eubacteria-specific probe. 41 *C. fetus* isolates originating from different sources were used for testing. 16 isoltesÂ originate from preputial washing of cattle, 2 from vaginal mucus of cattle, 4 from aborted calf fetuses, 1 from an aborted lamb fetus, 1 from intestinal swine content, 1 from intestinal calf content, 3 from reptile cloak swab and 13 from human blood culture. In total 20 *Cff* isolates, 14 *Cfv* isolates and 7 *Cft* isolates were used. Additionally we used thirty isolates of non-*C. fetus* species including *C. jejuni* ssp. *jejuni*, *C. jejuni* ssp. *doylei*, *C. coli*, *C. lari*, *C. upsaliensis* to test the specificity of the FISH probes.

Results: Following the optimization process of the hybridization conditions e.g. formamide concentration etc. the optimal fluorescence intensity was obtained for the two probes specific to *C. fetus*. The probe designed to bind in the 23s rRNA-encoding region proved to be highly specific for *C. fetus* showing no cross-reaction with other *Campylobacter* species. It was as well able to detect all three *C. fetus* subspecies. In contrast the second probe designed to bind in the 16s rRNA region recognized beside the intended *C. fetus* subspecies other *Campylobacter* species as well especially *C. jejuni ssp. doylei*.

Conclusion: It was possible to develop a highly *C. fetus*-specific FISH assay using the probe designed to bind in the 23s rRNA-encoding region. The FISH assay can be used to detect all three known *C. fetus* subspecies obtained from abortion and blood samples.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

322/ZOP

Competitive growth of *E. coli* O104:H4 strain with 5-*N*-acetyl-9-*O*-acetyl neuraminic acid as a carbon source

N. Saile[1], L. Schwarz[1], K. Eissenberger[1], J. Klumpp[2], F. Fricke[1], H. Schmidt*[1]

[1]*University of Hohenheim, Stuttgart-Hohenheim, Germany*

[2]*ETH Zurich, Zürich, Switzerland*

Sialic acids occur in high amounts in the intestinal mucus of mammals. *Escherichia coli* strains express three chromosomal *nan*-operons, that are responsible for the catabolism of sialic acids. One of the encoded proteins, NanS, is an *O*-acetyl esterase expressed by particular *E. coli* strains, that cleaves an acetyl residue from 5-*N*-acetyl-9-*O*-acetyl neuraminic acid (Neu5,9Ac$_2$), and therefore converts it to 5-*N*-acetyl neuraminic acid (Neu5Ac). This enzymatic reaction initiates the catabolism of Neu5,9Ac$_2$. Moreover, in pathogenic *E. coli* there are numerous further prophage-encoded Neu5,9Ac$_2$-esterases (NanS-p) described.

In this study, we investigated the occurrence of multiple *nanS*-p alleles in the EHEC O104:H4 strain LB226692, which caused the large outbreak of HUS in summer 2011 in Germany, and whether the *nanS*-p alleles offer a competitive growth advantage in relation to intestinal *E. coli*, using Neu5,9Ac$_2$ as a sole carbon source.

The genome of strain LB226692 has been determined using the PacBio platform. According to laboratory safety standards, we used the Shiga toxin-negative derivative C227-11Φcu for further laboratory analysis. This strain lacks the Stx2a-encoding phage. All *nanS*-p genes of C227-11Φcu were deleted and co-culture experiments with apathogenic *E. coli* strain AMC 198 in M9 minimal medium with Neu5,9Ac$_2$ as sole carbon source were performed.

Surprisingly, we could not detect the chromosomal *nanS* gene in LB226692 but in AMC 198. LB226692 carries 5 *nanS*-p genes in different prophages. The derivative C227-11Φcu still carries 4 *nanS*-p alleles since this variant was cured of its Stx2a-encoding phage, that encode one of the NanS-p proteins. With sequential deletion of the 4 *nanS*-p and simultaneous growth control on media with Neu5,9Ac$_2$, a gene-dose effect, detected by elongation of the generation times, could be recorded. In co-culture experiments, growth of AMC 198 was repressed by all mutant strains except that which did not carry any *nanS*-p gene. In that case AMC 198 overgrew O104:H4.

We concluded that prophage-associated *nanS*-p genes play a major role for O104:H4 in growth competition using Neu5,9Ac$_2$ as a carbon source.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

323/ZOP

Microevolution of epidemiological highly relevant non-O157 enterohemorrhagic *Escherichia coli* (EHEC)

I. Eichhorn[1], T. Semmler[2], A. Fruth[2], D. Pickard[3], M. F. Anjum[4], A. Mellmann[5], H. Karch[5], L. H. Wieler*[1,2]

[1]*Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany*

[2]*Robert Koch Institute, Berlin/ Wernigerode, Germany*

[3]*Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom*

[4]*Department of Bacteriology, Weybridge, Addlestone, Surrey, United Kingdom*

[5]*Institute for Hygiene, University Münster, Münster, Germany*

Background and Objectives: Many non-O157 EHEC have emerged as serious causes of hemolytic uremic syndrome (HUS) and bloody diarrhea worldwide. The most important non-O157 O serogroups causing one third of EHEC infections in Germany are O26, O103, O111 and O145. We have previously observed cluster formation of O26 and O111 EHEC in one single sequence type complex, STC29. STC29 also harbors aEPEC of the same O serogroups, which differ from EHEC by the absence of *stx*-converting bacteriophages, suggesting an ongoing microevolution of bidirectional-conversion, in which the phage-encoded *stx*-gene is transferred between aEPEC and EHEC [1].

Aim: Unravelling the microevolution of aEPEC and EHEC strains of STC29 focusing on epidemiologically highly important EHEC lineages of O serogroups O26 and O111.

Materials and Methods: 99 strains (aEPEC: n=20; EHEC: n=79; of human (n=53) and bovine (n=45) origin) were whole genome sequenced (WGS) to identify the maximum common genome (MCG), analyze single nucleotide polymorphisms (SNPs), define the presence of virulence associated genes (VAGs) as well as the occupation of insertion sites for mobile genetic elements.

Results: Three distinct clusters were identified based on the MCG-based SNP-analysis: Cluster 1 harbored O111 strains also designated as ST16 by MLST. Interestingly, the distinct Cluster 2 included only O26 aEPEC strains of ST29, while the more heterogeneous Cluster 3 combined EHEC as well as aEPEC strains of O serogroup O26 that were only merely separated into strains of ST29 and ST21. Analysis of the presences or absence of accessory

VAGs corroborated the SNP-analysis, and suggests a parallel evolution of the MCG of those strains and the acquisition of virulence genes. Furthermore, analysis of insertion sites for mobile genetic elements resulted in a similar relation of the analyzed strains. Our cumulative results of MLST, MCG SNP-analysis, VAG-analysis and insertion site identification enabled us to develop a microevolutionary model of EHEC of O serogroups O26 and O111, giving evidence that they developed as two distinct lineages from a common aEPEC ancestor of ST29 by lysogenic conversion with *stx*-converting bacteriophages. Furthermore, none of the features analyzed correlated with the host the strains had been isolated from.
Conclusions: EHEC of STC29 belonging to O serogroups O26 and O111 originate from a common aEPEC ancestor. The lack of host-specific features corroborates these aEPEC and EHEC strains as being *bona fide* zoonotic pathogens.

Reference
[1] Eichhorn, I., et al., *Highly virulent non-O157 EHEC serotypes reflect a similar phylogenetic lineage, giving new insights into the evolution of EHEC.* Appl Environ Microbiol, 2015.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

324/ZOP
Lysogenic conversion of atypical enteropathogenic *E. coli* (aEPEC) from different hosts with a Shiga Toxin-converting bacteriophage
I. Eichhorn[1], T. Semmler[2], D. Pickard[3], G. Dougan[3], H. Schmidt[4], H. Karch[5], L. H. Wieler*[1,2]
[1]*Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany*
[2]*Robert Koch Institute, Berlin/ Wernigerode, Germany*
[3]*Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom*
[4]*Institute of Food Science and Biotechnology, University of Hohenheim, Hohenheim, Germany*
[5]*Institute for Hygiene, University Münster, Münster, Germany*

Background and Objectives: The genes for Shiga Toxin 1 (*stx1*) and 2 (*stx2*) are encoded on lambdoid bacteriophages (*stx*-phage), which are integrated as prophage into the genome of enterohemorrhagic *E. coli* (EHEC), enabling toxin production. When host bacteria undergo environmental stress, the lifecycle of these phages switches from lysogenic to lytic cycle. The released phage particles can infect other *E. coli* such as aEPEC, which are distinguished from typical EHEC only by the absence of the *stx*-gene. Natural occurring transduction events of aEPEC with *stx*-phage have been detected in the gut of animals.
Aim: In a recent genomic study, we propose aEPEC as a common ancestor of two important non-O157-EHEC O-types (O26 and O111) of Sequence Type Complex STC29, in which the ongoing microevolutionary scenario of these important non-O157 EHEC is characterized by a bidirectional-conversion of the phage-encoded *stx*-gene. Here we thrive to proof these findings by functional *in vitro* experiments.
Materials and Methods: A modified *in vitro* plaque-assay was applied, using a high titer of a bacteriophage carrying a deletion in the *stx2* gene (Φ3538 *Δstx2::cat*) to increase the possibility of transduction events. Three strains, a murine aEPEC-strain MU/07/0324/209 (ST28, serotype Ont:H6), isolated from a striped field mouse in the surrounding of a cattle shed, and a human aEPEC-strain 910/10 (ST28, Ont:H6) were chosen as acceptor strains, as their close genomic relationship implies a high zoonotic potential. The third strain, bovine aEPEC IMT19981 also belonged to ST29, but was of serotype O26:H11.
Results: All three aEPEC could be lysogenized with phage Φ3538 *Δstx2::cat* . Integration of this bacteriophage into the bacterial host genome was confirmed by amplification of the gene *cat* as well as by Southern-Blot hybridization, using a DIG-labeled *cat*-DNA-probe. Analysis of the whole genome sequence of the lysogens showed that phage Φ3538 *Δstx2::cat* was inserted into the known tRNA integration site *argW* that is highly variable among *E. coli*.
Conclusion: The lysogenic conversion of aEPEC with a *stx*-phage *in vitro* was proven, emphasizing the validity of the proposed microevolutionary model and the important role of aEPEC as progenitors of typical EHEC. The host range of the aEPEC acceptor strains reflects the high risk of zoonotic transmission.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

325/ZOP
Comparison of manual and automated extraction of RNA to develop a mobile PCR for the detection of avian influenza viruses
K. Meissner*[1], T. Homeier-Bachmann[1], C. Sauter-Louis[1], F. J. Conraths[1]
[1]*Friedrich-Loeffler-Institut, Institut für Epidemiologie, Greifswald - Insel Riems, Germany*

Highly pathogenic avian influenza is a highly contagious disease causing substantial economic losses in poultry production worldwide. It is caused by influenza viruses of the subtypes H5 and H7, while other subtypes may induce inapparent infections or mild disease. Low pathogenic forms of the subtypes H5 and H7 may rapidly mutate into highly pathogenic avian influenza viruses.
Laboratory diagnosis at an early stage of an outbreak is crucial for effective disease control. Therefore, the aim of the study was the development of a mobile platform based on an RT-PCR approach. Moreover, an automated RNA extraction system was used to accelerate the diagnostic workflow.
Fecal homogenates of geese and turkeys were spiked with avian influenza viruses of the subtypes H5 and H9. Swab samples were taken and resuspended in lysis buffer. The samples were then vortexed and heat-inactivated. All experiments were performed in triplicate. A comparison of a manual and an automated isolation method of total RNA was conducted. Subsequently, a real-time PCR was performed on a portable instrument (Wernike, Beer et al. 2013)
Comparison of the manual and the automated extraction showed that it was possible to detect the virus in the samples with both methods, but manual extraction was more sensitive. However, in case of a suspicion of highly pathogenic avian influenza, samples will be taken from birds with clinical signs or dead animals, which are expected to harbour large amounts of virus. It is therefore highly likely that a safe diagnosis is also possible with the less sensitive automated method. Therefore, we conclude that the acceleration of the automated extraction method will be advantageous in the early diagnosis of an outbreak of highly pathogenic avian influenza.
In the next step, we will adapt a high-speed RT qPCR assay for the use in a mobile PCR unit on farms.

Reference
[1] Wernike, K., M. Beer and B. Hoffmann (2013). "Rapid detection of foot-and-mouth disease virus, influenza A virus and classical swine fever virus by high-speed real-time RT-PCR." J Virol Methods **193**(1): 50-54.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

326/ZOP
Molecular and functional characterization of IdeC, the IgG-specific protease of *Streptococcus canis*
A. M. Lapschies*[1], P. Krienke[1], I. Eichhorn[1], M. Fulde[1]
[1]*Institute of Microbiology and Epizootics/Freie Universität Berlin, Department of Veterinary Medicine, Berlin, Germany*

The beta haemolytic Lancefield group G *Streptococcus* (*S.*) *canis* is an opportunistic pathogen. Although naturally colonizing the mucosal surface of dogs and cats, *S. canis* has recently been described as an emerging zoonotic pathogen. Little is known about its pathogenesis and virulence factor repertoire.
In the present study, we characterized the Immunoglobulin G-degrading enzyme of *S. canis*, designated IdeC, with regard to its biochemical properties and phenotypic behaviour. Therefore, different biochemical and molecular biological techniques, e.g.

cloning and site-directed mutagenesis, recombinant protein expression, non-denaturing and denaturing SDS-PAGE, phenotypic protease- and protease-inhibition assays and EDMAN sequencing, were applied. Similar to other members of the IdeS protease family, IdeC is highly species-specific [1–3], with the exception that IdeC targets IgG molecules from two different hosts with high specificity, cats and dogs; IgGs from other species, such as human, equine, bovine and porcine IgG, remain uncleaved. Notably, although the cleavage sites of feline and canine IgG are identical as demonstrated by EDMAN sequencing, IdeC cleavage activity of feline IgG is significantly higher than of canine IgG. We further demonstrated that IdeC-dependent IgG cleavage is a two step process, occurring between the hinge region and Fc domain of the mature IgG molecule. By using several highly-specific protease inhibitors and metal-ion chelators, such as iodoacetamide and EDTA, and a recombinant IdeC protein that contains an amino acid exchange of a cysteine putatively located in the catalytic centre of IdeC, we characterized IdeC as a metal-ion independent cysteine-protease, as previously described for other streptococcal proteases [1–3].

In summary, although the basic properties of IdeC are in accordance with other members of the IdeS enzyme family, its broad cleavage activity against IgG molecules derived from more than one (naturally occurring) host species is a unique feature of IdeC and most properly based on the fact that *S. canis* is a commensal of both, dogs and cats. Further investigations are clearly needed to entirely explain the phenomenon of host specificity in proteases of the IdeS family.

References

[1] Von Pawel-Rammingen U, Johansson BP, Björck L. IdeS, a novel streptococcal cysteine proteinase with unique specificity for immunoglobulin G. EMBO J. 2002;21(7):1607–15.

[2] Spoerry C, Seele J, Valentin-Weigand P, Baums CG, von Pawel-Rammingen U. Identification and Characterization of IgdE, a Novel IgG-degrading Protease of Streptococcus suis with Unique Specificity for Porcine IgG. J Biol Chem. 2016;291(15):7915–25.

[3] Rungelrath V, Wohlsein JC, Siebert U, Stott J, Prenger-Berninghoff E, von Pawel-Rammingen U, et al. Identification of a novel host-specific IgG protease in Streptococcus phocae subsp. phocae. Vet Microbiol. 2017 Mar;201:42–8.

Presentation: Tuesday, February 20, 2018 from 17:00 – 18:30 in room Audimax Foyer Erdgeschoss.

H

I

M

N

O

T