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ABSTRACTS

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ABSTRACTS

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Fachgruppe (FG), Ständige Arbeitsgemeinschaft (StAG)

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Workshop 01
Microbial Pathogenesis (FG MP)
13. Sep. 2021 • 10:00–11:00

001/MPV

***Chlamydia trachomatis* inhibits apoptosis in infected cells by targeting the pro-apoptotic proteins Bax and Bak**

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Apoptosis acts in defence against microbial infection, and many infectious agents have developed strategies to inhibit host cell apoptosis. The human pathogen *Chlamydia trachomatis* (*Ctr*) is an obligate intracellular bacterium that strongly inhibits mitochondrial apoptosis of its human host cell but there is no agreement how the bacteria achieve this. We here provide a molecular analysis of chlamydial apoptosis-inhibition in infected human cells and demonstrate that the block of apoptosis occurs during the activation of the effectors of mitochondrial apoptosis, Bak and Bax. We use small-molecule Bcl-2-family inhibitors and gene targeting to show that previous models cannot explain the anti-apoptotic effect of chlamydial infection. Although the anti-apoptotic Bcl-2-family protein Mcl-1 was strongly upregulated upon infection, Mcl-1-deficient cells and cells where Mcl-1 was pharmacologically inactivated were still protected. *Ctr*-infection could inhibit both Bax- and Bak-induced apoptosis. Apoptotic Bax-oligomerization and association with the outer mitochondrial membrane was reduced upon chlamydial infection. Infection further inhibited apoptosis-induced conformational changes of Bak, as evidenced by changes to protease sensitivity, oligomerization and release from the mitochondrial porin VDAC2. Mitochondria isolated from *Ctr*-infected cells were protected against the pro-apoptotic Bcl-2-family proteins Bim and tBid but this protection was lost upon protease digestion. However, the protective effect of *Ctr*-infection was reduced in cells lacking the Bax/Bak-regulator VDAC2. These results identify a novel way of apoptosis inhibition, involving only the most downstream modulator of mitochondrial apoptosis and suggest that *Chlamydia* has a protein dedicated to the inhibition of apoptosis to secure its survival in human cells.

002/MPV

NAD(H)-mediated tetramerisation controls the activity of *Legionella pneumophila* phospholipase PlaB

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Introduction: *Legionella pneumophila* is an important lung pathogen causing a life-threatening pneumonia termed Legionnaires" disease. *L. pneumophila* produces a variety of phospholipases which may participate in lung destruction and progression of disease. Indeed, the virulence factor PlaB promotes lung colonization, tissue destruction, and intracellular replication of *L. pneumophila.* It is a highly active phospholipase exposed at the bacterial surface and shows an extraordinary activation mechanism by tetramer deoligomerization. To unravel the molecular basis for enzyme activation and localization, we determined the crystal structure of PlaB in its tetrameric form.
Methods: To decipher the molecular basis for PlaB"s unusual activation and to gain insight on how it associates with the OM, we have determined its crystal structure. Based on the structure, we introduced several mutations, performed protein purification, determination of lipolytic activities, liposome association, localization, and bacterial integrity experiments.
Results: We found that the PlaB tetramer is a dimer of identical dimers, and a monomer consists of an N-terminal α/β-hydrolase domain expanded by two non-canonical two-stranded β-sheets, β6/β7 and β9/β10. The C-terminal domain reveals a novel fold displaying a bilobed β-sandwich with a hook structure required for dimer formation and structural complementation of the enzymatic domain in the neighboring monomer. This highlights the dimer as the active form. Dβ9/β10 mutants showed a decrease in the tetrameric fraction and altered activity profiles. The variant also revealed restricted binding to membranes resulting in mislocalization and bacterial lysis. Unexpectedly, we observed eight NAD(H) molecules at the dimer/dimer interface, suggesting that these molecules stabilize the tetramer and hence lead to enzyme inactivation. Indeed, addition of NAD(H) increased the fraction of the tetramer and concomitantly reduced activity.
Discussion: Our data reveal structural elements and an unprecedented NAD(H)-mediated tetramerization mechanism required for spatial and enzymatic control of a phospholipase virulence factor.Since NAD(H) is a central cofactor of energy metabolism and confined to the intracellular milieu of the bacterium, we propose that the allosteric regulatory process identified here is suited to fine tune PlaB in a way that protects *L. pneumophila* from self-inflicted lysis while ensuring its activity at the pathogen–host interface.

003/MPV

Protein disulfide isomerase potentiates extracellular adhesion protein (Eap)-driven staphylococcal invasion into endothelial cells

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Question: *Staphylococcus aureus* is not only an extracellular but also an intracellular pathogen. The main internalization pathway is the binding of the bacteria to host cells, such as endothelial cells, via a fibronectin (Fn) bridge between *S. aureus* Fn binding proteins and α5β1-integrin, followed by phagocytosis. However, also other *S. aureus* adhesins, such as the secreted extracellular adherence protein (Eap), have been shown to promote cellular invasion. Eap also appears to have other roles in *S. aureus* pathogenicity. Previously, we demonstrated that Eap induces platelet activation by stimulating protein disulfide isomerase (PDI). In the present study we have investigated the interaction of Eap with PDI on endothelial cells and whether this affects staphylococcal invasion.
Methods: The amount of free ectosulfhydryls on endothelial cell line cells (EA.hy926 and HMEC-1) +/- Eap incubation was determined by flow cytometric analysis of the binding of Alexa 488 C5 maleimide. Di-eosin-oxidized-glutathione was used to determine PDI reductase activity. Uptake of *S. aureus* 8325-4 as well as of *S. carnosus* TM300 (an Eap-negative species) in host cells was analyzed +/- in presence of Eap after 1h of infection using lysostaphin protection assay followed by host cell lysis. Number of intracellular bacteria was assessed by plate counting. Bacitracin (removed again before addition of bacteria), rutin and DTNB as well as anti-PDI antibodies were used as PDI inhibitors. In addition, impact of PDI knockdown by siRNA was tested. Statistical analyses were performed using one-way analysis of variance.
Results: Eap increased the number of free ectosulfhydryls on host cells and potentiated PDI reductase activity. Adherence and invasion of *S. aureus* as well as *S. carnosus* in host cells was significantly increased in the presence of Eap. Inhibition of PDI by rutin and anti-PDI antibodies as well as PDI knock down reversed the enhancing effect of Eap. Both bacitracin and DTNB reduced staphylococcal invasion even without additional Eap. The uptake of *S. aureus* but not of *S. carnosus* into host cells was increased by addition of Fn during infection. Eap increased the binding of soluble Fn to host cells and enhanced the effect of Fn on *S. aureus* uptake. Uptake of *S. carnosus* was enabled by Eap alone, and further enhanced by simultaneous addition of Eap and Fn. The uptake was inhibited by bacitracin.
Conclusions: To our knowledge, our data provide the first evidence that PDI plays an important role in staphylococcal host cell invasion. *S. aureus* Eap is able to stimulate PDI on the host cell membrane. Therefore, Eap may play a dual role in host cell

invasion: Stimulation of PDI resulting in increased bacterial uptake, and formation of a molecular bridge - likely in concert with Fn - between staphylococci and host cell is thought to allow for host cell invasion even of bacteria otherwise unable to invade host cells such as *S. carnosus*.

004/MPV

The branched-chain amino acid transporter BrnQ1 is involved in *S. aureus* phagosomal escape in epithelial cells

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Introduction: *Staphylococcus aureus* (*S. aureus*) is a human commensal, but also an opportunistic pathogen and a notorious cause of hospital – acquired and epidemic infection. S.*aureus* shows a remarkable adaptation to a range of niches within its human host. Traditionally regarded as a bacterium which can online survive outside of human cells, *S. aureus* is by now recognised as a facultative intracellular pathogen, well adapted to reside inside virtually all types of human cells.
The fate of intracellular *S. aureus* depends vastly on the host cell type. While in professional phagocytes, such as macrophages, the bacteria replicate inside acidic phagosomes, in non-professional phagocytes, such as epithelial or endothelial cells, *S. aureus* escapes from phagosomes and replication is initiated within the host cell cytosol.
Question: The bacterial factors involved in the phagosomal escape of *S. aureus* in non-professional phagocytes are poorly understood. The staphylococcal alpha phenol-soluble modulins (PSMα) and the AusAB non-ribosomal peptide synthase have been described to play crucial roles in *S. aureus* phagosomal escape in epithelial cells. However, their presence, while necessary, is not sufficient to trigger bacterial phagosomal escape.
Here, we sought to identify additional bacterial factors involved in *S. aureus* phagosomal escape in epithelial cells.
Methods: A phagosomal escape screen employing high-content automated microscopy was conducted using a HeLa reporter cell line. Several *S. aureus* mutants in genes which are relevant to different metabolic processes were analysed for their capacity to escape phagosomal enclosure. The observed phenotypes were confirmed by *in trans* complementation studies and live cell imaging approaches, using various human epithelial cell lines. Additionally, bacterial intracellular replication assays were carried out and host cell death induced by intracellular bacteria was analysed.
Results: The screen approach revealed that the staphylococcal branched-chain amino acid (BCAA) transporter BrnQ1, but not the dedicated isoleucine transporter BrnQ2, plays an important role in the phagosomal escape and intracellular replication of *S. aureus.* Complementation *in trans* with a functional copy of the *brn*Q1 gene, as well as exogenous addition of BCAAs restore both phagosomal escape and the intracellular replication abilities of the *brn*Q1 mutant *S. aureus.*
Conclusions: Several mutants in genes which were found to be up- or downregulated during stringent response were screened for their ability to escape their phagosomal enclosure in HeLa cells. The BCAA transporter BrnQ1 was identified as a bacterial factor involved in the phagosomal escape of *S. aureus* in epithelial cells. Here, we provide for the first time, a link between transporter-mediated nutrient uptake and phagosomal escape of the facultative intracellular bacterium *S. aureus.*

005/MPV

***Staphylococcus epidermidis* biofilms alter the pro-inflammatory immune response in primary human macrophages**

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Question: Polarized macrophages are the first defense line of the human immune system. Since *S. epidermidis* evolved sophisticated mechanisms to escape the host"s immune response professional phagocytes are facing problems eradicating those pathogens. In order to understand why and how biofilms are able to persist during this study investigated the cellular effects of macrophages during infection.
Methods: Primary human macrophages isolated from buffy coats were infected with different *S. epidermidis* strains. Phagocytosis rates, macrophage polarization and TLR2 presentation were analyzed by confocal laser scanning microscopy and FACS analysis. For better understanding of the phagocyte"s reaction during infection transcription and protein levels of Il-1ß, TNF-α, Il-6 and Il-10 were assessed and a RNAseq experiment was carried out.
Results: Instead of being activated upon infection with bacteria embedded in a biofilm the macrophages" expression and secretion of pro-inflammatory cytokines is significantly reduced in contrast to contact with single cell bacteria. However, anti-inflammatory cytokines are highly upregulated. Along with these findings, the phagocytosis rate is dramatically decreased as soon as bacteria are protected in a biofilm. Interestingly, in an infection context the macrophages subtype is shifted towards anti-inflammatory M2 as confirmed in FACS analysis. The *S. epidermidis* recognition receptor is TLR-2. By blockage of this receptor the phagocytosis rate of biofilm positive strains can be recovered as well as the induction of the pro-inflammatory defense. When analyzing the overall gene expression pattern of macrophages during infection experiments it becomes clear that a dramatic dysregulation of the innate immune response takes place.
Conclusion: With the help of biofilm formation bacteria are able to evade phagocytosis by shifting the host"s immune response towards an anti-inflammatory profile. Understanding biofilm related immune escape mechanisms of *S. epidermidis* and other biofilm forming bacteria may pave the way towards novel therapeutic approaches in the future.

006/MPV

Induction of host cell death by intracellular *Staphylococcus aureus* is mediated by perturbation of host cell Ca^{2+} homeostasis and the staphylococcal cysteine protease staphopain A

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Despite being regarded as an extracellular bacterium, the pathogen *Staphylococcus aureus* can invade, survive and persist within human cells (1, 2). The intracellular niche is considered as hide-out from the host immune system and antibiotic treatment. Eventually, the pathogen escapes from the host cell resulting in host cell death, which is associated with tissue destruction and spread of infection. However, the exact molecular mechanisms employed by *S. aureus* to escape the host cell are still unclear.
We performed a genome-wide shRNA screen to identify host cell proteins and signaling pathways involved in *S. aureus* intracellular cytotoxicity and found the calcium signaling pathway to be involved (3). *S. aureus* induced a massive cytosolic Ca2+ increase in epithelial host cells after invasion and intracellular replication of the pathogen. This resulted from Ca2+ release from the endoplasmic reticulum and Ca2+ influx via the plasma membrane, leading to mitochondrial Ca2+ overload, activation of calpains and caspases and eventually to breakdown of the plasma membrane barrier function and cell death.
In addition, we identified the staphylococcal cysteine protease staphopain A expressed by intracellular *S. aureus* to induce cell death in epithelial cells (4). Loss of staphopain A function resulted

in delayed onset of host cell death and prolonged intracellular replication of *S. aureus*, but failed to prevent cytoplasmic Ca2+ overload. Overexpression of staphopain A by a non-cytotoxic strain in the host cell cytoplasm facilitated intracellular killing of the host cell even in the absence of detectable intracellular replication. In phagocytic cells, where intracellular *S. aureus* is exclusively localized to the phagosome, no cytotoxic effect of staphopain A was detected. We therefore conclude that staphopain A expressed by intracellular *S. aureus* induces host cell death after translocation to the host cell cytoplasm in epithelial cells. Moreover, staphopain A contributed to efficient colonization of the lung in a mouse pneumonia model. These findings reveal a novel, intracellular role for the bacterial protease staphopain A. Furthermore, we provide evidence that host cell death by intracellular *S. aureus* is not only a host cell-driven response induced by stress or cell defense but also pathogen-driven by staphopain A.
In summary, at least two independent cell death pathways are activated by intracellular *S. aureus* in epithelial cells. While initially staphopain A mediates *S. aureus*-induced host cell killing, cytosolic Ca2+ overload follows later and leads to the final demise of the host cell. We found evidence for both necrotic as well as apoptotic cell death pathways.

References:

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Workshop 02
Infection Control & SARS-CoV-2 (StAG HY/FG PR)
13. Sep. 2021 • 10:00–11:15

007/HYPRV
SARS-CoV-2 outbreaks and infection control in a tertiary care hospital

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Introduction: Implementation of hygiene measures have proven to be essential in containing the ongoing pandemic. Moreover, contact tracing and management of SARS-CoV-2 clusters are important to prevent viral spreading. Especially in a clinical environment, accurate surveillance of these clusters is crucial to protect patients as well as staff and to maintain service. We analysed notified clusters of COVID-19 in a tertiary care hospital to describe outbreak development in the course of the pandemic.
Materials and Methods: 20 SARS-CoV-2 clusters had been reported by infection control between March 2020 and May 2021. Infected individuals were categorised into three groups: patients, employees and others (e.g. accompanying persons).
We defined an outbreak as two or more epidemiologically linked PCR-confirmed SARS-CoV-2 cases. The cluster ended with the last laboratory-confirmed case and was monitored for the following 28 days.
Results: We recorded a total number of 101 laboratory-confirmed SARS-CoV-2 infections directly related to outbreaks. Patients accounted for 33% and employees for 64% of total infections. Cluster events had a median duration of 7 d (range 1-20 d). The number of observed outbreaks correlated with the regional incidence. Since April 2021, the number of clusters was comparatively low despite the third wave of infection, most likely due to a positive impact of the immunization campaign.
We identified a multitude of different causes for the cluster events, which were mainly induced by SARS-CoV-2 positive patients. Countermeasures included contact tracing and PCR-screenings as well as increased base hygiene measures.
Discussion: Despite stringent infection control measures, clusters could not be avoided during the COVID-19-pandemic. However, they were mostly well contained due to centralised outbreak management. While the index case of outbreaks was usually a patient, driving forces of outbreaks were staff members. Thus, the vaccination campaign, which commenced end of December 2020, had a major impact on protecting staff from nosocomial infection and on limiting disease spread considerably. Data on hospital outbreak control are important to motivate staff to receive a vaccine. Of note, outbreaks were not restricted to hospital wards routinely taking care of COVID-19 patients, but occurred in all settings.

008/HYPRV
Usage of medical face masks - A comparison between healthcare workers and the general population during the COVID-19 pandemic

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Introduction: Wearing of face masks, in addition to maintaining distance, is a key component of COVID-19 transmission prevention and is intended to protect potential contacts from ingesting pathogen-containing material. On 25.01.2021, a stricter mask requirement came into force in Germany[1]. As in medical facilities, only medical face masks (surgical face masks (SFM) and filtering face pieces Class 2 (FFP2 mask) or similar) were therefore mandatory in public areas (public transport and retail stores). The aim of the study was to analyse the wearing behaviour of face masks among healthcare worker (HCW) compared to the general population. In particular, it was observed which masks were used in public areas, whether the use was correct and which application errors were made.
Method: In an observational study, 490 HCW (309♀, 181♂) from different areas in the hospital and 245 persons (138♀, 107♂) in retail stores in the Cologne area were observed in the period between 15.02.–21.03.2021 ("3rd Corona wave") to record the mask types used as well as observed mask application errors. Based on the RKI notes "Respiratory Mask: Common Application Errors"[2] a checklist was created for systematic data collection that included mask type worn, observed gender, estimated age, and observed mask application errors. Wearing cloth masks was counted as not wearing a mask, as they were prohibited both in the hospital and in public areas.
Results:
A high overall wearing adherence of 99.6% was observed among HCW. 90.7% of the masks were worn correctly. A total of 48 application errors were observed. The most frequent application errors fell into the categories "mask not tight due to beard" (22.9%) "mask worn under nose" (20.8%) and "retaining straps too loose " (16.6%). The SFM was used incorrectly significantly more often than the FFP2 mask (p<.001). In the population, overall wear adherence was 87.8%. The mask was correctly applied 63.3% of the time. A total of 64 individual errors were observed. The most common error fell into the category of "nose clip not adjusted" (57.8%), followed by mask under nose (17.2%), and hair not tied (9.3%). Again, more errors were made when using SFM than when using FFP2 masks (p<.001).
Discussion: In general, HCW show higher wear adherence and lower number of errors when wearing masks than individuals do in the general population. This may be because HCW are trained in mask use and there have been few efforts to educate the general population on proper use. However, there is also a need for training among HCW in the use of SFM.
References:

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009/HYPRV

Infection control strategies for employees and companions during the COVID-19 pandemic in German hospitals – Results from a cross-sectional study

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Introduction: The hospital setting has potential risks for transmission of SARS-CoV-2 infection. Health care workers are regularly in close contact to patients. Visitors and persons accompanying patients (hereafter "companions") may also contribute to the spread of infections within the hospital setting. The Robert Koch Institute suggests infection control measures to reduce the risk of virus transmission in clinical areas, including personal protective equipment (PPE), visitor regulation, hand hygiene and keeping distance. The objective of the study was to identify infection control strategies for employees and companions in German hospitals.

Methods: In cooperation with the DGHM Working Group for Infection Prevention and Antibiotic Resistance, a cross-sectional, ethically-approved standardized questionnaire was developed and sent to infection control personnel (ICP) in 987 randomly selected hospitals in Germany in March and April 2021 using a cross-sectional, standardized online survey (Limesurvey). We report strategies in German hospitals pertaining to PPE for employees and companions, companion restrictions and possible exemptions.

Results: ICPs of 100 hospitals across Germany completed the survey (response rate:10%). Hospitals were grouped into small (≤ 249 beds; n=23), mid-sized (250-999 beds; n=55) and large (≥ 1000 beds; n=22) hospitals. While almost 60% of small and mid-sized hospitals recommended that their employees wear FFP2 masks as a matter of principle, only 4% of large hospitals did so, and the decision was often left to the employees (Table 1). In contrast, the use of face shields for aerosol-producing activities was recommended more often in large hospitals. Compared to small and mid-size hospitals, large hospitals mostly recommended the use of FFP2 masks when performing aerosol-producing activities (small 48%; mid-size 49%; large 82%) or treating COVID-19 patients (small 48%; mid-size 51%; large 77%). The classification of aerosol-producing activities (e.g. transesophageal echocardiography and dental procedures) varied according to hospital size. Using gloves when handling non-COVID patients was more often recommended in small and mid-size hospitals (small 35%; mid-size 35%; large 14%). More large (45%) than small (26%) hospitals prohibited companions (Table 2). Many hospitals made exemptions to companion restriction for specific reasons (e.g. birth or impending death). Most hospitals required companions to wear their choice of either a medical mask or FFP2 mask within the hospital area.

Discussion: There seem to be clear differences between the recommendations on the use of PPE and on visitor regulations between the hospital groups. Companion restriction seemed to be more rigorous in large hospitals. The classification of an aerosol-producing activity depended on hospital size – perhaps reflecting the extent to which these activities are carried out on site. Thus, harmonized implementation has not yet been achieved.

Fig. 1

Table 1. Personal protective equipment recommendations for employees (all values in percent).

	Small hospitals n=23	Mid-size hospitals n=55	Large hospitals n=22	Total n = 100
Masks recommendation for employees				
Only medical mask (EN 14683)	4.3	1.8	4.5	3.0 (-0.3 – 6.3)
Only FFP2 Mask (EN149)	52.2	61.8	**4.5**	47.0 (37.2 – 56.8)
Possible choice between medical mask or FFP2 mask	43.5	36.4	**90.9**	50.0 (40.2 – 59.8)
Recommended activities for using FFP2 masks (multiple answers possible)				
Aerosol-producing procedures	**47.8**	49.1	**81.8**	56.0 (46.3 – 65.7)
Contact with COVID-19 patients	47.8	50.9	**77.3**	56.0 (46.3 – 65.7)
Outbreak in ward	52.2	47.3	54.5	50.0 (40.2 – 59.8)
Emergency room	39.1	43.6	45.5	43.0 (33.3 – 52.7)
Hospitals which do not use FFP2 masks with an exhalation valve	95.7	94.5	90.9	94.0 (89.3 – 98.7)
Hospitals recommend using a face shield for certain activities	**78.3**	94.5	86.4	89.0 (82.9 – 95.1)
Recommended activities for wearing a face shield (multiple answers possible)				
Aerosol-producing procedures	**30.4**	61.8	54.5	53.0 (43.2 – 62.8)
Contact with COVID-19 patients	**17.4**	32.7	36.4	30.0 (21.0 – 39.0)
Swab procedures	**43.5**	25.5	**9.1**	26.0 (17.4 – 34.6)
Clinical procedures defined as aerosol-producing procedures (multiple answers possible)				
Oro- or nasopharyngeal swab procedures	91.3	90.9	90.9	91.0 (85.4 – 96.6)
Intubation	**78.3**	94.5	95.5	91.0 (85.4 – 96.6)
Bronchoscopy	**60.9**	92.7	100.0	87.0 (80.4 – 93.6)
Cardiopulmonary resuscitation	**73.9**	89.1	81.8	84.0 (76.8 – 91.2)
Esophageal-Gastro-Duodenoscopy	**52.2**	76.4	81.8	72.0 (63.2 – 80.8)
Transesophageal echocardiography (TEE)	**43.5**	76.4	**81.8**	70.0 (61.0 – 79.0)
Inhalation	56.5	56.4	31.8	51.0 (41.2 – 60.8)
Dental procedures	**26.1**	**27.3**	**90.9**	41.0 (31.4 – 50.6)
Coloscopy	**8.7**	20.0	13.6	16.0 (8.8 – 23.2)
Suction	4.3	5.4	0.0	4.0 (0.2 – 7.8)
Obligation to use gloves (multiple answers possible)				
Contact with COVID-19 patients	82.6	98.2	86.4	92.0 (86.7 – 97.3)
Contact with suspected COVID-19 patients	87.0	98.2	**77.3**	91.0 (85.4 – 96.6)
Contact with non-COVID-19 patients	34.8	34.5	**13.6**	30.0 (21.0 – 39.0)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

Fig. 2

Table 2. Infection control strategies for companions (all values in percent).

	Small hospitals n=23	Mid-size hospitals n=55	Large hospitals n=22	Total n = 100
Permitted companions				
Not permitted	**26.1**	45.5	45.5	41.0 (31.4 – 50.6)
One person	**69.6**	54.5	54.5	58.0 (48.3 – 67.7)
Two persons	**4.3**	0.0	0.0	1.0 (-1.0 – 3.0)
Exemptions to companion restriction (multiple answers possible)				
Patient with impaired mobility	26.1	32.7	**45.5**	34.0 (24.7 – 43.4)
Patient who need translation	**34.8**	50.9	54.5	48.0 (38.2 – 57.8)
Age-related reason	**26.1**	61.8	63.6	54.0 (44.2 – 63.8)
Patient with severe disability	**21.7**	45.5	50.0	41.0 (31.4 – 50.6)
Childbirth	**13.0**	49.1	**63.6**	44.0 (34.4 – 53.7)
Impending death	**65.2**	**92.7**	86.4	85.0 (78.0 – 92.0)
Do not implement companion restriction	**8.7**	0.0	4.5	3.0 (-0.3 – 6.3)
Companion by underage patient				
Only one parent/legal guardian	39.1	36.4	40.9	38.0 (28.5 – 47.5)
Only one parent/legal guardian but allow to switch	**21.7**	47.3	50.0	42.0 (32.3 – 51.7)
Both parents/two legal guardians	8.7	5.5	9.1	7.0 (2.0 – 12.0)
Mask recommendation for companions				
Only medical mask (EN 14683)	21.7	14.5	18.2	17.0 (9.6 – 24.4)
Only FFP2 Mask (EN149)	34.8	34.5	27.3	33.0 (23.8 – 42.2)
Possible choice between medical mask or FFP2 mask	**34.8**	50.9	54.5	48.0 (38.2 – 57.8)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

010/HYPRV

SARS-CoV-2 infection prevention strategies in German hospitals – Results from a cross-sectional study

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Background: Infection prevention knowledge and its implementation are important aspects for managing and containing the COVID-19 pandemic in hospitals. The objective of the study was to compare surveillance, separation- and information strategies in hospitals of different sizes across Germany.

Methods: In cooperation with the DGHM Working Group for Infection Prevention and Antibiotic Resistance, a cross-sectional, ethically-approved standardized questionnaire was developed, pre-tested and formatted as an online tool (LimeSurvey). We surveyed persons responsible for hygiene and infection prevention in 987 randomly-selected German hospitals in March and April 2021. We report descriptive statistics about the infection prevention strategies, especially spatial separation, employee breaks and information for hospital employees.

Results: In total 100 hospitals across Germany took part in the survey with a response rate of 10%. The participating hospitals were grouped into small (≤ 249 beds; n=23), mid-sized (250-999 beds; n=55) and large (≥ 1000 beds; n=22) hospitals.

About half of the hospitals surveyed (53%) separate cases, suspectd cases and others (Table 1). Incidental suspected cases of inpatients in the non-COVID area are usually left in this area and cared for in a single room, or else transferred directly to the area for suspected COVID cases. Most often, COVID treatment areas are located in separate COVID-only hospital wards (66%). In 54% of the responding hospitals, there are separate teams for COVID and non-COVID cases; larger hospitals are more likely to separate the staff only if a nosocomial case is confirmed or suspected.

The requirements for staff during breaks seem to be stricter in larger hospitals in terms of number of people and time without wearing masks (Table 2). Employees may remove their masks when they are alone in the room (small 70%; mid-sized 86%; large 91%) or for < 15 minutes (small 13%; mid-sized 13%; large 27%).

Most hospitals (93%) provide information to employees about the local development of the pandemic. The frequency of information distribution varies across size groups with 82% of large hospitals spreading the information at least weekly, followed by mid-sized (60%) and small hospitals (43%). The preferred form of communication in all hospitals is electronic (91%); differences occur between hospital sizes in the use of print media and audio-video podcasts.

Conclusion: Interestingly, there are no relevant differences in spatial separation strategies across hospitals beyond dealing with incidentally suspected cases. In contrast, information strategies seem to depend on hospital size. Especially relevant for transmission events, the guidelines for employees during breaks seem to differ. Future studies should examine if employee break guidelines are associated with outbreaks.

Fig. 1

Table 1. Spatial separation strategies for SARS-CoV-2 positive and suspected COVID-19 cases (all values in percent).

	Small hospitals n=23	Mid-size hospitals n=55	Large hospitals n=22	Total n = 100 (95% CI)
Existence of separated areas				
separated area for SARS-CoV-2 positive patients	21.7	47.3	31.8	38.0 (28.5 - 47.5)
separated area for suspected COVID-19 cases	52.2	50.9	59.1	53.0 (43.2 - 62.8)
Procedure for incidentally occurring suspected cases in the non-COVID area until clarification is obtained				
transfer to area for suspected COVID-19 cases	26.1	34.5	**4.5**	26.0 (17.4 - 34.6)
remain in area but in isolation / single room	**47.8**	56.4	**86.4**	61.0 (51.4- 70.6)
Organisation of ward for suspected COVID-19 cases				
shared rooms	13.0	9.1	9.1	10.0 (4.1 - 15.9)
single rooms only	43.5	40.0	50.0	43.0 (33.3 - 52.7)
Location of COVID-19 area				
separate building	0.0	3.6	0.0	2.0 (0.0 – 7.0)
separate wards	**52.2**	70.9	68.2	66.0 (56.7 -75.3)
separate area within same wards	17.4	23.6	22.7	22.0 (13.9 - 30.1)
Departments with completely separate areas for COVID-19 positive (multiple answers possible)				
normal ward	52.2	85.5	**86.4**	78.0 (69.9 - 86.1)
intensive care unit	**21.7**	56.4	**59.1**	49.0 (39.2 - 58.8)
Separate teams of healthcare workers for COVID-19 and non-COVID-19 cases				
Yes	43.5	58.2	54.5	54.0 (44.2 - 63.8)
No	21.7	25.5	**9.1**	21.0 (13.0 - 29.0)
Only if a nosocomial infection is confirmed or suspected	**4.3**	14.5	**27.3**	15.0 (8.0 - 22.0)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

Fig. 2

Table 2. Employee information strategies and guidelines for employees during breaks (all answers in percent).

	Small hospitals n=23	Mid-size hospitals n=55	Large hospitals n=22	Total n = 100 (95% CI)
Does your hospital provide information to employees about the development of the COVID-19 pandemic in the local area?				
Yes	**82.6**	94.5	**100.0**	93.0 (88.0 - 98.0)
Frequency of information for employees				
Daily	**13.0**	23.6	**36.4**	24.0 (15.6 - 32.4)
Weekly	30.4	36.4	45.5	37.0 (27.5 - 46.5)
Irregularly, e.g. when changes occur	21.7	20.0	**9.1**	18.0 (10.5 - 25.5)
Form of communication (multiple answers possible)				
Electronic (homepage, intranet, email)	**78.3**	92.7	**100.0**	91.0 (85.4 - 96.6)
Print (letter, poster, flyer)	26.9	23.6	**9.1**	22.0 (13.9 - 30.1)
Audio/visual podcast	8.7	9.1	**27.3**	13.0 (6.4 - 19.6)
Communication platform (multiple answers possible)				
In house communication platform	87.0	90.9	90.9	90.0 (84.1 - 95.6)
Hospital homepage	39.1	29.1	36.4	33.0 (23.8 - 42.2)
During breaks for food and drink: when may employees remove their masks? (multiple answers possible)				
Alone in the room	**69.6**	85.5	**90.9**	83.0 (75.6 - 90.4)
Air circulation in the room	60.9	58.2	59.1	59.0 (49.4 - 68.6)
Separated by at least 1.5 m (or empty chair)	60.9	60.0	45.5	57.0 (47.3 - 66.7)
At least 10m² per person	43.5	29.1	40.9	35.0 (25.7 - 44.3)
Time without mask is < 15 minutes	13.0	12.7	**27.3**	16.0 (8.8 - 23.2)
During cigarette breaks: what guidelines do employees need to follow? (multiple answers possible)				
Separated by at least 1.5 m	**69.6**	89.1	86.4	84.0 (76.8 - 91.2)
Alone in the smoking area	30.4	23.6	27.3	26.0 (17.4 - 34.6)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

288/HYPRV

Integrated Genomic Surveillance of SARS-CoV-2 as a prototype for other pathogenic microorganisms

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Integrated Genomic Surveillance refers to the integration of the genetic fingerprint of pathogens into the already established reporting systems. The current SARS-CoV-2 pandemic impressively illustrates the benefits of such extended pathogen surveillance, because only by sequencing the SARS-CoV-2 genomes can sufficient information be obtained to track the evolution of the pathogen in Germany and thus the emergence of new mutations as well as their entry from abroad. Variants that cause, for example, rapid transmissibility of the virus or a lower protective effect of vaccinations can thus be detected at an early stage and further investigations and measures to contain the spread can be initiated promptly.

Collecting all SARS-CoV-2 genome sequences across Germany via DESH, the associated epidemiological data from the patients via DEMIS, performing comprehensive bioinformatics analyses on this joint dataset and integrating all data and analyses in a high-performance data warehouse structure, allowed the RKI to perform and report constantly the relevant evolutionary markers of the virus and their emergence over time and geographical location as well as their functional consequences that are linked to the patients.
Across pathogens, linking the pathogen genome sequence to the reporting data enables the detection of infection chains even in the case of geographically extensive or temporally protracted spread of pathogens, even without immediately recognizable disease clusters. Examples include food borne outbreaks (e.g., caused by Listeria or EHEC) due to a contaminated food product distributed across regions, sometimes even internationally, or spread of resistant pathogens in hospitals. The improved ability to detect chains of infection also expands the scope for action to interrupt them. Knowledge about the emergence of antimicrobial resistances in microbial pathogens resulting in multi-resistant strains or the spread of highly virulent lineages as well as extended outbreak detection and transmission surveillance are examples for the great benefit that a broad genomic surveillance of pathogenic microorganisms in combination with a systematic collection of related epidemiological data can generate a very large added value.

Workshop 03
Microbiological diagnostics around Covid-19 (FG DKM/StAG DV)
FG Diagnostische und Klinische Mikrobiologie in Zusammenarbeit mit der StAG Diagnostische Verfahren
13. Sep. 2021 • 10:00–11:00

011/DKMV

Performance of three SARS-CoV-2 immunoassays, three rapid lateral flow tests and a novel bead-based affinity surrogate test for the detection of SARS-CoV-2 antibodies in human serum

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[5]Julius-Maximilians-University of Würzburg, Institut für Systemimmunologie, Würzburg, Germany

Question: For the control of immunity in COVID-19 survivors and vaccinated subjects there is an urgent need for reliable and rapid serological assays. Are there commercial assays with a satisfying performance compared to the virus neutralization assays for clinical antibody diagnostics?
Methods: Based on samples from 63 COVID-19 survivors up to seven months after symptom onset, and on 50 serum samples taken before the beginning of the pandemic, we compared the performance of three commercial immunoassays for the detection of SARS-CoV-2 IgA and IgG antibodies (Euroimmun SARS-COV-2 IgA/IgG, Mikrogen recomWell SARS-CoV-2 IgA/IgG, and SERION ELISA agile SARS-CoV-2 IgA/IgG) and three rapid lateral flow (immunochromatographic) tests (Abbott Panbio COVID-19 IgG/IgM, NADAL COVID-19 IgG/IgM, and Cleartest Corona 2019-nCOV IgG/IgM) with a plaque-reduction neutralization test (PRNT50) representing the gold standard. In addition, we report and validate a novel, non-commercial flow cytometry bead-based surrogate test.
Results: 57 out of 63 PCR-confirmed COVID-19 patients (90 %) showed neutralizing antibodies. The sensitivity of the seven assays ranged from 7.0 % to 98.3 %, the specificity from 86.0 % to 100.00 %.
Conclusions: Only one commercial immunoassay showed a sensitivity and specificity of greater than 98 %. These data indicate abundant interassay variability.

Fig. 1

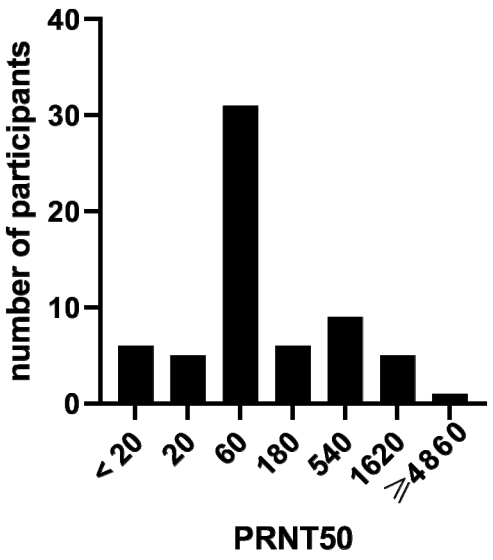
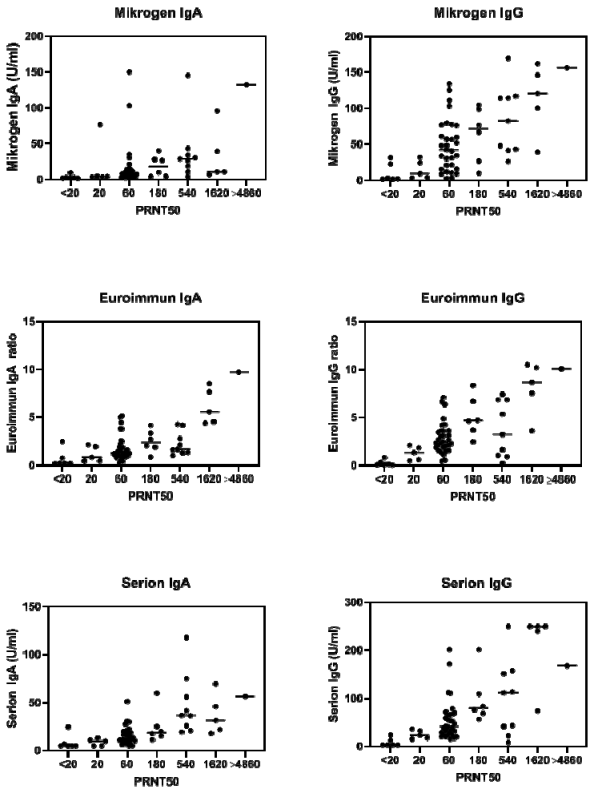


Fig. 2



012/DKMV

Clinical performance evaluation of SARS-CoV-2 rapid antigen testing in point of care usage in comparison to RT-qPCR

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Question: Antigen rapid diagnostic tests (RDT) for SARS-CoV-2 are fast, broadly available, and inexpensive. Despite this, reliable clinical performance data is sparse. In our study, we have analyzed if RDT show a sufficient analytical performance to replace or complement quantitative reverse transcription polymerase chain reaction (RT-qPCR) testing in clinical use.

Methods: In a prospective performance evaluation study, RDT from three manufacturers (NADAL®, Panbio™, MEDsan®) were compared to RT-qPCR in 5 068 oropharyngeal swabs for detection of SARS-CoV-2 in a hospital setting. Viral load was derived from standardized RT-qPCR Cycle threshold (Ct) values. The data collection period ranged from November 12, 2020 to February 28, 2021.

Results: Overall, sensitivity of RDT compared to RT-qPCR was 42.57% (95% CI 33.38%–52.31%), and specificity 99.68% (95% CI 99.48%–99.80%). Sensitivity declined with decreasing viral load from 100% in samples with a deduced viral load of ≥108 SARS-CoV-2 RNA copies per ml to 8.82% in samples with a viral load lower than 104 SARS-CoV-2 RNA copies per ml. No significant differences in sensitivity or specificity could be observed between the three manufacturers, or between samples with and without spike protein variant B.1.1.7. The NPV in the study cohort was 98.84%; the PPV in persons with typical COVID-19 symptoms was 97.37%, and 28.57% in persons without or with atypical symptoms.

Conclusions: RDT are a reliable method to diagnose SARS-CoV-2 infection in persons with high viral load. RDT are a valuable addition to RT-qPCR testing, as they reliably detect infectious persons with high viral loads before RT-qPCR results are available.

Fig. 1

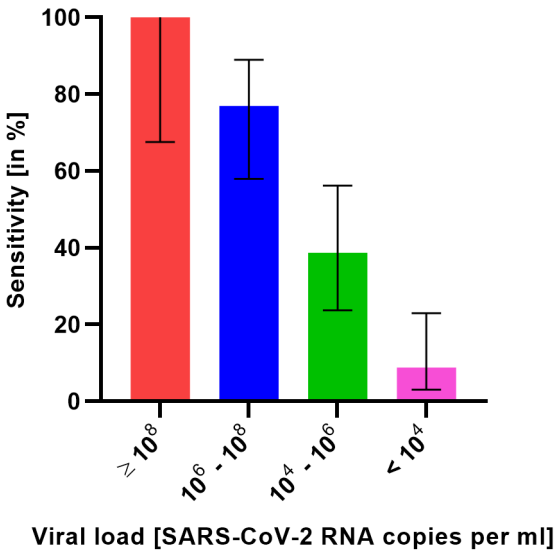
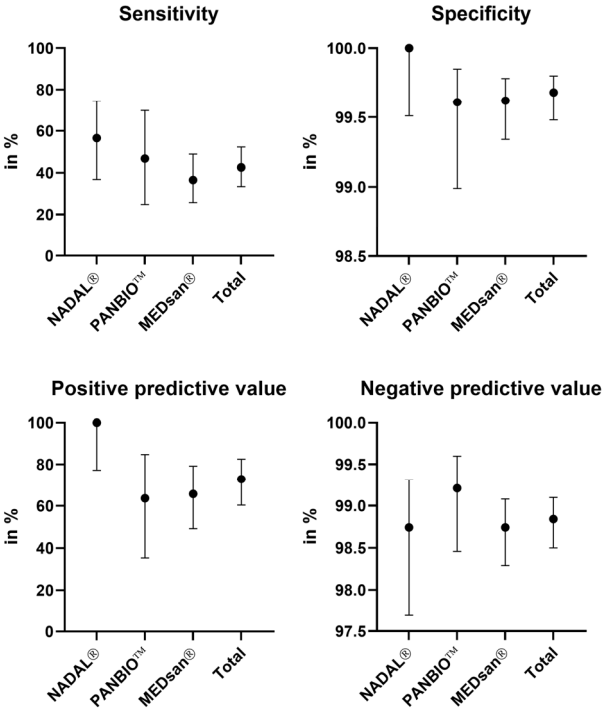


Fig. 2



013/DKMV

Is COVID-19 associated pulmonary aspergillosis (CAPA) a myth? Frequency of *Aspergillus* detection from respiratory samples in intensive care unit patients with and without COVID-19

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Introduction: COVID-19 associated pulmonary aspergillosis (CAPA) is reported to be an emerging disease with a mean incidence of 13.5% (range 3%-35%) and a mortality rate as high as 48%. Interestingly, most studies on CAPA focused exclusively on COVID-19 patients and it is currently unknown what the relative risk to develop CAPA in COVID-19 patients is. Therefore, we performed a study in order to compare the frequency of *Aspergillus* detection from respiratory samples and the rate of pulmonary aspergillosis in intensive care unit (ICU) patients with and without COVID-19.

Materials/Methods: We conducted a prospective observational study between November 2020 and May 2021 at the University Hospital Erlangen, a 1400-bed German tertiary care hospital. All patients from one surgical and two medical ICUs with a microbiological examination of a respiratory specimen (bronchoalveolar lavage fluid (BALF), bronchial and tracheal aspirate) were included. All specimens were analyzed by mycological culture, Blancophor® staining as well as by *Aspergillus* polymerase chain reaction (PathoNostics AsperGenius®) and by three galactomannan antigen assays (BioRad Platelia™ Aspergillus Ag EIA, Vircell Aspergillus Galactomannan Ag Virclia® Monotest, IMMY sona *Aspergillus* Galactomannan Lateral Flow Assay). Serum samples from patients with positive *Aspergillus* assays were additionally tested for galactomannan and (1→3)-β-D-glucan.

Results: 650 respiratory specimens (BALF 39.5%, bronchial secretion 34.0%, tracheal secretion 26.5%) from 262 patients were included in the study. 125 patients (47.7%) were treated for COVID-19. Surprisingly, the rate of patients with at least one positive *Aspergillus* assay was higher in the non-COVID-19 group (47.4% versus 40.0%, p=0.23). Correspondingly, the rate of positive *Aspergillus* assays was higher in the non-COVID-19 group for the Platelia™ Aspergillus Ag EIA (8.8% versus 6.5%, p=0-26), the Aspergillus Galactomannan Ag Virclia® Monotest (22.9% versus 14.2%, p=0.01), the sona Aspergillus Galactomannan Lateral Flow Assay (12.5% versus 9.3%, p=0.40) and for serum (1→3)-β-D-glucan (39.1 pg/ml versus 28.7 pg/ml, p=0.13). Only the rate of positive *Aspergillus* PCRs and serum Platelia™ *Aspergillus* Ag EIA was higher in the COVID-19 group (12.2% versus 8.4%, p=0.22 and 6.3% versus 0%, p=0.01). The mean levels of galactomannan and the mean ct-values of the *Aspergillus* PCR were comparable between COVID-19 and non-COVID-19 patients. These results remained consistent if only BALF samples were analysed.

Discussion: Positive results of different *Aspergillus* assays are not more prevalent in COVID-19 patients compared to non-COVID-19 patients. Our results question the hypothesis that pulmonary aspergillosis is more common in COVID-19 patients. However, the analysis of the clinical data of our study is pending.

014/DKMV

Electric Eye: a simple and accessible evaluation and automated documentation system for antigen test results

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Introduction: To efficiently monitor infectivity during the COVID-19 pandemic rapid antigen detection tests (RADTs) are widely employed. As these tests are often designed for easy point-of-care use, automated digital documentation is not a direct feature, yet often still a requirement. As our institute is a large scale user of RADTs, and as such of different brands depending on market availability. We therefore aimed to create a tool for simple and fast digital and manufacturer-independent documentation of such tests, which can be used downstream for further digital processes. Additionally a technical assessment and suggested test result was sought in order to reduce uncertainty when assessing RADTs.

Material/methods: We used a simple setup which can easily and rapidly be replicated for most settings, consisting only of a standard computer with an external webcam.

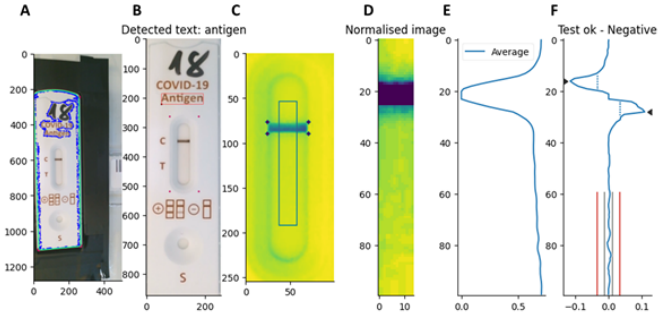
Results: With our software installed, the RADT together with a QR- or Barcode identifying the sample is placed in view of the camera. The operator can then press "N" for "negative", "P" for "positive", or "U" for "uncertain"/"invalid" test results. With the button press, a picture of the test is saved with the bar code and time stamp as file name. Additionally, for further use of the test result, a .csv file with the date, test abbreviation, and the operator determined test result is created. If the result was incorrect, a renewed button press with the same QR- or Barcode will overwrite the .csv file and create a new image. If testing is done, pressing "S" will upload the .csv file to another file path, after which already uploaded QR-/Barcodes will be rejected. For safety, saving without a recognised barcode leads to a visible warning.

Test results can additionally be assessed by the program itself if a RADT with one control bar is used (Fig. 1). The program can then display a brightness evaluation and, using the first derivative of the change in brightness, suggest a test result (Fig. 1 E-F). The program is accessible at: https://github.com/schwanbeck/UMG_Antigen_Evaluator

Discussion: We are presenting here "Electronic Eye", an accessible, cheap, yet reliable documentation tool that can be used for a wide variety of visually assessed qualitative tests. With the inbuilt QR/Barcode scanner, it allows for easy documentation with little hands-on time. Further, the electronic eye software has an inbuilt technical assessment function for RADTs (Figure 1). Results are stored as accessible .csv files for further use.

Figure 1 Camera aided antigen test evaluation. A: initial antigen test outline detection. **B**: Trapeze transformation to correct for misaligned outline. **C**: Antigen test control area detection (four black points) and test area indication (blue square). **D**: Test area after global brightness centring. **E**: averaged and smoothed brightness of test area. **F**: First derivative of brightness curve with indicated minima/maxima and thresholds for positive and intermediate test results.

Fig. 1



015/DKMV

Experiences made with a mobile app to provide clinical, diagnostic and therapeutic recommendations on infectious diseases in Germany

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Introduction: The development and implementation of specific guidelines pertaining to the diagnosis and treatment of infectious diseases is a key element of antimicrobial stewardship activities. However, many locally developed guidelines are not consistently used in daily clinical practice for a variety of reasons, e.g. the information in printed documents being rapidly outdated, or the lack of willingness among the intended recipients to carry additional booklets along while at work. Smartphone-based mobile applications may have the potential to overcome many of these shortcomings. Here, we present experiences made with such a mobile app at a large German University hospital.

Methods: Between August 2018 and October 2020, an interdisciplinary antimicrobial stewardship group at a University hospital in southwest Germany convened at regularly scheduled meetings to develop an antimicrobial diagnosis and treatment guideline in the format of a smartphone app. Guideline chapters were written by up to three individuals from different medical and surgical disciplines, and were subsequently subjected to internal peer review. During two final meetings, the content of all chapters was presented to members of the guideline committee, and open questions were discussed until consensus was reached. The technical implementation was elaborated by a medical software company, and the app was released for free in March 2021 for Android and iOS. The app is constantly being updated whenever new guidelines or significant clinical trial results become available.

Results: The mobile app comprises chapters on common infectious diseases of all body compartments, specific treatment recommendations for a variety of bacterial pathogens, and detailed fact sheets on frequently used antibiotics, which include information on a substance"s clinical spectrum, its potential use during pregnancy, and necessary dose adjustments in renal or hepatic insufficiency (Figures 1 and 2). The app was downloaded and installed more than 1400 times within three months of the official release, and it comprised content equal to 210 written pages (standard paper size). The app was rated as excellent by users on the app stores (average vote: 4.95/5). A survey performed at one University hospital elucidated that physicians stated that they were more likely to use the app than a "hard copy" guideline", mainly due to the app"s intuitive usability, its rapid search engine and the continuous updates of its content.

Conclusion: Our experience encourages wider use of and further research on smartphone-based applications to improve antimicrobial stewardship and antibiotic prescribing patterns among physicians in Germany and elsewhere.

Fig. 1

Fig. 2

016/DKMV

Combating the pandemic from a veterinary perspective

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The Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) has been supporting the health authorities in Lower Saxony in testing human medical samples for SARS-CoV-2 since April 2020 as one of the few purely veterinary laboratories nationwide without a human medicine department. LAVES is a central authority for consumer protection in Lower Saxony. It is responsible for the examination and assessment of official samples from all process and production stages in the food chain and for animal health in Lower Saxony. The PCR examinations in LAVES are carried out on the decision of the Lower Saxony state government as part of the administrative assistance for the Lower Saxony Ministry of Social Affairs, Health and Equality and thus for the public health service in Lower Saxony. Since May 11th, 2020 LAVES has been officially registered with the Robert Koch Institute (RKI) in Germany as an examination facility for corona. The implementation of the SARS-CoV-2 examinations in the LAVES has been successfully established since April 2020. In the LAVES laboratories over 110.000 "corona samples" were examined by the end of March 2021. In addition, since February 2021 VOC-PCR tests (investigation of Variants of Concern) have been carried out in LAVES. In parallel to the corona examinations, the LAVES IT department set up a laboratory and IT infrastructure very quickly in order to make the logistics for the submitting agencies as simple as possible. This enables, for example, fast and secure online transmission of the test results to the submitting agencies (especially health authorities). Looking back on the corona year 2020/21, it can be stated that veterinary laboratories were able to contribute to a significant increase in official laboratory capacities and thus to the clarification of outbreaks in the event of crises in the health sector, such as the corona pandemic.

Workshop 04
Eukaryotic Pathogens (with DMykG Lecture)
13. Sep. 2021 • 10:00–11:00

017/EKV

Evolutionary adaptations of environmental *C. albicans* to host-associated sugars lead to increased virulence and resistance

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Candida albicans is a pathogenic fungus that can cause both, superficial and severe invasive infections. As an opportunistic pathogen it is usually found in its commensal form, when it colonizes human mucosal surfaces such as the oral cavity, the vagina, and the gastrointestinal tract. As a result, it has rarely been isolated from the environment. Despite this, three independent environmental *C. albicans* isolates were found on old oak trees far from urban influences in 2016 (Robinson *et al. Ecology and evolution* **2016**, 1236-1250, *6*).

Because of the paucity of well-described *C. albicans* isolates from the environment and their potential relevance for understanding the pathogenicity of this fungus, we characterized and compared the three environmental strains to clinical isolates in regard to their virulence potential, growth, and antifungal susceptibility. Additionally, we investigated which host pressures are needed to adapt to, colonize and potentially infect the human host by performing an evolution experiment in sugar-rich medium.

By characterizing the environmental strains, we found one *C. albicans* oak tree isolate which exhibited a highly virulent phenotype, possibly evolved to counteract environmental threats such as environmental predators. Due to this so-called environmental virulence school, this environmental strain seems to have gained the ability to adhere, to invade, and to damage human host cells, which can most likely facilitate colonization and infection. Importantly, this strain was also intrinsically resistant to amphotericin B. Hence this environmental isolate could represent a new, highly virulent, and resistant *C. albicans* clade which can survive outside of the human host.

Additionally, we "forced" a less virulent, fluconazole-resistant *C. albicans* oak tree isolate with a micro-evolutionary approach to adapt to typical human dietary compounds, hence promoting its colonization ability in the human host. The evolution experiment yielded a host-adapted strain that shows a more flexible metabolism and increased virulence. Even more interestingly, this strain acquired a resistance to amphotericin B in addition to the increased fluconazole resistance. This could hint towards nutritional triggers in the western diet, such as sugars, that affect fungal virulence and can indirectly induce antifungal resistances during long-term evolution. Thus, carbon sources that are available within specific host niches may be crucial for *C. albicans* pathogenicity as well as drug susceptibility during colonization and infection.

018/EKV

Characterisation of *Giardia duodenalis*-induced barrier break down in organoid-derived duodenal monolayers

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Question: *Giardia duodenalis* is a major cause of gastrointestinal illness worldwide, but underlying pathophysiological mechanisms remain unclear. Current models to study pathogenicity mainly rely on cancer cell lines or on animal models. However, these models might not recapitulate the true series of events in human intestinal tissues. The aim of the presented study was to establish a reliable cell culture model based on primary human organoid-derived epithelia to study *Giardia duodenalis* pathogenicity that also mimics the complexity of intestinal epithelium.

Methods: We established a compartmentalized organoid-derived monolayers (ODMs) on a transwell system that mimics intestinal epithelium and its functions. Functional analysis of *Giardia duodenalis*-induced barrier breakdown in ODMs was performed at transcriptional and electrophysiological level, and in tight junction component function.

Results: Infection of ODMs with *Giardia duodenalis* induced a time- and parasite load-dependent breakdown of epithelial barrier function. Permeability after infection increased for medium and large-sized molecules as indicated by translocation of 332 Da fluorescein and 4 kDa FITC-dextran and loss of transepithelial electrical resistance (TEER). While we could exclude previously proposed parasite-dependent effects on pathways such as caspase-3-dependent apoptosis and rearrangements of the cellular skeleton by MLCK, analysis of differential gene expression indicated major transcriptomic changes in genes associated with ion transport and tight junction structure. Electrophysiological measurements showed reduced activity of solute carriers SLC12A2/NKCC1 and CFTR early after *Giardia duodenalis* infection. Changes in the organization, composition, localization and structure of key components of the tight junctional complex organization were confirmed by RT-qPCR, immunofluorescence, semi-quantitative western blotting and freeze fracture electron microscopy analysis.

Conclusions: Here, we show the establishment of an organoid-derived infection model suitable to study *Giardia duodenalis* infections *in vitro*. Using this model, a new series of events is suggested that ultimately leads to the loss of epithelial integrity. This model may help to further dissect and better understand the disease mechanisms in giardiasis.

019/EKV

The role of the complement system in a murine model of disseminated mucormycosis

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Introduction: Mucormycetes, a rather heterogeneous group of fungi, induce a life-threatening disease called mucormycosis. The prevalence of this disease, which shows high morbidity and mortality, increased within the last decade. Main risk factors for mucormycosis are immune deficiencies. An important link between innate and adaptive immunity is the complement system (C), which also provides several crucial functions in first-line defense against non-self-structures like fungi.
To enlighten the responsibility of C in the defense against mucormycosis, our objectives were, on the one hand, to compare the role of different parts of C in a murine model of disseminated mucormycosis for different species and on the other hand, to study the relevance of C for pathogenesis.
Material/Methods: Mice with a deficiency in complement C3 (DC3) or C6 (DC6) were intravenously infected with *Lichtheimia corymbifera* (LC), *Lichtheimia ramosa* (LR), *Rhizopus arrhizus* (RO), *Rhizopus microsporus* (RM), *Rhizomucor pusillus* (RmP) or *Mucor circinelloides* (M). Survival, clinical status, and immunological parameters were monitored over 14 days and compared to that of immunocompetent (wt) or neutropenic (DNeu) mice. Additionally, serum from healthy wt mice was analyzed for capacity to opsonize the fungi.
Results: When intravenously infected with M or RO, there is no difference between DC3, DC6, DNeu, and wt mice. C-deficiencies represent a risk factor for a lethal outcome in LC, LR, RM, and RmP. LC and RM lead to higher mortality in C-deficient mice, compared to DNeu. There is no significant difference between the lethality of DC3 and DC6 mice in intravenous infections with LC, M, RO, and RM. DC3 mice exhibited higher mortality than DC6 mice when infected with LR, whereas the opposite was the case in RmP infections.
Conclusion: Complement plays an important role in the murine model of disseminated mucormycosis. Mortality of the complement-deficient animals varies between the species. Further investigations have to be performed to fully understand the immunopathogenesis of mucormycosis and help to fight the high morbidity and mortality of this disease.

020/EKV

Identification of hit compounds with anti-schistosomal activity on *in vitro* generated juvenile worms in cell-free medium

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Introduction: Anthelminthic treatment options against schistosomiasis are limited. The current treatment relies almost exclusively on a single drug, praziquantel (PZQ). As a consequence, the development of resistance to PZQ and limited activity of PZQ against earlier development stages are respectively a risk and a limitation to achieving the goals of the new WHO roadmap towards elimination. For the discovery of new chemical starting points, the *in vitro* drug screening on *Schistosoma mansoni* against newly transformed schistosomula (NTS) is still the most predominant approach. The use of only NTS in the initial screening limits sensitivity to potential new compounds which are predominantly active in later developmental stages. Using our recently developed highly standardized, straightforward and reliable culture method that generates high rates of juvenile worms, we aimed to repurpose a subset of the NCATS Pharmaceutical Collection (340 compounds) to identify new hits using *in vitro* assay.
Methodology/Results: Cercariae were mechanically transformed into skin-stage (SkS) schistosomula and successfully cultured for up to four weeks to the liver stage (LiS). A commercial source of serum was identified, and decrease of NTS/well along with optimal drug testing conditions was established to test compounds on early and late LiS worms. The library was screened in 96-well format assays using PZQ as a positive control. Primary screening allowed a 5.9% hit rate and generated two confirmed hits on adult worms; a prophylactic antianginal agent and an antihistaminic drug.
Conclusion: With this robust and highly standardized *in vitro* assay, important developmental stages of *S. mansoni* up to LiS worms can be generated and maintained over a prolonged time. When exposed to a subset of the National Center for Advancing Translational Sciences (NCATS) Pharmaceutical Collection, 3 compounds yielded a defined anti-schistosomal phenotype on juvenile worms. Translation of activity on perfused adult *S. mansoni* worms was achieved only for Perhexiline (a prophylactic antianginal agent) and Astemizole (an antihistaminic drug).

Workshop 05
Molecular Pathogenesis of Zoonotic Infections (FG ZO)
13. Sep. 2021 • 10:00–11:00

021/ZOV

The A-subunit of Shiga toxin 2 of enterohemorrhagic *E. coli* intoxicates eukaryotic cells independent of its B-subunit

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Introduction: Shiga toxins (Stx) of enterohemorrhagic *E. coli* (EHEC) belong to the group of AB5 toxins. The A-subunit acts as rRNA-*N*-glycosidase, causing the depurination of the 28S rRNA in eukaryotic ribosomes, thereby blocking protein synthesis and leading to apoptosis of the target cells. The pentameric B-subunit is inevitably needed for receptor-mediated recognition of and uptake into the target cells. In a former study, we could show with another AB5toxin, the Subtilase cytotoxin, that the A-subunit alone was able to intoxicate eukaryotic epithelial cells *in vitro*.
Materials and Methods: In the current study, we investigated the cytotoxic effects of the Stx2A-subunit of enterohemorrhagic *E. coli* O157:H7 strain EDL933 with and without its corresponding B-subunits. Recombinant protein expression and purification strategies for the separate subunits were implemented. The identity of the respective subunit was proven by mass spectrometry and the biochemical characteristics assessed by circular dichroism (CD) spectroscopy and size exclusion chromatography (SEC). To evaluate the cytotoxicity, the Stx2A-subunit alone and in 1:5 molar combination with the B-subunit were applied to three different cell lines.
Results: His-tag affinity chromatography and gel filtration were used to purify StxA2-His and StxB2-His. Both subunits showed a single band in SDS-PAGE and were clearly identified with mass spectrometry. The secondary structure composition determined by far-UV CD spectroscopy matches the expectations based on the crystal structure for the Stx2 holotoxin. StxA2-His and StxB2-His showed one single species in SEC measurements each, indicating that both subunits showed stable oligomerization. Cytotoxicity assays with the cell lines Vero B4, HeLa, and HCT-116 of StxA2-His alone and in combination with StxB2-His showed that all cell lines are intoxicated independently of the presence of StxB2-His.
Discussion: The results of this study have shown that the A-subunit of Stx2 shows toxic activity to eukaryotic cells without its corresponding B-subunit. Further studies are needed to unravel the role of such a single A-effect in pathogenesis of EHEC-mediated diseases.

022/ZOV

Functional analysis of the effect of *Giardia duodenalis* on CRISPR/Cas9-modified human intestinal organoids

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Introduction: As a widespread protozoal parasite, *Giardia duodenalis* is a common cause for abdominal pain, malabsorption and diarrhea. Intestinal epithelial barrier defects are frequently observed in patients affected by the multifactorial disease giardiasis, which is characterized by "leaky" barriers due to the disturbance of the tight junction complex. While the exact mechanisms leading to epithelial barrier breakdown remain unknown, the proinflammatory cytokine TNF-alpha has been suggested to occupy a regulatory role during this host/parasite interaction. Thus, this project aims to elucidate the influence of TNF-alpha on barrier integrity during infection of human intestinal organoids with the parasite *G. duodenalis.*
Material/Method: Using the CRISPR/Cas9-technology, TNF-alpha knockout organoid cultures were generated and verified, followed by cultivation as organoid-derived monolayers (ODMs) in a compartmentalized transwell system. During infection of wildtype- and knockout-ODMs with *G. duodenalis*, changes in paracellular permeability were quantified via transepithelial electrical resistance (TEER) measurements, which serve as indicator of monolayer integrity.
Results: Due to the presumed regulatory role of TNF-alpha during this host/parasite interaction, it was hypothesized that the epithelial barrier of TNF-alpha knockout cultures remains intact during infection, indicated by consistent paracellular permeability and stable TEER-values. However, both wildtype- and knockout cultures showed comparable time- and MOI-dependent barrier defects upon infection. Consistent with these findings, the addition of recombinant human TNF-alpha to wildtype cultures did not compromise barrier function, but rather slightly strengthened TEER-values after 72 hours.
Discussion: Since the genetic depletion of TNF-alpha in human intestinal organoids could not prevent Giardia-induced TEER-loss after infection, we concluded that the multifunctional cytokine most likely is not involved in the regulation of epithelial barrier breakdown. Instead, the data even hint towards a barrier-protective role of TNF-alpha in intestinal epithelial cells.

023/ZOV

Analysing the fibronectin binding properties and genomic variation of *Bartonella henselae* adhesin A

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Introduction: *Bartonella henselae* infections can result in cat scratch disease, endocarditis, and vasculoproliferative disorders (e.g. bacillary angiomatosis). *Bartonella* adhesin A (BadA), an important virulence factor, is a trimeric autotransporter adhesin (TAA) and mediates bacterial adhesion to human endothelial host cells (EC) or extracellular matrix (ECM) proteins (e.g. fibronectin). Fibronectin is a high molecular weight glycoprotein facilitating the initial bacterial adhesion to host cells presumably via bridging to α5β1-integrins. The overall objective is to identify possible binding site(s) of BadA to fibronectin and, subsequently, to model and produce peptides (anti-ligands) that inhibit bacterial adhesion to ECs and ECM proteins.
Methods: Identification of potential fibronectin binding site(s) was performed via the construction and expression of truncated and modified BadA fusion proteins in a *B. henselae* BadA-knockout mutant. Affinity assays were carried out via ELISA and fluorescence microscopy. Furthermore, BadA-gene sequences of eight *B. henselae* strains were analysed using long-read PacBio SMRT sequencing, and BadA-expression was verified via electron microscopy, Western blotting, and immunofluorescence assays.
Results: ELISA fibronectin affinity assays using truncated BadA fusion proteins have shown the significance of the BadA length and the importance of certain domain(s) within BadA. Furthermore, anti-BadA antibodies were developed, and a clean BadA-deletion *B. henselae* mutant was constructed via Gibson cloning. Additionally, the genomic BadA sequences of various *B. henselae* strains were determined. BadA, derived from *B. henselae* Marseille, has even a higher molecular weight (3,972 amino acids) than previously demonstrated (3,082 amino acids).
Conclusion: The variable and repetitive nature of the BadA-genomic region might indicate frequent genome rearrangements which could contribute to differences in virulence. Previous research demonstrated the particular interaction between BadA and fibronectin in the initial attachment of *B. henselae* to human ECs and ECM proteins. Bacterial anti-ligands could function as a new class of antibiotics, for which an exact domain analysis of TAAs is decisive. These results represent a stable basis for upcoming methods to verify fibronectin-binding BadA-domains.

024/ZOV

Molecular modulation of Zur-regulated zinc deprivation response in mycobacteria

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Introduction: Zinc availability and maintenance of homeostasis in bacteria is essential for many biological processes and thus survival. Maintenance is achieved by the expression of specific systems, e.g. alternative ribosomal proteins (ARPs) and transporters. Expression of these systems is repressed by the global **z**inc **u**ptake **r**egulator Zur, when zinc is available. For *B. subtilis* (BS) a graded expression of zinc deficiency response genes regulated by Zur was reported[1]. Upon zinc starvation, ARPs expression was induced first, followed by zinc importers, and finally genes for ribosome assembly and folate synthesis. Graded expression is a consequence of decreasing zinc concentrations, which cause a partial or full loss of binding of the repressor, when Zur loses one or both of two functional zinc ions. This leads to partial or full expression of regulated genes. *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is the causative agent of Johne's disease, a chronical, fatal inflammation of the small intestine in ruminants. Upon zinc starvation MAP induces expression of ARPs and zinc transporters[2]. While many bacteria possess only one zinc importer, MAP possesses three uptake systems: a homologue of the common ZnuABC and additionally MptABC and the ZnuABC-like MAP3776-74[3]. The nonpathogenic, fast growing model organism *M. smegmatis* (MSMEG) is equipped with two zinc transporters ZnuABC1, ZnuABC2 and the porin MspD[4]. In this study we investigated time and concentration dependent expression pattern of MAP and MSMEG zinc deficiency systems and the impact of Zur on the expression of *map*3776-74 and *znu*ABC^{MAP}.
Methods: MAP and MSMEG were grown in MB and exposed to different concentrations of the zinc chelator TPEN or 10 µM TPEN for different time periods. Expression of *znu*ABC, *mpt*ABC, *map*3776-74 and the ARP *rpm*E2 in MAP or *znu*ABC1, *znu*ABC2, *msp*D and *rpm*G in MSMEG was analysed by qRT-PCR. Further promoters activity of *map*3776-74 and *znu*ABC^{MAP} promoters, fused to *lac*Z, was analysed in MSMEGwt and MSMEGΔ*zur* by β-galactosidase assay.
Results: Expression of all tested genes was time and concentration dependent. In MAP ARP *rpm*E2 was expressed first, followed by *mpt*ABC, *map*3776-74 and *znu*ABC. In MSMEG *znu*ABC1 was expressed earlier than *znu*ABC2, *rpm*G and *msp*D. The β-galactosidase assay clearly revealed a zinc and Zur dependent regulation of *znu*ABC^{MAP}. For *map*3776-74 a zinc dependent but Zur independent regulation was detected.
Discussion: Our data clearly indicate a time and concentration dependent induction of zinc deficiency responsive systems in MAP and MSMEG, most likely due to a graded Zur inactivation mechanism similar to BS. The order of induction in MAP was also similar to BS, however, in MSMEG ZnuABC1 seems to be predominant. Promoter analyses of *map*3776-74 indicate that MAP might have additional regulatory mechanisms for zinc uptake systems.

[1] doi:10.1038/ncomms12612
[2] doi:10.1186/1471-2164-15-1076
[3] doi:10.1128/JB.00049-21
[4] doi:10.1128/mSystems.00880-19

025/ZOV

Preclinical Evaluation of Oral Urolithin-A for the Treatment of Acute Campylobacteriosis in *Campylobacter jejuni* Infected Microbiota-Depleted IL-10$^{-/-}$ Mice

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Question: Human campylobacteriosis represents an infectious enteritis syndrome caused by *Campylobacter* species, mostly *Campylobacter jejuni*. Given that *C. jejuni* infections are rising worldwide and antibiotic treatment is usually not indicated, novel treatment options for campylobacteriosis are needed. Urolithin-A constitutes a metabolite produced by the human gut microbiota from ellagitannins and ellagic acids in berries and nuts, which have been known for their health-beneficial including anti-inflammatory effects since centuries.
Methods: We investigated potential pathogen lowering and immunomodulatory effects following oral application of synthetic urolithin-A during acute campylobacteriosis applying perorally *C. jejuni* infected, microbiota-depleted IL-10$^{-/-}$ mice as preclinical inflammation model. Treatment of mice with urolithin-A in drinking water was performed with a daily dose of 0.114 mg urolithin-A per kg body weight per day. Mice received access to the urolithin-A solution from two days after the first *C. jejuni* infection. The placebo control mice received autoclaved tap water instead.
Results: On day 6 post infection, urolithin-A treated mice harbored slightly lower pathogen loads in their ileum, but not colon as compared to placebo counterparts. Importantly, urolithin-A treatment resulted in an improved clinical outcome and less pronounced macroscopic and microscopic inflammatory sequelae of infection that were paralleled by less pronounced intestinal pro-inflammatory immune responses which could even be observed systemically.
Conclusions: This preclinical murine intervention study provides first evidence that oral urolithin-A application is a promising treatment option for acute *C. jejuni* infection and paves the way for future clinical studies in human campylobacteriosis.

026/ZOV

Host association of *Campylobacter coli* in Germany based on whole genome data

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Introduction: During the last two decades the zoonotic pathogen *Campylobacter* has become the main bacterial cause for food-borne infections in developed countries. Previous research was commonly focused on *C. jejuni*, since the species is associated with 85% of the recorded illnesses. However, a substantial number of campylobacteriosis cases of around 15% is currently caused by *C. coli*. While both *Campylobacter* species are common among the commensal gastrointestinal microbiota of the same host, their actual prevalence varies, and little is known about host-specific signatures in *C. coli* yet. Here, putative host associations of *C. coli* were analyzed and identified applying a genome-wide association study (GWAS) on whole genomes sequences of a representative study set.
Material and Methods: A stratified uniform random collection comprising 500 *C. coli* isolates from different sources across Germany, including 100 isolates from samples of human, chicken, duck, cattle and pig origin, were selected, and whole genome sequencing (WGS) was performed. Host-specificity was investigated by bootstrapping on top of a *k-mer* based GWAS to increase the accuracy of the identification of host specific determinates.
Results: Overall, the phylogeny of the *C. coli* WGS data was calculated based on the core genome comprising 1,255 genes, revealing a star-like population structure. In addition, phylogenetic distance calculation based on single nucleotide differences revealed a barrier of approximately 9,699 SNPs between genomes representing isolates of poultry (chicken, duck) and livestock (pig and cattle) origin. Bayesian analysis of the *C. coli* population structure revealed at least 14 distinct clusters probably reflecting several host-associated lineages. Of note, sequence type (ST) 827, associated with isolates of cattle origin, revealed a close relationship to a chicken-associated cluster, probably indicating a recent host jump. Clinical isolates of human origin clustered in distinct *C. coli* lineages, putatively indicating an increased pathogenicity associated with these phylogenetic backgrounds.
Conclusion: This study was designed to enhance the bioinformatic toolbox available for reliable and fast outbreak investigations facing the ongoing threat of *C. coli* infections in humans. Moreover, research on how pathogens, including *C. coli*, adapt to novel host niches and environments must include whole pan-genome analysis, since our recent research on *C. jejuni* indicated that the adaptation towards a specific host niche is most likely a long evolutionary and multifactorial process rather than the result of acquisition of single metabolic traits.

Workshop 06
Food Microbiology (FG LM)
13. Sep. 2021 • 13:15–14:15

027/LMV

Shiga toxin-producing *Escherichia coli* in flour – A german perspective

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Shiga toxin-producing *Escherichia coli* (STEC) can cause severe human infections which are often linked to food contamination. Human cases are frequently associated with contaminated beef and dairy products but recent outbreaks also highlight non-animal food as infection source. In the United States and Canada flour and undercooked or raw dough was identified as possible infection vehicle. In Germany, respective cases linked to flour or dough are not known so far; however, German flour was also found to be contaminated with STEC up to 39 % (PCR-positive) but isolates were only gained from 19 % of the samples. In our study, we investigated 123 STEC isolates from flour and products thereof, which were collected between 2015 and 2019 in Germany by Federal food inspection laboratories during standard food processing controls. Besides standard routine examination of serotype and shigatoxin subtype, isolates from 2018 were applied to whole genome sequencing (WGS) for phylogenetic analyses using (core genome) multi-locus sequence typing ((cg)MLST) and determination of virulence-associated genes (VAGs). Isolates were derived from wheat flour (61 %), rye flour (24 %), spelt flour (5 %) and other products thereof including ready-mixes (11 %). Overall, we found a high diversity of STEC

strains. Serotyping revealed 27 different serotypes including O157:H7, O145:H28, O146:H28 and O103:H2; however, otherwise rare serotypes like O187:H28 and O154:H31 were most prevalent. Comparison of serotype data from flour and human samples in Germany between 2015 and 2019 showed a high overlap. Flour and human isolates shared 14 distinct serotypes including high prevalent human serotypes O103:H2, O145:H21, O146:H21, O157:H7 and O8:H19. Furthermore, WGS revealed that strains of serotypes O157:H7 (ST11, *stx2c)*, O103:H2 (ST14, *stx1a*) as well as of O156:H25 (ST300, *stx1a*) harbour high numbers of VAGs. Among those were genes like *eae* and *nleB* as well as *est1a/sta1* indicating also strains as enterotoxic hybrid strains. Although contaminated flour products are yet not linked to human cases in Germany, we showed that flour can contain STEC strains with high pathogenic potential for human illnesses. Furthermore, investigation also on rare serotypes is needed to determine contamination sources of flour and products thereof.

028/LMV

Salmonella in Tahini: Occurrence and conservation

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Background

Tahini, a paste made from grounded sesame seeds is usually consumed without further inactivation steps in ready-to-eat products like hummus and sauces. In the past, it was associated with several *Salmonella* outbreaks caused by various serovars. Tahini is still of concern when investigating potential reservoirs of pathogens in low-moisture and high-fat foods. As occurrence and survival of *Salmonella* in Tahini play a crucial role considering the long shelf life of this product, data especially in Germany are still rare.

Material/Methods: To examine the microbiological status of Tahini in Germany, five different Tahini brands were tested for their total aerobic plate count (37°C, 24h) and the occurrence of *Salmonella* and pathogenic *E. coli* like enteropathogenic (EPEC) and Shigatoxin-producing *E. coli* (STEC). The survivability of pathogens in Tahini was investigated using two *Salmonella* strains originally isolated from Tahini (*S.* Havana and *S.* Senftenberg). The *S.* Havana has been linked to an outbreak associated with the consumption of Tahini. Samples of two different Tahini brands were spiked with inoculated sand (at 1.1-3.6×10^5 CFU/g, *S.* Havana, *S.* Senftenberg and EPEC) and analysed for their cell counts over time. All samples (incl. controls) were stored at 4°C for up to twelve weeks.

Results: The microbiological status of the Tahini products has been investigated for inoculation with the test organisms. The total aerobic plate count ranged from 2.8×10^1 to 1.4×10^3 CFU/g (av. 8.9×10^2 CFU/g). EPEC or STEC could not be isolated from all five Tahini. One Tahini sample harboured a *Salmonella* Tennessee strain.

After twelve weeks at 4°C the number of *Salmonella* in spiked Tahini samples were still comparable to the inoculum (2.8×10^4 CFU/g for *S.* Havana and 3.2×10^4 CFU/g for *S.* Senftenberg) while the numbers of *Salmonella* in spiked sand samples decreased (<200 CFU/g for both strains). In comparison, the EPEC strain showed a less persistent behaviour in both Tahini and spiked sand samples (<200 CFU/g). Here, the CFU dropped below LOQ within twelve weeks.

Discussion: Our results suggest a conservation of *Salmonella* within the matrix Tahini for a prolonged time, despite its low water activity and high fat content. This underlines the importance of hygienic practices, especially for the production of foods with a long shelf life consumed without further inactivation steps. Studies on contamination sources and characterizations of strains that occur in Tahini could help to improve the safety of tahini.

029/LMV

Growth behaviour of _Cronobacter_ spp in herbal infusions

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Introduction: Tea and herbal infusions are natural, non-sterile plant products consumed throughout the entire population and might contain pathogens. Due to an ubiquitous presence, resistance against dry stress and its ability to form biofilms, *Cronobacter sakazakii* and *Cronobacter* spp. are relevant foodborne pathogens, especially in dry food. *Cronobacter sakazakii* causes septicaemia, necrotizing enterocolitis and/or meningitis, especially in infants.

The present study focussed on the characterization of *Cronobacter* spp in commercially available infant teas with special emphasis on the survival and growth rates.

Material/Methods: The tenacity of *Cronobacter* spp strains in artificially contaminated tea (10E6-10E7 CFU) was investigated after brewing (at 24 °C, 60 °C, 80 °C and 100 °C) in various infusions at a volume of 200 mL, simulating different "at-home" brewing conditions.

Furthermore, 13 different *Cronobacter* spp. were cultivated in brewed herbal infusions (fennel bag and instant infusion and chamomile bag infusion) in 96 well plates over 20 hours followed by biofilm assays to asses variability in growth and attachment to plastic surfaces.

Results: The results of all experiments showed relevant differences of surviving different brewing temperatures and the growth in different infusions between all *Cronobacter* strains.

Depending on the brewing temperature, the chamomile bag infusion seems to have an inhibitory effect on *Cronobacter* spp., whilst both, bag and instant fennel infusions did not visibly inhibit the growth of *Cronobacter*.

The results showed a low chance of *Cronobacter* surviving brewing temperatures over 60 °C. However, the results of the *Cronobacter* growth curves showed, after a storage at room temperature, that in the biggest part of infusion-strain-combinations, a growth was detected between 10 and 15 hours of incubation. Whilst in some infusion-tea-combinations, no growth could be observed.

Conclusion: The results of the growth curves and behaviour especially lag time may help to evaluate the risk of preparation matrix of herbal infusions at consumer side as well as the effect of storage time of the reconstituted product on bacteria content.

The risk of *Cronobacter* surviving in herbal infusions may be very low after appropriate preparation and immediate consumption or storage at low temperatures. Nevertheless, if some cells may survive and the infusions are stored over some hours at room temperature, there may be a risk of *Cronobacter* growing in it, depending on the strain and sort of infusion.

030/LMV

That´s bitter: Investigating the impact of calcium on the proteolytic system of _Lactococcus lactis_ starter culture isolates

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Introduction: The fermentation of protein-rich dairy substrates yields products which exhibit bitter taste. It is hypothesized that the proteolysis of casein by *Lactococcus lactis* strains applied as starter cultures leads to the formation of bitter peptides. As this is not the case in products with regular protein contents, the increased calcium content was considered as a possible factor. The proteolytic system consists of the cell envelope peptidase PrtP, which cleaves casein, several transport systems for the uptake of di-, tri-, and oligopeptides, and intracellular peptidases. These are hypothesized to be repressed in their expression by the global transcriptional regulator CodY.

Materials and Methods: The genomic and plasmid DNA of two *Lactococcus lactis* subsp. *lactis* and *cremoris* starter culture isolates was isolated and sequenced using Illumina sequencing. The assembled genomes were compared to each other and to a set of reference genomes of the respective subspecies. The presence of genes encoding transport systems and enzymes of the proteolytic system was confirmed. Cells of one *prtP*-positive strain per subspecies were grown in media containing the same casein content under two conditions, either regular or double calcium content. Biomass was harvested for RNA preparation in the late exponential phase. Transcriptomic analysis *via* RNA-Seq was performed in biological triplicates.
Results: *L. lactis* ssp. *lactis* strains were genetically closely related to each other as well as the reference genomes, while *L. lactis* ssp. *cremoris* strains were not. For the two *prtP*-positive strains the individual genes encoding transport systems and enzymes of the proteolytic system as well as *codY* were not significantly regulated under high calcium conditions in comparison to regular calcium contents. However, gene set enrichment analysis revealed an upregulation of the genes of the proteolytic system in the *L. lactis* ssp. *cremoris* strain, while the respective gene set in the *L. lactis* ssp. *lactis* strain was not found to be significantly enriched. Here, in particular the peptide transporter genes were specifically enriched.
Discussion: Our results indicate that closely related strains react to changing environmental conditions in an individual manner and that the impact on the enzymatic level might outweigh that on the transcriptomic level. These findings stress the importance of an in-depth evaluation of selected strains for the application of starter cultures.

Workshop 07
Bacterial and Fungal Pathogenesis (FG MP/FG EK)
13. Sep. 2021 • 15:45–16:45

031/EKV
Dissecting the mechanisms causing intestinal damage and translocation by *Candida albicans*

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The opportunistic fungal pathogen *Candida albicans* thrives on human mucosal surfaces as a harmless commensal. Translocation across the gut barrier into the bloodstream by intestinal-colonizing *C. albicans* cells is thought to be the main source of disseminated candidiasis; however, the mechanisms behind this process remain unclear.
An *in vitro* model of intestinal epithelial cells grown in a transwell system was used to determine the association of fungal translocation with epithelial damage. The *C. albicans* peptide toxin, candidalysin, was essential for damage of enterocytes and subsequent fungal translocation[1].
To shed more light on intestinal epithelial infection, we performed dual-species RNA sequencing to identify differentially expressed genes (DEGs) of *C. albicans* and the host. The host response was mainly limited to late time points of infection, during which invasive fungal growth occurred. It included a fungal filamentation- and damage-driven up-regulation of FOS transcription factor and IL-8 gene expression.
The fungal response was dominated by a general hyphal transcriptional program. Early adhesion and invasion led to altered expression of cell wall-related genes, among them the fungal cell wall-remodeling chitinase-encoding gene *CHT2*. Deletion of the *CHT2* gene resulted in an increased damage potential of *C. albicans*, suggesting that Cht2 may prevent damage and promote fungal commensalism. DEGs during later invasion and damage were involved in filamentation regulation and fungal metabolism. Fungal zinc acquisition and storage genes, which would be induced upon zinc starvation, did not increase during infection, suggesting that *C. albicans* acquires this trace metal from the host. Correspondingly, deletion of zinc-related genes lowered host-cell damage during intestinal epithelial infection. Ongoing research aims to characterize how fungal metabolism and nutrient conditions impact epithelial damage.
In conclusion, our data indicate that in addition to filamentation and production of candidalysin, nutrient acquisition and surface properties impact *C. albicans* induced epithelial damage, translocation, and epithelial responses.

[1] Allert S *et al.* (2018) *Candida albicans*-induced epithelial damage mediates translocation through intestinal barriers. *mBio* 9(3), e00915.

032/EKV
The high osmolarity glycerol (HOG) pathway in *Aspergillus fumigatus*: a key player in stress resistance and a potential drug target

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The high osmolarity glycerol (HOG) pathway in *A. fumigatus* is an important regulatory system that enables the fungus to resist external stress conditions, e.g. osmotic shock or oxidative stress. In the model organism *Saccharomyces cerevisiae* the hybrid histidine kinase (HHK) Sln1p is the major sensor protein of the HOG pathway. In filamentous fungi however, the initial sensors of this pathway are so-called group III HHKs. In *A. fumigatus,* the corresponding HHK is TcsC which is part of a phospho relay system consisting of the phosphotransfer protein Ypd1 and the two response regulators Skn7 and SskA. Skn7 is a transcription factor, which represents the alternative branch of the HOG pathway whereas SskA initiates the classical part of the HOG pathway, which finally terminates in the activation of the Hog1 ortholog SakA. This pathway can either be activated by environmental stressors, or by pharmacological compounds like fludioxonil, which is approved for agricultural use to protect crops from fungal infection. Since resistances against fludioxonil are rarely observed, the HOG pathway appears to be a promising target for antifungal treatment. In *A. fumigatus* this substance induces a rapid growth arrest, a reorganization of the cell wall and a dramatic swelling of the cells which finally results in lysis and cell death.
For a more detailed understanding of the HOG pathway we used deletion mutants in *sskA*, *skn7*, *sakA* and *tcsC*. The application of fludioxonil leads to a massive increase of the intracellular glycerin concentration in wild type *A. fumigatus*. Although this effect is strongly reduced in a SakA deficient strain, this mutant is still sensitive to fludioxonil and shows the typical swelling of the cells. In absence of the transcription factor Skn7 a partial resistance could be observed whereas a double knockout of *skn7* and *sakA* leads to a completely resistant phenotype. Our data furthermore suggest that Skn7 mediates the fludioxonil-induced cell wall reorganizations. This leads to a reduced rigidity, that in combination with an increased intracellular pressure triggers the antifungal effect of fludioxonil and mediates the lethal expansion of the fungal cells.
To further investigate the impact of fludioxonil, we are currently analyzing the role of the phosphotransfer protein Ypd1 in the antifungal activity of fludioxonil and its interaction with the response regulators Skn7 and SskA.

033/MPV
CipA of *Acinetobacter baumannii* inhibits the alternative pathway by interacting with multiple complement components

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Question: *Acinetobacter baumannii* belongs to the group of ESKAPE pathogens known to be the leading causative agent of severe nosocomial infections worldwide. To establish an infection, *A. baumannii* developed a range of strategies to successfully

overcome eradication by the human host as well as recognition by adaptive and innate immune system. Powerful strategies established by this multidrug resistant pathogen involve biofilm formation, resistance to antibiotics, colonization and invasion of human cells as well as resistance to complement. Previously, we identified CipA as a novel multifunctional, pathogenicity factor of *A. baumannii* that inhibited the alternative pathway by recruitment of activated plasminogen and by interacting with C3 and C5.
In the present study, we set out to elucidate the molecular principles of the interaction of CipA with different complement components leading to serum resistance of *A. baumannii*.
Methods: CipA was tested for its ability to bind components C3, C3b, C5, Factor B, Factor H, and Factor I as well as the C3 proconvertase of the alternative pathway (AP). To assess the impact of CipA on complement, hemolytic and ELISA-based activation assays have been investigated. To identify the domains responsible for binding, a number of CipA variants as well as a CipA knockout strain were generated and used for further analyses.
Results: By employing complement activation assay, CipA inhibited the activation of the AP and the classical pathway in a dose-dependent fashion, while no impact on the lectin pathway or the formation of the MAC could be observed. Assuming that CipA preferentially interact with the AP, binding of C3, C3b, C5, Factor B, Factor I, and Factor H was assessed. With the exception of Factor B and Factor H, CipA bound to all other components in a dose-dependent fashion. A calculated IC50 value of 14,5 µM revealed a strong inhibitory activity of CipA on the AP. Utilization of diverse CipA variants showed that the bacterial protein contains at least two separate binding sites for C3b while the Factor I-interacting domain could be localized to the C-terminus of CipA suggesting that both complement components could simultaneously be bound by CipA. In addition, the interaction of C3b with CipA appears to be primarily mediated by the TED domain. Moreover, CipA did not impacted the formation of the C3 convertase of the AP. In the absence of CipA, the *A. baumannii* knockout strain did not bind serum-derived Factor I indicating that CipA is the key determinant for the recruitment of this particular complement component.
Conclusion: By functional characterization of the CipA-C3b/Factor I interaction, here we show that CipA of *A. baumannii* inhibits activation of the AP by binding to the TED domain of C3b, and via its C-terminus to factor I.

034/MPV

Development of an advanced multi-cell *in vitro* model of the meningeal blood-CSF barrier to study *Neisseria meningitidis* infection

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Introduction: *Neisseria meningitidis* (*Nm*) is a human-specific pathogen that can gain access to the central nervous system (CNS) by crossing the meningeal blood-cerebrospinal fluid barrier (mBCSFB), and cause meningitis. The human-specific nature of Nm has presented unique challenges in modeling this complex host-pathogen interaction. Mechanisms that govern *Nm* penetration of the mBCSFB and subsequent interactions with other cell types such as leptomeningeal cells (LMCs) have remained largely unknown. Here we sought to develop a multi-cell mBCSFB model that is entirely human based to examine *Nm* interaction *in vitro*.
Methods: Human brain endothelial-like cells (BEC) derived from induced pluripotent stem cells (iPSC), commercially available hCMEC/D3 BECs, and LMCs derived from tumor biopsies were used in model-development. Transmission electron microscopy (TEM), confocal and super-resolution microscopy methods were utilized for model characterization and localization of Nm in the human mBCSFB *in vitro* model. Gentamicin protection and transmigration assays were conducted to estimate *Nm* adherence, invasion, and barrier penetration. Barrier integrity was evaluated utilizing sodium fluorescein permeability assays and impedance measurements. qPCR was used to determine the cellular immune response to infection.
Results: We observed characteristic expression of cellular markers, including tight junction components such as ZO-1 and Occludin, in the co-culture set-up. We detected modest amounts of *Nm* adherence and relatively little invasion. We were able to visualize the infection models and resolve cellular structures via TEM and fluorescence imaging. We observed an increase in endothelial barrier integrity upon addition of the LMCs, and preliminary results suggest barrier deterioration upon prolonged bacterial challenge, accompanied by an increase in *Nm* transmigration.
Discussion: Our work highlights the usefulness of advanced multi-cell *in vitro* models that more accurately mimic the meningeal microenvironment for the study of *Nm* interaction at the human mBCSFB. Potential future applications include study of other pathogens that interact with the barrier and cause meningitis such as *S. pneumoniae or S. agalactiae*, as well as pharmacological studies. Further advancement of the model can be achieved by adding other relevant cell types such as immune cells or introducing more physiological parameters such as shear stress.

035/MPV

Licensed to kill: T6SS effectors of *Pseudomonas aeruginosa* modulate infection dynamics and immune response in *Galleria mellonella* larvae

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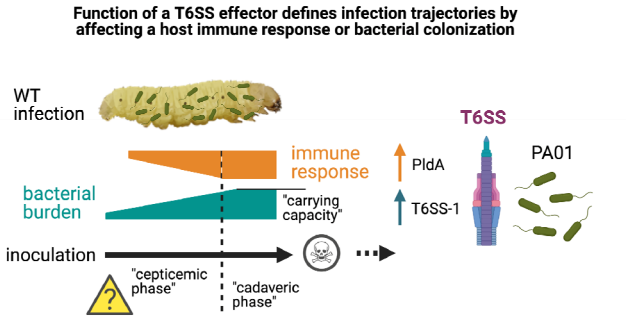
Introduction: *Pseudomonas aeruginosa* is an opportunistic human pathogen capable of forming biofilms and readily colonizing wounds, burns, and lungs of patients with cystic fibrosis. *P. aeruginosa* possesses many virulence factors including exotoxins, redox-active pigments, and specialized secretion systems. The type 6 secretion systems (T6SSs) of *P. aeruginosa* comprise a spear-like multi-component injection apparatus facilitating contact-dependent killing and nutrient acquisition. T6SSs enable the delivery of multiple effectors (toxins) with anti-bacterial and with anti-eukaryotic properties to a neighbouring "victim" cell. Previous studies highlighted an important role of T6SSs in inter- and intraspecific bacterial competition. Moreover, it was shown that certain T6SS effectors contribute to *P. aeruginosa* virulence. However, the mechanisms behind T6SS-mediated pathogenesis *in vivo* are very poorly understood.
Objective: We aimed to investigate the role of T6SS effectors in an acute *P. aeruginosa* infection of an insect host.
Methods: We utilized the *Galleria mellonella* larva infection model. Survival of the larvae systemically infected with either *P. aeruginosa* WT, or with one of the isogenic strains lacking the T6SS effector PldA (D*pldA*) or an entire T6SS-1 cluster (DT6SS-1) was monitored. Bacterial colonization levels were assessed at various time points post-infection (p.i.) as well as post-mortem (p.m.) by plating larval homogenates on a selective medium. Expression levels of T6SS-related genes were measured by qPCR. Innate immune responses in *G. mellonella* were evaluated by qPCR, and apoptosis of larval hemocytes was assessed by flow cytometry.
Results: We could confirm Hcp1 (the structural component of T6SS-1) production in the larva host by using immunohistochemistry and by qPCR. *G. mellonella* larvae infected with *P. aeruginosa* D*pldA* and with DT6SS-1 strains displayed a significantly prolonged median survival times (+1.75H and 0.75H, respectively), but significantly higher bacterial burdens p.m., when compared to WT-infected animals. We also detected T6SS-dependent upregulation of host genes encoding the anti-microbial peptides, hemolin and gloverin, at 11 hours p.i. Notably, infection dynamics was reversed at the earlier time points of infection: the larvae infected with *P. aeruginosa* D*pldA* and DT6SS-1 strains harboured significantly less *Pseudomonas* at 5, 7, and 9 hours p.i.

Every *P. aeruginosa* strain was characterized by a unique, distinct infection trajectory (CFUs vs. time p.i.). However, the differences in trajectories between the tested strains were abrogated when a high inoculum size was used (16000 *P. aeruginosa* cells per larva, corresponds to bacterial burdens at 7 p.i.).
Conclusion: Collectively, our data indicate that T6SS effectors are potent virulence factors of *P. aeruginosa* that promote host colonization by a pathogen, trigger innate immune responses, modulate infection dynamics, and affect survival of an insect host.

Fig. 1



036/MPV
Lipid trafficking at membrane contact sites during mycobacteria infectio
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Question: Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. The high lipid content of this pathogen accounts for many of its clinical manifestations. Using *Dictyostelium* as surrogate macrophage and *M. marinum* as pathogen, we found that mycobacteria mobilize host lipid droplets (LDs) to scavenge fatty acids. Here, we hypothesize that mycobacteria not only hijack LDs, but also components of the lipid trafficking machinery (LTM).
Methods: We have launched a first systematic effort to unravel the mechanisms by which mycobacteria acquire host lipids. Combining mass spectrometry-based lipidomics with the application of functionalized lipids and advanced imaging, we will map lipid flows between mycobacteria and their host at the subcellular and ultrastructural level. Additionally, we screen host lipid transporters for pathogen-induced alterations in localization and analyse their role in infection.
Results: A recurrent strategy is the induction of membrane contact sites (MCS) between host organelles and bacteria-containing vacuoles (BCV) to create focal points for lipid exchange. We found that mycobacteria selectively recruit components of the host LTM to the BCV: Oxysterol binding protein 8 is located in the cytosol, at the Golgi and the perinuclear ER and is recruited to ER-BCV-MCS during infection. OSBP8 is not mobilized by bacteria that lack the T7SS ESX1, indicating that bacterial effector proteins are involved in recruiting OSBP8 or alternatively, impact on the membrane composition of the BCV to recruit OSBP8 indirectly. Many lipid transfer proteins are recruited to MCS via interaction with phosphoinositides. We have found that the BCV accumulates PI4P and postulate that OSBP8 mediates sterol/PI4P counter transport at ER-Golgi-MCS analogous to yeast Osh4. Consequently, during mycobacteria infection OSBP8 may deliver sterols from the ER to the BCV in exchange for PI4P providing the bacteria with sterols. Interestingly, mycobacteria growth is accelerated in OSBP8- cells in which the distribution of the sterols and PI4P is completely disrupted. We hypothesize that the block in ER-BCV-lipid transport is compensated by an accumulation of sterols in endosomes that finally fuse with BCV.
Conclusion: By disclosing how mycobacteria exploit the host LTM at the molecular level, these efforts may lead to new starting points for anti-Tb therapies and will provide a basis for elucidating other aspects of the persistent lifestyle of mycobacteria.

Workshop 08
Infection Control of MDRO and other Nosocomial Pathogens (StAG HY/FG PR)
13. Sep. 2021 • 15:45–16:45

037/HYPRV
One year of routine screening for linezolid resistance in enterococci in a German tertiary care hospital
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Introduction: In 2018, the German Commission for Hospital Hygiene and Infection Prevention (KRINKO) recommended hygiene procedures to prevent infections and spread of enterococci with special antibiotic resistances, i. e linezolid resistance. While screening for vancomycin-resistant enterococci (VRE) is well established in many hospitals, screening for linezolid-resistant enterococci (LRE) is not available. In 2019, we established a screening procedure of rectal swabs for LRE based on broth enrichment and consecutive plating. Starting January 1st 2020, the routine VRE screening programme was supplemented by the LRE component.
Material and Methods: The LRE screening procedure comprised an enrichment culture of rectal swabs in enterococcosel broth with linezolid and subsequent plating of positive cultures on enterococcosel agar with linezolid. Linezolid resistance was verified by the VITEK® 2 system (bioMérieux) and gradient agar dilution. Whole genome sequencing (WGS) was applied to all linezolid resistant isolates in 2020 (NextSeq™ 500 system (Illumina)). The sequences were analysed by core genome multilocus sequence typing (cgMLST) using the Ridom SeqSphere+ software. For detection of 23S rRNA mutations as well as the optrA, cfr, cfr(B) and poxtA genes the LRE-finder of the Center for Genomic Epidemiology, Lyngby, Denmark, was used.
Results: A total of 10,557 rectal swabs of 3,714 patients were screened in 2020. Linezolid resistant enterococci (80 LRE and 4 LVRE) were detected from 78 patients resulting in a prevalence of 2.1% in this cohort under screening. The MIC values ranged from 6 to 64 µg/ml. 23S rRNA mutations were identified in 30 isolates. poxtA was detected in 38 isolates and optrA in 19. No cfr genes were identified. The 65 *E. faecium* isolates belonged to 27 sequence types (ST; MLST) and 38 complex types (CT; cgMLST). CT3122 was the most frequent CT (n=15) comprising ST78 isolates. The 19 *E. faecalis* isolates belonged to 13 STs and 18 CTs.
Discussion: The prevalence of linezolid resistant enterococci in the VRE screening programme was appr. 2%. Linezolid resistance mostly occurred in *E. faecium*, LVREs were rare. A pitfall of our current procedure is the long time to result.

038/HYPRV
Hospital-acquired infections due to enterococci in Europe: A systematic review and meta-analysis
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Background: Hospital-acquired infections (HAIs) caused by *Enterococcus spp*., especially vancomycin-resistant *Enterococcus spp*. (VRE), are of concern in Europe due to rising resistance proportions and limited treatment options. This study summarizes recent data on the incidence, mortality, and vancomycin resistance proportion of all HAIs and hospital-acquired bloodstream infections (HA-BSI) caused by *Enterococcus spp*. in Europe.

Methods: A systematic literature search in MEDLINE and EMBASE for articles published between 01/2010 and 2/2020 was conducted according to a protocol published a priori in the Prospective Register for Systematic Reviews (PROSPERO 2020 CRD42020166863). Statistical calculations with random-effects meta-analyses were performed to obtain pooled estimates.
Results: Of the 6,069 studies identified, 75 studies were included in this study. *Enterococcus spp.* and vancomycin-resistant *Enterococcus spp.* accounted for 10.9% (95%CI 8.7-13.4%, individual study range: 6.1-17.5%) and 1.1% (95%CI 0.21-2.7%, range: 0.39-2.0%) of all pathogens isolated from patients with HAIs, respectively. Hospital wide, the pooled incidence of HAIs caused by *Enterococcus spp.* ranged between 0.7 and 24.8 cases per 1000 patients (pooled estimate: 6.9 [95%CI 0.76-19.0]). The hospital-wide pooled incidence for HA-BSI ranged between 0.18 and 1.1 cases per 1000 patients (pooled estimate: 0.62 [95%CI 0.34-0.99]). In ICUs, the pooled incidence of HAIs due to *Enterococcus spp.* and vancomycin-resistant *Enterococcus spp.* was 9.6 (95%CI 6.3-13.5, range: 0.39-36.0) and 2.6 (95%CI 0.53-5.8, range: 0-9.7) cases per 1000 patients, respectively. Regarding HA-BSI due to *Enterococcus spp.* and vancomycin-resistant *Enterococcus spp.* in ICUs, the pooled incidence was 6.1 (95%CI 1.9-12.3, range: 0-24.7) and 0.06 (95%CI 0.0-2.1, range: 0-9.9) cases per 1000 patients. Hospital wide, the pooled vancomycin resistance proportions among *Enterococcus spp.* HAIs isolates was 7.3% (95%CI 1.5-16.3%, range: 2.6-11.5%), while the pooled vancomycin resistance proportions among *Enterococcus spp.* HA-BSI isolates was 3.0% (95%CI 0-9.2%, range: 0-33.3%). In ICUs, the VRE proportion among *Enterococcus spp.* HAIs isolates was 11.5% (95%CI 4.7-20.1%, range: 0-40.0%) and 12.6% (95%CI 0.7-31.5%, range: 0-66.7%) among *Enterococcus spp.* HA-BSI isolates. Among patients with HA-BSI with *Enterococcus spp.*, the pooled all-cause mortality was 21.9% (95%CI 15.7-28.9%, range: 14.3-32.3%); whereas the all-cause mortality due to VRE was 33.5% (95%CI 13.0-57.3%, range: 14.3-41.3%).
Conclusion: HAIs caused by *Enterococcus spp.,* and VRE are frequently identified among hospital patients and associated with high mortality. Continuous monitoring and the improved implementation of infection prevention and control programs as well as antibiotic stewardship measures are essential to reduce the burden of HAIs due to enterococci.

039/HYPRV

Infection control for two cases of *Candida auris* at a COVID-19 intensive care unit, Germany

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Introduction: *Candida auris* is an emerging pathogen in hospital infections that can present multi-resistance to antifungals and causes outbreaks. Hospital outbreaks with high case numbers and long courses are reported worldwide. Despite extensive infection control measures outbreaks are difficult to control.
Methods: Identification of yeast isolates was performed by MALDI-TOF and confirmed by ITS sequencing. Infection control measures were decided by a multi-disciplinary ad hoc outbreak panel. Patient screening once or twice a week and extensive environmental testing for *C. auris* was conducted. For the patient screening swabs were taken from: Axilla, groin, wound if applicable, throat/nose or a sample of the TBS if applicable, and a urine sample in catheterized patients.
Results: *Candida auris* was isolated from a urine sample of a COVID-19 patient who had been transferred from an Egyptian hospital to our COVID-19 intensive care unit (ICU). Immediately, disinfection routine was changed, because *C. auris* is insensitive to quaternary ammonium compounds. The patient had already been isolated from admission due to evidence of 4MRGN *Klebsiella pneumoniae*. Six days after confirmation of *C. auris* in the index patient, a second COVID-19 patient was identified with *C. auris*. Both patients were isolated in a separated area of the ICU. Strict hygiene and infection control measures were implemented promptly, such as: Allocation of one nurse for the two *C. auris* patients, providing medical equipment for only these two patients, intensifying of the disinfection measures, admission stop, and provision of information material on *C. auris* for the health care personnel. The two *C. auris* patients had been intubated using the same video laryngoscope seven days apart. Although the equipment and the spatulas had been manually reprocessed using chlorine dioxide-soaked wipes they might serve as transmission vehicle. Therefore, it was recommended to use disposable spatulas. In the nine weeks from initial confirmation of *C. auris* and discharge of the two affected patients, *C. auris* was repeatedly identified in clinical samples of them. However, it was not detected in any other patient on the ICU (n=7) or discharged from it (n=13) nor in any environmental sample (n=129).
Conclusion: A rapid confirmation of a *C. auris* in the laboratory and the immediate implementation of adequate hygiene measures on the ward are crucial in order to prevent transmission of *C. auris* to other patients.

040/HYPRV

Performance of Fourier-transform infrared spectroscopy typing: a monocentric study using a prospective two-year collection of 284 clinical extended-spectrum β-lactamase-producing *E. coli*, *K. pneumoniae* and *E. cloacae* complex isolates from three clinical wards with high risk of outbreaks

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Background: Fourier-Transform InfraRed spectroscopy (FTIR) is a developing rapid, simple typing technology. In this study, we assessed the accuracy of the FTIR-based IR Biotyper (Bruker Daltonics, Bremen) to classify the most frequently recovered ESBL-producing Enterobacteriaceae (ESBL-E) species in high-risk clinical wards of our hospital at the sequence type (ST) level.
Methods: To reach this aim, we used a prospective collection of 284 consecutive ESBL-E clinical isolates, including 120 *E. coli* (ECOL), 83 *K. pneumonie* (KP), and 81 *E. cloacae* complex isolates(ECC, 72 *E. hormaechei*, 7 *E. cloacae* and 2 *E. kobei*). Isolates were recovered in 2019 and 2020 from patients hospitalized in 2 adult Intensive care units and in neonatology, excluding replicates in a same patient. Multi locus sequence typing (MLST) was performed by in silico analysis after whole genome sequencing (WGS). Clonality within STs was assessed by SNP typing (<20). FTIR was performed using the IR Biotyper System running the IR Biotyper software with default settings, unaware of WGS typing results. A FTIR type was defined by a cluster of isolates or by a single isolate not clustering with others at the threshold clustering level automatically defined. We used the Simpson diversity index, the adjusted Rand"s index and the Wallace coefficient to assess the discriminatory power and correspondence level of FTIR typing compared to MLST.
Results: FTIR types diversity varied according to species, reflecting significant variations in genomic diversity of isolates at the ST level: 55 STs for *E. coli*, 19 STs for KP and 14 STs for ECC isolates. *E. coli* ST131, KP ST405, and ECC ST66 were the most frequent clonal group (28% of ECOL, 44% of KP and 43% of ECC isolates, respectively). Agreement between the two methods was satisfactory for KP and ECC but not for *E. coli* (Fig. 1), probably reflecting a high frequency of KP and ECC nosocomial

acquisitions/outbreaks (< 20 SNPs) compared to *E. coli* isolates (none) during the 2-year study period.
Conclusion: FTIR typing shows to be a real-time first-step powerful technique for surveillance of MDR KP and ECC outbreaks in high-risk clinical wards. Suspected clonal outbreaks should be confirmed by WGS.

Fig. 1

	Method	N° of types	Simpsons (95% CI)	Adjusted Rand	Adjusted Wallace (95%CI)
E. coli	FTIR	52	0.890 (0.849-0.931)	0.420	0.361 (0.286-0.437)
	MLST	55	0.918 (0.876-0.960)		0.502 (0.336-0.668)
K. pneumoniae	FTIR	22	0.768 (0.679-0.856)	0.862	0.853 (0.696-1.000)
	MLST	19	0.772 (0.688-0.855)		0.873 (0.755-0.990)
E. cloacae complex	FTIR	13	0.743 (0.668-0.818)	0.884	0.800 (0.695-0.905)
	MLST	14	0.781 (0.701-0.861)		0.987 (0.971-1.000)

N° of types: FITR types and sequence types. Simpsons: diversity index. Adjusted rand: global correlation index between the two methods. Adjusted Wallace: directionality of the correspondences

Workshop 09
Advances in Pathogen Diagnostics (StAG DV/FG DKM)
FG Diagnostische und Klinische Mikrobiologie in Zusammenarbeit mit der VAAM-FG Qualitätssicherung & Diagnostik sowie der StAG Diagnostische Verfahren
13. Sep. 2021 • 15:45–16:45

041/DKMV
Saving time in microbiological blood culture diagnostics: A prospective evaluation of an enrichment procedure for accelerated Identification (ID) and antimicrobial susceptibility testing (AST)

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Background: Global sepsis incidence and mortality rates have painted a grim picture: A study from 2017 reported an incidence of 677 per 100.000 and estimated that of all global deaths in 2017, 11 Million or 19.7% were sepsis-related (1). Time to obtain identification (ID) and antimicrobial susceptibility test (AST) results from positive blood culture bottles to guide appropriate therapy remain crucial prognostic factors. We evaluated a new device, the FAST-Prep™ PBC System (Qvella, Ontario, Canada), that promises to reduce time-to-results by isolating and concentrating microorganisms from a positive blood culture (PBC) aliquot producing a Liquid Colony (LC). The system requires a 2ml aliquot of a PBC to deliver a bacterial "Liquid Colony" after 30 minutes of processing, which can be used directly for downstream processes.
Methods and Materials: We analyzed 223 samples collected from January 2021 to May 2021, each from unique patients, for evaluation using the "Liquid Colony" for ID and AST. We determined concordance with routine ID and AST results, which were produced using overnight subcultures. Species ID was performed with the MALDI-TOF-MS (Bruker) and AST with the VITEK®2 system (bioMerieux, France).
Results: Out of 223 blood cultures, 30 (13.5%) were excluded from analysis. 29 (13%) of them were polymicrobial and 1 (0.5%) did not produce growth on agar plates, leaving 193 (86.5%) samples to be analyzed. Of those, 183 (95.5 %) resulted in correct ID and 10 (4.5%) failed to deliver an ID using MALDI. Mean time-to-results for ID and AST after positivity was 12.9h and 26.98h respectively for the FAST-Prep™ PBC, while for routine workflow time-to-results were 37.02h and 53.31h respectively. Mean MALDI scores when tested directly using the "Liquid Colony" were 2.03 for gram-positive and 2.16 for gram-negative bacteria. Routine MALDI scores were 2.08 for gram-positive and 2.25 for gram-negative bacteria. Categorical analysis was calculated comparing the AST results using the LC to the standard of care overnight subculture results. One hundred and forty nine out of 162 samples (92%) were included in the analysis. Among the excluded samples, 11 (6.8%) had inadequate biomass to reach a McFarland value of 0.5, 1 (0.6%) produced no result on the Vitek and 1 (0.6%) was mixed containing 2 different morphotypes of the same species, leaving 2258 bug-drug combos. We observed a categorial agreement of 97.7% for gram-positive and 99% for gram-negative bacteria.
Conclusion: Through our prospective evaluation of the FAST-Prep™ PBC System, we were able to show its advantages to save substantial time compared to standard of care methods employed today without sacrificing quality in identifying pathogens and their susceptibility profiles. This promising new system can enable the user to save time of one day or more in blood culture diagnostics.
References:

- Rudd KE, et al. Lancet. 2020, 395(10219):200-211.

042/DKMV
Fluorescence in situ hybridisation (FISH) for molecular imaging of microorganisms – Quality Control in diagnostic microbiology

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Question: Fluorescence in situ Hybridization (FISH) is increasingly used to visualize microorganisms directly with the spatial context of the surrounding host tissue. Here, for research as well as diagnostic application, quality control measures for reliable high-quality FISH results are of utmost importance.
Materials/Methods: We routinely use FISH in combination with PCR/sequencing (Sanger and Microbiome analysis) for the analysis of clinical tissue sections, a tool we call FISHseq. We apply FISHseq for the diagnosis of biofilm-associated infections including infective endocarditis, oral biofilms, device-associated infections, and infections due to fastidious or yet uncultured microorganisms like *Treponema* spp., *Tropheryma whipplei*, *Bartonella*, *Coxiella burnetii*, or *Brachyspira*.
Results: We defined quality control measures for the entire FISH procedure: pre-analytics, sample fixation and embedding, FISH probe selection, FISH probe labelling, FISH probe optimization, FISH analysis including epifluorescence microscopy, interpretation of FISH results and, finally, their documentation and diagnostic assessment. We also highlighted pitfalls for possible misinterpretation of FISH results.
Conclusions: In diagnostic microbiology, FISH needs technical as well as medical expertise. Diagnostic quality control standards are mandatory for sensitivity and specificity of FISH probes, control of the microscopy performance, standard operation procedures (SOPs), and documentation in accordance with quality control requirements. Using these, FISH provides "insights" into the infection landscape that are otherwise unattainable and may guide therapy algorithms. In the future, artificial intelligence may facilitate the introduction of FISH into routine application that is for now restricted to specialized laboratories.

043/DKMV
Diagnostic performance of the novel Fungitell STAT assay in comparison to the classical Fungitell assay and the Fujifilm Wako β-glucan test in patients with invasive fungal disease

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Introduction: Most data on the performance of the fungal biomarker (1→3)-β-D-Glucan (BDG) was generated using the Fungitell assay (FA) and to a lesser extent the Wako β-glucan test (GT). Direct comparison of both assays showed a superior

sensitivity of the FA. However, the FA has disadvantages concerning the workflow when small sample sizes are analysed. It is performed most economically with 21 patients per run while the GT is designed for multiple- or single-sample use. Recently, the Fungitell STAT assay (FA-STAT) was launched with a similar workflow as the GT. The objective of this study was to examine if the FA-STAT combines the high sensitivity of the FA with the workflow of the GT.
Materials/Methods: We performed a case-control/cohort study on three patient groups, namely patients with blood culture-proven candidemia (n=150 plus 50 control patients), with proven/probable invasive aspergillosis (n=47) and with *Pneumocystis jirovecii* pneumonia (PCP, n=63). Aspergillosis was categorized according to the EORTC/MSG criteria 2019. Diagnosis of PCP required a compatible clinical and radiological presentation and the detection of the pathogen. For calculation of diagnostic performance the manufacturers' cutoffs were used (FA=60 pg/ml, FA-STAT=0.74, GT=7.0 pg/ml).
Results: The sensitivity and specificity in the candidemia group were 91.3% and 66.7% for the FA, 93.0% and 65.2% for the FA-STAT and 57.0% and 92.0% for the GT. There was no difference between the area under the ROC curve (AUC) of the FA and the FA-STAT. However, the AUC of the FA-STAT was significantly higher than the AUC of the GT (p=0.03). The sensitivity of the FA, FA-STAT and GT in the aspergillosis group was 76.6%, 79.5% and 54.5% and in the PCP group 100.0%, 100.0% and 90.5%. There was again no significant difference in sensitivity between the FA and the FA-STAT. In all three patient groups there is a very strong correlation between the quantitative BDG results of the FA and the FA-STAT.
Discussion: The FA-STAT combines the excellent sensitivity of the FA with the convenience of the GT for small sample throughput. In terms of sensitivity but not specificity the FA and the FA-STAT are superior to the GT in all three patient groups.

044/DKMV

Development of an innovative detection assay for STEC/EHEC based on the enzymatic activity of Shiga toxin

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Introduction: Shigatoxigenic (STEC) and its more virulent form enterohemorrhagic *E. coli* (EHEC) are important pathogens causing disease ranging from diarrhea to severe hemolytic uremic syndrome (HUS). STEC/EHEC are found in association with animals and food and may produce large outbreaks accompanied by high economic costs. Ten years after the EHEC O104:H4 outbreak, timely and qualified detection of STEC/EHEC, including isolate recovery in patients, animals and food, remains of high importance but still challenging and work-intensive. Isolate recovery is essential for risk profiling, infection cluster and infection chain elucidation. Thus, the availability of a reliable rapid test for the identification of STEC/EHEC would be a tremendous advance. STEC/EHEC are a group of pathogens with high variation in marker genes, but the major virulence factor, the Shiga toxin, is found in all STEC/EHEC. Therefore, in the here presented study, we designed and evaluated a detection method based on the catalytic activity of Shiga toxin (Stx).
Methods: Different EHEC strains harboring genes for Stx 1 and/or 2 were selected for analysis. Firstly, the Stx production was analyzed by testing different media as well as bacterial stress response inductors. The Stx production was monitored by Western blotting, ELISA and Vero cell cytotoxicity analysis. Secondly, for the detection based on enzymatic Stx activity, several fluorescently-labeled Stx oligonucleotide substrates were designed, and reaction conditions were optimized. Furthermore, specificity was validated.
Results: Growth in nutrient-rich media with stress response triggering agents are established conditions for Stx production. Several fluorescent dye-labeled oligonucleotide enzyme substrates were analyzed, yielding robust and specific Stx detection within 30 to 60 minutes depending on Stx quantity.
Conclusion: We established a rapid detection assay for EHEC based on the enzymatic activity of the Stx as the major virulence factor.

045/DKMV

A unique single nucleotide polymorphism in multi-copy 16S rRNA genes and transcripts facilitates sensitive identification of *Bacillus anthracis* by real time PCR

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Introduction: Among the *Bacillus* species, the anthrax pathogen *Bacillus anthracis* poses the greatest risk to human and animal health. Identification of *B. anthracis* by polymerase chain reaction (PCR) or other methods is challenging because of the bacterium"s close genetic relationship to other species of the *Bacillus cereus sensu lato group* (such as *Bacillus cereus* or *Bacillus thuringiensis*). Thus, molecular detection and identification is depending on the use of a limited set of species-specific gene targets (e.g., *dhp61* or *PL3*) or unique single nucleotide polymorphisms (SNPs) (e.g., in *rpoB* or *plcR* genes). All of these represent single-copy targets within a *B. anthracis* genome equivalent. Here, we made use of an *in silico* validated multi-copy target, a species-specific SNP. This SNP is conserved and present in up to four loci of the 16S rRNA gene sequence of every *B. anthracis* quality genome analyzed to date (n=959).
Methods: From this knowledge-base, a hydrolysis probe-based PCR assay was developed and experimentally validated. In this assay, the *B. anthracis* specific SNP is interrogated for by a fluorescently-labeled probe; the alternative SNP-state is masked by a fluorescently dark "competitor" probe. In an effort to push the detection limit, the assay was further adapted for reverse transcription PCR targeting 16S rRNA transcripts. These transcripts are found in cells in concentrations 3-4 log units higher than multi-copy 16S rRNA operons. Thus, the assay targets the SNP in genomic DNA and RNA employing identical primers and probes.
Results: The assay was specific as only *B. anthracis* DNA or RNA yielded positive results. DNA detection performed linear over 9 log10 units and was sensitive with a limit of detection (LoD) of 2.9 copies/reaction (i.e., about one genome equivalent). For *B. anthracis* 16S rRNA the assay was also specific and linear over 8 log10 units with a LoD of 6.3 copies/reaction.
Discussion: In this study we introduced the first combined chromosomal DNA- and ribosomal RNA-targeting multi-copy real time PCR assay for *B. anthracis*. Harnessing unique SNPs in 16S rRNA genes and their transcripts strongly highlights the great potential that such genomic variations have not only for identification of *B. anthracis* and for diagnostics of anthrax disease. Potentially this approach is also applicable to other pathogens which are otherwise difficult to discriminate from their less notorious relatives.

046/DKMV

Determination of a tentative dalbavancin epidemiological cut-off value (TECOFFs) for *Enterococcus faecium*

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Question/Background: Dalbavancin is a semi-synthetic lipoglycopeptide antibiotic that shows potent activity against gram-positive bacteria including staphylococci, streptococci and supposedly *vanB*-positive enterococci. Although extensive data are available on the *in vitro* activity of dalbavancin for *E. faecalis*, comprehensive data on *E. faecium* are generally scarce due to the global dissemination of *vanA*-positive and glycopeptide resistant

VRE. In Central Europe, however, a continuing shift from *vanA*-type vancomycin-resistance to *vanB*-type resistance has been observed from 2006 onwards. Therefore, dalbavancin may become a possible treatment option. We aimed to investigate the *in vitro* activity of dalbavancin against different *van* genotypes, with particular focus on *vanB*-type *E. faecium*, in order to find a dalbavancin TECOFF for *E. faecium*.

Methods: Susceptibility testing for 25 *van*-negative, 50 *vanA*-positive and 101 *vanB*-positive clinical *E. faecium* isolates was performed using broth microdilution (BMD) as a reference method. In addition, dalbavancin MICs were determined by MIC gradient strips. All isolates were routinely sequenced and typed by MLST and cgMLST. (T)ECOFFs were determined by the use of ECOFFinder and a threshold of 99%.

Results: For *vanB* type *E. faecium* isolates, dalbavancin MICs were similar to those of vancomycin-susceptible isolates reaching values not higher than 0.125 mg/L (Table 1). ECOFFs for *van*-negative and *vanB*-positive isolates were calculated to be 0.5 mg/l and 0.25 mg/L, respectively (BMD). In contrast, *E. faecium* possessing *vanA* predominantly showed dalbavancin MICs >8 mg/L, therefore preventing the determination of an ECOFF. Considering the total distributions of dalbavancin MICs, the TECOFF would be 0.25 mg/L.

Conclusion: Dalbavancin demonstrated potent in vitro activity against vancomycin-susceptible and *vanB*-type *E. faecium*. On the basis of the observed total MIC distribution, 0.25 mg/L can be suggested as dalbavancin TECOFF to distinguish *E. faecium* without and with a phenotypically detectable acquired vancomycin resistance mechanism.

Fig. 1

Table 1: Distribution of dalbavancin MICs for *E. faecium* isolates (n= 176). MIC gradient strip values were converted into doubling dilution as found in BMD and values of 16, 32 and >32 mg/L were downsized to >8 mg/L (maximum measurable value by BMD). The vertical line demonstrates the CLSI breakpoint for *E. faecalis* and the tentative ECOFF for *E. faecium* of 0.25 mg/L.

species / method	n	MIC [mg/L] ≤0.08	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	>8	MIC_{50} [mg/L]	MIC_{90} [mg/L]	ECOFF* [99%]	CLSI** [% S]
vanA, BMD	50	1		1		3		1			1	21	22	8	>8	-	10
vanA, strip	50			1	1	1	1	1					45	>8	>8	-	8
vanB, BMD	101	1	6	64	27	3								0.032	0.064	0.25	100
vanB, strip	101	6	45	44	6									0.016	0.032	0.125	100
van-, BMD	25		3	6	11	5								0.064	0.125	0.5	100
van-, strip	25		6	11	7		1							0.032	0.064	0.25	100

Legend: BMD, broth microdilution; strip, MIC gradient strip test.

*ECOFFs were identified by the use of ECOFFinder (EUCAST)

**we applied the susceptibility breakpoint for vancomycin-susceptible *E. faecalis* as provided CLSI.

Workshop 10
Microbiota (FG PW)
13. Sep. 2021 • 15:45–16:45

047/PWV
Exploring the interaction network of a synthetic gut bacterial community

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A key challenge in microbiome research is to predict and understand functionality from microbial community composition. As central microbiota functions are determined by complex bacterial community networks it is important to gain insight into the principles that govern bacteria-bacteria interactions. In this line, we focused on metabolic interactions of the Oligo-Mouse-Microbiota (OMM^{12}) synthetic bacterial community, which is increasingly used as model system in gut microbiome research.

So far, little is known about the ecological structure and metabolic capabilities of this synthetic bacterial community, both of which determine community assembly, population dynamics and bacterial community functionality. Using a bottom-up approach, we uncovered the directionality of strain-strain interactions in mono- and pairwise co-culture experiments, as well as in community batch culture. Metabolomics analysis of spent culture supernatant of individual strains in combination with genome-informed pathway reconstruction provided insights into the metabolic potential of the individual community members. Thereby, we could show that the OMM^{12} interaction network is shaped by both, exploitative and interference competition *in vitro*. In particular, *Enterococcus faecalis* KB1, a low-abundant member of the mammalian gut microbiota, was identified as important driver of community composition by affecting the abundance of several other consortium members *in vitro*.

Together, our work provides a detailed understanding of the mode of *in vitro* strain-strain interactions within the OMM^{12} consortium, which serves as a knowledge base for future mechanistic studies and the fundamental basis for targeted community manipulation to probe a wide range of microbial community functions *in vivo*.

048/PWV
Low-abundant bacterial commensals are the key determinants of a healthy airway metagenome in the early years of life

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Introduction: The default filtration of DNA background contamination in shotgun metagenomics is connected with a loss of information on the rare species inhabiting the environment of interest. Consequently, the role of rare taxa in the healthy and cystic fibrosis (CF) airway bacteriome remains understudied. Also, probiotic intervention strategies are investigated to treat or prevent events of microbial dysbiosis which have been associated with pathological conditions in the human body. However, studying the probiotic effect on microbial communities by *in vitro* or *in vivo* studies is difficult due to the complex living conditions of microbes. We developed a python tool (raspir) based on Fourier transforms and spectral comparison to implement high-confidence species predictions and study the role of rare commensals in the early development of the airway microbial community. We used kernel-based machine learning and in silico simulations to modulate the effect of probiotic intervention strategies on the chronically diseased microbial community and identify the best therapeutic approach for stabilising the CF network.

Patients and Methods: We collected deep cough swabs from children with CF (n = 41) and healthy (n = 52) children in three age groups (0, 1-3, 4-6 years of age), performed random DNA sequencing (Illumina, NextSeq) with reference-based alignment.

Results: In healthy airways, most of the rare and core bacteria were detected across all age groups. In this age-independent background network, core and rare commensals were equally important in maintaining the network structure and integrity. In CF children, the background network was prone to fragmentation and rare species were underrepresented. The presence or absence of rare species was the key variable in distinguishing a healthy from a CF metagenome. Probiotic simulation studies revealed a stabilisation effect of the CF background network, the higher the number and diversity of transferred healthy airway commensals. Transferring a few healthy species with high frequencies destabilised the CF network.

Conclusion: Our study revealed the benefit of "airway microbiome transplantation" strategies to rescue vulnerable bacterial airway networks indicating the importance of reviewing current probiotic applications for treatment or prevention. Rare species should be included in future investigations of the human microbiome to obtain a complete picture on microbial community signals.

050/PWV

RNA landscape of the emerging cancer-associated microbe *Fusobacterium nucleatum*

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Question: *Fusobacterium nucleatum*, long known as a constituent of the oral microflora, has recently garnered renewed attention for its association with several different human cancers. The growing interest in this emerging cancer-associated bacterium contrasts with a paucity of knowledge about its basic gene expression features and physiological responses. As fusobacteria lack all established small RNA-associated proteins, post-transcriptional networks in these bacteria are also unknown.
Methods: Here, using differential RNA-seq (dRNA-seq), we generate high-resolution global RNA maps for five clinically relevant fusobacterial strains - *F. nucleatum* subspecies *nucleatum*, *animalis*, *polymorphum* and *vincentii* as well as *F. periodonticum* – for early, mid-exponential growth and early stationary phase. In addition, we developed new genetic tools to study the function of discovered sRNAs in *F. nucleatum*.
Results: These data are made available in an online browser, and we use these to uncover fundamental aspects of fusobacterial gene expression architecture and a suite of noncoding RNAs. Developing a vector for functional analysis of fusobacterial genes, we discover a conserved fusobacterial oxygen-induced small RNA, FoxI, which serves as a post-transcriptional repressor of the major outer membrane porin FomA.
Conclusion: Our findings provide a crucial step towards delineating the regulatory networks enabling *F. nucleatum* adaptation to different environments, which may elucidate how these bacteria colonize different compartments of the human body.

051/PWV

Combating *C. difficile* infection through combinatorial microbiome editing and microbiome-sparing antibiotics

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Introduction: The mammalian microbiota consists of several hundred species of bacteria that are performing biotransformation of diverse molecules along the gastrointestinal tract. In the undisturbed state, this community has the capability to protect the host against invading pathogens in a process called "colonization resistance" (CR). Antibiotic treatment can disrupt the microbiota affecting its composition and functionality, potentially leading to a weakened CR. Nosocomial pathogen like *Clostridioides difficile* can expand into these newly created niches and cause pseudomembranous colitis, which is further complicated by high a recurrence rates. In order to reduce the risk of starting the vicious cycle of antibiotic treatment, breakdown of CR and infection, novel approaches strengthening CR by microbiome editing or microbe sparing antibiotics should be explored.
Methods: We modified the OMM12 gnotobiotic mouse model with several commensal bacteria, including the secondary bile acid (secBA) producing bacteria *Extibacter muris* to determine their role in *C. difficile* outbreak and pathogenesis. In parallel, we are investigating the effect of a new antibiotic candidate against *C. difficile*, Chlorotonil A, on the microbiota, the intestinal bile acid pool, infection susceptibility, as well as their therapeutic potential to treat CDI. To assess the microbiota composition and *C. difficile* count we utilized 16S rRNA gene sequencing and plating assays. The disease state and intestinal bile acid pool of infected mice were determined by ELISA and HPLC metabolomics.
Results: OMM12 mice are naturally susceptible to CDI infection as its members lack the potential to convert primary bile acids into *C. difficile* inhibiting secBA. In line with this observation, colonization with a single 7α-hydroxylating bacteria *E. muris* significantly increases the amount of secBAs in the intestinal tract, therefore promoting CR against CDI. Colonization with additional six commensal strains, without the genetic potential to produce secBAs, significantly boosts secBA production by *E. muris*. Ongoing metabolomics analysis addresses the role of these six commensal bacteria on intestinal bile acid conversion and their contribution to the colonization resistance against *C. difficile*. While we demonstrated, that reconstitution of CR by microbiota editing is sufficient to prevent CDI; in a therapeutic approach, we could show that a new candidate antibiotic Chlorotonil A successfully ameliorates disease state of CDI in mice. Compared to broad-spectrum vancomycin treatment, we did not observe CDI recurrence in Chlorotonil A-treated mice.
Discussion: Bile acid homeostasis is a key aspect of preventing or alleviating severe CDI. By microbiota editing and utilizing microbe sparing antibiotics, we are trying to understand the functional contribution of commensal bacteria to intestinal bile acid metabolism, which will provide new strategies in probiotic development and antibiotic treatment.

052/PWV

The intestinal mycobiome in premature born infants

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Question: Assembly and differentiation of the microbiome in infants is influenced by many external factors, including nutrition and antibiotic therapy. While the establishment and development of the microbiome has been studied in mature born infants, little is known about preterm infants. Furthermore, microbiome analyses often focus on the bacteriome, neglecting other microbiota.
Methods: In this study we analysed the mycobiome of preterm infants. 52 human faecal samples were collected from 17 infants with a gestational age < 37 weeks and a birth weight between 1,250 and 1,750 g. The infants were grouped by probiotic and antibiotic treatment: 7 infants received probiotics, 6 probiotics and antibiotics, and 4 no treatment. DNA was extracted from faecal samples and used for ITS sequencing. The bioinformatics analysis was performed with PIPITS and QIIME. The most abundant detected phyla were Ascomycota and Basidomycota.
Results: At the genus level Candida, Aureobasidium, Aspergillus and Penicillium were the most abundant. The mycobiome composition showed high variability within groups and no significant differences between groups. However, some differences were observed between treatment groups regarding abundances at the genus and species level. For example, Aspergillus was less abundant in infants treated with probiotics alone compared to infants treated with a combination of antibiotics and probiotics or infants without treatment. The mycobiome composition appeared to be quite stable over time.

Conclusion: We observed high variability of the mycobiome between individuals with comparatively little differences between treatment groups and over time, suggesting that the mycobiome in preterm infants is specific and relatively stable for each individual.

Workshop 11
Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens (FG ZO/FG MS)
13. Sep. 2021 • 15:45–16:45

053/MSZOV

German wildlife as a common source for *E. marmotae* - Epidemiology, diversity and impact of isolates of the recently described novel *Escherichia* species

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Introduction: In Germany, the national zoonosis monitoring includes the screening of the antimicrobial resistance dynamics of commensal *Escherichia coli* from retail meat, as well as faeces from livestock slaughterhouses and wild animals. Based on phenotypic resistance against highly critically important antimicrobials, selected isolates are subjected to whole-genome sequencing (WGS) for characterization of the resistance mechanisms. *In silico* characterization of colistin-resistant *E. coli* lead to the detection of two *E. marmotae* isolates, representing members of a recently identified species originally obtained from wild Marmots in the Tibetan Plateau, China. Both isolates were collected in 2016 from wild boars from different regions in Germany. Interestingly, both *E. marmotae* exhibited confirmable MALDI-TOF MS scores <2.3, which did not allow reliable species identification. Thus, this study aims to determine the impact of German wildlife as a common source of *E. marmotae* currently misidentified as *E. coli* due to a lack of resolution of the standard typing methods.
Material/Methods: To identify additional *E. marmotae* isolates in German wildlife, we screened our *E. coli* collection for isolates achieving MALDI scores <2.3. Selected isolates were further analyzed for their phenotypic and genotypic properties in comparison to an *E. marmotae* reference strain.
Results: Screening of our *E. coli* collection yielded in 15 possible isolates of wildlife origin exhibiting an average MALDI score of 2.07. WGS identified 14 additional *E. marmotae* and one *E. albertii* isolate, all obtained from faeces of wild boars. Here we describe the characteristics of in total 16 *E. marmotae* originating from eight different German federal states collected in 2016 and 2020. Interestingly, nine isolates exhibited phenotypic resistance to colistin, a last-resort drug in human medicine. According to *in silico* typing, ST133 was the most prevalent sequence type among *E. marmotae*, while two isolates exhibited a yet unknown type. Phenotypic characterization of *E. marmotae* showed a close relationship of the API reactions and of the growth properties on different culture media. However, the *E. marmotae* exhibited substantial differences in their phylogenetic relationship and the content of virulence-associated genes. Furthermore, electron microscopy revealed strong differences in the expression of fimbriae of the individual isolates.
Discussion: Our study revealed the presence of a diverse population of the newly described species *E. marmotae* in German wildlife. About half of the isolates carry colistin resistance, the origin of which is still being investigated. Furthermore, our study underlines the importance of reliable species identification in routine diagnostics. Based on the results of the *in silico* analysis a human pathogenic potential of *E. marmotae* cannot be excluded. Thus, the transmission from wildlife products to human might pose a risk for colonization and/or infection.

054/MSZOV

Agricultural fertilisation with poultry manure results in persistent environmental contamination with the pathogen *Clostridioides difficile*

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Introduction: *Clostridioides difficile* can be found at high abundance in the intestinal tract of livestock animals, including chickens. Consequently, fertilization of agricultural land with livestock manure may cause environmental contamination with *C. difficile* spores. Our aims were to test for the presence and survival of manure-derived *C. difficile* in fertilized soil and to investigate the risk of aerogenic spread of viable *C. difficile* in association with agricultural dust.
Methods: We conducted a field experiment, where 12 tons of manure from broiler fattening facilities in Germany was spread on 2.1 hectars of agricultural land[1]. *C. difficile* was isolated from manure, from fertilized soil, and from dust particles that were captured during the experiment. Genomes of these isolates were sequenced and analyzed using the platform EnteroBase[2].
Results: We detected a high diversity of *C. difficile* isolates in broiler manure samples, including PCR ribotypes common from human disease. Isolates genomically indistinguishable from manure isolates were recovered from fertilized soil more than two years after fertilization, and they were also found in dust samples collected at the application site.
Conclusion: We present evidence of long-term contamination of agricultural soil with manure-derived *C. difficile* and demonstrate the potential for airborne dispersal of *C. difficile* through dust emissions during manure application. *Clostridioides* genome sequences virtually identical to those from manure had been recovered from chicken meat and from human infections in previous studies, suggesting broiler-associated *C. difficile* are capable of zoonotic transmission.

1. Frentrup M, Thiel N, Junker V, *et al*. Agricultural fertilization with poultry manure results in persistent environmental contamination with the pathogen *Clostridioides difficile*. *Environ. Microbiol*. 2021; in press.
2. Frentrup M, Zhou Z, Steglich M, *et al.* A publicly accessible database for *Clostridioides difficile* genome sequences supports tracing of transmission chains and epidemics. *Microb Genomics*. 2020;6(8):e000410.

055/MSZOV

Comparative analysis of genomic diversity and mobile resistomes of broiler strains of *Salmonella* Infantis and *E. coli*

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Question: In the last decade the multiresistance of *S.* Infantis has grown into a global problem of the broiler industry. Because multiresistant (MDR) strains of *S.* Infantis and *E. coli* frequently coexist is broilers, it seems to be logical to ask, how much the mobile resistomes of these MDR enteric bacteria overlap. Therefore, we compared genomic diversity and mobile resistomes of *S.* Infantis and *E. coli* to understand possible genomic relations and to gain a first insight into the potential exchange of mobile resistance determinants between these two enteric bacterial species.

Methods: For this, whole-genome sequences of 56 *S.* Infantis and 90 *E. coli* strains were analysed. The collection was established to allow a genomic comparison of antibiotic resistance in *S.* Infantis and *E. coli* from different broiler sources and humans in Hungary. For molecular epidemiological analysis the Ridom SeqSphere+ software was used. Sequence types (STs) were determined by multilocus sequence typing (MLST), the relatedness of *S.* Infantis and *E. coli* strains was further analysed by cgMLST. Web-based tools ResFinder and PlasmidFinder were used for the detection of acquired resistance determinants. Integrons were analysed on the basis of the genomic contigs by using the Geneious Prime software.
Results: The cgMLST analysis revealed a high genomic heterogeneity of broiler *E. coli*. This highly discriminatory genotyping tool also provided the first insight into the internal genomic structure of the PFGE clone B2 of *S.* Infantis, which is epidemic in Hungary. We also identified new MDR sequence types for *S.* Infantis (ST7081 and ST7082) and for *E. coli* (ST8702 and ST10088). Our results from comparative mobile resistome analysis describes broiler *E. coli* as genetically diverse with a high prevalence and diversity of plasmids and of mobile resistance genes, while resistomes of *S.* Infantis proved to be less diverse. For both species the MDR genotype *tet*(A)-*aadA1-sul1* was predominantly identified, conferring resistance to tetracyclines-aminoglycosides-sulphonamides, while the ampicillin resistance gene *bla*TEM-1 was detected mostly in *E. coli* strains. Gene *aadA1* was commonly identified as part of a 1kb class 1 integron. IncF plasmids were carried almost exclusively by *E. coli,* while IncI1 plasmids were predominantly identified in *S.* Infantis, but this plasmid family was also frequently detected in *E coli* strains.
Conclusions: This is the first comparative genomic analysis of contemporary human and broiler strains of *S.* Infantis and *E. coli* isolated in Hungary. The diversity of mobile resistomes suggests that commensal *E. coli* could be potential reservoirs of resistance for MDR *S.* Infantis, but so far it seems that IncI1 plasmids and class 1 integrons could have the greatest contribution to the genetic interaction between *E. coli* and *S.* Infantis.
Acknowledgements. Financial support of the Heinrich Hertz-Foundation and of the project NKFI K 128600 is acknowledged.

056/MSZOV
Intimate association of *Campylobacter jejuni* clinical isolates with *Enterococcus faecium*
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Background: *Campylobacter* is responsible for the majority of food-borne bacterial infections in several continents and resistance to first-line antibiotics is on the rise. Reduced susceptibility to carbapenems has not yet been reported but would pose a serious threat.
Materials&Methods: Phenotypic AMR testing was performed using broth micro dilution according to EUCAST. Bacterial morphology and colony structures were assessed by fluorescence microscopy and scanning electron microscopy. Whole genome sequencing was performed with Illumina NextSeq, yielding paired end sequence reads.
Results: During *Campylobacter* surveillance two *C. jejuni* clinical isolates with a multi resistance phenotype including resistance to carbapenems were found. Genome analysis revealed that the samples though derived from single colonies also contained ci.10% of *Enterococcus faecium* DNA reads. Indeed, scanning electron microscopy analysis confirmed the presence of coccoid bacteria in addition to spiral-shaped *Campylobacter.* The images revelaed coaggregation between the bacteria surrounded by a filamentous network. Genomic data of further 23 out of 273 clinical *C. jejuni* and *C. coli* isolates contained reads of another intestinal bacterial species, most frequently *Enterobacter cloacae*, for which carbapenem-resistance has already been described.
Discussion: Unusual antimicrobial resistance patterns in *Campylobacter* clinical isolates may be due to concomitant/by-caught intestinal bacteria, such as multi-resistant *Enterococcus* species. Detection was possible by genome analysis and strain purification required implementation of additional sub cultivation steps. The here observed intimate attachment of *Campylobacter* and *Enterococcus* may not only confuse conclusions on antibiotic resistance but may also promote horizontal gene transfer of resistance determinants.

057/MSZOV
Dynamics of quinolone- and cephalosporin-resistant *Escherichia coli* carriage along the production chain in Thuringian pigsties
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Question: Due to rising levels of antimicrobial resistance (AMR), the German government restricted veterinary usage of fluoroquinolones (FQ) and certain cephalosporins (CS) in 2018, leading to a subsequent reduction in their sales figures. AMR determinants can, however, be maintained independent of the extent of antimicrobial (AM) usage via co-selection and cross-resistance. Therefore, we studied the persistence of genetic elements coding for AMR and strains carrying them within a fattening run (FR) and from one FR to another in Thuringian pigsties.
Methods: Pooled faecal samples from three consecutive FRs of one conventional (C2) and two organic (B1, B2) farms were collected over a period of 16 months and screened for indicator *E. coli* on Gassner plates containing enrofloxacin, ceftiofur or cefquinome. After determining the percentage of FQ-/CS-resistant bacteria by colony counting, the resistance profiles of single strains were phenotypically assessed using a commercial diagnostic system with 16 AMs (13 classes according to WHO categorization) important in veterinary medicine. For genotypic comparison, strains were subjected to MLVA-PCR and plasmid profiling. Individual clones were then selected for whole genome sequencing (WGS).
Results: A total of 301 strains, resistant to one or more of the three above-mentioned AMs, were isolated from all pigsties (n=142 from C2, 111 from B1, 48 from B2). We identified the underlying determinants as plasmid-encoded (*qnrS* and *aac(6')-lb-cr*) or mutational (*gyrA*, *parC* and *parE*) for FQ resistance, while CS resistance was conferred by *blaCTX-M* (70% *blaCTX-M-1*, 30% *blaCTX-M-15*). WGS data even revealed the presence of additional genes encoding AMR against a variety of AM classes. Despite the strains" heterogeneity, reflected by 60 different MLVA-patterns (similarity > 98%), all isolates classified as multidrug-resistant (MDR) with strains being 30/42/60% 3-6MDR and 70/58/40% 7-11MDR on farm C2/B1/B2, respectively. Resistance levels were generally higher in piglets and declined towards the end of the fattening period. Notably, less than 1% of the strains were resistant to colistin, tulathromycin or amoxicillin-clavulanic acid.
Conclusion: The strong variations in occurrence of AMR *E. coli* between farms, compartments, age of pigs and FRs will be interpreted with respect to farm metadata, e.g. AM usage or cleaning and disinfection routines. The final goal is to identify potential dominant clones and plasmids that drive the maintenance and spread of AMR on these farms.

058/MSZOV
Nasal colonisation of dogs and their human household contacts with *Staphylococcus aureus*/ MRSA and the question of intrafamilial transmission
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Introduction: *Staphylococcus aureus* is widely disseminated as nasal colonizer of humans and has less frequently also be observed in dogs. Nosocomial infections with MRSA are known from human as well as from small animal hospitals. The study reported here aimed to answer questions on *S.aureus*/MRSA colonization of dogs living in families together with colonized humans.
Materials and ethods: Study participants baseline characteristics were assessed by questionnaires.

Processing of nasal swabs without pre-enrichment, species diagnostics, antibiotic susceptibility testing, *spa*-typing and attribution to MLST-clonal complexes was performed as described previously (1). Selected isolates were analyzed in detail with Ridom SeqSphere+, using WGS data generated by Illumina MiSeq sequencing. The study was approved by the ethical committee of the medical faculty of Magdeburg University (#33/14).

Results: The sample: 179 persons participating in the study represented members of 83 families, there were 116 dogs living in these families.

Nasal carriage: *S. aureus* was detected in 66 participants (36,9%). *S.aureus* nasal colonization was significantly more frequent among persons suffering from eczema. Among dogs 9 (7,8%) revealed as colonized.

Intrafamilial transmission: *S. aureus* carriage was found in 46 (55,4%) of the 83 households. In 19 of these households two and more persons were concomitantly colonized (39 of 54 persons living in these households). In 16 of these households isolates from different persons exhibited identical typing patterns which suggests intrafamilial transmission between humans.

In 8 households concomitant *S. aureus* carriage by dogs and humans was observed. The isolates exhibited *spa*-typing typing patterns identical to the isolates which were detected in humans which suggests intrafamilial transmission between humans and dogs. cgMLST analyses indicated the existence of a cluster of closely related isolates for each family, containing both human and animal *S. aureus* strains.

Antibiotic resistance: Two among the 66 isolates from humans revealed as MRSA (ST22, *mec*A; ST130, *mec*C), there was no MRSA among the isolates from dogs.

Discussion: The observed frequency of *S.aureus* nasal colonization was significantly higher as that observed in previous recent studies in Central and Northern Germany. Conditions predisposing to *S.aureus* carriage were not more frequent as in previous studies (1). The possible influence of living together with dogs on the nasal microbiome and consequences on *S.aureus* colonization resulting therefrom remain to be explored. Dogs were more rarely colonized. As only 8 among the 83 households were affected, it seems that transmission between humans and dogs occurs, but the owners are the reservoir.

References:

Cuny, C. et al., Toxins (Basel). 2019. PMID: 30935022

Workshop 12
Technical Hygiene (StAG HY/FG PR)
14. Sep. 2021 • 10:00–11:00

059/HYPRV

Looking for a needle in a haystack: SARS-CoV-2 Screening in patients and employees

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Background: Testing people without symptoms or exposition can identify non- or pre-symptomatic SARS-CoV-2 infected persons. Prompt isolation measures can thus reduce the risk of transmission. This may be particularly relevant in hospitals to protect vulnerable groups. Although plausible, the effectiveness and efficiency of this approach are not known yet.

Materials/Methods: Positivity rates of SARS-CoV-2 testing in patients and employees without symptoms and contact person history were investigated at two University Hospitals (UK1 and UK2) in a low to moderate incidence region and in a moderate to high incidence region, respectively, from 03/2020 until 03/2021. In addition, positivity rates of employees with symptoms or contact person history at UK2 were collected from 12/2020 to 03/2021.

The following groups were defined:

a. In-patients at admission
b. In-patients during hospital stay
c. Out-patients e.g. before surgery
d. Employees without symptoms and contact person history
e. Employees with either symptoms or contact person history (as control)

For a.-c. and e., screening was directly performed by PCR tests from pharyngeal swabs, for d., an antigen assay from a nasal swab was used initially (in case of positivity followed by a PCR assay).

Results: A total of 140195 analyses were included. Positivity rates in the different groups were low to very low with a. 181/72063 (0,25%), b. 119/25364 (0,47%) c. 169/32721 (0,52%) d. 65/4923 (1,32%), and e. 59/5124 (1,15%). That leads to the following numbers needed to test (NNT): 389; 213; 194; 76; 87, respectively. For correlation of these incidentally identified people with regional 7-day case counts see figure 2.

A cost calculation performed for SARS-COV-2 screening at UK2 points out the following costs: for a.-c. averaging 34,87€ per PCR, for d., 11,75€ per antigen assay, and for e., there is an invest of 39,79€ per PCR. Thus identification of an infected patient or employee without symptoms or contact person history goes along with a total of 8299,22€.

Figure 1: Absolute number of positive cases per week

Figure 2: Correlation of positivity rates with regional case counts. Kendall"s tau: UK1 Patients 0,36*; UK1 Staff 0,38*; UK2 Patients 0,48*, UK2 Staff 0,38*; *p<0,000

Conclusions: Our study characterizes the positivity rates of additive testing and surveillance strategies in hospitals during the SARS-CoV-2 pandemic. Although risk minimization in hospitals always seems to make sense, it is at least reasonable to question general screening and testing regimes due to the low positivity rates. Regional case counts seem to be correlated with admission screening positivity rate, thus it may be used for guidance. There are, however, several limitations yet to be discussed. The effectiveness of early or additional identification of patients or employees depends inversely on the degree of implementation of additional hygiene measures such as masks, spacing rules, ventilation, etc.

Fig. 1

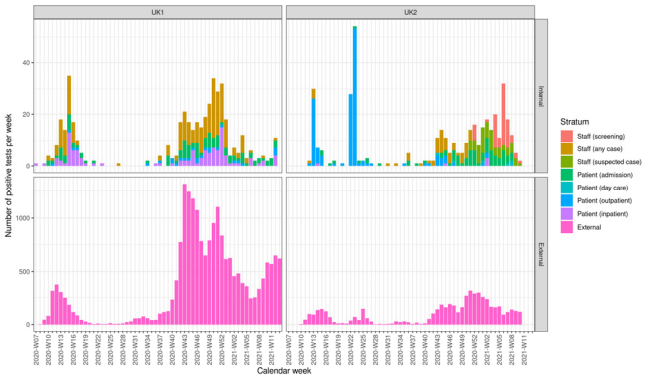
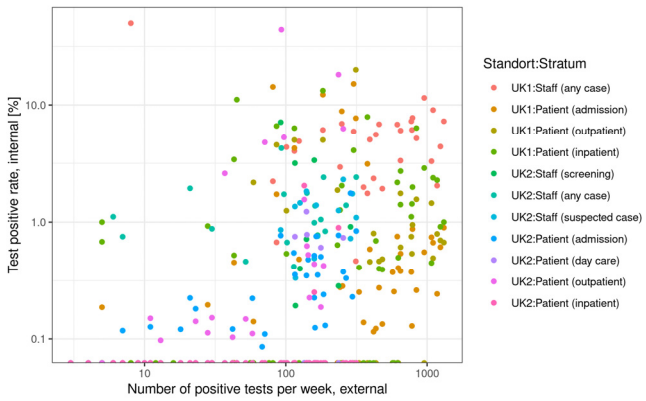


Fig. 2



060/HYPRV

COVID-19 surveillance and testing strategies for patients in German hospitals – Results from a cross-sectional study

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Background: During the COVID-19 pandemic, hospitals were challenged to develop effective surveillance and test strategies. To gain a better understanding of these measures in university hospitals and non-university hospitals in regions with relatively high and low incidence figures in Germany, we surveyed infection control specialists regarding their hospital"s surveillance strategies. We focus our report on measures directed towards hospital inpatients.

Methods: As part of the B-FAST project of the Network University Medicine (NUM), we surveyed university hospitals (UKs) as well as non-university hospitals in a federal state with higher (Bavaria; "BY") and lower (Lower Saxony; "NI") COVID-19 incidence density, using a cross-sectional, standardized, ethically approved online questionnaire (LimeSurvey) in March 2021. We focused upon the questions asked to assess patients" infection risk (including temperature checks) at admission, test strategies for patients and different screening indications (e.g. upon admission, distinct patient groups) for testing patients without symptoms and contact anamnesis for SARS-CoV-2 infection.

Results: In total, we received 100 responses (1 per hospital). In university hospitals, the response rate was 92% (33/36), in Lower Saxony 32% (37/115) and in Bavaria 11% (30/261). Upon admission, symptoms (95%) and contact to a person who tested positive for COVID-19 (94%) were the most frequent questions (Table 1). Hospitals in Bavaria asked more often about contact to persons with COVID-19 symptoms. Hospitals in Lower Saxony more often asked questions about profession and travel. With regards to temperature measurement, hospitals in Bavaria were more likely to check patient"s temperature at admission. In all hospitals, patients were regularly tested for SARS-CoV-2 infection (Table 2). Most hospitals tested patients with PCR tests (87% NI; 90% BY; 100% UK) and many hospitals used antigen tests for patients. Antibody testing of patients was only common in university hospitals (22% NI; 13% BY; 67% UK). In university hospitals, specific patient groups were more often tested for an asymptomatic SARS-CoV-2 infection than in Lower Saxony or Bavaria, such as especially vulnerable patients (19% NI; 20% BY; 39% UK) and patients unable to wear a mask during treatment.

Conclusion: Surveillance and testing strategies for hospital inpatients showed large overlap. Even so, there are some differences. University hospitals use more PCR and antibody testing than non-university hospitals. Hospitals in a region of higher incidence were more concerned about contact to persons with COVID-19 symptoms and patient temperature. In contrast, hospitals in a region with lower incidence often asked screening questions about possible exposition via travel or profession. These results give first insights to surveillance and testing strategies in German hospitals.

Fig. 1

Table 1. Topics asked in a patient survey upon hospital admission, including actively measuring the patient's temperature (all values in percent).

Screening at admission	Univ. hospitals n=33	Lower Saxony n=37	Bavaria n=30	Total n = 100 (95% CI)
COVID-19 symptoms	97.0	91.9	96.7	95.0 (90.7 – 99.3)
Contact to a person who tested positive for COVID-19	97.0	91.9	93.3	94.0 (89.3 – 98.7)
Contact to a person with COVID-19 symptoms	75.8	81.1	**93.3**	83.0 (75.6 - 90.4)
Recent stay in a high-risk region outside of Germany	72.7	83.8	70.0	76.0 (67.6 - 84.4)
Current quarantine due to SARS-CoV-2 exposition	**84.8**	64.9	73.3	74.0 (65.4 – 82.6)
Current isolation due to SARS-CoV-2 infection	**81.8**	67.7	70.0	73.0 (64.3 – 81.7)
Patient temperature measurement	**39.4**	48.6	**70.0**	52.0 (42.2 - 61.8)
Recent stay in a German region with higher incidence	36.4	**56.8**	40.0	45.0 (35.2 – 54.8)
COVID-19 vaccination status	15.2	27.0	20.0	21.0 (13.0 – 29.0)
Profession	**3.0**	**24.3**	13.3	14.0 (7.2 – 20.8)
Participation in larger cultural events (e.g. concerts, sports)	3.0	5.4	3.3	4.0 (0.2 – 7.8)
Influenza vaccination status	3.0	5.4	3.3	4.0 (0.2 – 7.8)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

Fig. 2

Table 2. SARS-CoV-2 testing methods and screening strategies for hospital patients in different situations (all values in percent).

Testing methods for patients and additive screening strategies after admission	Univ Clinics n=33	Lower Saxony n=37	Bavaria n=30	Total n = 100 (95% CI)
Testing methods used for inpatients				
PCR test	**100.0**	**86.5**	90.0	92.0 (86.7 – 97.3)
Antigen test	72.7	83.8	76.7	78.0 (69.9 – 86.1)
Antibody test	**66.7**	**21.6**	**13.3**	34.0 (24.7 – 43.3)
Screening strategies for inpatients in different situations				
At inpatient admission	97.0	94.6	93.3	95.0 (90.7 – 99.3)
During patient information talks (e.g. before an operation)	42.4	54.1	43.3	47.0 (37.2 – 56.8)
By especially vulnerable patients (e.g. dialysis, oncology)	**39.4**	18.9	20.0	26.0 (17.4 - 34.6)
By persons coming from high-risk regions outside of Germany	21.2	13.5	13.3	16.0 (8.8 – 23.2)
In selected samples of patients	9.1	5.4	10.0	8.0 (2.7 – 13.3)
By persons who cannot wear a mask during medical treatment	**12.1**	5.4	3.3	7.0 (2.0 – 12.0)
By persons coming from German regions with higher incidence	9.1	5.4	6.7	7.0 (2.0 – 12.0)
Depending upon the regional incidence	9.1	2.7	3.3	5.0 (0.7 – 9.3)
Depending upon the positive rate within the clinic	3.0	8.1	3.3	5.0 (0.7 – 9.3)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

061/HYPRV

Source of SARS-CoV-2 infections among hospital employees in three hospitals in Cologne

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Introduction: During the SARS-CoV-2 pandemic many health-care workers became infected. Whereas during the first wave of the pandemic not enough personal protective equipment (PPE) was available, later on still many new cases of infected health-care workers occurred, despite increasing knowledge about infection prevention, control measures and availabilities of PPE.

Objective: To investigate whether and to which extent hospital employees (HE) aquired SARS-CoV-2 nosocomially, and whether and to which extent they were infected by infected patients or infected colleagues.

Methods: All new SARS-CoV-2 infected employees of three different hospitals in Cologne city were asked if they remembered relevant contacts to known SARS-CoV-2 positive persons, and if so, what the contact was like (duration, room, distance, PPE) by infection control personal (ICP). Cases from March 2020 until April 2021 were included. Definitions for "proven nosocomial infection", "probable nosocomial infection" and "community-acquired infection" were set up.

Results: In hospital A, B and C 98,150 and 45 HEs were affected, out of whom 51 (52%), 89 (59%) and 22 (49%) were community-acquired cases, respectively. Probable nosocomial cases were 13 (13%), 30 (20%) and 10 (22%) and proven nosocomial cases 34

(34%), 31 (21%) and 13 (29%). In all three hospitals 18 (27%) of all proven nosocomial cases were infected by a contact to a patient, the remaining 73% by a contact to infected colleagues. In hospital A, B and C 32, 24 and 12 were nosocomial cases with an association to a total of 18 different clusters (6, 9 (2 in non-medical sections of the hospital (NMS) and 3 (2 NMS), respectively), accounting for 23% of all positive HEs in all three hospitals.
Discussion: Hospital employees (HEs) were considerably often infected by SARS-CoV-2. Infection opportunities were the community outside the hospital, patients or infected colleagues. Most employers were infected in the community and were obviously not able to prevent transmission in their private context. In 6% of all cases a transmission from a patient could be proven by spatial and temporal relation and the kind of the contact. A fifth of all infected employers were affected in context with a cluster. Our data demonstrate a highly complex transmission setting in and outside the hospitals for their employees. For infection control personal many different contact possibilities of HEs are very challenging in respect to a timely analysis of each individual case for implementing tailored infection control measures to prevent further spread.

062/HYPRV
Increased incidence of *S. aureus*-bloodstream infections in COVID-19-ICU-patients with previous respiratory colonisation
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Introduction: Super-infections have been reported to be a complication in COVID-19-patients (Musuuza, 2021). We describe an increase of *S. aureus*-bloodstream infections (Sa-BSI) in a high-care ICU specialized on ECMO-treatment (An-ICU), mainly in male COVID-19 patients associated with the second and third waves of SARS-CoV-2 infections in Germany.
Methods: Descriptive study of a Sa-BSI cluster. A case was defined as a patient with an *S. aureus* positive blood culture during a stay on An-ICU between 01.11.2020 and 25.05.2021
Results: We registered 16 cases matching the case definition. With an average of 2.3 cases/month during the investigation period this clearly exceeds the baseline expectancy of 0.4 cases/month, based on data from 01/2018 to 10/2021. The median age of the cases was 62 years and 15 patients were male. Fourteen patients were admitted to the ICU due to severe COVID-19 disease. Nine patients died during their ICU-stay and seven were discharged to another hospital. In 15 patients, *S. aureus* was also detected in respiratory samples. No other focus was identified for any of the patients. Sa-BSI was nosocomial in nine patients, whereof three were already colonised in the respiratory tract on admission. Spa-typing was performed for 14 isolates, revealing high diversity with only two spa types occurring twice.
Additional control measures were initiated and continued until time of writing. These included Chlorhexidine-washing and use of chlorhexidine-patches for all patients, as well as respiratory screening for *S. aureus* and use of mupirocin ointment for positive patients. Thereafter, the majority of cases were already admitted with Sa-BSI.
Discussion: We describe a series of Sa-BSI, primary in male COVID-19-patients, at a high-care ICU. Route of entry was most likely the respiratory tract. Early recognition and treatment of *S. aureus*-colonisation might improve the outcome. Therefore, *S. aureus*-screening on admission and subsequent decolonisation should be considered in COVID-19-patients requiring intensive care treatment.
Reference: Musuuza JS, Watson L, Parmasad V, Putman-Buehler N, Christensen L, Safdar N (2021) Prevalence and outcomes of co-infection and superinfection with SARS-CoV-2 and other pathogens: A systematic review and meta-analysis. PLoS ONE 16(5): e0251170. https://doi.org/10.1371/journal.pone.0251170

063/HYPRV
Exploration of the spreading of a specific B.1.1.7 strain in Europe
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Introduction: Although B.1.1.7 is the predominant SARS-CoV-2 lineage during the third wave in early 2021 in most European countries, not all the strains circulating in Europe can be traced back to the B.1.1.7 strains circulating in UK last December. In several European countries, such as Austria, Czech Republic, Slovakia and Germany, one B.1.1.7 strain with several extra mutations accounts for 30% - 90% of B.1.1.7 related COVID-19 cases. The source and spreading route of this specific B.1.1.7 strain remains unclear.
Methods: With genomic epidemiology and a large number of SARS-CoV-2 genome sequences deposited in GISAID from all the European countries, we investigated the spreading history of this B.1.1.7 strain. Lineage group assignment of SARS-CoV-2 genomes was performed with software package **P**hylogenetic **A**ssignment of **N**amed **G**lobal **O**utbreak **LIN**eages (Pangolin). Phylogenetic maximum likelihood (ML) and time trees were constructed using the SARS-CoV-2-specific procedures taken from github.com/nextstrain/ncov. We used R package ggplot2 and ggtree to clean and plot data and trees.
Results: The earliest samples of this B.1.1.7 strain were identified in November 2020 in a few European countries, such as Switzerland, France and Denmark. Although only a small number of cases related to this strain had been reported at that time, it had quickly spread to several other European countries. In early January 2021, it was frequently detected in Czech Republic and became the dominant B.1.1.7 strain there. This widely-spread B.1.1.7 strain is responsible for more than 90% COVID-19 cases in Czech since February 2021. In Austria, this strain accounted for more than 60 % of B.1.1.7 related cases; in Hungary, 69.0%; in Slovakia: 45.2% (until 15.05.2021). In Germany, the strain is responsible for 34.5% B.1.1.7 related cases, and we have observed new spike mutant accumulation in this stain.
Discussion: The results of phylogenetic analysis indicate this strain might be the only B.1.1.7 variant (or one of a few variants) imported to Czech in last December or early January 2021, but the consequence of it is dramatic. The wide spread of this strain was the major driving force for the high COVID-19 7-day incidences in Czech Republic in February 2021, and partly responsible for the third wave in Germany in March 2021. This finding emphasized the importance of international collaboration on virus mutant surveillance, not only for SARS-CoV-2, also for other epidemic viruses or bacteria.

Workshop 13
Clinical Microbiology (StAG DV/FG DKM)
14. Sep. 2021 • 10:00–11:00

064/DKMV
The relationship between ciprofloxacin blood-levels, prophylaxis failure and death among patients with allogeneic stem-cell transplantation – A single centre observational prospective study
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Background: Ciprofloxacin (CIP) is applied during prophylaxis of patients undergoing allogeneic stem-cell transplantation (alloSCT). We aimed at investigating the relationships between CIP blood-levels, (suspected) prophylaxis failure and death during a patients' stay at the alloSCT-unit.

Materials/Methods: A single-center, observational study was performed. CIP was administered (q12) either orally (500 mg) or by intra-venous infusion (400 mg). CIP-treatment was halted upon developing febrile conditions (>38.2 °C) suspecting infection, requiring alternative antibiotic therapy (tshift) and blood cultures to be ordered (BC). We thus used tshift as an indicator of prophylaxis failure. CIP-Blood-samples were usually drawn at three time-points (triplets): 1h, 6h after the first daily-dose and before next-dose. Samples were collected after initial administration plus every following Monday and Thursday. CIP-levels were measured using a HPLC-protocol. Triplet CIP-AUC0-24h (AUC) and average patient CIP-AUC (avAUC) were calculated in mg•h/l using the linear trapezoid method. Achieving prophylaxic CIP-levels was assumed at AUC/MIC ≥ 72 (MIC = 0.5, ECOFF for *Pseudomonas aeruginosa*). The study received ethics approval by the local committee (file no. 15/10/2017).

Results: 63 patients were included (90% of enrolled, Figure 1) and 316 triplets obtained. While 78% of all triplets failed to achieve prophylaxic levels, a broad range of intra-patient AUC variability was observed (Figure 2). 54 patients required tshift. Comparing group-medians, gender-specific phenomena were observed as avAUC were higher among females (p=0.001), who displayed lower odds to achieve low (< median) avAUC (OR=0.24 [0.08-0.69]-CI95%). Seven patients died (11.1%) and their avAUC did not differ from those of survivors (p>0.1). Patients with tshift had a 13% [4%-22%]-CI95% lower probability to survive compared to those without, while the avAUC did not differ between patients with and without tshift (p>0.1). However, triplets that occurred ultimately before tshift/ BC displayed higher odds for low AUC (OR=2.08; [1.13-3.83]-CI95%). This value dropped to 1.55 [0.95-2.54]-CI95% when the penultimate triplets were added to analysis.

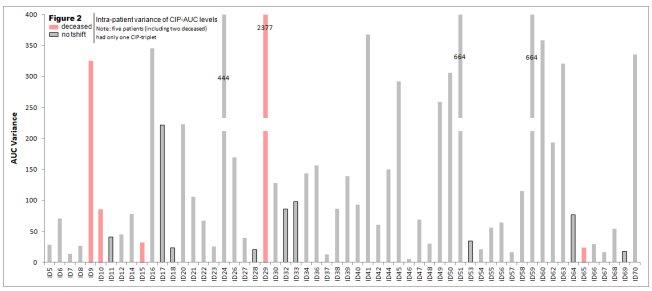
Conclusions: Our study describes the relationships between CIP-levels, prophylaxis failure and death-risk among alloSCT-patients. While no coherent conclusions could be drawn from patient-level data, triplet-level analysis hints at lower CIP-levels at the onset of suspected infection, a surrogate of prophylaxis failure. The onset of suspected infection was in turn associated with poorer patient outcome. Therefore, monitoring CIP-levels might be of advantage, especially since different levels of intra-patient variability were observed. Further analysis is needed to investigate whether any causality between low CIP-levels and patient-outcome exists. Depending on this, current dosing-strategies might require adapting CIP-prophylaxis to patient characteristics.

Fig. 1

Figure 1 Descriptive Statistics of patient characteristics.

Parameter	
Females	27/63 (43%)
Age [years] range (24 - 78)	58.6 ± 12.3
BMI [kg/m²]	26.7 ± 5.2
LOS [days]	35 ± 10
No. CIP episodes per patient	1.8 ± 0.6
Pat. with positive blood cultures	26/63 (41%)
Positive blood cultures	80/167 (48%)

Fig. 2



065/DKMV

***Staphylococcus aureus* nasal colonisation among dental health care workers in Northern Germany (StaphDent study)**

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) can colonize dental patients and students, however, studies on the prevalence of MRSA and methicillin-susceptible *S. aureus* (MSSA) among dental health care workers (DHCW) including use of personal protective equipment (PPE) are scarce. We conducted an observational study (StaphDent study) to (I) determine the prevalence of MRSA and MSSA colonization in DHCW in the region of Mecklenburg Western-Pomerania, Germany, (II) resolve the MSSA population structure to gain hints on possible transmission events between co-workers, and (III) clarify use of PPE.

Methods: Nasal swabs were obtained from dentists (n=149), dental assistants (n=297) and other dental practice staff (n=38). Clonal relatedness of MSSA isolates was investigated using *spa* typing and, in some cases, whole genome sequencing (WGS). PPE use was assessed by questionnaire.

Results: While 22.3% (108/485) of the participants were colonized with MSSA, MRSA was not detected. MSSA prevalence was not associated with size of dental practices, gender, age, or duration of employment. The identified 61 *spa* types grouped into 17 clonal complexes and four sequence types. Most *spa* types (n=51) were only identified once. In ten dental practices, a specific *spa* type occurred twice. WGS data analysis confirmed a close clonal relationship for 4 of these 10 isolate pairs. PPE was regularly used by most dentists and assistants.

Conclusions: To conclude, the failure to recover MRSA from DHCW reflects the low MRSA prevalence in this region. Widespread PPE use suggests adherence to routine hygiene protocols. Compared to other much higher regional HCW MRSA rates the absence of MRSA in DHCW indicates that consequent usage of PPE is protective.

066/DKMV

The mosaic mtr locus as major genetic determinant of azithromycin resistance of Neisseria gonorrhoeae, Germany, 2018

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Introduction: Azithromycin resistant Neisseria gonorrhoeae (NG) isolates increased from 4.3% in 2016 to 9.2% in 2018 within the German Gonococcal Resistance Network (GORENET) NG sample collection. Using whole genome sequencing (WGS) of NG isolates in combination with clinical and epidemiological data, we aim to understand this observed increase.

Methods: GORENET was set up in 2013 as a laboratory network to monitor NG infections in Germany by collection of NG isolates, epidemiological and clinical data. In 2018, isolates with reduced susceptibility to azithromycin (MIC ≥ 0.25 mg/L) were analyzed by WGS followed by assignment of sequence types based on NG multiantigen sequence typing (NG-MAST) and multilocus sequence typing (MLST), detection of antimicrobial resistance determinants and generation of a core SNP distance-based neighbor-joining phylogenetic tree. Comparison with published isolates was performed based on a custom ad-hoc cgMLST scheme and calculation of a minimum spanning tree.

Results: Whole genome phylogenetic analyses resulted in 4 major clades corresponding to NG-MAST genogroups G2400, G3779 (G1407), G5441 and G12302. The clade comprising G12302 accounted for the majority (60.5%, 23/38) of isolates with azithromycin resistance (MIC > 0.5 mg/L) and was characterized by the presence of the recently described Neisseria lactamica-like mosaic mtr locus. In addition, strains in this clade were significantly associated with rectal infection site (17.4% (4/23) vs. 3.8% (4/105), $p< 0.05$) and younger age (median age: 27 years [IQR 23-33] vs. 36 years [IQR: 27-46], $p<0.05$). Comparison with published isolates revealed similarity between a US and a German isolate of MLST ST9363 (12 nucleotides difference) and between a US and a German isolate of MLST ST11422 (21 nucleotides difference).

Discussion: Our data indicate the recently observed increase in isolates resistant to azithromycin in Germany coincides with clonal expansion of NG-MAST genogroup G12302 and suggest that, together with horizontal gene transfer of resistance determinants and well-established point mutations, international spread of resistant lineages plays a major role regarding azithromycin resistance in Germany.

067/DKMV

The conjugative relaxase TraA – a key enzyme for pIP501 transfer

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Introduction: Misuse and overuse of antibiotics have led to an increasing dissemination of multi-resistant bacteria over the last decades. Horizontal gene transfer drives the spread of antibiotic resistances with conjugative plasmids as the major players in this process. Gram-positive (Gram+) conjugative plasmids which are members of the incompatibility (Inc) 18 group are frequently detected in streptococci and enterococci. Inc18-type plasmids are broad-host-range plasmids encoding for several antibiotic resistance genes. Given that plasmid-encoded transfer genes enable conjugative transfer also among unrelated bacteria makes the study of these mobile genetic elements even more interesting in order to combat the spread of multi-resistant bacteria. The conjugative Inc18 plasmid pIP501 from *Streptococcus agalactiae* serves as a model to examine conjugative transfer mechanisms in Gram+ bacteria. pIP501 transfer is mediated by a plasmid-encoded Type IV Secretion System (T4SS) which enables the translocation of single-stranded DNA from a donor to a recipient cell across the bacterial envelope. Protein key players must be elucidated to better understand T4SS-dependent transfer mechanisms. The relaxase TraA of pIP501 is the first-encoded protein on the pIP501 transfer (*tra*) operon. The enzyme is essential for the binding and cleaving of single-stranded DNA prior to conjugative transfer and tunes early steps of *tra*-operon expression, presumably together with the small soluble protein TraN TraA can be selected as a prime target for conjugative transfer inhibition because of its central and conserved role in all conjugative systems.

Materials and Methods: A markerless *traA* gene knockout was generated in pIP501 to investigate its role in conjugative pIP501 transfer by *in vivo* biparental mating assays. To exclude polar effects on downstream *tra* genes of the *tra*-operon, the deletion mutant was complemented with the wildtype *traA* gene. TraA exhibits a two-domain structure where the relaxase function was predicted to reside in the N-terminal domain, the function of the C-terminal domain is still unknown. Two complementation variants containing one of the respective domains were constructed to partially complement the *traA* deletion mutant.

Results: Biparental mating assays revealed that TraA is essential for pIP501 transfer as no transfer was detected with the pIP501Δ*traA* deletion mutant as donor. The transfer rate was below the detection limit of the assay of 4.4 × 10-8 transconjugants per recipient. Complementation of the *traA* deletion strain with the *traA* wildtype gene showed full recovery of transfer capacity. Complementation with respective *traA* domains revealed no pIP501 transfer. Hence, none of the domains are active alone *in vivo*. Further *in vivo* studies with TraA are in progress to define the role of the TraA C-terminal domain and to elucidate possible protein-protein interactions between the two regulatory T4SS proteins, TraA and TraN.

068/DKMV

Identification of the novel intrinsic *Acinetobacter*-derived cephalosporinase ADC-194 and oxacillinase OXA-822 from *Acinetobacter calcoaceticus*

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Introduction: Bacteria of the *Acinetobacter calcoaceticus-baumannii* complex have the ability to acquire an extended antibiotic resistance. Here, we characterized the novel *Acinetobacter*-derived cephalosporinase ADC-194 and the novel intrinsic oxacillinase OXA-822 from a carbapenem-resistent *Acinetobacter calcoaceticus* clinical isolate.

Material/Methods: Species affiliation was carried out employing MALDI-TOF MS, typing of house keeping genes (16S rDNA, *rpoB*, *recA*, *blaOXA-213-like*) and whole genome sequencing (WGS) with subsequent phylogenetic analyses. Antibiotic susceptibility was evaluated by broth micro dilution. The genes encoding for ADC-194 and OXA-822 were cloned with its putative promotor into the expression vector pMW, and the impact on antimicrobial susceptibility was determined in *Escherichia coli*, *A. calcoaceticus* and *A. baumannii*. OXA-822 was purified employing affinity chromatography and gel filtration and enzyme kinetics were measured using spectrometry.

Results: Species identification revealed the clinical isolate AC38 as *A. calcoaceticus* as determined by WGS and phylogenetic analyses as standard methods for identification failed. AC38 harbored the novel Ambler class D resistance gene encoding OXA-822, which belongs to the intrinsic OXA-213-family. Phylogenetic analyses of the 63 variants of the intrinsic OXA-213-family showed a bifurcation into two subgroups, whereas one was associated to *Acinetobacter pittii* and the second to *A. calcoaceticus* which does not allow a distinct identification. Analyses of resistome of AC38 revealed the novel intrinsic gene encoding for the Ambler class C beta-lactamase ADC-194.

Presence of ADC-194 led to increased MICs for ampicillin in all tested species and elevated MICs for penicillins and cephalosporins especially in *A. baumannii*. In contrast presence of OXA-822 resulted in reduced susceptibility only to carbapenems. Purified OXA-822 displayed the highest substrate affinities for carbapenems. For imipenem a 10-fold reduced affinity was measured compared to other carbapenems. Molecular modelling indicated the absence of an interaction of the negatively charged side chain of OXA-822 with imipenem.
Discussion: We characterized the clinical isolate AC38 as *A. calcoaceticus* and identified the novel intrinsic beta-lactamases ADC-194 and OXA-822. Presence of ADC-194 conferred a high level resistance against penicillins and cephalosporins in *A. baumannii,* whereas OXA-822 elevated MICs species dependent for carbapenems. The purified enzyme OXA-822 showed penicillinase and carbapenemase activity. Rapid species identification as well as detection of novel beta-lactamases of clinical isolates are crucial in microbiological diagnostics and standard protocols need to be consistently adapted.

069/DKMV
Investigation of minimal bactericidal concentrations of sodium bituminosulfonate for *Staphylococcus aureus*
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Introduction: Resistance of bacteria to antibiotics is steadily increasing. Therefore, and in view of limited development pipelines, alternative substances are being tested for their antibacterial effectiveness. A possible use of old substances for this new indication is an important concept for combating antibiotic-resistant bacteria. Sodium bituminosulfonate, which is the active ingredient derived from the sulfur-rich oil shale and widely known as Ichthyol, may represent such an alternative antimicrobial agent. In this study, we investigated the *in vitro* bactericidal activity of sodium bituminosulfonate against *Staphylococcus aureus* as determined by minimal bactericidal concentrations (MBC).
Material/Method: According to the recommendation of CLSI, minimal inhibitory concentrations (MIC) and MBC of sodium bituminosulfonate were determined for 20 methicillin-susceptible (MSSA) and 20 methicillin-resistant (MRSA) consecutive clinical isolates of *S. aureus*. Only one isolate per patient was eligible. For MIC investigation, the broth microdilution method was used. Sodium bituminosulfonate was tested in double dilution concentration steps ranging from 0.03 g/L to 256 g/L. For determination of MBC, 10 µl samples were subcultured from the clear wells and colonies were counted after overnight incubation. Based on the initial inoculum, cell reduction of at least 99.9% was considered as MBC. All MIC and MBC determinations were performed in triplicate and median value was calculated for analysis.
Results: A total of 40 *S. aureus* strains were included for MIC and MBC determination of sodium bituminosulfonate. For MSSA, MIC_{50}, MIC_{90} and MIC range were 0.125 g/L, 0.25 g/L and 0.06 – 0.25 g/L, respectively; MBC_{50}, MBC_{90} and MBC range amounted to 0.5 g/L, 1.0 g/L and 0.125 – 1.0 g/L; $(MBC/MIC\ ratio)_{50}$, $(MBC/MIC\ ratio)_{90}$ and the range of the MBC/MIC ratio were 4, 4 and 1-8. Among MRSA, MIC_{50}, MIC_{90} and MIC range were 0.125 g/L, 0.25 g/L and 0.06 – 0.25 g/L, respectively; MBC_{50}, MBC_{90} and MBC range were 0.5 g/L, 1.0 g/L and 0.06 – 1.0 g/L; $(MBC/MIC\ ratio)_{50}$, $(MBC/MIC\ ratio)_{90}$ and the range of the MBC/MIC ratio were 4, 4 and 1-8, respectively.
Discussion: For sodium bituminosulfonate, bactericidal activities against MSSA and MRSA were demonstrated on a representative collection of clinical isolates. The encouraging results warrant further detailed characterization by time-kill kinetics and an extension of the MBC investigations to other pathogens.

Workshop 14
Gastrointestinal Zoonotic Diseases (FG ZO/FG GI)
14. Sep. 2021 • 10:00–11:00

070/MPV
Investigating the role of tetraspanin CD81 in *Salmonella* pathogenesis
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Salmonella is a Gram-negative, rod-shaped facultative intracellular pathogen that causes gastroenteritis or enteric fever and represents public health risk worldwide. Following an oral route of infection, the bacteria eventually reach the small intestine through which they can come into contact with the host epithelial cells. Adhesion and invasion of the intestinal epithelium is a critical step from which further cellular pathogenesis takes place. Tetraspanins are a group of transmembrane proteins with a canonical structure of four transmembrane domains and two protruding extracellular loop domains, which have been implicated to participate in various biological roles. They associate with other tetraspanins and proteins through primary and secondary interactions which, in recent years, have been linked to play a role in facilitating the infection processes of microbial pathogens (i.e. as adhesion platforms, receptor signaling platforms, etc.). In this direction, we explore the role of tetraspanins (in particular, human CD81) and their contribution to *Salmonella* invasion of host cells. We demonstrate, that overexpression of hCD81 in hepatoma cells (HepG2) enhanced the adhesion and invasion of both wild-type typhoidal and non-typhoidal *Salmonella* strains. Furthermore, knock-out of hCD81 in intestinal epithelial cells, Caco2-Bbe1, and HT29-MTX cells by CRISPR technique led to a significant reduction in *Salmonella* invasion and replication. In addition, we show that incubation of wild-type host cells with synthetic peptides derived from the large extracellular loop of human hCD81 further increased the invasion of *Salmonella* to host cells. Imaging studies provided insights into the localization of tetraspanin clusters and show that hCD81 is co-recruited along with actin ruffles of active *Salmonella* invasion sites. Interestingly, its association seemed to be abrogated by inhibiting actin ruffling. Furthermore, we provide evidence that this effect is species-specific, as overexpression of rat CD81 in HepG2 cells does not facilitate an increase in invasion and replication of *Salmonella,* like that of overexpression of hCD81. Which host and bacterial factors further contribute to this process are still under investigation, as candidate host proteins and bacterial effectors will be explored accordingly.

071/ZOGIV
Resveratrol and curcumin prevent *Campylobacter jejuni*-induced epithelial barrier dysfunction in models for the leaky gut
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Introduction: The zoonotic *Campylobacter jejuni* infection is the most common cause for foodborne gastroenteritis worldwide and a serious health problem. The aim of the study was to characterize the barrier-protective and anti-inflammatory properties of the polyphenols resveratrol and curcumin to treat *C. jejuni* infections.
Methods: Beside the monoculture of human intestinal epithelial HT-29/B6 cells, we additionally established a co-culture consisting of HT-29/B6 monolayers together with basolateral localized THP-1 immune cells. This co-culture considers direct epithelial and/or immune-mediated barrier defects resulting from the infection. Moreover, depending on the filter pore size of the cell monolayers and the primary infection side (basal versus apical), indirect bacterial effects can be distinguished from direct host cell interactions. For validation, germ-free IL-10-/- mice were orally infected with *C. jejuni* and treated with either resveratrol or

curcumin. Murine colon samples were mounted into Ussing chambers for functional electrophysiological measurements. Barrier function was determined by measuring changes in transepithelial electrical resistance (TER) and permeability for marker molecules like fluorescein (332 Da).

Results: *C. jejuni* caused a barrier disturbance in the *in vitro* models and in the *in vivo* model, indicated by a decrease in TER and an increase in permeability to fluorescein. *In vitro,* further barrier compromising mechanisms were identified for tight junction (TJ) protein expression changes, especially of the barrier-forming TJ protein claudin-1 and the pore-forming TJ protein claudin-2. Moreover, localization changes of TJ proteins as claudin-4 and -8 and epithelial apoptosis induction significantly contributed to the barrier dysfunction. Co-culture studies showed the contribution of the immune response in the development of the barrier disturbance due to increased pro-inflammatory cytokine secretion of TNF-α, IL-1β, and IL-6. All mechanisms together allow the entry of antigens into the epithelium and subsequently into the submucosa, leading to the leak flux type of diarrhea. Both compounds inhibited the *C. jejuni*-induced barrier defects indicated by an increased TER and a decreased fluorescein permeability. Also, the TJ protein expression and the subcellular redistribution of TJ proteins was restored. The apoptotic ratio diminished after the treatment with resveratrol and curcumin. However, resveratrol acts immune-modulating, whereas curcumin showed rather direct anti-inflammatory properties.

Conclusions: *In vitro* in the epithelial monoculture or in the co-culture containing THP-1 immune cells as well as *in vivo* in the IL-10-/- mouse model, the tested polyphenols resveratrol and curcumin showed effective barrier-protective, anti-inflammatory, immune-modulating, and anti-apoptotic action, although with a different mechanism. Thus, the phytopharmaceuticals may be useful therapeutic agents in campylobacteriosis.

072/ZOGIV

Resveratrol alleviates acute *Campylobacter jejuni* induced enterocolitis in a preclinical murine intervention study

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Question: The polyphenolic compound resveratrol has been shown to exert health-beneficial properties. Given globally emerging *Campylobacter* infections in humans, we addressed potential anti-pathogenic, immuno-modulatory and intestinal epithelial barrier preserving properties of synthetic resveratrol in the present preclinical intervention study applying a murine acute campylobacteriosis model.

Methods: Two days following peroral *C. jejuni* infection, secondary abiotic IL-10−/− mice were either subjected to resveratrol or placebo via the drinking water. Treatment with synthetic resveratrol started two days after the initial *C. jejuni* infection. Resveratrol was dissolved in 2% carboxy-methyl-cellulose (to a final concentration of 0.05%) and provided to mice by adding the solution to the autoclaved tap water. The final concentration of the resveratrol solution was 300 mg/L (resulting dose of 60 mg per kg body weight per day). Placebo control animals received vehicle only.

Results: Whereas placebo mice suffered from acute enterocolitis at day 6 post-infection, resveratrol treatment did not only lead to improved clinical conditions, but also to less pronounced colonic epithelial apoptosis as compared to placebo application. Furthermore, *C. jejuni* induced innate and adaptive immune cell responses were dampened in the large intestines upon resveratrol challenge and accompanied by less colonic nitric oxide secretion in the resveratrol versus the placebo cohort. Functional analyses revealed that resveratrol treatment could effectively rescue colonic epithelial barrier function in *C. jejuni* infected mice. Strikingly, the disease-alleviating effects of resveratrol could additionally be found in extra-intestinal and also systemic compartments at day 6 post-infection.

Cpnclusion: For the first time, our current preclinical intervention study provides evidence that peroral resveratrol treatment exerts potent disease-alleviating effects during acute experimental campylobacteriosis.

073/ZOGIV

Beyond Shiga toxin production – the Stx2 phage modulates *E. coli* O104:H4 gene expression and metabolism

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Question: Shiga toxin (Stx)-encoding phages can infect, lysogenize and convert susceptible bacteria to Stx producers, thus either transforming commensal strains into pathogens or increasing the virulence of already pathogenic bacterial hosts. In addition, there is growing evidence that Stx phages modulate bacterial host gene expression. We recently demonstrated that Stx2 phage carriage has a profound impact on transcription in the commensal *E. coli* K-12 strain MG1655. Among other effects, Stx phage carriage by this strain causes a significant decrease in the ability to assimilate gluconeogenic substrates. Here, we further investigated the Stx2 phage-dependent effects on gene expression and phenotype in the exceptionally pathogenic 2011 *E. coli* O104:H4 outbreak strain.

Methods: RNA-seq was performed with total RNA from wild type *E. coli* O104:H4 and from the Stx2 phage cured derivative (*E. coli* O104:H4 fcu) cells grown in LB medium at 37°C to mid-log phase. Directional cDNA libraries were sequenced on an Illumina platform. Raw data were processed using READemption and differences in the gene expression were determined by DESeq2. Protein-encoding genes homologous to *E. coli* K-12 MG1655 were analysed using the STRING database of protein-protein interactions. Phenotype microarray assays were performed with BIOLOG PM1 Carbon Sources plate.

Results: On average 11 million reads were sequenced per library and at least 96% of them were mapped to the reference genome of *E. coli* O104:H4. DESeq2 analysis revealed 466 upregulated and 356 downregulated genes in wild type *E. coli* O104:H4 in comparison to its Stx2 phage-cured derivative. Further analysis using the STRING database of protein-protein interaction revealed that the majority of genes upregulated by the Stx2 phage carriage in *E. coli* O104:H4 were involved in metabolic pathways, e.g. amino acid metabolism, TCA cycle, gluconeogenesis, while genes encoding proteins of biosynthesis pathways were found to be downregulated. Indeed, BIOLOG phenotype microarray experiments revealed that these changes in the gene expression were reflected by enhanced respiration of *E. coli* O104:H4 wild type when compared to the Stx2 phage negative derivate with 36 of the 95 tested substrates. In particular, the carriage of Stx2 phage resulted in the significant increase in the assimilation of gluconeogenic and TCA cycle substrates, e.g. amino acids, carboxylic and dicarboxylic acids, nucleosides.

Conclusions: Our study revealed a profound impact of the Stx2-encoding phage carriage on *E. coli* O104:H4 gene expression. Phenotypically we found above all an enhanced respiration with gluconeogenic and TCA cycle substrates. Interestingly, this is the opposite of what we previously observed in *E. coli* MG1655. Thus, the effect of Stx2 phage carriage on metabolism appears to be strongly dependent on the genetic background of the bacterial host.

074/ZOGIV

A new recombineering system for the efficient genetic manipulation of enterohemorrhagic *Escherichia coli*

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) are Shiga Toxin (Stx) encoding *Escherichia coli* (*E. coli*) that can cause the life-threatening hemolytic-uremic syndrome. EHEC have caused large outbreaks of foodborne infections in the past and continue to pose a threat, especially to children and elderly. A causative therapy for EHEC infections is currently not available as the use of antibiotics is still controversial. A prerequisite for the development of new therapeutic strategies to cope with this dangerous zoonotic pathogen is the establishment of efficient methods to manipulate its genome. However, in comparison to other *E. coli*, EHEC are particularly resistant to the genetic manipulation with classical recombineering tools. Even though some empirical protocols have already been established, the successful genetic manipulation is often still sporadic. The mutagenesis of EHEC can be therefore time consuming and frustrating. We hypothesized here that the use of classical protocols may result in phage activation and subsequent cell lysis, which could explain the overall low efficiency of these recombineering tools. Therefore, we have developed a new recombineering system based on the inhibition of the phage activator protein RecA and could demonstrate that it is reproducibly superior to classical methods.
Methods: We have constructed a derivative of the Datsenko and Wanner plasmid pKD46 that co-expresses the RecA inhibitor RecX. We systematically compared the efficiency of the plasmid pKD46recX in the genetic manipulation of three genetic loci to pKD46 in EHEC O157:H7 EDL933 Δ*stx1/stx2* and *E. coli* K-12 MG1655. In addition, we tested the plasmids in a SOS-response reporter strain.
Results: We show that when compared to pKD46 the use of pKD46recX increased significantly the recombination efficiency in EHEC O157:H7 EDL933 Δ*stx1/stx2*, but not in *E. coli* K-12 MG1655. We also have already used pKD46*recX* successfully to genetically modify EHEC O157:H7 and EHEC O104:H4 wild type strains. However, the plasmid was not superior to pKD46 in the inhibition of a ciprofloxacin induced SOS-response in *E. coli* K-12 MG1655.
Discussion: We have developed a new recombineering system that is superior to classical methods in EHEC. The exact mechanism that resulted in the improvement of recombination efficiency remains so far elusive, as the co-expression of RecX together with the recombination enzymes was apparently not further repressing SOS-induction and therefore eventually phage activation. However, the system was applied already repeatedly and successfully in otherwise hard to manipulate EHEC wild type strains.

075/ZOV

Genome-wide association study revealed host-adaptive genes in *E. coli*.

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Background: *Escherichia coli* is an opportunistic pathogen that can colonize or infects various host species. However, there is a significant gap in our understanding regarding whether lineages of *E. coli* are adapted/restricted to certain hosts. Furthermore, genomic determinants underlying this are also unknown.
Methods: 1,198 *E. coli* strains were isolated from five host-species (human, cattle, chicken, pig, and wild boar) between 2003 and 2018, comprising both healthy and diseased hosts from four countries (Germany, Spain, UK, and Vietnam). Genes associated with each host were identified by applying a microbial genome-wide association study using PySEER.
Results: We found enrichment of phylogroups A within pig, B1 within cattle, B2 within human and chicken, and G within chicken isolates in our collection. Certain Sequence types, such as ST95, ST117 were enriched within strains from the chicken host, and ST131 was enriched within human strains. A novel cluster of *nan* genes in combination with *axeA* genes, that were characterized *in-silico* and predicted to be involved in sialic acid (Sia) metabolism, was associated with human hosts. *In-vitro* analysis, using the Δ*nan/axeA* mutant strain showed impaired growth when only sialic acid (Neu5Ac) or mucus (containing various sialic acids) was available as a carbon source. On the other hand, an association of homologs of the omptin family of proteases with cattle and chicken hosts was enlightening.
Conclusions: We identified genes in the *E. coli* genus associated with the colonization of human, cattle, and chicken but not with pig hosts. Our key finding, a novel *nan* gene cluster in combination with *axeA* genes was mainly found in *ExPEC* lineages. Our *in-vitro* analysis indicated that these genes might be involved in catabolizing sialic acid. The omptin family of proteases was reported to be involved in resistance against different host secreted antimicrobial peptides. Taken together, our findings provide an exhaustive overview of genetic determinants of host specificity in *E. coli*, which could be useful for risk analysis and diagnostic/monitoring purposes or predicting and preventing the spread of antimicrobial-resistant *E. coli*.

Workshop 15
Molecular Infection Epidemiology and Pathogen Genome-Based Prediction of Antibiotic Resistence (FG MS)
14. Sep. 2021 • 10:00–11:00

076/MSV
Transmission of pre-XDR/XDR-TB in the Mumbai metropolitan region, India

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Background: Multidrug-resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* complex (MTBC) strains are a great challenge for tuberculosis (TB) control in India. Still, factors driving the MDR/XDR epidemic in India are not well defined.
Methods: Whole genome sequencing (WGS) data from 1 852 MTBC strains obtained from patients from a tertiary care hospital laboratory in Mumbai were used for phylogenetic strain classification, resistance prediction, and cluster analysis (12 allele distance threshold). Factors associated with pre-XDR/XDR-TB were defined by odds ratios and a multivariate logistic regression model.
Results: 1 017 MTBC strains were MDR, out of which 69.2 % (n=704) were pre-XDR, and 4.4 % (n=45) were XDR. Lineage 2 (L2) strains represented 45.1 % of the MDR, 79.9 % of the pre-XDR, and 66.7 % of the XDR strains, and were significantly associated with pre-XDR/XDR-TB ($P < 0.001$). Cluster rates were high among MDR (57.8 %) and pre-XDR/XDR (79 %) strains with three dominant L2 strain clusters (Cl 1-3) representing 54.1 % of the pre-XDR and 42.2 % of the XDR-TB cases. Cl 1 strains accounted for 28.6 % of the pre-XDR/XDR MTBC strains. Transmission could be confirmed by identical mutation patterns of particular pre-XDR/XDR strains.
Conclusions: High rates of pre-XDR/XDR strains among MDR-TB patients require rapid changes in treatment and control strategies. Transmission of particular pre-XDR/XDR L2 strains is the main driver of the pre-XDR/XDR-TB epidemic. Accordingly, control of the epidemic in the region requires measures with stopping transmission esp. of pre-XDR/XDR L2 strains.

077/MSV
PHIMS-TB: Implementation of public health integrated molecular tuberculosis surveillance in Germany

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Introduction: Tuberculosis (TB) control measures require in-depth epidemiological data e.g. obtained by integrated molecular surveillance (IMS) systems to be effective even in low incidence countries. IMS should effectively combine high resolution strain typing by next generation sequencing (NGS) methods with epidemiological data from routine TB notifications. This combination allows for detailed analysis of transmission within the population, and in turn for development of efficient intervention and control policies. To establish a nationwide generic approach in Germany, the Robert Koch Institute (RKI) and the Research Center Borstel (RCB) started the PHIMS-TB project to develop and implement the necessary workflows and structures allowing evaluation of the impact of country-wide molecular tuberculosis surveillance in Germany.
Material and Methods: *Mycobacterium tuberculosis* complex (Mtbc) strains obtained from TB patients living in Germany have been sent to the RCB for NGS, and, partially, conventional diagnostics. NGS data were analyzed with standard pipelines (resistance prediction, phylogenetic classification, cluster analysis) and results will be linked with epidemiological TB notification data at the RKI.
Results: In the pilot phase of the study, we analyzed Mtbc strains from a third of German culture positive TB cases notified in 2020. The majority (64%) of strains belonged to lineage 4, followed by strains of lineages 3, 2, and 1. Most Mtbc strains (78%) were found to be likely fully sensitive against antibiotics employed in TB treatment. The proportion of resistant Mtbc strains was remarkably higher for lineage 2 strains. Overall, 39% of strains investigated were grouped in clusters, with clustering rates being higher both for resistant and lineage 2 strains.
Discussion: Country wide TB surveillance potentially allows for rapid and comprehensive detection of transmission networks and outbreaks, and high-resolution measurement of resistance levels. Optimal communication channels with local health authorities are essential and expected to facilitate fast and targeted contact tracing. Rapid NGS analysis may also support patient care especially of drug resistant TB patients.

078/MSV
Molecular surveillance of listeriosis in Germany: Identification of large listeriosis outbreaks by whole genome sequencing

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Introduction: *Listeria monocytogenes* causes foodborne infections with high mortality. The majority of cases are either sporadic or occur in small disease clusters. However, large outbreaks may occasionally occur, leading to a significant burden of disease.
Materials and Methods: *L. monocytogenes* isolates from approximately two-thirds of all mandatorily notified German listeriosis cases are sent to the Consultant Laboratory for *L. monocytogenes* at the Robert Koch Institute, which runs a subtyping program for identification of listeriosis outbreak clusters (1). Whole genome sequencing (WGS) and core genome multi locus sequence typing (cgMLST) were used for subtyping of more than 2,500 human *L. monocytogenes* isolates collected in Germany between 2015 and 2021. Presumable food vehicles for listeriosis infections were identified through matching of cgMLST subtyping results from human isolates and food isolates collected by the National Reference Laboratory at the German Federal Institute for Risk Assessment. For selected cgMLST clusters, patients were interviewed on their food consumption habits to add epidemiological evidence.
Results: Two thirds of all human isolates grouped into more than 220 different cgMLST clusters. Among them were several large clusters (2-4) including one with 112 cases (4), which has been among the largest European listeriosis outbreaks for more than 25 years. For some clusters, case-control studies and WGS-typing of isolates from suspected food sources provided the key for identification of the source of infection.
Discussion: Our work illustrates the importance and suitability of a comprehensive WGS-based national surveillance system comprising human and food isolates in order to stop and to prevent listeriosis outbreaks.
1 - Halbedel *et al. J Clin Microbiol.* 2018 May 25;56(6):e00119-18.

2 - Lachmann *et al. Clin Microbiol Infect.* 2020 Sep 23:S1198-743X(20)30572-3.
3 - Lüth et al. *Emerg Microbes Infect.* 2020 Dec;9(1):1600-1608.
4 - Halbedel *et al. Emerg Infect Dis.* 2020 Jul;26(7):1456-1464.

079/MSV

Context-aware genomic surveillance reveals hidden transmission of a carbapenemase-producing *Klebsiella pneumoniae*

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Genomic surveillance can inform effective public health responses to pathogen outbreaks. However, integration of non-local data is rarely done. We investigate two large hospital outbreaks of a carbapenemase-carrying *Klebsiella pneumoniae* strain and show the value of contextual data. By screening more than ten thousand genomes, 500 thousand metagenomes, and two culture collections using *in silico* and *in vitro* methods, we identify a total of 431 closely related genomes partly reported in 28 studies. We identify the relationship between the two outbreaks through time-dated phylogeny, including their respective origin. One of the outbreaks presents extensive hidden transmission, with descendant isolates only identified in other studies. We then leverage the genome collection from this meta-analysis to identify genes under positive selection. We thereby identify an inner membrane transporter (*ynjC*) with a putative role in colistin resistance. Contextual data from other sources can thus enhance local genomic surveillance at multiple levels and should be integrated by default when available.

Fig. 1

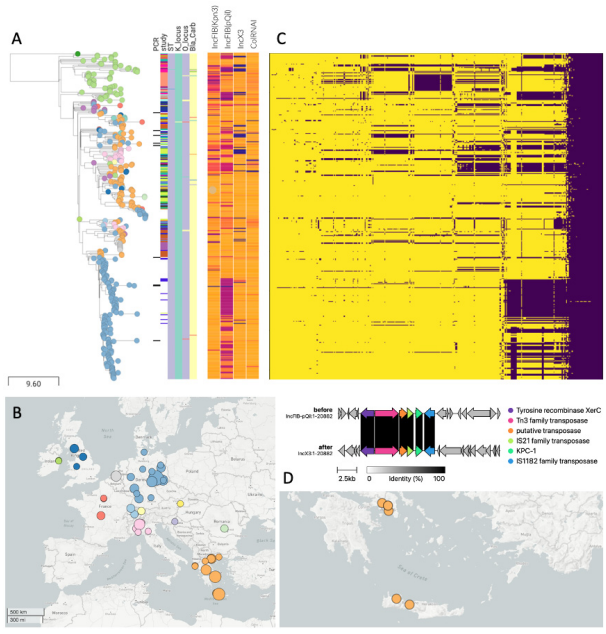
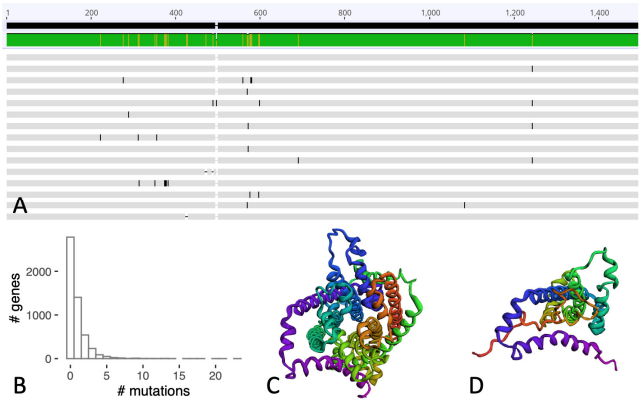


Fig. 2



080/MSV

Mutations in the *gdpP* gene are a clinically relevant mechanism for β-lactam resistance in methicillin resistant *Staphylococcus aureus* lacking *mec* determinants

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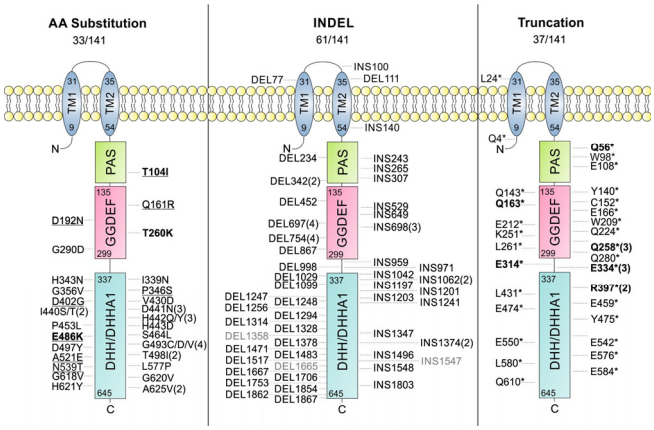
Question: In *Staphylococcus aureus,* resistance to β-lactamase stable β-lactam antibiotics is commonly mediated by the *mecA*-encoded penicillin binding protein 2a (PBP2a) or the *mecC*-encoded PBP2c. However, a continuous increase of methicillin resistant isolates lacking known *mec* genes (MRLM) has been observed during the past years. The MRLM phenotype has been previously associated with a hyperproduction of *blaZ*-encoded β-lactamase, mutations in native PBPs leading to reduced affinity to β-lactams or increased peptidoglycan crosslinking due to overexpression of PBP4. In this study, we used comparative genomics as well as genome wide association (GWAS) approaches to detect genetic polymorphisms associated with the MRLM phenotype in clinical *S. aureus* strains.
Methods: Minimal inhibitory concentrations to oxacillin and cefoxitin were determined by broth microdilution for 141 MRLM isolates and 142 methicillin susceptible controls of the same clonal backgrounds. The absence of *mec* was confirmed via PCR. Absence of β-lactamase hyperproduction was confirmed by oxacillin sulbactam testing. *Spa*-type, multi locus sequence type (MLST) and core genome MLST complex type (cgMLST CT) were deduced from whole-genome sequence data. The microbial pan-GWAS tool SCOARY was applied to link patterns of gene presence or absence with the MRLM phenotype.
Results: SNP-analyses of *pbp*1-4 genes revealed 57 different amino acid substitutions in 44 isolates (31.2 %) that occurred exclusively in MRLM strains. In two additional isolates, deletions in the *pbp4*-promoter were identified, which led to a 17-fold increase in transcription of *pbp4* in one isolate. 131 MRLM isolates (92.9 %) carried mutations in the phosphodiesterase gene *gdpP,* leading to truncations, insertions, deletions and amino acid substitutions in the gene product (Fig.1). GWAS was able to significantly associate these mutations with the MRLM phenotype. Furthermore, a gene enrichment analysis confirmed that the *gdpP* gene had on average 4.6 times more mutations in MRLM strains. Mutations in *gdpP* had no effect on transcription levels of *pbp4*. However, we observed reduced cell sizes as well as decreased growth rates.
Conclusions: We report mutations in *gdpP* as a clinically relevant mechanism for β-lactam resistance in MRLM isolates. This observation is of particular clinical relevance, since MRLM are easily misclassified as MSSA, which may lead to unnoticed spread of β-lactam resistant isolates and subsequent treatment failure.

Fig. 1



081/MSV

The power of SARS-CoV-2 genotyping and SNP-based clustering for contextual outbreak assessment

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Introduction: The COVID-19 pandemic has triggered an unprecedented increase in viral genome sequencing for the purpose of molecular surveillance. Since January 2021 over 150,000 SARS-CoV-2 genomes have been sequenced in Germany and over 1.5 million genomes have been sequenced globally and uploaded to the GISAID EpiCov database[1]. These datasets are ideally suited for outbreak identification but also to enrich and better understand local outbreak events with additional associated sequences. Outbreaks are often detected by grouping together isolates whose pairwise genomic distance is below a threshold, e.g. 21 SNPs to discriminate between hospital clusters of K. pneumoniae[2]. However, alignment-based approaches are computationally costly and impractical given the current amount of data[3]. Also, standard clustering approaches can overcomplicate what could be simple comparisons of mutation profiles between SARS-CoV-2 genomes.
Material and Methods: To rapidly identify putative outbreaks and transmission clusters in this data we developed BREAKFAST, a tool for rapid sequence clustering in the specific context of SARS-CoV-2, and applied it to Germany-wide sequences as well as global sequences from GISAID. Our approach, which instead of computing pairwise alignments, derives transmission clusters from SNP occurrences, is justified by the low mutation rate of SARS-CoV-2 and the absence of complex structural variants. We implemented simple parameters such as the maximum edit distance between two sequences to distinguish clusters that facilitate easy interpretation and decision making.
Results: We show how basic SNP-based clustering methods can be applied to a large pool of genomic sequences. Using pre-computed mutation profiles we can cluster 114,042 sequences in 1.5 minutes using 80 cores and a peak of 1.45 GB of RAM. Beyond the detection of new outbreaks, we can also identify all isolates linked to a known outbreak. By applying our tool to a set of 150,000 sequences from Germany we were able to enrich an outbreak investigation with additional sequences and identify a potential missing link.
Discussion: These results show that simple methods, which leverage a pathogen"s specific properties, can be used in conjunction with large data sets to provide key insights into the ongoing COVID-19 pandemic. We found that it is important to have a method that is fast, with parameters that are easy to understand and communicate. These methods were used to add individuals to known outbreaks, and trigger follow-up epidemiological investigations of transmission clusters.
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Workshop 16
Infection Immunology (FG II)
14. Sep. 2021 • 10:00–11:00

082/IIV

Clinically used antibiotics compromise pulmonary defense against subsequent infection with multidrug resistant *Klebsiella pneumoniae* by disturbing the gut microbiota

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Broad-spectrum antibiotics are widely used in critically ill patients, many of whom develop hospital-acquired pneumonia. Although preceding antimicrobial therapy is known as a major risk factor for infections with multidrug-resistant bacteria, the underlying mechanisms remain incompletely understood. We employed conventionally colonized and microbiota-depleted wild-type and *Ffar2/Ffar3*-/- mice, animals treated with short-chain fatty acids (SCFAs), and mice transplanted with a microbiota derived from human patient donors to study infection with KPC-producing *K. pneumoniae*. Our results showed that microbiota-depleted mice and animals lacking SCFA receptors are more susceptible to *K. pneumoniae* infection then conventionally colonized wild-type mice. Oral treatment of microbiota-depleted mice with acetate or butyrate partly rescued the antibacterial defense in the lung. Moreover, human microbiota transfer experiments indicate that clinical used antibiotics (e.g. Meropenem & Tazobactam) can influence antibacterial defense in the lung, presumably via depletion of SCFA-producing gut microbes. These data suggest that clinically used antibiotics reduce the gut microbiota´s capacity to produce short chain fatty acids and thereby compromise pulmonary defense against subsequent bacterial infection. Ongoing experiments are focusing on the specific microbiota alterations induced by broad-spectrum antibiotics and on the immune cells that are modulated by SCFA depletion.

083/IIV

The impact of *Streptococcus* on host immune responses triggered by *Pseudomonas aeruginosa* infection in the context of cystic fibrosis

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Introduction: Inflammatory responses triggered by *Pseudomonas aeruginosa* infections play an important role in the pathogenesis of a variety of diseases, e.g. cystic fibrosis (CF). Several studies have shown that the airway microbiome in people with CF can either be dominated by one typical CF pathogen, such as *P. aeruginosa*, or show a polymicrobial profile characterized by the prevalence of commensal oropharyngeal bacteria, such as species belonging to the genus *Streptococcus*. In these studies, samples with a one-pathogen dominance profile were associated with stronger inflammation and worsened lung function, whereas samples with a

polymicrobial profile were associated with improved lung function, suggesting that certain commensal bacteria might protect host from pathogen related inflammation. Our previous study with the human epithelial cell line BEAS-2B has shown that a few commensal *Streptococcus* strains isolated from CF airway microbiota may exert inhibotory effects on *P. aeruginosa* triggered inflammation. To understand the mechanism of the inhibitory effects on host immune responses, in the current study, further investigations were performed with *ex-vivo* Precision Cut Lung Slices (PCLS) from mice.

Methods: PCLS from mice were used to study commensal-pathogen-host interactions. Following mono- or co-infection, representative inflammatory markers were assessed at mRNA level by qPCR and at protein level by ELISA and multiplex assay. To achieve more comprehensive information about the impact of *Streptococcus* on host immune responses, we also examined *P. aeruginosa/Streptococcus* interaction-triggered transcriptomic changes in the host with deep RNA sequencing.

Results: The results obtained by ELISA and multiplex assay showed significant reductions of several cytokines/chemokines in *S. mitis/P. aeruginosa* co-infected PCLS compared to *P. aeruginosa* mono-infected samples, and differential expression of the candidate genes was observed with qPCR in *S. mitis/P. aeruginosa* co-infected PCLS compared to *P. aeruginosa* mono-infected samples. With deep RNA sequencing, we compared PCLS transcriptomic changes in response to *P. aeruginosa* mono-infection, *S. mitis* mono-infection and *S. mitis/P. aeruginosa* co-infection, and the results showed that more than 1000 genes were significantly downregulated in co-infection compared to *P. aeruginosa* mono-infection. Functional enrichment analyses indicated that several signalling pathways involved in the pathogenesis of CF were downregulated in co-infection compared to *P. aeruginosa* mono-infection, such as neutrophil extracellular trap formation, mTOR-signalling pathway and Toll-like receptor signalling pathway.

Discussion: The results indicated that selected commensal *Streptococcus* strains may inhibit the inflammatory response triggered by *P. aeruginosa* infection. Moreover, RNA-sequencing provided insights into the underlying mechanism of the inhibitory effects.

084/IIV

TNF is essential for upregulation of DECTIN-2 family C-type lectin receptors on human macrophages after mycobacterial challenge

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Question: Tumour Necrosis Factor (TNF) is an important proinflammatory cytokine. Since TNF contributes to the pathology in several autoimmune diseases, TNF-blockers are used successfully as therapy to inhibit inflammation. However, TNF blockade increases the risk for infections, including the reactivation of latent tuberculosis.

The DECTIN-2 family C-type lectin receptors (CLR) MINCLE, MCL and DECTIN-2 bind several mycobacterial ligands and activate myeloid cells. We recently found that in mice upregulation of DECTIN-2 family CLR and cytokine production triggered by stimulation with mycobacterial cord factor or whole *M. bovis* BCG was impaired in TNF-deficient macrophages. Based on these findings, we investigated whether the TNF-dependence of CLR expression also applies to human myeloid cells.

Methods: Human CD14+ monocytes were obtained from healthy donors and differentiated into macrophages, followed by stimulation with the synthetic cord factor analogue Trehalose-dibehenate (TDB), BCG and TNF for 24h in the presence or absence of the TNF-blocker Etanercept. Expression of DECTIN-1 and of the DECTIN-2 family CLR MINCLE, MCL and DECTIN-2 was analyzed on the mRNA and protein level using qRT-PCR and FACS. In addition, cytokine production was measured by ELISA.

Results: Etanercept significantly inhibited mRNA expression of MINCLE, MCL and DECTIN-2 in response to various stimuli including BCG. In contrast, Etanercept did not reduce DECTIN-1 mRNA expression. Regarding the protein level, only MCL was upregulated after TNF and BCG stimulation, while MINCLE, DECTIN-2 and DECTIN-1 were not significantly affected. Consistent with this pattern, Etanercept inhibited only the MCL protein expression, while MINCLE, DECTIN-2 and DECTIN-1 were not impaired. Moreover, Etanercept downregulated the enhanced cytokine production of IL-8 and CCL4 after stimulation.

Conclusions: These results demonstrate that the essential function role of TNF in upregulation of the DECTIN-2 family CLR and cytokine production is conserved between mouse and human macrophages. In a next step, it should be tested whether TNF-blockade in patients with chronic inflammatory diseases causes downregulation of myeloid CLR expression *in vivo*. If so, impaired CLR expression could contribute to suboptimal detection and immune responses against mycobacteria in patients receiving TNF-blockers.

085/IIV

The role of mitochondrial modulation in innate immunity and tumorigenesis during *Helicobacter pylori* infection

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Introduction: *Helicobacter pylori* frequently colonizes the human stomach and is the major risk factor for stomach adenocarcinoma. To date, pathophysiologic mechanisms remain unclear in substantial parts. Many environmental stressors including infections may affect mitochondrial function and structure, have short- and long-term effects on mitochondrial and host physiological processes and can lead to various pathologies. Previous studies have shown DNA-damage-induction by *H. pylori in vitro* and affection of mitochondrial functions and structure. We hypothesized that a low-level activation of mitochondrial apoptosis signaling in the absence of cell death may contribute to the DNA-damage-induction, mitochondrial modulation, and host cell response.

Methods: We generated several human epithelial cell lines with specific defects in components of the apoptosis-system or depleted of their mitochondrial DNA to investigate their potential contribution to DNA-damage and mitochondrial modulation induced by *H. pylori*, as well as *H. pylori* strains deficient in various virulence factors to define the ones that interact with the mitochondrial apoptosis system.

Results: We found that *H. pylori* induced DNA-damage in cell culture as reported previously, which was dependent on a low-level activation of the mitochondrial apoptosis system and the caspase-dependent DNAse (CAD) and occurred in the absence of cell death. Depleting the cells from mitochondrial DNA had similar impact on DNA-damage. Inflammatory cytokine secretion during *H. pylori* infection was partly dependent on low-level apoptosis activation. Mitochondrial DNA depletion attenuated the mitochondrial sublethal apoptosis signaling as well as cytokine production. *H. pylori* triggered oxidative stress distinct by deregulation of antioxidants and an increase in mitochondrial superoxides. Low-level mitochondrial apoptosis activation and alteration of mitochondrial dynamics can therefore be a part of the immune reaction to *H. pylori*-infection.

Conclusions: Our data demonstrate low-level activation of the mitochondrial apoptosis apparatus and deregulation of mitochondrial dynamics in human epithelial cells by *H. pylori*. We hypothesize that this activation serves the purpose of bacterial immune recognition but can lead to DNA-damage, which may play a role in *H. pylori* induced oncogenesis.

086/IIV

Expression of arginase 1 by myeloid cells prevents resolution of cutaneous lesions in *Leishmania mexicana*-infected mice

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Introduction: Control of intracellular *Leishmania* (*L.*) parasites requires IFNγ-dependent induction of type 2 nitric oxide (NO) synthase (NOS2) converting L-arginine into citrulline and leishmanicidal NO. NOS2 activity is counteracted by arginase (Arg) 1 and 2, both of which cleave L-arginine into urea and ornithine. The latter is a precursor of polyamines that are essential for both immune cell and parasite proliferation. NOS2 is induced by Th1 cytokines, whereas Arg1 is upregulated in a Th2 micro milieu. Recently, we observed that the expression of Arg1 and Arg2 steadily increased in *L. mexicana*-infected BALB/c and C57BL/6 mice during disease progression. Here, we tested the hypothesis that host arginases promote inflammation and prevent resolution of chronic cutaneous leishmaniasis (CL) caused by *L. mexicana*.

Material/Method: C57BL/6 wild-type (WT), germ-line knockout ($Arg2^{-/-}$) or cell-specific conditional knockout mice ($Tie2cre^{+/-}Arg1^{fl/fl}$, $Tie2cre^{+/-}Arg1^{fl/fl}Arg2^{-/-}$, $Cx3cr1cre^{+/-}Arg1^{fl/fl}$, $CD4Cre^{+/-}IL10^{fl/fl}$) were infected into the skin with 3×10^6 stationary-phase *L. mexicana* promastigotes. Infected skin tissue was collected at different time points post infection (p.i.) and processed for mRNA (RT-qPCR), protein (Western blot, ELISA, FACS, confocal microscopy), metabolite (LC-MS) and single-cell RNAseq analyses. One-way ANOVA test was used for statistical analysis.

Results: WT ($Arg1^{fl/fl}$) control mice developed non-healing chronic CL, which was delayed in $Arg2^{-/-}$ mice. In contrast, $Arg1^{\Delta Tie2}$ and $Arg1^{\Delta Tie2}Arg2^{-/-}$ mice showed strongly reduced pathology and ultimately resolved their skin lesions despite parasite persistence. A similar phenotype was observed in mice deficient for Arg1 in monocytes and macrophages ($Arg1^{\Delta Cx3cr1}$), indicating that myeloid Arg1 accounts for chronic CL. Using $IL\text{-}10^{\Delta Cd4}$ mice, $CD4^+$ T-cell-derived IL-10 was identified as Arg1 inducer. Arg1 mRNA expression in the infected skin was already present at day 20 p.i. and further increased until day 60 p.i. when pathology became prominent in WT mice. As shown by LC-MS metabolomics, high amounts of Arg1 led to a depletion of L-arginine and a significant rise in polyamines in the infected skin tissue. Interestingly, the clinical cure of Arg1-deficient mice did not result from increased NOS2 activity during the healing phase, because the NO levels detected in WT and $Arg1^{\Delta Tie2}$ and $Arg1^{\Delta Cx3cr1}$ tissues were comparable. Single cell RNAseq data from infected skin of WT and Arg1-deficient mice at day 35 p.i. rather revealed that alterations in the T cell compartment and myeloid cell recruitment are involved in Arg1-dependent induction of pathology.

Discussion: The present work demonstrates that Arg1 acts as anti-resolvin in a NO-independent manner during chronic CL infection. We propose that changes in functional subpopulations of macrophages and T cells as well as in the skin micromilieu account for the Arg1-mediated effect.

087/IIV

Fetomaternal immune crosstalk modifies T cell priming through sustained changes to DC function

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Background: Prenatal exposure to infections can modify immune development. These environmental disturbances during early life potentially alter the incidence of inflammatory disorders as well as priming of immune responses. Infection with the helminth *Schistosoma mansoni* is widely studied for its ability to alter immune responsiveness, and associated with variations in co-infection, allergy, and vaccine efficacy in endemic populations.
Objective: Exposure to maternal schistosomiasis during early life, even without transmission of infection, can result in priming effects on offspring immune responses to bystander antigenic challenges as relate to allergic responsiveness and vaccination, with this work seeking to clarify further effects and underlying immunological imprinting.

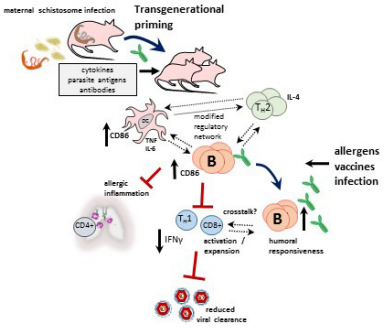
Methods: Here, we combine a chronic maternal schistosomiasis infection model with a thorough analysis of subsequent offspring immune responses to allergy and vaccination models, including viral challenge and steady state changes to immune cell compartments.

Results: We demonstrate that maternal schistosomiasis alters CD4+ responses during allergic sensitization and challenge, in a skewed IL-4/B-cell-dominant response to antigenic challenge associated with limited inflammatory response. Beyond that, we uncover previously unidentified alterations to CD8+ T cell responses during immunization, dependent upon vaccine formulation, that have functional impact upon the efficacy of vaccination against viral infection in a murine Hepatitis B virus model.

Conclusion: Alongside steady-state modifications to CD4+ T cell polarization and B cell priming, we trace these modified CD8+ responses to an altered dendritic cell phenotype sustained into adulthood, providing evidence for complex priming effects imparted by infection via fetomaternal crosstalk.

(J Allergy Clin Immunol. 2021 Mar 5 PMID: 33684437)

Fig. 1



Workshop 17
Food Hygiene (FG LM)
14. Sep. 2021 • 13:15–14:15

088/LMV

Investigational tracing as method to identify the source of a *Listeria monocytogenes* outbreak in 2019 in Germany

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Introduction: Since sequence typing of *Listeria monocytogenes* isolates was established, the number of detected listeriosis outbreaks is increasing across Europe. Due to the nature of the disease listeriosis outbreaks are difficult to solve. A simple and rapid method to inform outbreak investigations is the use of investigational tracing. This approach was applied in 2019 to generate evidence to stop a prolonged listeriosis outbreak in Germany.
Methods: Starting point for the investigational tracing were nine health care facilities (HCF) across Germany at which single cases stayed before onset of disease. Data on companies that delivered foods to the HCF and on ready-to-eat foods consumed there were collected. Following a successive approach data analysis identified similarities in the food supply of HCF. The data collected were heterogeneous and needed to be standardised. Different brand names and changing article numbers were challenging aspects during the identification of manufacturers.
Results: The initial analysis of delivering companies revealed no similarities. Detailed information on consumed foods with a high risk for Listeria contamination became available for six HCF. All served a large number of different cold cut meat products to their in-patients. Investigational tracing revealed that only meat products of one out of 29 food business operators had been consumed in all six HCF. Following this evidence, further activities of the competent food safety authorities enabled identification of the outbreak strain on food products and in the processing environment of this company. A product recall and measures taken stopped the listeriosis outbreak.
Conclusions: Investigational tracing can be key for the investigation of listeriosis outbreaks at selected locations with profound food records.

089/LMV

Antimicrobial activity of glycolic acid and glyoxal against biofilm cells

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Introduction: Surfaces seem to provide adequate conditions for microbial survival, particularly as biofilms. The presence of biofilms on surfaces is particularly critical due to their high resistance to control approaches. Even after surface cleaning and disinfection, bacteria survive and persist on surfaces, which supports the demand for new biofilm control strategies. This study aimed to understand the antibiofilm activity of glycolic acid (GA) and glyoxal (GO), which have been recently reported as active biocides against planktonic cells. Benzalkonium chloride (BAC) and peracetic acid (PAA) were used as reference biocides.
Methods: The antimicrobial activity of the selected biocides was characterized against biofilm cells of *Bacillus cereus* and *Pseudomonas fluorescens.* For this purpose, 48 h-old biofilms were produced in microtiter plates and as colony biofilms. The antimicrobial effects were assessed in biofilm culturability, removal and inactivation. Biofilm extracellular components (*i.e.* proteins and polysaccharides) were tested as chemical interfering agents in antimicrobial tests. Moreover, the limitation of biocide penetration by reaction-diffusion interactions was evaluated in colony biofilms.
Results: Biofilm cells were more tolerant to selected biocides than the planktonic counterparts. *B. cereus* biofilms were only effectively reduced (at least 3-log CFU/cm^2 reduction) by PAA (with minimum biofilm eradication concentration (MBEC) of 10,000 µg/mL). The effective biofilm control of *P. fluorescens* was achieved for >10,000 µg/mL of PAA (MBEC=20,000 µg/mL) and GA (MBEC=40,000 µg/mL), and >20,000 µg/mL of GO, being BAC not effective. In terms of biofilm removal and inactivation, BAC and GO supported high biofilm removal (60-97%) even under low concentrations (<100 and <1000 µg/mL, respectively), while PAA and GA caused high biofilm inactivation (90-99% at >100 and >1000 µg/mL, respectively). In general, biofilm components negatively affected the antimicrobial activity of the selected biocides, being BAC the most affected. Finally, despite its high antibiofilm activity, PAA was strongly diffusion-reaction limited (80-90% of penetration limitation).
Conclusions: This study demonstrates that GA and GO had promising antimicrobial activity against biofilm cells, being at a low level limited by physicochemical interactions with biofilm components. Both GA and GO demonstrated remarkable effects in potentiating BAC and PAA action in biofilm control.

090/LMV

Characterisation of novel *Campylobacter*-specific bacteriophages from Germany – Identification of promising candidates for use along the food production chain

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Introduction: Campylobacteriosis is the most frequently reported foodborne bacterial gastroenteritis in the EU and an important zoonosis worldwide. Symptoms including watery to haemorrhagic diarrhea, abdominal pain, but severe long-term sequelae like Guillain-Barré syndrome can also occur. Thermophilic *Campylobacter* (*C.*) secies like *C. jejuni* and *C. coli* asymptomatically colonize the chicken intestine. Most human infections in industrial countries have been attributed to the consumption of chicken meat or cross contaminated products. Bacteriophages (phages) are viruses that specifically infect bacterial genera, species or strains. Current estimations predict that reducing of *Campylobacter* in chicken caeca by 2 log units could diminish the risk for human infection, arising from consumed broiler meat, by 42% (EFSA 2020). The use of phages at different stages of the food production chain could reduce the risk of bacterially contaminated food, but isolation and characterisation of novel phages is a prerequisite for effective phage application.
Results: In this project, 19 *C. jejuni* and 18 *C. coli* phages have been isolated from 301 chicken samples. Based on the host range analysis of the phages using 23 *Campylobacter* isolates, four *C. jejuni* myoviruses were chosen for further characterization. Tests at different pH and temperature conditions, that could inactivate phages along the food chain, identified phage vB_CjM-LmqsCP1-4 to be the most stable and a promising candidate for a wide variety of applications. Results from efficacy testing against *C. jejuni* field isolates in liquid culture led to the rejection of two other phages because they were unable to reduce the growth of one of the selected field strains at medium and low phage doses.
Further efficacy testing of three other *C. coli* and one *C. jejuni* myoviruses with broad host spectra against two *C. jejuni* and one *C. coli* field isolates included tests with different phage combinations. Results revealed that a mixture of *C. coli* virus vB_CcM-LmqsCP218-2c2 and *C. jejuni* virus vB_CjM-LmqsCP1-1 was effective against both *Campylobacter* species, making it a promising combination for practical application in food production settings.
Out of several phage DNAs that were subjected to WGS, only some genomes could be derived. The technique might be vulnerable for the composition of the phage DNA or a probable DNA modification persists among the majority of the members.

Conclusions: The obtained results emphasise the need for kinetic tests on different *Campylobacter* to elucidate overall phage performance, as host range analysis does not allow for a rational choice of the most effective phage or phage combination, while WGS of phage DNA is challenging.

EFSA (2019). "The European Union One Health 2018 Zoonoses Report." EFSA journal **17**(12): e05926.
EFSA (2020). "Update and review of control options for Campylobacter in broilers at primary production." EFSA journal **18**(4): e06090.

091/LMV

Photodynamic inactivation of bacteria (PIB) used for decontamination of surfaces in food industry

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Introduction: Contaminated surfaces in meat production are a source of bacteria transmission from surface to surface of different equipment (e.g. conveyor belts, housings). PIB needs a dye molecule (photosensitizer), which generates mainly singlet oxygen upon light exposure. Thereby bacteria are oxidative destroyed regardless their type and resistance profile. PIB works in suspension[1] and as antimicrobial coating of surfaces[2]. Here, the inactivation of food relevant bacteria was investigated in suspension, antimicrobial coatings, and in micro-emulsions.
Material and Methods: Overnight cultures of *L. monocytogenes* DMSZ 15675 or *S. enterica* DMSZ 11320 were re-suspended to achieve up to 10^8 bacteria per mL. Curcumin, flavin, or perinapthenone photosensitizers (TriOptoTec GmbH, Germany) were added to suspension or micro-emulsions with different concentrations. Perinapthenone was added to a lacquer material yielding a coating on PU-plastic (conveyor belt) or stainless steel (housings) samples. In case of surface experiments, 50 µL of bacteria suspension was applied as droplets on the surface, allowing bacteria to dry for 60 minutes. Photosensitizers were activated by visible blue light of LEDs (DELO GmbH, Germany). After irradiation, the number of viable cells was determined by plating bacteria on agar and counting the colony forming units. The results were calculated as $\log_{10}$ reduction using internal references. Experiments were repeatedly performed yielding mean values ± standard deviation.
Results: Regardless of the photosensitizer used, *L. monocytogenes* and *S. enterica* could be inactivated in suspension experiments with increasing concentration of the respective photosensitizer. The maximal antimicrobial efficacy showed the perinapthenone photosensitizers with a reduction of up to 6.8 $\log_{10}$ ± 0.1. After inoculation of bacteria on coated stainless steel or PU-plastic, bacteria were inactivated on the surface upon light exposure yielding a reduction of maximal 4.8 ± 0.8 $\log_{10}$. The surface of inoculated PU-plastic samples were sprayed with the micro-emulsion containing photosensitizers. Irradiation of sample surfaces for five seconds could reduce the number of bacteria by 3.9 ± 0.8 $\log_{10}$.
Discussion: PIB enables a very efficient inactivation of typical food pathogens. On one hand, the antimicrobial coating can inactivate bacteria on surfaces of food processing equipment, permanently and autonomously. On the other hand, the antimicrobial micro-emulsion can be sprayed on surfaces of food processing equipment to inactivate food pathogens.

[1] Schreiner M et al, Br J Dermatol. 179(6):1358-1367, 2018.
[2] Eichner A et al, J Hosp Infect 104(1):85-91, 2020.

Workshop 18
Prevention of Nosocomial Infections (StAG HY/FG PR)
14. Sep. 2021 • 15:45–16:45

092/HYPRV

Descriptive analysis of COVID-19 outbreaks in a large tertiary care university hospital: experiences from the first year of the COVID-19 pandemic

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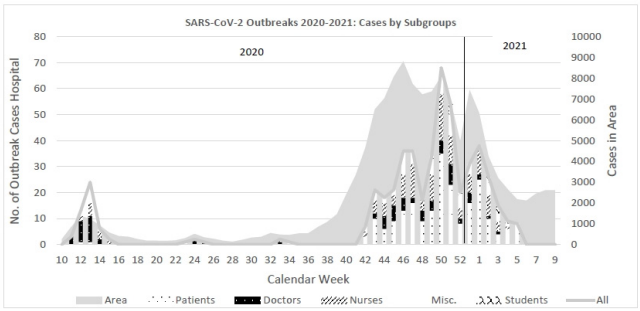
Introduction: SARS-CoV-2 has been described as causative for outbreaks in health care settings multiple times. Undiagnosed health care workers (HCW) and/or patients can be causal for numerous and/or large outbreaks in in- and outpatient care. Here, we analyze outbreaks and clusters that occurred in a tertiary care university hospital setting since start of the pandemic and provide insights into dynamics and epidemiology of healthcare-associated SARS-CoV-2 clusters.
Methods: We analyzed data from March 2020 to February 2021. Inclusion criteria: clusters, with at least (a) 1 healthcare-associated patient case plus any HCW cases; (b) at least 2 healthcare associated patient cases or (c) at least 3 HCW cases with direct patient contact. The assessment of the probable health care association and association with a specific cluster was made by the responsible infection prevention and control (IPC) team. We excluded clusters that involved only non-patient care hospital staff as well as HCW clusters on COVID wards due to different outbreak dynamics.
Results: We included 43 outbreaks into our analysis. In total 514 cases, which comprise 216 patients (< 0.2% of all cases treated during this period) and 298 HCW. Of all outbreaks, 35 occurred on wards. A total of 30 wards were involved including 2 intensive care units (3 wards were affected twice, 2 wards were affected three times). Among the 35 ward outbreaks, 22 affected medical wards, 7 affected surgical wards and 6 affected interdisciplinary wards. 4 ward outbreaks did not include patients. The other 8 outbreaks occurred in outpatient care units (n =3) or functional areas (n=5). No affected patients were identified in any of these non-ward outbreaks. In 11 of 43 outbreaks more than one patient care unit (either in-or outpatient care) was affected. The outbreak duration was 31 days on average (range: 14-73). The average number of involved cases was 12 (range: 2-56). Transmission routes were estimated and analyzed by the IPC team and were considered to be predominantly HCW to HCW plus HCW to patient and vice versa. Transmissions between patients occurred when sharing the same room. The probable index cases were often unsuspected and/or asymptomatic patients with a high need for care. Peaks of outbreak cases corresponded with incident cases in the region (Fig. 1).
Fig 1: Subgroup analysis of all cases (stacked columns) in relation to the urban surrounding area (filled area).
Discussion: Since the beginning of the pandemic outbreak management was a major aspect of IPC daily routine. While in-house outbreak control was very successful in the low-incidence situation after the first wave, outbreak-associated patient cases with increasing numbers in the second wave could not be reliably prevented despite increasing experience with the virus. These findings support the assumption that effective protection of risk groups does not work without good case control in the community.

Fig. 1



093/HYPRV

Hand hygiene compliance during surgical ward rounds after tailored interventions targeting infection prevention and control (IPC) teams and other IPC stakeholders: bivariate crosstabular analyses from the cluster-randomised controlled WACH-trial taking into account the onset of the COVID-19 pandemic

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While there is little research on hand hygiene compliance during surgical ward rounds, the most recent study showed a positive effect of a tailored training intervention targeting physicians in a tertiary care university hospital.[1] The present paper reports a corresponding analysis from the cluster-randomized WACH-trial ("Wound Infections and Antibiotics Use in Surgery", 2018-20; German Clinical Trials Register-ID: DRKS00015502), in which, by adapting the psychological tailoring approach of the PSYGIENE-trial (DRKS00010960), interventions targeted infection prevention and control (IPC) teams in non-university hospitals to increase surgical site infection-(SSI-)preventive compliance.

After briefing, in each of N=6 hospitals in-house staff observed hand hygiene (N=3820 opportunities) during N=1034 ward rounds in general/visceral and/or orthopedic/trauma surgery before and after a 3-4 months intervention ("tailoring"-study arm) or "usual practice"-period. The interventions consisted of written reports and two-day workshops including team coachings, and were tailored based on empirical assessments of SSI-preventive compliance and its determinants (COM-B-model) using mixed methods. Cluster-level data analysis was confined to opportunities by physicians and nurses (N=3392), and OpenEpi 3.01 used for compliance estimations, and chi-square and Breslow–Day tests.

While in the "usual practice"-study arm, compliance did not significantly change from pre- to post-intervention (physicians: 59.9 to 59.2%, nurses: 60.3 to 60.6%), increases were observed in the "tailoring"-arm (physicians: 54.8 to 63.1%, p=0.003, and nurses: 58.4 to 74.5%, p<.001, respectively). At the same time, compliance increased after tailored interventions in orthopedic/trauma surgery only (49.2 to 68.5%, p<0.001), however starting from a lower baseline (general/visceral surgery: 63.9 to 67.2%; p=0.285; Breslow–Day test of interaction: p<0.001). As post-intervention observations in the "tailoring"-arm were partly conducted after the onset of the COVID-19 pandemic, stratified analyses were performed. This revealed that the significant overall compliance increase after tailoring (56 to 67.9%, p<0.001) pertained to data before pandemic onset (post-intervention: 70.7%; p<0.001) as well as afterwards (67.2%; p<0.001; interaction: p=0.408).

Effectively adapting psychological tailoring from tertiary care to IPC teams in non-university hospitals is possible. At the same time, hand hygiene tended to be emphasized both in the WACH-interventions and by their addressees, which may have contributed to the overall positive result. The latter was not affected by the COVID-19 onset in this study. Differences between general/visceral and/or orthopedic/trauma surgery need further scrutiny.

[1] Schuchardt J, Chaberny IF, Schock B. [Infection control training for physicians to improve hand hygiene on surgical round: The more the better?]. Unfallchirurg. 2020;123(7):541-6. doi: 10.1007/s00113-019-00760-y

094/HYPRV

Hygienic hand disinfection compliance in the operating theatre of an orthopedic university clinic: results of an observational study

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Introduction: Hand hygiene is a cornerstone in surgical site infection prevention. There are numerous opportunities for hygienic hand disinfection in a surgical area during perioperative patient care. Especially cross-transmission via contaminated hands of staff and repetitive aseptic tasks is a challenge. This study aimed to determine hygienic hand disinfection compliance in orthopedic surgery.

Materials and Methods: An observational study was conducted in July/August 2020 in a tertiary care university clinic in Germany with six operation theatres. One trained observer assessed hygienic hand disinfection compliance of surgical and anesthesia staff via direct observation according to the WHO"s 5 moments for hand hygiene (WHO-5). N=16 patients were followed continuously in the surgical area, i.e. during the whole perioperative care sequence from arrival to discharge from the surgical area. Nearly all hand hygiene opportunities per sequence were assessed. Statistical analyses including logistic regressions were conducted with IBM© SPSS© v26.

Results: In total, 1,145 hand hygiene opportunities were observed, of which 184 were attributable to surgeons, 374 to anesthesiologists, 158 to surgical nurses, and 429 to anesthesia nurses. The WHO-5 distribution was: before patient contact: 190 (16.6%), before aseptic task: 277 (24.2%), after body fluid exposure risk: 198 (17.3%), after patient contact: 221 (19.3%), and after contact with patient surroundings: 259 (22.6%). The overall compliance rate was 40.8% (95%-CI: 37.9-43.6%), with a larger difference pertaining to surgical vs. anesthesia staff (28.4% vs. 46.1%, p<0.001) than to physicians vs. nurses (38.5% vs. 42.9%, p=0.13). Adjusting for gender and place of observation (e.g. operating vs. induction room), logistic regression analyses revealed a significant interaction between medical specialty and professional group (p<0.001). Specifically, the odds for compliance were higher for anesthesia compared to surgical staff among physicians only (OR=3.9, 95-CI 2.5-6.0, p<0.001), reflecting a difference between 47.9% for anesthesiologists and 19.6% for surgeons. Comparisons by WHO-5 were obstructed due to no opportunities before aseptic tasks for surgeons.

Discussion: Overall, hygienic hand disinfection compliance in the surgical area was just about 41%. Notably, surgeons performed worse than anesthesiologists did. One hypothesis might be that surgeons predominantly focus on surgical hand disinfection and tend to neglect hygienic hand disinfection in the operating theatre, while this difference between medical specialties does not apply to nurses (albeit with rates below 50% for their compliance as well). These first results indicate that hand hygiene behavior in this special setting needs to be improved. Tailored target group-specific interventions (e.g. in form of on-site educational training sessions) might be an appropriate way to address each professional group"s needs best.

095/HYPRV

Do patients need advice and information to prevent infections?

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Introduction: Opinions differ on how in-patients should be involved in infection control. While some authors emphasize the importance of patient empowerment and link it to successful care delivery, others point out that involvement can lead to overtaxing the patient. To address this issue, the present study was conducted as a quantitative survey to obtain data on possible patient advice and information needs.
Methods: We performed standardized personal interviews in which patients i) undergoing elective total arthroplasty (TEP) surgery, and ii) those tested positive for methicillin-resistant *Staphylococcus aureus* (MRSA) were included. The survey included multiple response options. Table 1 shows the points of interest concerning the following topics: Antiseptic washing and/or decolonization, hospital stay, influence of MRSA on health, and home environment. Ethics approval was obtained by the local committee (33/8/19).
Results: 425 patients were enrolled and 163 included after written consent as follows: 98/183 (53.6% TEP) and 65/242 (26.9% MRSA). 73% of MRSA patients were not interviewed due to illness, including speech or affect orientation (n=76; 43%), discharge (n=60; 34%), language barrier (n=15; 8.5%), no interest (n=11; 6%) and other (n=15; 8.5%). Reasons for TEP patients non-response (n=85; 46%) were concurrent medical examination or no interest. Main results of the study are summarized in Table 2. There is a tendency that MRSA patients have an increased need for advice compared to TEP patients (MRSA: n=26; 40% vs. TEP: n=22; 22.4%), as measured by open questions regarding MRSA/hospital hygiene. In addition to the high level of acceptance for antiseptic washes by TEP patients, the majority of respondents showed willingness to bear the costs for them (n=58; 59%).
Conclusion: Our data suggest that the majority of patients had a need for advice/information. Implementation should be target group specific and timely. This potentially can underline the idea of patient empowerment.

Fig. 1

Table 1. Points of interest of the survey

Status quo		Need and implementation of advice		
Open questions	Used sources of information	Main topics of interest	Kind/form of information and advice	Timeframe of the consultation

Fig. 2

Table 2. Main topics and desired kind of information/advice with respect to the corresponding group.

Patients (n=163)	Main topics [n / (%)]				Desired kind of information/advice [n / (%)]				
	Antiseptic washing and/or de-colonization	Hospital stay	Influence of MRSA on health	Home environ-ment	Personal advice	Tele-phone hotline	Via email / internet	Via bro-chure	Advice at least within 24 h
with TEP (n=98)	77 (79%)	56 (57%)	n.d.	n.d.	49 (50%)	38 (39%)	46 (47%)	51 (52%)	71 (73%)
with MRSA (n=65)	45 (69%)	30 (46%)	41 (63%)	36 (55%)	37 (57%)	21 (32%)	22 (34%)	31 (47%)	43 (66%)

n.d.: not done

096/HYPRV

Nosocomial outbreaks caused by endoscopes in gastroenterology

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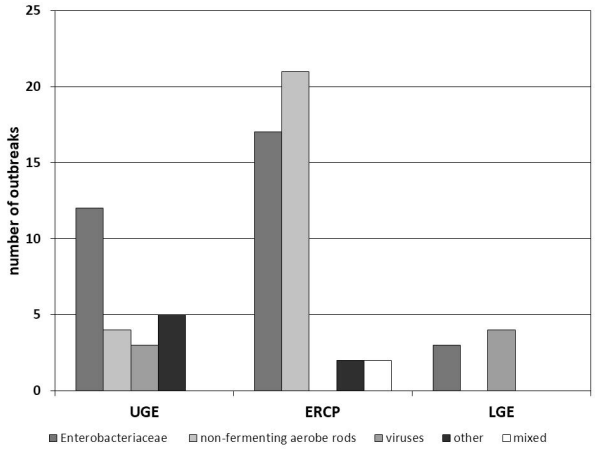
Background: Upper gastrointestinal endoscopy (UGE), endoscopic retrograde cholangiopancreatography (ERCP) and lower gastrointestinal endoscopy (LGE) are frequently performed procedures. However, there are several reports on pathogen transmissions via endoscopes causing nosocomial outbreaks (NO). This study provides information on the reasons for such transmissions, endoscope-specific risks and the distribution of pathogens.
Methods: Systematic search: Worldwide Outbreak Database, PubMed and Embase. Data retrieved: demographic data of patients, attack rate, mortality, type of microorganism, method of reprocessing after endoscope use.
Results: 73 NO with transmissions to 7,353 patients got included. Details are provided in the table. Data on patients at risk was available for 33 NOs (358 transmissions in 7,079 patients). LGE was associated with the highest attack rate of 12.8%. The figure shows the distribution of pathogens according to the type of endoscope. LGEs were associated with the transmission of *Enterobacteriaceae* and viruses while UGE and ERCP also showed spread of non-fermenting aerobic rods. The main reason for contaminated endoscopes was insufficient reprocessing of the device. Noteworthy, the quality of manual reprocessing was inferior to automatic reprocessing.
Discussion: This study demonstrates the risk of nosocomial transmissions and subsequent infections by endoscopic procedures with emphasis on breaches in standard operation procedures during manual reprocessing. Therefore, critical appraisal of the reprocessing work flow is highly recommended whenever there is suspicion of an inappropriate handling of endoscopes. Furthermore, staff should always be aware of the possibility of pathogen spread in general – even at low levels – to detect infection control failures at an early most time point. A rather large proportion of included NOs were due to multi drug resistant pathogens, but this is most likely due to publication bias. It seems safe to assume that the true level of transmissions in endoscopy remains largely underestimated. Moreover, we repeatedly noticed gaps of information on relevant parameters in articles, e.g. <50% of the articles provided the total number of patients at risk which necessary to determine an attack rate. Thus, for future NO reporting we recommend strict adherence to the checklist on NO characteristics as provided in the Outbreak Reports and Intervention Studies Of Nosocomial infection (ORION) statement.

Fig. 1

	UGE	ERCP	LGE
Outbreaks [#]	24	42	7
Multi drug resistant pathogens involved [#; %]	7; 29.2%	25; 59.5%	0; 0.0%
Exposed patients [#]	4,103	3,172	78
Patients affected by transmissions [#]	247	449	12
Mortality [%]	9/143 =6.3%	26/205 =12.7%	1/10 =10.0%
Attack rate [%]	143/4,103 =3.5%	205/2,898 =7.1%	10/78 =12.8%
Type of reprocessing			
Manual [#; %]	12; 50.0%	8; 19.0%	3; 42.9%
Automatic [#; %]	5; 20.8%	21; 50.0%	2; 28.6%
Not mentioned [#; %]	7; 29.2%	13; 31.0%	2; 28.6%
Cause of transmission			
a) Deviations in reprocessing / human failure [#; %]	11; 45.8%	12; 28.6%	4; 57.1%
b) Damage of endoscope / technical failure [#; %]	1; 4.2%	8; 19.0%	0; 0.0%
Multiple failures from category a) and b) [#; %]	3; 12.5%	9; 21.4%	1; 14.3%
Other causes identified [#; %]	9; 37.5%	13; 31.0%	2; 28.6%

Fig. 2



Workshop 19
Microbial Viruses: Complexity in Biology and Potential in Diagnostics and Treatment (FG MV)
14. Sep. 2021 • 15:45–16:45

097/MVV
Idleness of the *Staphylococcus aureus phage* Sb-1 and *Pseudomonas aeruginosa* phage NP-3 can be overcome by the antibiotic meropenem

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Introduction: The antimicrobial potential of bacteriophages (briefly phages) has been mostly studied by focusing on single bacterial species. However, chronic infections, such as wound infections are typically mixed infections, where, besides others, *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) can play a major role. The goal of this study was to investigate the efficacy of two species-specific phages when applied as single or combined agents with or without the antibiotic meropenem against individual species or a two-species system.
Material/Methods: Multi-drug resistant clinical isolates of SA and PA where challenged with the therapeutic SA-phage Sb-1, and the PA-phage NP-3. Antibacterial efficiency of the phages in Lysogeny broth (LB) was compared with phage activity in equine serum by measuring changes in the optical densities of bacterial populations every 20 minutes for 16 h. As a follow-up experiment SA and PA were grown as a co-culture in equine serum. Samples were taken after 2, 4, 6, and 8 hours, and then every 24 hours for seven days, and after two weeks for quantifying bacteria and phages using real-time quantitative PCR (RT-PCR) along with CFU and PFU determination.
Results: In LB-media, both phages suppressed susceptible bacteria substantially within 16 h, which could mostly be amended with co-addition of meropenem. This was different to equine serum, where the single phage Sb-1 entirely failed, and NP-3 suppressed PA only after 72h of incubation. However, reduction of PA enabled SA to stronger multiply due to the disappearance of the other bacterial competitor. This could be prevented by co-addition of meropenem, which showed positive interactions with the single phages Sb-1 and NP-3, respectively, leading to an improved bacterial suppression. When phages were given as a pair to a SA/PA co-culture, no or little suppression of both bacteria occurred. This idle situation could be overcome, by also adding meropenem to the phage mixture, leading to a substantial suppression of both bacterial species.
Discussion: Antibacterial activity of phages in serum is strongly reduced compared to LB medium, which needs to be considered, when selecting phages for phage therapy. Treating a mixed infection with a mono-phage therapy may allow the non-targeted bacteria to flourish, which might negatively affect the treatment success. In addition, when treating a mixed infection with a phage cocktail of two or more phages, possible negative interactions between phages can occur, again negatively impacting the therapeutic success. Synergistic interactions of the antibiotic with either the single phages or with both phages together enable the most effective suppression of SA and PA. As a conclusion, the composition of phage cocktails should be well considered in order to minimize negative outputs with phages during a real application.

098/MVV
Bacteriophages against Staphylococcus lugdunensis and their inhibitory effect on biofilms

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Introduction: Although Staphylococcus lugdunensis belongs to the coagulase-negative staphylococci, it has a similar pathogenicity profile to Staphylococcus aureus. Treatment of periprothetic Staphylococcus lugdunensis infections is challenging due to an extensive biofilm growth and the emergence of antibiotic resistant clinical isolates. The arising field of bacteriophage therapy represents a promising alternative treatment approach.
Methods: In this project, bacteriophages specifically against S. lugdunensis were isolated for the first time. Four bacteriophages (phSL9509, phSL9509.2, phSL9571 and phSL9579.3) specific against S. lugdunensis were isolated and characterized. The therapeutical potential was assessed by replication parameters, host range and biofilm prevention and inhibition efficacies in static microtiter plate assays as well as under continuous flow conditions in flow cells.
Results: Bacteriophages phSL9509 and phSL9579.3 were identified as Myo- or Herellevirus while phSL9571 was characterized as a rare Staphylococci-infecting Podovirus. All four bacteriophages exhibited similar replication parameters comparable to other Staphylococcus bacteriophages. All four bacteriophages show a broad intra-species host spectrum. Three of the four bacteriophages also exhibit broad interspecies activity against clinical and laboratory S. aureus and MRSA strains. Biofilm experiments under static conditions or continuous flow conditions confirmed the efficiency of biofilm prevention for all four phages and showed a significant degradation of existing biofilms.
Discussion: Based on the results, bacteriophage phSL9579.3 was rated as potentially suitable for a therapeutic application, phages phSL9509, phSL9509.2 and phSL9571 were assessed to exhibit a very high therapeutic potential.

Workshop 20
Pathogenesis of Gastrointestinal Infections (FG MP/FG GI)
14. Sep. 2021 • 15:45–16:45

099/GIV
Proteomic adaptation of *Clostridioides difficile* to treatment with the antimicrobial peptide nisin

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Introduction: *Clostridioides difficile* is the leading cause of antibiotic-associated diarrhea represents an enormous financial burden for the health care system due to its increasing incidence and mortality. As antibiotic-resistant *C. difficile* strains are advancing, alternative treatment options are heavily needed.

Antimicrobial peptides (AMPs) are an interesting alternative to classic antibiotics and information on the effects of AMPs on *C. difficile* will not only enhance the knowledge for possible biomedical application but may also provide insights in mechanisms of *C. difficile* to adapt or counteract AMPs.
Methods: We applied state-of-the-art mass spectrometry methods to quantitatively investigate the early proteomic response of *C. difficile* 630Δ*erm* to sublethal concentrations of the AMP nisin, thereby investigation the cytosolic and the membrane-associated proteomes.
Results: The results of our study do not only point at a heavy reorganization of the cellular envelop but also determined pronounced changes in central cellular processes such as carbohydrate metabolism. Further, the number of flagella per cell was altered during the adaptation process suggesting a role of these cellular structures in combating this particular AMP, which is independent from pure cell motility.
Discussion: For *C. difficile* it is tempting to compare the proteomic response to nisin to those towards bile acid, which also exhibit an amphiphilic structure and hence a saponaceous character. However, the correlation of the data was very low if it exists at all. Hence, the proteomic response to nisin seems to be specific to the antimicrobial action of this peptide and combats way more effects of nisin than just the detergent-like effect.
Moreover, the data point at a time-resolved adaptation to AMPs, which involves different proteins in the early and the late phases of adaptation and resistance development. In case of nisin the expression of flagella seems to be an important feature at the onset of nisin-adaptation.

100/GIV
Characterisation of the interaction of *H. pylori* with members of the annexin family
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Introduction: *Helicobacter pylori* infects approx. half of the world"s population and establishes persistent colonization in the gastric mucosa. Infection with this bacterium is associated with an increased risk of developing severe gastric pathologies. *H. pylori* has developed many adaptions to evade the immune system and to establish a chronic infection, *e.g.* modifications in the structure of lipopolysaccharide (LPS). Recently, we discovered that *H: pylori* can bind annexin A5 (ANXA5), a protein ubiquitously expressed throughout the human body.
Methods: ANXA5 binding to different *H. pylori* strains was measured using ANXA5 Alexa-647 (Thermo Fisher) by flow cytometry. Binding to LPS was analyzed by dot blot analysis. Influence on CagA translocation was measured using the HiBiT translocation assay. Toll-like receptor 4 (TLR-4) activation was analyzed using the HEK-Blue hTLR-4 reporter cell line and QuantiBlue (Invivogen). Tissue sections of human stomach biopsies were imaged using a Leica TCS SP5 confocal microscope and analysis was performed with Fiji software.
Results: According to our dot blot results, ANXA5 binds *H. pylori* via LPS. Therefore, we analyzed different LPS mutant strains for ANXA5 binding. The *rfaE* deletion mutant showed a significant increase in binding. The LPS structure of this mutant is highly truncated and consists only of lipid A and a KDO residue. On these grounds, we analyzed different lipid A mutants for ANXA5 binding. In fact, deletion of the lipid A-specific phosphatases *lpxE/F* resulted in increased ANXA5 binding. These knock out mutants are known to elicit an increased TLR-4 response compared to wild type lipid A (Cullen, 2011). This TLR-4 activation was decreased by pre-incubation of bacteria with annexin A5. Interestingly, strains also showed increased ANXA5 binding after mouse passage. In addition, CagA translocation was moderately decreased by pre-incubation of the bacteria with ANXA5. Furthermore, the analysis of stomach biopsies of *H. pylori* positive patients showed an increase in ANXA1, ANXA2 and ANXA5 levels compared to *H. pylori* negative individuals.
Discussion: In this study, we identified and characterized the previously unknown interaction of *H. pylori* with annexins. LPS, more specifically lipid A, was identified as a binding partner for ANXA5, because alterations in the LPS structure changed binding patterns to *H. pylori.* ANXA5 binding to an *lpxE/F* deletion mutant reduced TLR-4 activation in a reporter cell line, which hints at a possible biological mechanism. While it is known for *H. pylori* lipid A to be modified to evade recognition by TLR-4, data for human conventional dendritic cells suggest that they are able to recognize *H. pylori* via TLR-4 (Neuper, 2020). Therefore, we suggest that *H. pylori* can mask recognition by TLR-4 via binding of ANXA5.

Cullen, Giles, Wolf, Ecobichon, Boneca, Trent (2011). PLoS Pathog 7(12)
Neuper, Fauenlob, Sarajlic, Posselt, Wessler, Horejs-Hoeck (2020). Int J Mol Sci 21(11)

101/GIV
Mucosal inflammatory responses to *H. pylori* are mediated by gland base cells
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The gastric mucosa frequently encounters bacteria and their toxins that are deactivated in the stomach, without causing mucosal injury and inflammation. *H. pylori* can colonize and persist in the stomach. While most *H. pylori* are either free-swimming or in contact with surface epithelial cells, a subpopulation establishes micro-colonies deep in gastric glands in contact with stem cells, that are characterized by high Wnt signaling, which is controlled by stromal R-spondin 3. These gland-associated bacteria trigger gland hyperplasia that can further progress to premalignant and malignant gastric lesions. The mechanisms by which stem cell colonization causes tissue responses are not well understood.
We aimed to analyze the role and interplay of R-spondin and its receptors in antral glands upon infection with *H. pylori* by using conditional knockout mice and gastric 3D-organoids. R-spondin knockout mice, or mice lacking R-spondin receptors, were infected for 2 weeks or 2 months with *H. pylori* and analyzed by confocal microscopy, quantitative real-time PCR, and microarray. Wildtype bacteria and bacteria (ΔCagE mutants) that are not able to inject ADP heptose, an *H. pylori* virulence factor activating NF-κB, into the cells were used. Organoids grown with or without supplemented R-spondin were treated with ADP heptose and analyzed by confocal microscopy and quantitative real-time PCR.
We found that R-spondin signaling enables epithelial cells in the gland base to respond to *H. pylori*. R-spondin signaling primes gland base cells and enables rapid activation of NF-κB signaling upon encountering *H. pylori* or ADP heptose.
Our data reveal that gland base cells act as sensors and effectors of *H. pylori* and provide an intriguing link between epithelial stem cell homeostasis and mucosal immunology.

102/GIV
Human probiotic *Escherichia coli* Nissle 1917 mediates epigenetic regulation of innate immunity in insects to combat enteropathogens
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Introduction: The probiotic *Escherichia coli* strain Nissle 1917 (EcN) confers a health benefit to humans suffering from acute and persistent diarrheal diseases. Despite the widespread medical application of EcN, the underlying mechanisms of communication between the probiotic and its hosts remain largely elusive. Most likely, the beneficial activities of EcN result from complex interactions of the bacterium with the innate immune system of the

intestinal tract. We investigate epigenetic regulation of innate immunity by EcN during enteropathogenic *E. coli* (EPEC) infection. In addition, we aim to establish the insect model *Galleria mellonella* as an *in vivo* surrogate system for assessing probiotic intervention against gut infection at 37°C.
Material/Method: The probiotic strain EcN or *E. coli* strain DH5α were administered to *Galleria mellonella* larvae by force-feeding, and after 24 h, the same larvae were similarly infected with EPEC. The foregut, midgut, and hindgut of larvae with and without probiotic application were dissected 24 h after EPEC force-feeding for DNA, RNA, and histone isolation. We analyzed DNA methylation, histone acetylation, and expression of antimicrobial defense genes by ELISA and RT-PCR.
Results: The mortality of larvae by EPEC was reduced following EcN, but not DH5α administration. Bacterial colonization in the larval gut was restricted to the peritrophic matrix consisting of chitin, mucins, and peritrophins. EcN, but not DH5α administration markedly changed the expression of antimicrobial peptides and epigenetic enzymes, namely histone deacetylases, histone acetyltransferases, and DNA methyltransferases mainly in the midgut of EPEC infected larvae. We correlated changes in gene expression in the infected larval gut with the differential regulation of histone acetylation (H3K9 and H4K5 acetylation) and DNA methylation with or without probiotic intervention.
Discussion: We are the first to establish the *G. mellonella* larvae as an ethically acceptable, inexpensive oral infection model for assessing the protective effects of potential probiotics against enteropathogens at 37°C alternatives to vertebrate models. We identified new bacterial factors and conserved host epigenetic modifications together constituting a probiotic effect in *G. mellonella*. We present evidence that the probiotic EcN interferes with histone acetylation and DNA methylation in larvae to induce innate immunity-related gene expression that confers protection against EPEC infection. Our results may be relevant for understanding the bacteria-host interaction at the human gut barrier after the ingestion of probiotic therapeutics to prevent intestinal infections.

103/GIV
Recent evolvement of a highly pathogenic EHEC O104:H4 masked as O181:H4
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Introduction: Enterohemorrhagic *E. coli* (EHEC) cause the severe clinical manifestation hemolytic uremic syndrome (HUS). Worldwide serovar O157:H7/H- is of highest relevance, but the large HUS outbreak in 2011 was caused by an EHEC strain of the rare serovar O104:H4.
Methods: During molecular surveillance, we identified and further characterized a highly virulent EHEC strain by whole genome sequencing. Further, virulence features, phylogenetic context, and a hypothesis concerning strain emergence were investigated.
Results: We describe a novel hybrid EHEC strain of the rare serovar O181:H4 associated with HUS. The strain interestingly shares MLST ST678 and virulence markers except the O antigen (OAG) with the O104:H4 outbreak strain. Both strains exhibited comparable cytotoxicity, adhesion pattern, and a very close phylogenetic relationship. Only a few differences were found in their chromosomes and a partially distinct plasmid repertoire. Close relatedness of the strains suggested that O104 to O181 OAG conversion might have occurred recently. We identified further scenarios of possible recent serovar conversion among MLST ST300, ST101, and ST301 *E. coli* strains.
Discussion: Closely related HUS-associated EHEC strains can possess distinct OAG gene clusters likely acquired by horizontal gene transfer. Strains similar to the O104:H4 outbreak strain are still around causing severe infections and may be masked by low risk serovars.

104/GIV
Phenotypic and functional characterization of clinical EPEC isolates using a novel neonatal *in vivo* model
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The small intestinal pathogen enteropathogenic Escherichia coli (EPEC), a major cause of diarrhea, remains associated with infant mortality in developing countries. Hallmarks of EPEC infection in humans include age-dependent susceptibility, intestinal colonization and the formation of attachment and effacement (A/E) lesion based focal microcolonies. A recent genomic study of 70 clinical isolates collected during the Global Enteric Multicenter Study (GEMS) could not identify any specific bacterial virulence factors that could explain the wide range of clinical outcomes (lethal, symptomatic and asymptomatic) observed in children. Here, thirteen typical EPEC strains isolated during the GEMS study were investigated in a highly controlled and comparative manner *in vivo*. The formation of bacterial microcolonies and the induced antimicrobial host response were studied in a novel murine neonatal infection model using immunofluorescence microscopy and transcriptomic analysis of isolated epithelial cells. Despite efficient colonization by all strains, the numbers of bacterial microcolonies attached to the small intestinal mucosa differed dramatically between the different isolates. Consistently, the analysis of the transcriptional profiles of small intestinal epithelial cells isolated from infected animals using real-time PCR as well as Massive Analysis of cDNA Ends (MACE)-Sequencing showed a vastly different host response between different isolates. Strikingly, the expression level of genes such as *Il18* and *Reg3g* directly correlated with the strain"s ability to form microcolonies. As our mouse model excludes host genetic differences, our data suggests that yet unknown bacterial factors drive the host epithelial response *in vivo*. An in-depth genomic analysis of the 13 strains allowed for the identification of new putative bacterial factors important for EPEC pathogenesis *in vivo*. Our findings could help to better understand EPEC pathogenesis *in vivo* and develop new therapeutic strategies to reduce infant mortality in developing countries.

Workshop 21
Public Health Microbiology: Contributions of Reference and Consulting Laboratories (StAG RK)
14. Sep. 2021 • 15:45–16:45

105/RKV
Changes in the epidemiology of invasive meningococcal and *Haemophilus influenzae* infections during the COVID-19 pandemic in Germany
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Introduction: The present pandemic may have also influenced infections other than COVID-19. We compared prevalence data of invasive *Neisseria meningitidis* (Nm) and *Haemophilus influenzae* (Hi) infections for 12 months under containment measures in Germany to the preceding year"s period to analyse epidemiologic changes. The data extend a recent international analysis covering the months until June 2020 [1].
Materials and Methods: Besides notification according IfSG, diagnostic laboratories send invasive Nm and Hi isolates to the NRZMHi. Submissions were analysed for 01.04.2019 to 31.03.2020 (pre-pandemic period, pre-PP) and 01.04.2020 to

31.03.2021 (pandemic period, PP). Coverage was assessed by comparing the NRZMHi cases with the number of notified cases (SurvStat@RKI).

Results: Invasive Nm was detected in 210 cases in pre-PP. The number dropped to 45 cases in PP, which equals a decrease by 79%. Nm invasive infection incidence for pre-PP and PP according to IfSG notifications was 0.30/100,000 and 0.07/100,000, respectively. The coverage of cases analysed at the NRZMHi was 84% for the pre-PP, and 83% for the PP.

The NRZMHi received invasive Hi isolates from 738 cases in pre-PP, compared to 154 cases in PP. The number of invasive Hi cases was reduced by 79%. Incidence rates dropped from 1.19/100,000 (pre-PP) to 0.28/100,000 (PP). The reference laboratory coverage was 76% for pre-PP, and 78% for PP.

There were no signs for alterations of serotype/serogroup distribution in PP, although due to small numbers this has to be interpreted carefully

Discussion: Reduced submissions to the NRZMHi correlated with pandemic response measures. Whereas submissions were comparable to the previous year in the first months of 2020, they decreased significantly after March 2020. Declined notification rates were reported by the RKI for many infectious diseases in 2020 except tick-borne encephalitis.

Infection protection measures against COVID-19 seem to have a significant impact on both, invasive Hi and Nm infections. Since invasive bacterial infections such as meningitis and sepsis are life threatening events and the coverage for Nm and Hi did not change significantly, a bias by unnotified cases seems unlikely. In light of these dynamics infection surveillance and epidemiologic analysis are important to monitor further developments as the pandemic is ongoing. It will be important to carefully observe the epidemiology after restrictions have been lifted, since the effects of reduced transmission on natural immunity of the population are unknown.

[1] Brueggemann AB et al. Lancet Digit Health. 2021; 3: e360-e370

106/RKV

First cross-national outbreak of foodborne botulism observed in the EU due to a commercial product

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Between November and December 2016, a cluster of four cases of foodborne botulism type E in persons of Russian and Kazach backgrounds was documented in Germany. Consumption of salt-cured, dried roach (*Rutilus rutilus*) was identified as the source of the outbreak. The implicated batches had been distributed by an international wholesaler and were recalled from Europe-wide outlets of a supermarket chain and other independent retailers. After reporting the outbreak via the European Epidemic Intelligence Information System for Food- and Waterborne Diseases and following a notification through the EU Rapid Alert System for Food and Feed, two further epidemiologically linked cases of foodborne botulism type E were identified in Spain. In all cases *Clostridium botulinum* type E and/or botulinum neurotoxin type E was detected from clinical samples as well as available fish samples from the affected households. The outbreak of foodborne botulism cases reported here, which affected six patients from five unrelated households altogether, is the first cross-national outbreak of foodborne botulism observed in the European Union involving the consumption of a commercial product. Additional cases were likely prevented by a broad product recall, underscoring the importance of timely public health action.

107/RKV

Identification of immunodominant *Bartonella bacilliformis* proteins for serodiagnostic and vaccine development

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Question: *Bartonella bacilliformis* is the causative agent of Carrión"s disease, a vector-borne biphasic illness restricted to the South American Andes. In the acute phase, the bacteria infect erythrocytes causing severe hemolytic anaemia and transient immunosuppression with high fatality rates (40-90%). *B. bacilliformis* is transmitted by the bite of sandflies (*Lutzomyia* spp.) and asymptomatic infections are assumed to represent the source of new outbreaks. For disease prevention and surveillance strategies, the identification of those asymptomatic carriers is of particular significance especially in the light of climate change and potential expansion of the vector. Therefore, a reliable serodiagnostic tool and a vaccine are urgently needed. However, with only limited knowledge of the immune response to *B. bacilliformis* infections, antigen candidates for a vaccine are also widely unknown. This study aims to identify immunodominant proteins of *B. bacilliformis* for serodiagnostic use and vaccine development.

Methods: Based on the genomes of *B. bacilliformis* strains KC583 and KC584, a reverse vaccinology approach in combination with heterologous genomic expression libraries was used to identify immunodominant proteins. Antigen candidates were recombinantly expressed and their reactivity was systematically assessed by Western blotting, line blotting and ELISAs with a serum collection of Peruvian patients suffering from *B. bacilliformis* infections.

Results: In total, 21 potentially immunodominant proteins were identified, recombinantly expressed and analysed by Western blotting using a pool of patient sera. Fourteen antigens were found to be immunoreactive with patient sera and were further analysed by line blotting using sera of 26 Carrión"s disease patients and 96 healthy German blood donors. Results indicated the use of three antigens as sero-markers to detect IgG antibodies against *B. bacilliformis*. Based on these findings a diagnostic ELISA with a sensitivity of 81% and a specificity of 95% was developed.

Conclusions: The combination of reverse vaccinology and heterologous genomic expression libraries has been proven to be effective for the identification of immunodominant proteins. The herein developed line blot assay and ELISA represent useful serodiagnostic tools for future epidemiological studies in endemic areas and provide a solid basis for future vaccine development to prevent the highly lethal Carrión"s disease.

108/RKV

Whole-genome comparisons of vancomycin-resistant *Enterococcus faecium* from bloodstream infections in Germany reveal predominance of a *vanB* clone of ST117/CT71

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Background: Vancomycin-resistant enterococci (VRE) are important multidrug-resistant nosocomial pathogens. At the local, regional, national and international level VRE vary in terms of prevalent *van* genotypes and the predominance of clonal variants. In order to assess corresponding dynamics, isolates from invasive infections submitted to the National Reference Centre for Staphylococci and Enterococci between 2015 and 2019 were analyzed by means of whole genome sequencing.
Methods: Antibiotic susceptibility testing and species identification for all enterococcal isolates were determined by broth microdilution and species PCRs. The *van* genotypes were inferred from multiplex PCR as part of the daily routine and deduced from Next-generation sequencing (NGS) data. NGS was performed for a total of 543 invasive *E. faecium* isolates that were sent to the NRC between 2015 and 2019. All isolates originated from patients with blood stream infections. Genome sequences were reconstructed by *de novo* assembly and subsequently typed by MLST and cgMLST. For the reconstruction of *vanB* transposon elements, we used both NGS and Nanopore long-read sequencing technology.
Results: Based on the molecular typing of invasive *E. faecium* isolates a sharp increase in the prevalence of *vanB*-type ST117/CT71 isolates was observed from 2015 onwards (2015: 1.8%), reaching percentages as high as 39% in 2019. First appearing in 2016, ST80/CT1065 (*vanB*) has been the second most common strain type since 2018 (9%; 2019: 8%). MLST types such as ST203 (*vanA*) or ST192 (*vanB*), which were prominent during recent years occurred only sporadically in 2019. For ST117/CT71 isolates, cgMLST provided only limited resolution, as apparently independent isolates with no epidemiological link differed only by a few alleles (n <10). Tn*1549* transposon typing of 152 *vanB*-type ST117/CT71 revealed only minor sequence variations. Thus, typing resolution could not be increased by including parts of the accessory genome content, which makes outbreak investigations for this strain type even more challenging.
Conclusion: Our study shows that formerly prominent genetic lineages of VRE in Germany were largely displaced by isolates of ST117/CT71(*vanB*) in recent years. Epidemiologically unrelated isolates of this strain variant differ by less than 10 alleles and are thus not resolvable by cgMLST. Also, the integration of *vanB* transposon analysis did not increase discriminatory power. These results suggest a nationwide spread of this strain variant by unknown routes. The reasons for the epidemiological success of the *vanB*-type ST117/CT71 strain have yet to be determined and are subject of ongoing investigations.

109/RKV

Source-tracking impossible? The dominant *Legionella pneumophila* clone in the Berlin area shows low molecular diversity

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Introduction: Within the Berlin LeTriWa project we conducted an intensive source-tracking of community acquired Legionnaires" disease (CALD) in Berlin by comparing clinical and environmental Isolates. The predominant clone in the Berlin region is *L. pneumophila* sequence type (ST)182, subtype Knoxville. The same ST182 caused a nosocomial outbreak that occurred 2003 in Frankfurt/Oder.
Intensive genotyping of *L. pneumophila* isolates by using the current gold standard method, the seven gene sequence-based typing scheme (SBT), revealed limitations in the discrimination of several ST which could not be compensated for by additional phenotypic typing scheme. In practical terms, this means that ST182 strains are disproportionately frequently found in both patients and water systems, and in both cities, and cannot be distinguished by commonly used methods.
Since most isolates belong to the aforementioned clone ST182, we used whole genome multi locus sequence typing (wgMLST) to obtain a higher resolution and to assign sources to CALD cases.
Methods: We sequenced (Illumina, coverage > 35), assembled (SPAdes) and annotated (Prokka) 39 isolates. The wgMLST was generated using roProfile. Most isolates were sampled in Berlin in 2017 – 2019. Ten isolates were sampled during the Frankfurt/Oder outbreak in a psychiatric ward in 2003 including two follow up samples in 2009. In addition, we had another three clusters of epidemiologically related isolates. The wgMLST scheme was imported into the Bionumerics software for further analysis. Subsets of common genes present in all isolates were identified to generate a core genome MLST (cgMLST). Cluster analysis was done using Unweighted Pair Group Method with Arithmetic mean (UPGMA). Single nucleotide polymorphisms (SNP) and recombination events were analyzed using Gubbins.
Results: The wgMLST revealed 3893 alleles and 2026 alleles for the cgMLST, respectively. The wgMLST give a rather unclear picture of clusters that do not necessarily reflect the epidemiologically linked isolates. Most of these linked isolates have > 50 allelic differences. This allows the exclusion of environmental isolates as potential source for CALD on the molecular level. However, epidemiologically unrelated strains also form distinct clusters, which indicates a common source of infection. By using the cgMLST scheme, almost all isolates are linked to each other. The difference between adjacent isolates is five or less alleles (except two isolates with 15 and 25 allelic differences). The SNP and recombination event analysis support the findings. The genomes of the two isolates with 15 and 25 allelic differences revealed several recombination events.
Discussion: The *L. pneumphila* clone ST182, Knoxville, is genetically very homogenous. The application of whole genome based typing methods does not allow to break down this clone into subclones. The surveillance and the source tracking of ST182 CALD cases is highly difficult.

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Molecular epidemiology of *Mycobacterium abscessus* isolates recovered from german cystic fibrosis patients

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Infections due to *Mycobacterium abscessus* are a major cause of mortality and morbidity among patients suffering from cystic fibrosis (CF). Moreover, for CF patients *M. abscessus* has been suspected to be involved in person-to-person transmissions. The presence of dominant global clonal complexes indicates that closely related isolates may occur among CF patients. The aim of this work is to elucidate the epidemiology of *M. abscessus* among German CF patients and to evaluate these data within a global context. In this study, we performed whole genome sequencing on a set of about 150 early and sequential isolates of *M. abscessus* recovered from respratory secretions of patients treated in 14 German CF centers. We used the MTBseq pipeline with the reference genome *M. abscessus* ATCC19977 to identify clusters of closely related isolates and correlate those with global findings. In addition, subspecies distribution and genotypic drug susceptibility for macrolides and aminoglycosides was assessed for all isolates by characterisation of the *erm*(41)*, rrl,* and *rrs* genes. So far, we could identify representatives of all circulating global clonal complexes (Absc. 1, Absc.2 and Mass. 1, as described by Bryant *et al*. Science 2016). Further, regarding patients from Frankfurt University Hospital we performed a contact analysis. According to our knowledge, this is the largest study about phylogenetic relations of *M. abscessus* CF isolates in Germany.

Workshop 22
Case Studies Clinical Microbiology – TED-Session (StAG KM)
14. Sep. 2021 • 15:45–16:45

111/KMV

Lethal Waterhouse-Friderichsen Syndrome caused by *Capnocytophaga canimorsus* in an asplenic patient

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We report a case of Waterhouse-Friderichsen Syndrome (WFS) due to bacteremia with *Capnocytophaga canimorsus* and mycetal superinfection in a 61-year-old splenectomised woman.
Four days before admission, the patient suffered a dog bite on the third finger of the right hand, and developed cephalgia, neck pain, nausea and vomiting. Blood samples revealed gram-negative bacilli, but cultures remained negative despite repeated culturing. Ceftriaxone and ampicillin were administered but her condition did not improve.
The patient was transferred to our tertiary intensive care unit with the clinical presentation of Waterhouse-Friderichsen Syndrome (WFS). On arrival, she was in fulminant septic shock with ubiquitous purpura fulminans. She had massive cytokine release syndrome (IL-6 140000 ng/ml), a procalcitonin of 138 ng/ml, Type A lactic acidosis, and severe disseminated intravascular coagulopathy (DIC). The patient received extensive therapy, including mechanical ventilation, antibiotic coverage with meropenem, vasopressor therapy, continuous renal replacement therapy (with additional Seraph® 100 Microbind® Affinity Blood Filter, ExThera Medical), therapeutic plasma exchange, and multiple transfusions of blood products. Septic cardiomyopathy was managed by implantation of a veno-arterial extracorporeal membrane oxygenation (vaECMO).
In the disease course, the patient developed dry perioral and acral necrosis on the right and left hands, and died ten days after the dog bite due to refractory septic shock with WFS.
Blood culture collected on the day of her demise was positive for *Candida albicans* likely originating from the abdomen. Post-mortem, blood culture bottles obtained at the transferring hospital were forwarded to our microbiology laboratory for detailed analysis. Fine gram-negative bacilli were confirmed and identification based on MALDI-TOF revealed *Capnocytophaga canimorsus* on Columbia blood agar (incubation at 37°C for 48 hr).
The autopsy showed bilateral hemorrhagic necrosis of the adrenal cortex, septic embolism to heart, kidneys, and liver with mycetal superinfection. A minor, superficial skin defect in the area of the right middle finger after a dog bite was considered to be the portal of entry for the pathogen. *C. canimorsus* are gram-negative bacilli that belong to the family Flavobacteriaceae and are members of the microflora in the oral mucosa of dogs and cats. Infections with *C. canimorsus* are rare, and the diagnosis is often complicated and prolonged.
In our opinion, the occurrence of a fulminant sepsis after a dog bite should always urge the attending physician to keep the differential diagnosis of *C. canimorsus* at the back of his mind. However, despite maximum therapy, the prognosis of a *C. canimorsus*-induced septic shock remains very poor especially in splenectomised patients. A specific therapeutic approach would be highly desirable but is unfortunately not to be expected due to the rarity of the disease.

Fig. 1

Postersession 01
13. Sep. 2021 • 11:15–12:45

Microbial Pathogenesis (FG MP)

112/MPP
SARS-CoV-2 infections reveals a broad organ tropism with a systemic inflammatory response without the direct damage of extrapulmonary tissues

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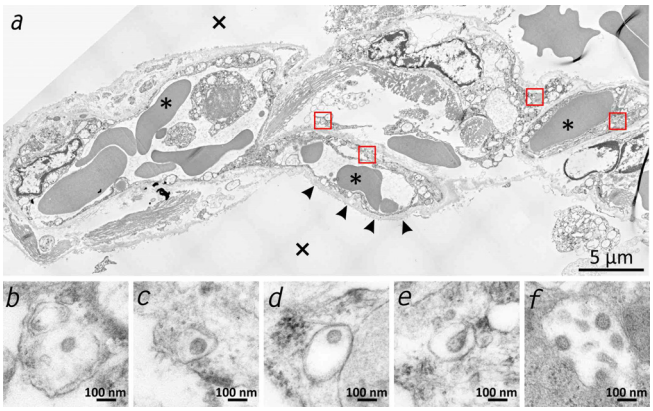
Introduction: The coronavirus disease 2019 (COVID-19) indicates infections with the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) as a systemic disease. Therefore, to understand the pathogenesis of SARS-CoV-2, we analyzed the viral load and the innate immune response *in vitro* and *in vivo*.
Material/Methods: We investigated the viral distribution within the human body and the correlation with the tissue damage in deceased COVID-19 patients. In addition, the organ tropism was analyzed in the human alveolus-on-a-chip system, composed of epithelial/endothelial cells and macrophages, to investigate the viral load and the immune response.
Results: Our results demonstrate high viral loads in most of the lungs of deceased COVID-19 patients measured by real-time PCR. We were even able to verify viral particles in the lung tissue by using transmission electron microscopy (Figure 1) and figured out severe lung damage in a majority of the cases. Additionally, viral RNA was detected throughout various extrapulmonary tissues and organs, e.g., in the gastrointestinal and cardiovascular systems, to a much lower extent than in the lung. Despite the presence of viral RNA, the histological analysis did not reveal severe tissue damage. Besides, our *in vitro* data in the human alveolus-on-a-chip system show that SARS-CoV-2 efficiently infects epithelial cells. In contrast, the adjacent endothelial layer was neither infected nor did it show productive virus replication. Nevertheless, the endothelial-epithelial barrier function was activated by a robust inflammatory response and severely damaged, contributing to the viremia observed in many patients.
Discussion: Our study demonstrates the dissemination of viral RNA throughout the body, which supports the hypothesis that a maladaptive host response fails to clear the viral particles, resulting in viremia and multiorgan dysfunction. Based on our i*n vitro* data, we suggest that although the efficient replication of SARS-CoV-2 depends on the epithelium, the neighboring endothelial cells are affected by the epithelial cytokines. This systemic response results in the damage of the epithelial/endothelial barrier function and strong viral dissemination.
Figure 1. TEM lung tissue. (a) Alveolar septum showing capillaries with erythrocytes (asterisk). The blood-air barrier is damaged (arrowheads). (a) SARS-CoV-2 in plasmatic vesicles of alveolar fibrocytes. (f) Reference image of SARS-CoV-2 in Vero-76 cells.
Fig. 1



113/MPP
Impact of glycosphingolipids on meningococcal pathogenicity at the nasopharyngeal epithelial barrier

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Introduction: *Neisseria meningitidis* (*Nm*) is a major cause of bacterial meningitis and sepsis. A critical step in the pathogenesis of *Nm* is the passage through the epithelial barrier of the nasopharynx, a step that is still poorly understood. Recent published data on human brain microvascular endothelial cells proved that *Nm* heavily interacts with GM1, a glycosphingolipid (GLS) strongly abundant on the epithelium. This interaction seems to be essential for the ability of *Nm* to invade the cells.
The aim of this study was to investigate the role of GLS on the ability of *N. meningitidis* to overcome the epithelial barrier in the nasopharynx. We hypothesized that interaction between *Nm* and GLS (e.g. GM1) induce a clathrin-mediated internalization of the bacteria into the epithelial cells and with that, allows *Nm* to cross this barrier and reach the blood stream.
Methods: Human lung epithelial cells (Calu-3) were grown on permeable cell culture inserts as air – liquid interface model which was evaluated by immunofluorescence imaging, Transepithelial electric resistance (TEER) and permeability (NaF and Fitc-dextran) measurements. The cells were exposed to different *Nm* strains and the ability to cross the barrier was determine by transmigration experiments in the presence or absence of cholera toxin b subunit (CtxB), blocking the interaction between *Nm* and a specific GLS subset, or clathrin mediated endocytosis inhibitors. Localization of the bacteria through the epithelial barrier were carried out by SIM and EM imaging.
Results: Here we demonstrate that the Calu-3 air – liquid interface model shows distinct epithelial barrier characteristics, like their multilayer structure and mucus production. The ability of *Nm* to overcome this barrier strongly depends on the strain used and relies on the clathrin-mediated endocytosis of the bacteria as shown by an approximately 80 % reduction of transmigrated bacteria after 24 h in the inhibitor experiments. In addition, pretreatment of the cells with CtxB showed a similar effect on bacterial transmigration through the epithelial barrier.
Discussion:
The results demonstrate that the ability of *Nm* to initially cross the epithelium of the nasopharynx relies on the interaction between *Nm* and host GLS. This might be followed by clathrin-mediated

endocytosis of the bacteria, a pathway already shown for other pathogenic bacteria.

114/MPP

Neisseria meningitidis regulates Sphingosine kinase activity to favour entry into brain endothelial cells

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Introduction: Sphingolipids, originally known for their function as membrane constituents, are receiving growing attention after discovering, that down-stream metabolites of these structuring components also act as intra- and extracellular effector molecules. These bioactive sphingolipids, i.e. Ceramide (Cer) and Sphingosin-1 phosphate (S1P) play a role in various diseases, e.g. cancer and cardiovascular disease. In recent years, sphingolipids also gained attention in the field of medical microbiology, since involvement of sphingolipid metabolites in infection and host defence against different human pathogens was delineated. *Neisseria meningitidis (Nm),* a gram-negative diplococcus, that invades the cerebrospinal fluid (CSF) and causes meningitis, was shown to induce Cer production through acid sphingomyelinase (ASM) activation, which enables *Nm* to reach the CSF. In our study, we are focusing on S1P, a down-stream metabolite of Cer. S1P is formed by two kinase isoforms Sphingosine kinase (SphK) 1 / 2 and it is known to regulate vascular stability. Therefore, we investigate whether *Nm* also affects S1P signalling to facilitate invasion into brain endothelial cells.
Methods: We measured sphingolipid levels of brain endothelial cells (hCMEC/D3) over time course after infection with *Nm* using LC-MS/MS to get a complete picture of sphingolipid homeostasis during *Nm* infection. We established an ATP-based assay and used qPCR to observe SphK activation during *Nm* infection. SphK activity assay was adapted to *Nm* mutant strains, lacking different virulence factors (Opa, Opc, pili and capsule expression) to determine bacterial effectors for SphK activation. We used SphK inhibitors to determine the role of the different kinases in *Nm* adherence and invasion in hCMEC/D3 cells.
Results: *Nm* wildtype (*wt)* led to activation of SphK, with a peak activation 4h post-infection. Consequently, intracellular S1P Levels were transiently increased, reaching their maximum at 4h post-infection. Infection with mutant strains identified pili as the major bacterial effector. Inhibition of SphK led to significant reduction of *Nm* invasion in hCMEC/D3 cells, while there was no effect on bacterial adherence.
Discussion: Our study showed that sphingolipid down-stream metabolite S1P plays a significant role in *Nm* pathophysiology. Inhibition of S1P-producing SphKs led to significant reduction in *Nm* invasion, which might be a possible pharmacological target to prevent meningococcal meningitis.

115/MPP

Adaptation strategy of *Staphylococcus aureus* upon contact with human skin using an *ex vivo* skin model

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Introduction: The human epidermis provides physical protection and is normally impenetrable to pathogenic microbes. Nevertheless, bacteria such as *Staphylococcus aureus* are able to colonize the skin surface, which sometimes leads to serious infections. *S. aureus* is equipped with several interactive regulatory systems, which might orchestrate appropriate virulence gene expression during colonization and infection. We hypothesize that gene expression might differ significantly once the *S. aureus* changes its habitat from the nose to the skin and that such regulatory switches contribute to successful colonization capacity of the organism. On the long-run, knowledge on the specific gene expression pattern can give major insights into the importance of highly expressed factors and the importance of underlying regulatory systems.
Methods: We established an *ex vivo* skin explants model to investigate *S. aureus* adaption to human skin. The model allows to monitor changes in gene expression over up to 8 days after the initial contact.
Results: From the analysis of regulatory loci we found evidence for a significant down regulation of the global virulence regulator *agr* directly after the initial contact with skin, regardless of the growth phase from which *S. aureus* originated. In contrast, the alterative sigma factor B (*sig*B) and the antimicrobial peptide-sensing system (*gra*RS) were actively transcribed 6 hours after epidermal contact suggesting a role of these regulatory elements. Accordingly, tissue adherence was primarily mediated by the *sig*B target genes clumping factor A (*clf*A) and the fibrinogen and fibronectin binding protein A (*fnb*A). At later timepoints, wall teichoic acid (WTA) also contributed to the adhesion process. Besides the expression of adhesive molecules immune evasion via the staphylococcal complement inhibitor (*scn*) and the staphylokinase (*sak*) implicated the colonization capability of *S. aureus*. In agreement with other studies, we detected a strong involvement of proteases from all three catalytic classes during the entire colonization process.
In summary, *S. aureus* gene expression during colonization of human skin differs significantly from nasal colonization. In both scenarios, however, defined individual factors are expressed. Taken together, colonization of healthy skin led to a uniform response of the pathogen to the surrounding milieu independently of the human host and regardless of the growth phase from which *S. aureus* originates.

116/MPP

The influence of extracellular serine proteases on pneumococcal persistence in the nasopharynx

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Introduction: *Streptococcus pneumoniae* is a pathobiont causing life-threatening diseases including pneumonia, sepsis, meningitis. Pneumococci express up to four trypsin-like or subtilisin-like family surface-associated serine proteases: HtrA, SFP, PrtA, and CbpG. Studies focusing on the deletion of single pneumococcal serine proteases are difficult to interpret due to the compensatory effects of the other serine proteases. Here, we analyzed the impact of double and triple mutants of non-invasive serotype 19F_EF3030 and invasive serotype 4 on bacterial fitness and pneumococcal pathogenesis.
Methods: Bacterial fitness of constructed mutants was assessed *in vitro* growth experiments using complex and chemically defined media. Host-pathogen interactions have been investigated using *in vitro* cell culture-based infection assays with human nasopharyngeal epithelial cells (Detroit-562). Host cells were infected with nonencapsulated TIGR4 or 19F wild-type and isogenic serine protease mutants expressing only CbpG, SFP, HtrA, or PrtA. The influence of single serine proteases on nasopharyngeal colonization, lung infection and systemic dissemination was analyzed in experimental *in vivo* mouse infection models of colonization (19F) and pneumonia (TIGR4).
Results: Mutants deficient for serine protease activity were unaffected in bacterial growth. *In vitro* adherence assays showed that mutant strains without or with only one functional protease are significantly reduced in adherence to human respiratory epithelial cells. Moreover, *in vivo* infection of mice with the colonizing strain 19F lacking serine protease activity or expressing only one functional serine protease indicated a significant reduction of nasopharyngeal colonization. Mice infected with bioluminescent TIGR4*lux*Δ*htrA*Δ*cbpG*Δ*sfp* expressing only PrtA showed a significantly prolonged survival time in a murine pneumonia

model, suggesting a decreased virulence of the pneumococcal mutant strain.

Conclusion: Our data suggest that extracellular serine proteases contribute to pneumococcal adherence and colonization in experimental models. However, the deletion of pneumococcal serine proteases has only a moderate effect on pneumococcal virulence in a murine pneumonia model. Hence, these results implicate that serine proteases may be important in pneumococcal colonization and may facilitate the overcome of tissue barriers to cause severe systemic diseases.

Reference:

Ali, M.Q., et al. (2021). doi: 10.3389/fcimb.2020.613467.

117/MPP

Deciphering the function of a small CRISPR-associated RNA in *Neisseria meningitidis* in host cell adhesion

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Introduction: *Neisseria meningitidis* (Nme) is a gram negative, commensal pathogen which colonize the mucosa of the human nasopharynx and can cause life-threatening infections such as sepsis and meningitis. They possess a type II-C CRISPR/Cas systems that contains a CRISPR RNA array (crRNA), a trans-activated crRNA (tracrRNA), genes encoding canonical Cas1, Cas2 and Cas9 proteins and an uncharacterized small CRISPR-associated RNA (scaRNA) (1,2). Here, we address whether and how scaRNA along with Cas9 affects meningococcal adhesion to human nasopharyngeal cells.

Methods: To validate the expression of scaRNA we performed northern blots analyses in different growth conditions. We generated scaRNA knock-out strains and isogenic complemented strains and used these strains in adhesion assays with human nasopharyngeal cell lines. Rifampicin assays and electrophoretic mobility shift assays (EMSAs) were used to test for potential interactions between scaRNA and Cas9. To further identify potential targets for scaRNA-dependent regulation, differential RNA-seq and quantitative proteomics were performed.

Results: We could show that Cas9, tracrRNA (3) and scaRNA, but not crRNAs, are required for adhesion to human epithelial cell lines (Figure 1). Via transcriptomic and proteomic comparisons, we could show that Cas9 and scaRNA regulate the expression of predominantly metabolic genes at the transcriptional and/or posttranscriptional level, notably comprising genes involved in inorganic ion uptake (*efeUOB*), glutamate metabolism (*gdhA*/*gdhB*), proline metabolism (*proA*), the methylcitrate cycle (*prpC*) and, surprisingly, the CRISPR/Cas system-associated endonuclease (*cas9*). Northern blot analyses showed that the scaRNA is constitutively expressed and its half-life was unaffected by Cas9 as shown by rifampicin assays, although EMSA experiments revealed that the meningococcal scaRNA can interact with NmeCas9 (Figure 2).

Discussion: The effect of Cas9/scaRNA on cell adhesion was independent of any crRNAs which suggests a novel mechanism for Cas9/scaRNA-mediated regulation of predominantly metabolic genes. In line with these unexpected findings, genome-wide surveys in recent years have provided evidence for an indeed intimate link between amino acid and energy metabolism and host cell adhesion (4,5). Therefore, this observation provides a starting point to study the mechanisms of virulence regulation by the type II-C CRISPR/Cas system in Nme.

(1) Zhang *et al*., Mol Cell, 2013
(2) Heidrich *et al*., Nucleic Acids Research, 2017
(3) Heidrich *et al*., RNA Biology, 2018
(4) Capel *et al.*, mBio, 2016
(5) Schoen *et al.*, Front Cell Infect Microbiol., 2014

Fig. 1

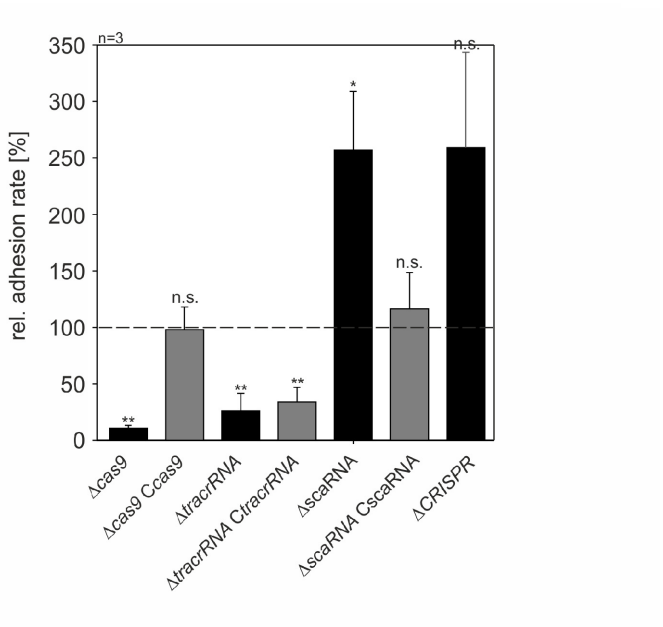
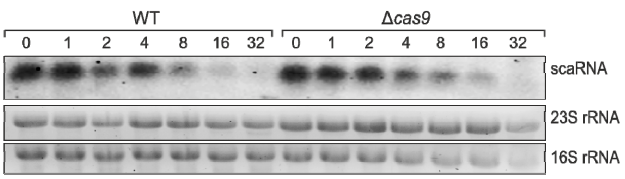
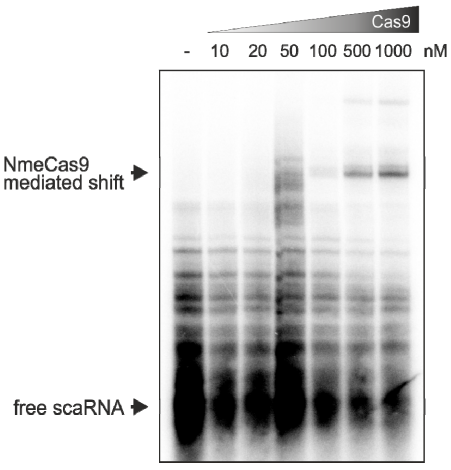


Fig. 2

Rifampicin assay



EMSA



118/MPP

The *Legionella pneumophila* GDSL hydrolase PlaA, a highly active lysophospholipase, is processed and activated by the zinc metalloproteinase ProA

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Introduction: The facultative intracellular pathogen *Legionella pneumophila* is the causative agent of Legionnaire"s disease, a potentially fatal pneumonia. The pathogen colonizes lung macrophages and epithelial cells where it blocks maturation of the phagosome and thus evades degradation in the lysosome. Instead, the *Legionella* containing vacuole (LCV) is established which allows bacterial replication. During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II and type IVB secretion systems. At least 15 phospholipases A, including the group of three GDSL hydrolases, are encoded in the genome. The GDSL hydrolase PlaA was previously described as a factor promoting bacterial exit under certain conditions.
Objectives: We here focus on the characterization of the phospholipase PlaA which belongs to the family of GDSL hydrolases. In the presented project, we investigate the mode of secretion, activation mechanism, and 3D structure of PlaA.
Materials and Methods: The mode of secretion was assessed by Western blotting. For detection of enzymatic activity, recombinant PlaA was purified and subjected to lipid hydrolysis assay. Additionally the effect of the zinc metalloproteinase ProA on PlaA integrity and activity was determined. Moreover, the 3D structure of PlaA was analyzed via crystallization.
Results: We show that PlaA is secreted via the type II secretion system and exhibits lysophospholipase A activity. Moreover, PlaA is processed by the *L. pneumophila* zinc metalloproteinase within a disulfide loop after secretion which increases its lysophospholipase A activity. Additionally, we present the 3D structure of PlaA which reveals that the uncleaved disulfide loop stabilizes a lid covering the catalytic triad and therefore explains enzyme activation after disulfide loop cleavage.
Conclusion: As PlaA is secreted via the type II secretion system and thus present within the LCV lumen, we propose that its lysophospholipase activity is directed towards the LCV membrane and thus may contribute the cell egress of *L. pneumophila*.

119/MPP

PlaD, a novel type IVB secreted effector protein of *Legionella pneumophila*

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Introduction: The facultative intracellular pathogen *Legionella pneumophila* is the causative agent of Legionnaire"s disease, a potentially fatal pneumonia. *L. pneumophila* is ubiquitous in aqueous habitats and amoebae are a natural host. However, the pathogen also colonizes lung macrophages and epithelial cells. *L. pneumophila* blocks maturation of the phagosome and thus evades degradation. Instead, the *Legionella* containing vacuole (LCV) is established which allows replication. During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II (Lsp) and type IVB (Dot/Icm) secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins and the GDSL hydrolases, are encoded in the genome.
Objectives: We here focus on the characterization of the phospholipase PlaD which belongs to the family of GDSL hydrolases and shows various differences to the other GDSL hydrolases PlaA and PlaC. We aim to understand the importance, secretion path, and mode of action of PlaD in infection.
Materials and Methods: We investigated the mode of secretion of PlaD by means of Western blotting and protein translocation assay. Additionally, we determined its binding to various lipids and its interactions with eukaryotic proteins by means of lipid-protein-overlay assays, proximity ligation, and pull down assays. Further, we analyzed the localization of PlaD during infection via immunofluorescence microscopy.
Results: We showed that, during infection, PlaD is Dot/Icm-dependently injected into the host cell cytoplasm where it localizes to distinct organelles. Moreover, we demonstrated that PlaD binds to a subset of phosphoinositide species and interacts with a class of regulatory proteins of the host cell. Additionally, our data revealed that the C-terminal half of PlaD is essential for its secretion and phosphoinositide binding but dispensable for interaction with the regulatory proteins.
Conclusion: Based on its Dot/Icm dependent injection into the host cell cytoplasm, we classify PlaD as a novel type IVB secreted effector protein of *L. pneumophila*. We propose that PlaD is involved in the regulation of host cell signaling cascades during infection.

120/MPP

Hydrogen peroxide is to a greater extent than pneumolysin responsible for rapid microglial cell death during *in vitro* infection with *S. pneumoniae*

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Introduction: *Streptococcus (S.) pneumoniae* (pneumococcus) is the most common cause of bacterial meningitis and meningoencephalitis in humans with a mortality rate of 20-30 %. Among the major pneumococcal virulence factors are the capsular polysaccharide (CPS), the cytolysin pneumolysin (Ply) and hydrogen peroxide (H_2O_2), which is primarily produced by the enzymatic activity of pyruvate oxidase (SpxB). The role of these virulence factors in the pathogenesis of meningitis is still not fully understood. To characterize the response of specific brain phagocytes (microglia) to pneumococcal infection, a comparative analysis of primary microglia cells and primary bone marrow-derived macrophages (BMDM) was performed.
Material/Methods: Primary microglia and BMDM were infected with wild-type *S. pneumoniae* D39 as well as with mutants deficient for CPS, Ply, or SpxB. The survival of phagocytes during the infection was visualized by fluorescence microscopy using Hoechst and propidium iodide staining, while bacterial growth was determined via plating. In addition, an antibiotic protection assay was performed to determine bacterial intracellular survival. Furthermore, both primary phagocyte cultures were exposed to different concentrations of either recombinant Ply or H_2O_2, and cell survival was measured.
Results: Primary microglia were killed during pneumococcal infection independently of Ply, whereas viability of BMDM was not affected by pneumococci. The Antibiotic protection assays indicated that BMDM are more efficient in killing pneumococci than microglia. Moreover, treatment of cells with recombinant Ply led to a dose-dependent cytotoxic effect in both phagocyte types. However, very high concentrations of Ply, starting at 250 hemolytic units (HU)/ml, were required for this effect. In contrast to that, there were less than 10 HU/ml formed during pneumococcal infection of microglia. Interestingly, exogenously added catalase protected microglia during pneumococcal infection suggesting a role for hydrogen peroxide. Indeed, Infection of microglia with pneumococci deficient in SpxB led to reduced killing of microglia. Exogenously added hydrogen peroxide, similar to concentrations produced by pneumococci, was sufficient

to kill microglia. All microglia were killed after 6 hours when incubated with H_2O_2 concentrations as little as 0.25 mM, while pneumococci were able to form up to 2.5 mM H_2O_2 after 6 hours when grown under the same conditions.
Discussion: Because microglia are not as effective in killing of pneumococci as BMDM, they are subjected to higher doses of toxins and accumulating metabolic by-products such as hydrogen peroxide. Microglia react very sensitive towards hydrogen peroxide, whereas they are relatively tolerant towards pneumolysin.

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Influence of Teichoic Acid D-Alanylation on Cytokine-Induction and interaction of *Streptococcus suis* with porcine Leukocytes

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Introduction: *Streptococcus suis (S. suis)* is a common swine pathogen but also poses a threat to human health. Especially in Asia, there have been outbreaks with severe cases of streptococcal toxic shock-like syndrome (STSLS). Therefore, it is crucial to understand how *S. suis* interacts with the hosts immune system during bacteremia. *S. suis* has the ability to attach D-alanine to the poly-glycerophosphate chains of its lipoteichoic acids (LTA) [1]. Although the role of LTA as a ligand of pattern recognition receptors is controversially discussed [1, 2], it has been suggested that D-alanylation of LTAs modifies the immune response of the host [3, 4]. Here, we investigated the impact of D-alanylation of LTA on the interaction with porcine leukocytes in a model of bacteremia.
Methods: We performed loss-of-function experiments with a *dltA*-mutant incapable of introducing D-alanine into its LTA. Read out parameters included measurement of phagocytosis and oxidative burst response of porcine granulocytes, survival of bacteria, the cytokine response and the association of bacteria with porcine blood monocytes. Whole porcine blood or isolated leukocyte fractions were incubated with Far Red-labeled *S. suis* wild type (WT) and *dltA*-mutant bacteria. The amount of Far Red-positive granulocytes and monocytes was determined by flow cytometry. Porcine interleukin (IL)-1β and tumor necrosis factor (TNF)-α were evaluated by ELISA.
Results: Phagocytosis and oxidative burst of porcine granulocytes, as well as interaction of bacteria with monocytes were significantly increased for the *dltA*-mutant, whereas IL-1β but not TNF-α levels were significantly reduced in whole blood infected with the mutant, suggesting a possible influence of LTA D-alanylation on inflammasome regulation. This finding could not be attributed to the alanine content of purified LTA, as preparations obtained of the WT and the mutant strain induced IL-1β to a similar extent. However, the observed increase in association to monocytes and the decreased IL-1ß levels in response to the *dltA*-mutant, indicate a negative influence of bacterial association with monocytes on IL-1β secretion.
Discussion: Modifying the surface by attaching D-alanine to its LTA helps *S. suis* to avoid phagocytosis and reduces the association to monocytes but promotes the induction of IL-1β, which is a double-edged sword for the host. Pro-inflammatory cytokines play an important role for the severity of an infection. While IL-1 signaling was shown to be beneficial to control and clear streptococcal burden [5] an exacerbated inflammatory response due to inflammasome activation is able to induce STSLS [6].

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122/MPP

A new and simple bioassay for quantitative autoinducer-2 analysis

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Question: Gram-positive and Gram-negative bacteria shed and react to inter-species signalling molecules called autoinducer-2 (AI-2), which is important for the regulation of virulence related traits. AI-2 quantification is therefore essential for understanding population density-dependent changes in bacterial behaviour and pathogenicity. However, currently available quantification bioassays require rather complex protocols, have low detection ranges or are sensitive to trace components of e.g. growth media. In order to facilitate and improve the detection of AI-2 we have developed an *Escherichia coli*-based bioassay that is sensitive, cheap, fast, robust and reliable in the quantification of biological active AI-2.
Methods: We have constructed a fluorescent reporter gene-based cassette that can be chromosomally integrated in *Escherichia coli (E. coli)*, but also in a low copy plasmid for the use in other Gram-negative bacterial species. The cassette encodes the *lsrRK* operon from *E. coli* and a *lsrA::yfp* reporter encoded on the opposite strand. Internalized AI-2 induces YFP (**Y**ellow **F**luorescent **P**rotein) expression in a concentration-dependent manner, which can be detected in real-time in a plate reader instrument.
Results: The chromosomally integrated as well as the low-copy plasmid-based reporter cassette was systematically tested in *E.* coli K-12 MG1655 and compared to existing AI-2 quantification methods. AI-2 quantification was possible in a concentration range from 200 nM to 100 µM. Calibration curves were used to calculate biological active AI-2 amount in cell-free bacterial samples of different *E. coli* strains at several time-points. The determined relative concentration measurements were highly reproducible in between biological replicates. In addition, assay interference of several sugars was systematically tested, enabling a critical interpretation of the reliability of the quantification results. The plasmid-based reporter cassette was also tested in clinical *E. coli* isolates and was almost as sensitive as *E. coli* K-12 MG1655 in the quantitative detection of AI-2.
Conclusions: We have developed a new, sensitive and easy to handle bioassay for the measurement of AI-2, an important signalling molecule in infections of Gram-positive and Gram-negative bacterial pathogens. The new model has a high detection range and is less sensitive to interfering compounds, i.e. sugars, which is a major advantage in analysing potential AI-2 concentrations in complex samples as compared to already established methods. Moreover, reporter strain interchangeability results in versatile and interesting application opportunities, including real-time AI-2 production screenings of clinical isolates in co-culture experiments. In addition, the measurement of AI-2 mimicking molecules produced by eukaryotic host cells may be in the future directly possible with the bacterial reporter strain used for infection experiments, instead of time-consuming external measurements.

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BioID assay identifies host cellular ribosomal proteins in close proximity to SNX1 in Chlamydia trachomatis serovar D-infected HeLa cells

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Introduction: Chlamydia trachomatis is an obligate intracellular bacterial pathogen that resides and replicates within a membrane-bound vacuole termed "inclusion" wherefrom the bacterium selectively recruits proteins from the host cell. Among recruited proteins are sorting nexins (SNX) of the human retromer complex. In particular, SNX5 and SNX1 are highly enriched in the host cell-derived inclusion proteome.
Methods: Using a proximity-dependent biotinylation assay (BioID), we identified host cellular and bacterial factors associated with SNX1 during C. trachomatis serovar D infection.
Results: We identified close proximity of 40 proteins to ectopically expressed SNX1 from statistical analysis of the BioID data. Among these novel SNX1-associated proteins were ten cellular ribosomal proteins. Recruitment of ribosomal proteins to the chlamydial inclusion was species-specific, time- and serovar-dependent. Depletion of SNX1/2 or SNX5/6 abolished localization of ribosomal proteins to the inclusion. Finally, RPL13a-silenced cells showed increased C. trachomatis serovar D inclusion size, number of bacteria and infectious progeny.
Discussion: Our data show an important role of host cellular ribosomal proteins in C. trachomatis infection and indicate a SNX-dependent recruitment mechanism of ribosomal proteins to the chlamydial inclusion. These findings represent an interesting example of how C. trachomatis manipulates the host cell and reveal a novel role of cellular ribosomal proteins in controlling bacterial infections.

Part 1: General and Hospital Hygiene (StAG HY): Antimicrobial Resistence and Drugs, Infection Prevention (FG PR)

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Adaption of peroxyacetic acid (PAA) aerosol-based disinfection protocols to field conditions

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Background: After establishing suitable aerosol-based disinfection protocols in high-containment facilities, presented in 2019, we started to investigate the field suitability of this method. Actual, this question is of pivotal importance caused by the tense situation of the spreading SARS-CoV-2 and African swine fever virus in Europe as well as quick response after bio terroristic attacks. Thereby, the influence of multiple different climate conditions on the disinfection efficacy has to be evaluated.
Materials and Methods: Spore forming bacteria, mycobacteria and mould spores were inoculated on stainless steel carriers according to quantitative carrier testing protocols (DVG, RKI). The carriers were placed at different locations within a provisory sealed radio shelter (20.56 m³) that was aerosolized with an ultrafine aerosol (~7.5 µm) of a ~100 ml solution containing 1.3 % PAA. After an incubation time up to 60 min and 1 h aeration phase, the microorganisms were recovered and the inactivation efficacy was determined as log10 reduction. Remaining germ titers were determined by using standard titration techniques and a growth based photometric measurement device in parallel. The tenacity of the used surrogates where compared to BSL 3 virus and bacteria strains used in bio terroristic attacks or from recent outbreaks.
Results: The adapted and validated protocols resulted in a ≥ 4 log10 reduction of all tested microorganisms. An incubation time of 60 minutes was adequate for low temperature ranges (10 to 0°C). Warmer surfaces (ΔT ≥ 4°C) are protected by boundary layers independent of the absolute temperature and lead to reduced inactivation efficacies. The tested automated device is suitable to determine remaining germ titers within 19 h. The used surrogates had a similar or even higher tenacity compared to the BSL3 pathogens.
Conclusion: The PAA aerosol-based disinfection method is applicable under field conditions and highly effective in rendering contaminated areas or devices safe to handle. Suitable automated devices will simplify the analyses and significantly shorten readout times.

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Airborne contamination and decontamination of medical equipment with bacterial and viral surrogates

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Introduction: Patients affected by highly pathogenic infectious diseases are regularly treated in isolation wards in Germany. However, if the number of patients exceeds the capacity, treatment in normal wards may be necessary. Medical equipment located near the patient may be contaminated by airborne pathogens during the patient's stay and might act as a potential transmission source to following patients. This potential transmission path may also be of relevance in the ongoing COVID-19 pandemic. Therefore, this hypothesis as well as the evaluation of an airborne decontamination of the medical equipment by hydrogen peroxide fumigation was systematically investigated in aerosolization experiments.
Material and Methods: The experimental series were performed for four surrogates: *Staphylococcus aureus, Geobacillus stearothermophilus* spores, the non-enveloped MS2-bacteriophage, and the enveloped feline coronavirus (ongoing experiments). As a first step, the airborne tenacity of each surrogate was validated for relative humidities of 30-70%. In the contamination experiments, five medical devices were exposed to a defined airborne surrogate concentration and sampled internally and externally. In the decontamination experiments, the devices were equipped with germ carriers with a defined surrogate contamination and the addition of organic substances and exposed to hydrogen peroxide vapor. Subsequently, the surrogate reduction on the germ carriers was quantified.
Results: Significant tenacity differences were observed between the aerosolized surrogates. *S. aureus* showed a significantly increased tenacity at relative humidity <50%. The MS2-bacteriophage showed significantly decreased tenacity at intermediate RH around 50%.
In the contamination experiments, all devices were strongly contaminated externally. We also detected lower concentrations of all surrogates internally. The decontamination efficiency showed a high variance depending on the investigated parameters (surrogate, sampling location, organic substance). It was significantly reduced inside the devices, when blood was added as an organic substance and for *G. stearothermophilus*.
Conclusion: The results strongly indicate that internal and external contamination of medical devices with pathogenic bacterial and viral agents may occur regularly, in some cases to a high degree, in isolation wards. It was also revealed that fumigation with hydrogen peroxide does not always ensure sufficient decontamination.

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Establishing semi-automated infection surveillance in obstetrics and gynaecology

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Introduction: Surveillance of hospital acquired infections is an inherent part of hospital infection control. (Semi)automated surveillance reduces manual workload while maintaining high sensitivity by preselecting patients. While semiautomated systems

have been described for a variety of applications only few data exist for obstetrics and gynaecology. The aim of this study was to assess sensitivity and workload-reduction provided by parameters applied to filter patients for manual evaluation for surgical-site infection (SSI) after mastectomy and caesarean delivery (CS).
Methods: The retrospective, single centre study was performed at a tertiary care hospital, Germany, department for obstetrics and gynaecology. SSIs were defined according to standard case definitions. 416 mastectomies conducted in 2018 were retrospectively screened for SSI to generate a reference standard. For CS, data from WebKess were used as reference standard. 3438 patients were included between 5/2013 and 12/2019. Indicators such as antibiotic prescription, microbiological and administrative data were analysed for sensitivity, number needed to identify one case (NNI), and potential workload reduction.
Results We identified 9 cases with SSI among 416 mastectomies (2.16%). Antibiotic prescription post-surgery detected all SSI (sensitivity 100%, NNI 10). Diagnosis codes detected 8 SSI (sensitivity 88.9%, NNI 5) and collection of microbiological samples 6 SSI (sensitivity 66.7%). The combination "diagnosis code or microbiological sample" reached a sensitivity of 100% and a NNI of 7. Reference standard of CS was 3,438 procedures with 54 SSI (1.57%). The sensitivities were 35.2%, 5.6%, 85.2% and 90.7% for collection of microbiological samples, diagnosis codes, readmission/post-hospitalisation care, and the combination of all indicators, respectively.
Discussion: We confirm that using a semiautomated surveillance system reduces workload by maintaining high sensitivity depending on type of surgery and most likely on local circumstances. Surveillance of mastectomies in our hands would benefit from the use of the indicators antibiotic prescription and diagnosis codes. Surveillance of CS could be performed with satisfying sensitivity by the analysis of patients who were readmitted or taken care of after discharge. Semiautomated systems depend on local accuracy of documentation and thorough digitalization. The results need to be confirmed by analysis of larger datasets in multi-centre studies.

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Outbreaks within the outbreak – intrahospital MRSA-clusters during the COVID-19 pandemic

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Introduction: During the ongoing COVID-19 pandemic changes of infection control practices to prevent SARS-CoV-2 transmissions could be observed. Among others, these comprise the use of medical masks, intensified hand hygiene, visitors" reductions and distance keeping in hospitals. So far, it remains unclear, whether a change of these every-day infection control practices will modulate numbers of nosocomial infections.
Methods: Two MRSA clusters occurred during June 2020 and March 2021 on an obstetrics/gynecology and a medical intensive care unit (ICU) ward only treating COVID-19 patients, respectively. Subsequently, outbreak investigations were initiated in both scenarios, including methicillin-resistant *Staphylococcus aureus* (MRSA) screening of health care workers (HCW) and environmental sampling of contact surfaces for source detection. All found MRSA isolates were subjected to whole genome sequencing to determine their genetic relatedness.
Results: In total, three patients and four patients were detected MRSA positive on ICU and obstetrics/gynecology unit, respectively. Environmental sampling revealed five contact surfaces to be MRSA positive on the ICU ward, but remained negative on the obstetrics/gynecology ward. MRSA screening of 301 HCWs resulted in one and five MRSA positive samples, respectively. Verified by WGS –based typing, in both scenarios environmental and/or HCW samples were genetically related to isolates obtained from patients.
Discussion: Here, we demonstrate two intrahospital MRSA clusters during the ongoing pandemic. The close genetic relatedness between MRSA originating from HCWs, patients and their environment indicates the direct or indirect transmissions of this pathogen on both wards. These clusters highlight the problem of increased nosocomial transmissions in spite of the adoption of COVID-19 infection prevention strategies, also suitable for preventing the spread of multi drug resistant organisms (MDROs). Possible reasons for the occurrence of MDRO outbreaks within the prevailing COVID-19 outbreak situation are the diversion of hospital resources as well as decreased awareness for MDROs among HCWs.

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Assessment of the antimicrobial activity of iodine cyanide

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Introduction: Public health is under threat in view of antimicrobial resistance (AMR), a challenge amplified by the increasing prevalence of biocide and multidrug resistance. This potentially affects our society negatively, as many bacterial and fungal infections are becoming untreatable. In addition, due to the loss of biocide efficacy via cross-resistance, disinfection can become increasingly difficult. Biocide resistance is a particular problem in hospitals, other healthcare settings, agriculture, the food industry, and particularly vulnerable communities., Hence, novel antimicrobial compounds are needed that can tackle AMR and biocide-resistant pathogens. One potential candidate is the antimicrobial agent iodine cyanide (ICN). This compound is synthesized in trace amounts within the human body, but data on its antimicrobial properties are scarce.
Material/Method: Therefore, this study evaluated the antimicrobial capacity of iodine cyanide against several representative microorganisms, including Gram-positive (*Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli*) and fungi (*Candida parapsilosis*, *Aspergillus fumigatus*). This was initially done using a combination of disk diffusion assays and gas phase inhibition tests for the selected strains. Thereafter, the antimicrobial properties if iodine cyanide were additionally confirmed by growth inhibition experiments, and its ability to inhibit bacterial biofilm formation using the crystal violet assay along with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).
Results: Iodine cyanide inhibited the growth of selected strains of *Enterococcus faecalis*, including a type strain (ATCC 29212) and two multidrug-resistant environmental isolates, and *Escherichia coli* (e.g., *E. coli* type strain ATCC 29522 and two antimicrobial-resistant environmental isolates). The growth of the tested *Enterococcus faecalis* and *Escherichia coli* strains was inhibited in rich medium to ≥ 50% at iodine cyanide concentrations of ≥ 100µM. Interestingly iodine cyanide inhibited the growth of these strains even when applied via the gas phase. In addition to planktonic cells, iodine cyanide inhibited biofilm formation by the tested strains of *E. coli* and *E. faecalis,* as was demonstrated using the crystal violet assay, SEM and CLSM. Furthermore, iodine cyanide showed antifungal properties as it inhibited the growth and affected the vitality of *Candida parapsilosis* (ATCC 22019), a common nosocomial pathogen, via the gas phase. Iodine cyanide even inactivated conidia of *Aspergillus fumigatus* (ATCC 204305).
Discussion: Iodine cyanide is a promising biogenic biocide candidate for controlling the growth and biofilm formation of Gram-positive and Gram-negative bacteria and the growth of fungi. Its ability to inhibit MDR bacteria, fungal conidia and microbial biofilm formation highlights the potential of this compound.

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Identification of novel CD8+ T cell epitope candidates in SARS-CoV-2 proteins

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Introduction: Novel corona Virus SARS-CoV-2 has emerged as a critical pathogen determining the current pandemic situation worldwide. One hallmark of the anti-viral adaptive immune response is the activation and subsequent differentiation of CD8+ T cells into cytotoxic T lymphocytes (CTL). This requires the interaction of MHC class I and the T cell receptor complex. MHC class I epitopes are produced by proteasome-mediated degradation of intracellular virus proteins through either peptide hydrolysis or peptide splicing. The resulting peptides are transferred to the MHC class I complex and transported to cell surface where the MHC-bound epitopes are recognized by specific CTLs. To verify the involvement of a CD8+ T cell response in the elimination of pathogens, it is a prerequisite to determine which MHC class I epitopes are processed by infected cells. In general, *in silico* epitope prediction provides information about sequences with the ability to bind MHC class I. However, T cell responses to these predicted sequences in patient samples appeared to be very heterogeneous. Therefore, we investigated the proteasome-mediated peptide hydrolysis and splicing of several regions in the spike protein with focus on specific MHC class I epitope candidates. In addition, we are currently analyzing these epitopes for their ability to elicit a CD8+ T cell response.
Method: Based on the results of the *in silico* analysis with netMHCpan, synthetic polypeptide substrates containing several possible MHC class I epitopes were synthesized. Subsequently, these substrates were processed *in vitro* with purified proteasomes. The resulting non-spliced and spliced peptide products were measured by Liquid Chromatography–Mass Spectrometry (LC-MS) and identified through bioinformatics. The putative epitopes are tested for MHC binding and for their ability to activate CD8+ T cells of convalescent COVID-19 patients.
Results: We analyzed synthetic precursor peptides from the spike protein of SARS-CoV-2 containing predicted epitopes for their specific proteasomal cleavage pattern. Thus, we identified spliced and non-spliced epitope candidates, which are localized in the receptor binding region and the C-terminus of the SARS-CoV-2 spike protein. Furthermore, the identified epitope candidates bind to MHC class I. The potential epitope-specific CD8+ T cell analysis is currently being processed.
Discussion: The identification of SARS-CoV-2 epitopes generated by the proteasome for MHC class I presentation may enable us to highlight specific regions of the spike protein which may play an important role in the host immune response. Furthermore, the results may give us the opportunity for future analysis of putative mutations within these regions, which is an issue for the efficacy of SARS-CoV-2 vaccines. Therefore, these results not only foster the understanding of the host-pathogen interaction, but also provide the basis for improvement of current vaccines and vaccination strategies.

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Establishment of a surrogate system for determining the potency of air purifiers on Biosafety Level-3 pathogens

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Question: The spread of viruses through air poses a high risk for health e.g. influenza and coronaviruses. Reducing the burden of airborne pathogens is therefore an effective measure to minimize the exposure. The potency of the air purifier is greatly affected by the ambient condition as well as differences in the biophysical properties of viruses. Moreover, with new emerging viruses, e.g. SARS-CoV-2, a high level of biosafety lab is necessary (BSL-3). This, however, is not widely available and of high maintenance cost. Therefore, in this study, feline coronavirus (FCoV) was used as a surrogate virus for SARS-CoV-2 to test the effectiveness of seven different air disinfection devices which are based on different disinfection principles.
Methods: The efficacy of ionization system, Ultraviolet C (UVC), electro-, hepa- and moss-based-filters as well as UVC/electrofilter with or without hepafilter on FCoV were examined. Using an aerosol generator with 5 bars, FCoV (approx. 1e7 TCID50/m3 air) was nebulized in a closed container. Air was drawn through a gelatin membrane filter at two sampling points, at the virus entry site and after filtration and/or ionization. The reduction in infectivity (TCID50) of the FCoV was determined on cell culture according to the Spearman–Kaerber method.
Results: Measurement at the point of virus entry revealed a 2 log10 reduction in FCoV infectivity due to handling and nebulization. The most potent systems (approx. 100% reduction) were the ones including hepafilter and/or UVC. The efficacy of air ionization system varied greatly depending on the number of emitters and the point of measurement (0-78%). With electro- or the moss-based filter, virus reductions of 80 and 20% respectively, were reached.
Conclusions: The use of hepafilter as well as UVC can be considered the best method to inactivate infectious coronavirus particles in air.

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From raw sequences to alert: bioinformatics infrastructure and targeted method development in response to SARS-CoV-2

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Introduction: From the beginning of last year, the world has witnessed the emergence and unprecedented spread of SARS-CoV-2 which has so far claimed 3.5 million lives worldwide (June 2021, WHO). During the pandemic, viral genome sequencing and subsequent bioinformatics data analyses proved to be essential to track the spread of the virus and to detect emerging mutations as well as variants of concern that can be associated with antibody escape or higher transmissibility[1-4]. To accomplish these tasks, bioinformatics methods must be particularly agile, versatile, efficient, reliable, and scalable, even on rapidly growing and diverse data sources, to provide much-needed unambiguous information.
Material and Methods: Here, we describe our SARS-CoV-2 bioinformatics workbench covering all steps from short- and long-read genome reconstruction, data and consensus QC, genomic profiling, and outbreak detection. Optimized genome reconstruction[5] and virus lineage assignment tools were complemented by novel methods for SARS-CoV-2 mutation screening, positive selection detection, sequence clustering, phylogenomics, as well as genomic incidence estimation[6] to rapidly identify and report concerning evolutionary developments as they unravel. Our tools are publicly available and implemented using state-of-the-art workflow management systems, robust container and software packaging systems, and open-source software.
Results: Based on data from 1,500 in-house sequenced SARS-CoV-2-positive samples and 150,000 whole-genome sequences collected in Germany since January 2021, we highlight how the different analysis steps are combined and automated to enable rapid alert systems for emerging viral variants and mutations. We demonstrate the performance of such a genomic surveillance

system using selected sequence clusters and outbreak scenarios that have been analyzed and monitored in recent months.

Discussion: The ongoing pandemic has demonstrated the crucial importance of viral sequencing and bioinformatics. Genomic surveillance is currently an integral part of the national response to the virus and will allow retaining the upper hand as vaccine roll-out steers us on a path towards ending the pandemic.

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COVID-19 vaccination and immunisation surveillance for employees in German hospitals – Results from a cross-sectional study

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Background: With the fast development and approval of different vaccines acting against SARS-CoV-2, hospitals were urged to develop strategies not only for surveillance and testing, but also vaccination of employees. Since needs and resources of small, mid-sized and large hospitals differ markedly we surveyed hospitals regarding their experience with respect to surveillance and vaccination of employees, with the ultimate goal of developing recommendations of action.

Methods: The data come from a cross-sectional, standardized, ethically approved online survey (LimeSurvey) of German hospitals in March 2021 as part of the B-FAST project of the Network of University Medicine (NUM). The sample included nationwide university hospitals as well as non-university hospitals from two federal states. The focus is on questions about COVID-19 vaccination and immunization of employees. Descriptive statistics were applied. The hospitals were grouped according to the number of hospital beds: small (<250 beds, n=30), mid-size (250-999 beds, n=39) and large hospitals (≥1000 beds, n=31).

Results: We received 100 responses comprising 33 (92%) of university hospitals and 67 (18%) participating hospitals in two federal states. Two-thirds of the large hospitals (64.5%) started with the vaccination of their employees in December 2020, while most of the small (63.3%) and mid-sized (56.4%) hospitals started in January 2021. 80% of respondents have not yet decided on conducting a boost vaccination in autumn of 2021. Relevant information for decision making include e.g. vaccine effectiveness (77%), variants of concern with potential for immune escape (75%) and vaccine availability (61%). Regardless of complete vaccination, 41% of hospitals do test their employees for SARS-CoV-2 (small 36.7%; mid-sized 38.5%; large hospitals 48.4%). Most often testing is offered in case of symptoms (88%) and exposition (78%), respectively. Of course, testing is offered for employees in COVID-19 areas (51%), but also regardless of the area of work (45%) (table 1). In case of infections in completely vaccinated employees, 72% will initiate further diagnostics, e.g. strain sequencing (61%) and anti-SARS-CoV-2 antibody quantification (46%), respectively. Only 18% of respondents definitely plan to test for SARS-CoV-2 antibodies in vaccinated employees regardless of an infection; 6% plan to determine neutralizing antibodies in employees (table 2).

Conclusion: Our data suggest a rapid implementation of vaccination possibilities for employees in hospitals of all sizes. However, there seems to be a broad range of testing and surveillance strategies in completely vaccinated employees. Based on the growing knowledge, common recommendations addressing this issue would be of benefit, even more in cases of newly occurring variants of concern.

Fig. 1

Table 1. SARS-CoV-2 testing and surveillance strategy for vaccinated employees in German hospitals. All values are presented in percent and stratified according to hospital size.

	Small hospitals n=30	Mid-size hospitals n=39	Large hospitals n=31	Total n=100 (95% CI)
PCR-testing of vaccinated employees	36.7	38.5	48.4	41.0 (31.4 – 50.6)
Testing strategies for vaccinated employees				
Symptom-induced testing	**76.7**	89.7	96.8	88.0 (81.6 – 94.4)
Exposition-induced testing	70.0	79.5	83.9	78.0 (69.9 – 86.1)
Regular testing in COVID-19 areas	56.7	46.2	51.6	51.0 (41.2 – 60.8)
Regular testing regardless of area of work	53.3	51.3	**29.0**	45.0 (35.2 – 54.8)
As part of clinical trials	**0.0**	10.3	**16.1**	9.0 (3.4 – 14.6)
Further diagnosis of infections in vaccinated employees	70.0	69.2	77.4	72.0 (63.2 – 80.8)
Strain sequencing	56.7	59.0	67.7	61.0 (51.4 - 70.6)
Anti-SARS-CoV-2 Ab quantification	46.7	33.3	**61.3**	46.0 (36.2 - 55.8)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

Fig. 2

Table 2. Time and form of testing for SARS-CoV-2 antibodies in vaccinated employees in German hospitals. All values are presented in percent and stratified according to hospital size.

	Small hospitals n=30	Mid-size hospitals n=39	Large hospitals n=31	Total n=100 (95% CI)
Time of testing for SARS-CoV-2 antibodies				
Defined time after second vaccination	**6.7**	7.7	9.7	8.0 (2.7 – 13.3)
Before winter season 2021	6.7	5.1	**0.0**	4.0 (1.1 – 9.9)
Another defined time	-	-	-	6.0 (1.3 – 10.7)
Unsure/undecided	-	-	-	12.0 (5.6 – 18.4)
No testing planned	73.3	74.4	61.3	70.0 (61.0 – 79.0)
Form of testing for SARS-CoV-2 antibodies				
All antibodies	10.0	10.3	19.4	13.0 (6.4 – 19.6)
Neutralizing antibodies	6.7	5.1	**6.5**	6.0 (1.3 – 10.7)

Sub-group proportions outside of the 95% confidence interval for the entire sample are presented in **bold type**.

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Impact of weekly screening on a flexible testing strategy for inpatients

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Introduction: Testing strategies in acute care hospitals during the SARS-CoV-2 pandemic should be flexible and adaptable to both local epidemiology and transmission dynamics. The role of infectious yet asymptomatic individuals has already been described. Rapid identification of presymptomatic and wholly asymptomatic individuals is crucial for effective infection control in a hospital setting, as constant mask wearing by patients cannot be realized.

Method: To evaluate the efficacy of a weekly SARS-CoV-2 screening via PCR for all inpatients, we compared the results of identified active cases during two separate periods with differing incidence rates within the COVID-19 pandemic. The first period is defined from March 12th, 2020 to December 13th, 2020, and the second period is defined from December 14th, 2020 to May 10th, 2021. The 7-day notification rate in the area exceeded 100 cases

per 100,000 inhabitants for 5 weeks in the first observation period and for 16 weeks during the second. We compared the number of cases identified via screening upon admission as well as in alignment with recommendations from the Robert Koch Institute (RKI) with the number of cases detected by the additional weekly screening of all inpatients (implemented on December 14th, 2020). Additionally, we classified all cases according to the severity of symptoms.

Results: We examined 1,059 cases of laboratory-confirmed SARS-CoV-2 infection from inpatients at a university hospital. In 565 cases (53.4%), the infection was diagnosed for the first time; 453 patients tested positive prior to or upon admission, whereas 112 patients underwent screening for various other indications. Among the 453 cases, 108 patients (23.8%) had no COVID-19-associated symptoms at time of screening. 33 of the 49 cases (67.3%) identified via weekly screening during the second observation period exhibited no COVID-19-associated symptoms at time of screening.

In Table 1, we compared the rates of identified active cases during two time periods with regard to the manner of implemented screening.

Tab. 1 Screening indication and case detection rate

	Total N =	Screening prior to or upon admission N =	Screening according to RKI recommendations N =	Regular weekly screening
Period 1	178	164 (92.1%)	14 (7.9%)	---
Period 2	387	289 (74.7%)	49 (12.7%)	49 (12.7%)
Total	565	453 (80.2%)	63 (11.2%)	49 (12.7%)

Conclusion: While community screening is integral to the management of the SARS-CoV-2 pandemic, it is equally important to conduct thorough and intensive screening in hospitals when there is widespread community transmission. Screening upon admission revealed the highest case detection rate independent of the notification rate. Moreover, the regular weekly screening of all inpatients detected as many cases as the screening measures identified and recommended by the RKI. Additional studies are needed to define an incidence threshold at which expanded screening measures should be established, if necessary.

135/HYPRP

Targeted molecular detection of multi-resistant bacteria on clinical surfaces

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Background: Here, we describe an integrative method to detect carbapenemase-producing gram-negative bacteria (gn-Cp) on surfaces/fomites in the patient environment. For sampling, we examined patient rooms from different wards of a 1400-bed university hospital in Germany. From December 2018 to June 2020, we examined environmental samples from 28 patient rooms occupied with patients who were proven to be colonized with gn-Cp by rectal screening.

Methods: Depending on the duration of the patient´s hospitalization, we took samples after 24 hours, 72 hours and one week. For sampling, we divided the patients´ environment into four parts and took samples from near- and extended patient areas. To obtain a representative bacterial swab from a larger surface, such as the patient cabinet, we used Polywipes. To enrich the bacteria within the wipe, we used CASO boullion. The bacterial DNA was isolated. Carbapenemase was detected with specific qPCR primers.

Results: With this culture- and molecular-based approach, we could control the effectiveness of cleaning and disinfection in everyday clinical practice in and around the patient room. Therefore, we could track the spread of gn-Cp within the patient room. From the 28 patients colonised with gn-Cp, we detected three colonised with *Pseudomonas aeruginosa,* ten with *Citrobacter freundii,* eight with *Escherichia coli,* two with *Enterobacter cloacae,* three with *Klebsiella pneumoniae*, one with *Klebsiella oxytoca,* and one with *Enterobacter hormaechei*. The carbapenemases found were 16 VIM, five OXA-48, five NDM, one IMI, and one KPC.

The number of positive detections fluctuated between 30.5 % (mean value positive results after 72 hours) and 35.2 % (after 24 hours and one week). Interestingly, there was no difference in the exposure to gn-Cp between the areas with hand contact (area "hand" mean value positive results, 27.4 %) and areas without hand contact (area "extended" mean value positive results, 28.9 %), although the disinfection cycle also differs in these areas.

Discussion: The method used to detect multidrug-resistant bacteria in the environment of patients by using Polywipes is reliable and can therefore be used as an effective, new tool in hygiene and infection control. Our results indicate that precise detection tools are needed as a basis to prevent nosocomial transmission of gram-negative bacteria.

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Staphylococcus aureus and *Staphylococcus schweitzeri* from fomites in Nigeria

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Abstract

Introduction: Fomites are inanimate objects that transmit pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), to humans. Recently, the *S. aureus*-related complex (*S. aureus*, *S. argenteus*, and *S. schweitzeri*) was established based on phylogenetic and taxonomic analyses. However, phenotypic characteristics are insufficient in the delineation of these species. In this study, we describe the *S. aureus*-related complex from inanimate surfaces in Nigeria.

Methods: Fomite samples (n=239) in Obafemi Awolowo University, Nigeria, were initially screened for *S. aureus*. The sampling period was from October 2015 to June 2016. Delineation of the *S. aureus*-related complex was determined by MALDI-TOF, PCR of the *S. aureus* specific thermonuclease, and the nonribosomal peptide synthetase genes. Characterization of the isolates was based on antimicrobial susceptibility, *spa* typing, multilocus sequence typing, and virulence gene detection (*lukS*/*lukF-PV*, *chp*, *sak*, *scn*). Representative isolates were selected for whole-genome sequencing.

Results: *S. aureus* (n=14) and *S. schweitzeri* (n=2) isolates were identified, including three MRSA. The *S. aureus* isolates were classified as ST8/CC8, ST30/CC30, ST15/ST5875/CC15, ST508/ST5876/CC45, ST121/CC121, ST152/CC152, and ST3961. All the CC30, CC121, and CC152 isolates were *lukS*/*lukF-PV* positive. The MRSA isolates (PVL+) were assigned with CC152. Phylogenetic analysis revealed that the *S. schweitzeri* isolates were closely related to those from the fruit bat (*Eidolon helvum*) in Nigeria.

Conclusions: MALDI-TOF and PCR achieved the differentiation of *S. aureus* from *S. schweitzeri*. Fomites are a reservoir for *S. aureus* and *S. schweitzeri* that was so far recovered primarily in African wildlife.

Keywords: Fomite, *Staphylococcus aureus*, *Staphylococcus schweitzeri*, Genotype, Animal

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Evaluation of software tools to determine outbreak-specific genotypic markers for a rapid and cost-efficient surveillance system for *Acinetobacter baumannii* outbreaks

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Question: *Acinetobacter baumannii* is a bacterial pathogen that causes hospital-associated infections worldwide. Its high environmental tolerance and increasing multi-drug resistance frequently contribute to nosocomial outbreaks, leading to enhanced mortality of patients. Thus, rapid detection and tracking of *A. baumannii* outbreaks is important to improve patients' safety. Nowadays, whole-genome sequencing (WGS) enables perfect

outbreak surveillance, but the time-consuming and expensive method is not applicable in every hospital. To reduce turnaround time and enhance cost efficiency and accessibility of outbreak tracking, we are currently developing a system that combines WGS and real-time PCR detection of *A. baumannii* outbreak strains by targeting outbreak-specific single nucleotide polymorphisms (SNPs) previously detected bioinformatically.

Methods: To find the most suitable SNP detection tool, we evaluated performance and outcome of the software tools SeqSphere, RUCS, Gegenees and Find differential Primers, of which the latter was developed to directly find primers targeting outbreak-specific SNPs. We investigated to which extend the different tools were able to detect outbreak specific SNPs or primers, using the sequenced index isolate of a former *A. baumannii* outbreak consisting of 15 isolates as target genome and two collections comprising 481 and 228 *A. baumannii* genomes as non-target genomes, respectively. We determined run time of the tools and screened the resulting SNPs (up to 20 chosen randomly) for their presence in the remaining 14 outbreak isolates and their absence in other *A. baumannii* genomes via alignment analysis.

Results: Analysis time and results varied considerably between the tools. Find differential Primers did not detect any outbreak-specific primers. In contrast, SeqSphere, RUCS, and Gegenees found putative outbreak-specific SNPs within 1 h, 1 h, and 31 h, respectively. Using the collection with 481 non-target genomes, only SeqSphere and RUCS detected two and 24 SNPs. Whereas both SNPs identified with SeqSphere were outbreak-specific, only two of 20 SNPs detected by RUCS and randomly selected for alignment analysis were exclusively present in all 15 outbreak isolates and absent in other *A. baumannii* genomes. Applying the smaller collection, SeqSphere, RUCS, and Gegenees found nine, 65 and 11 SNPs, respectively, of which two, two and one SNP(s) of maximally 20 SNPs screened by alignment analysis were unique to all outbreak isolates. All remaining SNPs tested were unspecific, i. e. absent in some outbreak isolates or present in non-outbreak strains.

Conclusions: SeqSphere and RUCS were the most suitable SNP detection tools as they found outbreak-specific SNPs within 1 hour. To avoid the detection of unspecific SNPs, analysis should be done with a large non-target genome collection. This evaluation will serve as a basis for the development of outbreak-specific SNP detection assays by real-time PCR in the laboratory.

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Adherence to hand hygiene procedures in the context of toilet usage of school children at German primary schools

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Introduction: Semi-public areas such as schools pose an increased risk of the transmission of various infectious diseases, in particular respiratory and gastrointestinal infections. One of the most effective measures for the prevention of infectious disease transmission is hand hygiene. However, data on children´s hand hygiene are scarce. In particular, there is a lack of insight into such hygiene aspects associated with the use of toilets facilities. In a pilot study, we investigated the frequency of sanitary facilities by primary school children with focus on the use of touchless soap dispensers at school.

Materials and Methods: In this feasibility study, electronic door contacts at the entrance doors of sanitary facilities and touchless soap dispensers with usage recording capability were installed to analyze the frequencies of toilet use in relation to children´s handwashing. Four primary schools (PSs) of the German federal state North-Rhine Westphalia were included. The data collection took place during the school year 2018/2019. The overall proportion of handwashing after toilet use (i.e. handwashing compliance) was calculated as ratio of dispenser activations/door activations.

Results: Overall, 916 children were included with a girl/boy ratio of 449 (49.0%)/467 (51.0%) varying between 90 (PS-A; 44/46), 162 (PS-B; 77/85), 289 (PS-C; 141/148), and 375 (PS-D; 187/188) children. The overall mean value (SD) of soap dispenser activations per day were 71.9 (59.2) on average ranging between schools from 19.7 (12.4) in PS-A, 51.6 (30.0) in PS-B, 98.8 (36.3) in PS-C to 153.9 (53.4) in PS-D and overall mean value (SD) of door activations per day (entry and exit count as one operation) were 138.8 (59.7) on average ranking from 69.1 (13.9) in PS-A, 121.3 (18.4) in PS-B, 176.1 (21.8) in PS-C to 213.4 (42.5) in PS-D. The mean compliance (SD) is 0.49 (0.37) over all schools (PS-A, 0.29 (0.21); PS-B, 0.43 (0.23); PS-C, 0.56 (0.21); and PS-D, 0.79 (0.59)). A stratified analysis by gender revealed that girls from three schools washed significantly (Wilcoxon-test) more often their hands when visiting the toilet facilities (mean values compliance girl/boy; PS-A, 0.28/0.86, P=0.554; PS-B, 0.49/0.44, P<0.001; PS-C, 0.63/0.51, P=0.001; and PS-D, 1.3/1.1, P<0.001).

Discussion and conclusion: Our study results provided novel insights into the usage of sanitary facilities by school children at PSs and their adherence to hand hygiene procedures. With regard to the calculated compliance, a significant gender difference was observed in the majority of the schools. Further studies are warranted in the school setting to analyze how an increase in the use of soap dispensers and a better compliance with hygiene procedures in general can be achieved. Our feasibility study revealed that our study design is capable of reliably collecting anonymous data on hand hygiene procedures associated with the usage of toilet facilities as precondition for future intervention studies.

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Molecular epidemiology of *Vancomycin resistant enterococci* (VRE) at a tertiary care medical centre 2004-2010

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Introduction: Due to the rise in the number of invasive VRE infections in Germany, there is a need to understand the way VRE spreads in hospitals. In this study, we investigate the molecular epidemiology of VRE isolates from the first accumulation of VRE at our institution in 2004 until 2010. In a retrospective analysis of all first VRE isolates from each patient, we used Whole Genome Sequencing (WGS) and Multilocus Sequence Typing (MLST)/core genome (cg) MLST to explore the question whether a hyperendemic outbreak took place or whether different sequence types/complex types contributed to the rise in cases. A significant influencing factor in our analysis is the introduction of VRE screening that started in 2007.

Methods: Our analysis includes all first VRE isolates of each patient at our hospital collected during the years 2004-2010. We also recorded basic patient data and type of specimen. We performed repeated testing for species determination (MALDI-TOF), antibiotic resistances and PCR for detection of *vanA/B*. Species other than *E. faecium* were not included. In a first project, we performed conventional MLST. As part of a second project, WGS was performed for cgMLSTyping.

Results: Until 2006 detections of VRE remained singular cases, however, in 2007 there was a sharp rise in cases reaching a peak in 2008, until a decrease in 2009 and 2010. Of 167 VRE first isolates from 2004-2010 95% had a *vanB* resistance cluster and only 5% *vanA*. Overall, ten different sequence types were detected. ST17 (2008: 28%) and ST192 (2008: 54%) were mainly responsible for the sharp increase in VRE in 2007-2008. From 2009-2010 ST17 and ST192 still formed a significant part of the sequence types but ST117 as a new sequence type was identified in equally high numbers (each about 30%). By cgMLST a more detailed

epidemiological analysis was possible: Thereby we could identify CT3256 as the dominant complex type within ST17, and CT10 as the dominant complex type within ST192. However, it also became evident that there were several other, additional complex types within each sequence type so that the spread was not as monoclonal as could be understood by MLST analysis only. In the early years of this study (2004-2006) nearly all the analyzed VRE stem from clinical specimens, whereas since 2007 about half of the VRE were detected by screening. Concerning the distribution of sequence types within the hospital, we found that ST192 dominated in intensive care as well as in normal wards while we detected ST17 more commonly in normal wards.

Discussion: MLST and cgMLST analysis point at a spread of sequence types ST17/CT3256 and ST192/CT10 as the main cause for the first peak in VRE cases in 2007/2008. The trigger for the spread of these sequence types in our hospital might have been an outbreak in two intensive care units.

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Surveillance and characterisation of carbapenem-resistant Gram-negative bacteria in liver transplant candidates using whole genome sequencing

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Objectives: In patients with liver cirrhosis, colonization and infection with carbapenem-resistant Gram-negative bacteria (CRGN) are associated with high mortality. Although crucial for a customized prevention strategy, epidemiological context and resistance determinants of these isolates often remain elusive. To pinpoint resistance determinants and unravel potential in-hospital transmission routes, we employed whole genome sequencing (WGS) of CRGN patient isolates obtained from our center between 2010 and 2018.

Methods: A total of 351 patients on the waiting list for liver transplantation were screened for CRGN upon listing, transplantation or admission to ICU. We identified 24 CRGN isolates of which 18 were further analyzed. Using WGS and bioinformatics genotypes, resistome, mobile genetic elements and phylogenetic relatedness were explored. Potential epidemiological links were assessed by a detailed analysis of patient charts.

Results: In total, carbapenem-resistant (CR) *Klebsiella pneumoniae* (n=9) and CR *Pseudomonas aeruginosa* (n=7) were the predominating pathogens among others (n=2). Four different carbapenemase genes were identified *in silico* (encoding VIM-1, VIM-2, OXA-232 and OXA-72) while the majority of isolates belonged to the group of non-carbapenemase producing CRGN. Core genome phylogenetic analysis proved a broad genetic variety, with three *K. pneumoniae* isolates being in closer relation to one another. Analysis of the respective patient records for these three patients revealed no epidemiological context. Further, analysis of porin sequences known to be involved in carbapenem resistance unveiled individual mutations (insertion of transposon, premature stop codon and frameshift mutation) for these three isolates.

Conclusion: Our data demonstrate a high diversity of CRGN in our cohort of patients with liver cirrhosis, excluding transmission of CRGN or any evidence for plasmid-mediated spread of carbapenemases within the group. For the three closely related isolates, an individual acquisition of carbapenem resistance appears likely. Therefore, the data incentivize targeted screening for certain multidrug-resistant isolates prone to subsequent acquisition of carbapenem resistance in combination with antibiotic stewardship programs for this vulnerable patient cohort.

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OXA-484, a new carbapenemase of the OXA-48-like class D beta-lactamase family identified in *Escherichia coli*

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Introduction: OXA-48 is one of the most prevalent carbapenemases in Enterobacterales worldwide. Several OXA-48 variants (OXA-48-like) with diverse genetic background, hydrolysis activity and dissemination pattern have been described. Here, we investigated the so far uncharacterised OXA-48 variant OXA-484 from a clinical *Escherichia coli* isolate regarding antibiotic resistance pattern, horizontal gene transfer (HGT) and genetic support of $bla_{\text{OXA-484}}$.

Methods: Clinical isolates harbouring *bla* genes encoding for OXA-484, OXA-48, OXA-181, OXA-232 and OXA-244 were characterised by whole genome sequencing. The encoding genes were cloned into an pCR-Blunt II-TOPO expression vector and *E. coli* and *Klebsiella pneumoniae* recipients were transformed. Transconjugation frequencies of $bla_{\text{OXA-48-like}}$ were determined by liquid mating assays utilising the sodium azide-resistant strains *E. coli* J53 and *K. pneumoniae* PRZ as recipients. The resistance phenotype caused by OXA-48-like carbapenemases were compared by analysing minimal inhibitory concentrations (MICs) of transconjugants (Tc) harbouring natural plasmids and transformants (Tf) harbouring expression vectors carrying $bla_{\text{OXA-48-like}}$.

Results: OXA-484 was identified in a clinical *E. coli* isolate, which showed slightly elevated carbapenem MICs but did not grow on screening agar plates used for detection of carbapenem-resistant Enterobacterales. The isolate belonged to the high-risk clone ST410, which has been described as a worldwide distributed extraintestinal pathogenic lineage causing nosocomial outbreaks. OXA-484 differed by the amino acid substitution 214G in the β5-β6 loop compared to the most closely related variants OXA-181 (214R) and OXA-232 (214S), which is essential for hydrolysis activity. $Bla_{\text{OXA-484}}$ was encoded on a self-transmissible 51.5 kb IncX3 plasmid (pOXA-484) with high sequence similarity to widely spread plasmids harbouring $bla_{\text{OXA-181}}$. Frequencies of intraspecies and intergenus HGT of pOXA-484 (1.4×10^{-7} to 2.1×10^{-6}) were similar to pOXA-181 (5.2×10^{-8} to 3.6×10^{-5}) but lower compared to pOXA-48 (8.6×10^{-5} to 1.1×10^{-3}). OXA-484 elevated MICs of temocillin and carbapenems in Tc and Tf similar to OXA-232 and OXA-244 but lower compared to OXA-48 and OXA-181 resulting in a difficult to detect antibiotic resistance phenotype.

Conclusions: OXA-484 combines properties of OXA-181-like IncX3 plasmid support and transferability and beta-lactamase activity of OXA-232. Low MICs for temocillin and carbapenems and consequently the difficult detection by screening agars in combination with the capability for HGT might promote the silent dissemination of OXA-484 carrying Enterobacterales.

Postersession 02
14. Sep. 2021 • 11:15–12:45

Part 2: General and Hospital Hygiene (StAG HY): Antimicrobial Resistence and Drugs, Infection Prevention (FG PR)

143/HYPRP
Survival of nosocomially relevant bacteria on different inanimate surfaces

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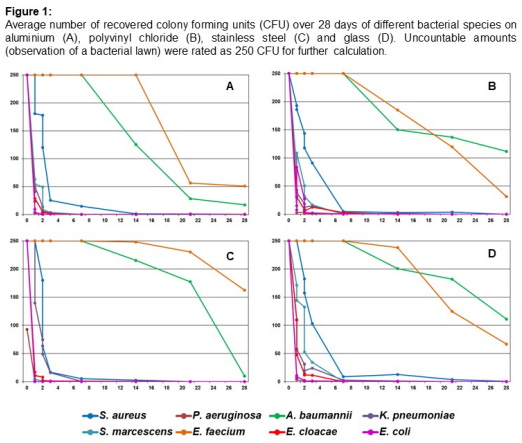
Background: The likelihood of bacterial transmission in the hospital setting depends on the pathogen´s tenacity. Unfortunately, data on this topic are rather scarce and rely largely on estimations from nosocomial outbreak reports only [1].
Methods: We determined survival kinetics of the eight most prevalent nosocomial species (*Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27853, *Acinetobacter baumannii* DSM30011, *Klebsiella pneumoniae* ATCC700603, *Serratia marcescens* DSM12485, *Enterococcus faecium* ATCC19434, *Enterobacter cloacae* ATCC13047, and *Escherichia coli* ATCC25922) on the four types of material mainly used in hospitals (aluminum, stainless steel, glass, and polyvinyl chloride (PVC)). Bacterial suspensions were standardized by a McFarland turbidity of 1.0 and then plated on ten separate spaces in volumes of 25 µL each on the above mentioned surfaces. Surfaces were thereafter stored uncovered at room temperature in order to maintain conditions as given in routine daycare of patients. Contact plates (Replicate Organism Detection And Counting (RODAC)) were used for sampling over a period of 28 days at ten defined time points. All experiments were performed in independent triplicates (overall 960 samples).
Results: *Acinetobacter baumannii* and *Enterococcus faecium* showed the highest survival capability regardless of the type of surface tested. Viable bacteria of those species remained detectable even at the end of the entire observation time period. In contrast, survival of other species was limited to approximately seven days only. Figure 1 summarizes the outcomes of bacterial recovery from the different surfaces over time.
Conclusion: These results are important information for all staff in the field of infection control as they explain late events of nosocomial transmissions. They stress the importance of thorough cleaning and surface disinfection measures in order to prevent pathogen spread and nosocomial outbreaks.
References:
1. Kramer A, Schwebke I, Kampf G. How Long Do Nosocomial Pathogens Persist on Inanimate Surfaces? A Systematic Review. BMC Infect Dis 2006;6:130. (doi:10.1186/1471-2334-6-130)

Fig. 1



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Surgical site infection-preventive compliance with 14 pre-, intra- and postoperative measures after tailored interventions for infection prevention and control (IPC) teams and other IPC stakeholders: An overview of the cluster-randomised controlled WACH-trial's results in six non-university centres

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Question: Preventing surgical site infections (SSI) implies compliance with numerous measures.[1] Few randomized studies have addressed compliance promotion in this context. The cluster-randomized trial WACH ("Wound Infections and Antibiotics Use in Surgery", 2018-20; German Clinical Trials Register-ID: DRKS00015502) aimed at improving SSI-preventive compliance by adapting the psychological tailoring approach of the PSYGIENE-trial (DRKS00010960) from interventions addressing clinical staff to infection prevention and control (IPC) teams and other stakeholders in non-university hospitals.
Methods: After briefing, in each of N=6 hospitals in-house staff observed compliance for 14 measures in N=1034 ward rounds and N=905 surgical procedures (general/visceral and/or orthopedic/trauma surgery) before and after a 3-4 months intervention ("tailoring"-arm) or "usual practice" period. Interventions consisted of written reports and 2-day IPC-workshops incl. coaching, and were tailored based on appraisals of compliance and its determinants (COM-B [2]) using mixed methods. Data analysis (cluster-level) were performed using OpenEpi 3.01 for compliance estimations, chi-square and Breslow–Day tests.
Results: In the "tailoring"-arm, a compliance increase was observed for all measures (mean: 11%), with $p<0.05$ in 11 cases. However, for seven of these measures baseline compliance rates were lower than in the "usual practice"-arm (with these differences ranging between -50% and -6%), while in the latter study arm, one increase and three decreases were significant (mean overall change: -1%). Given similar baseline, "tailoring" was superior for hand hygiene during ward rounds (+12% vs. +3%; interaction: $p=0.007$) and wearing surgical cap (+22% vs. -13%; $p<0.001$).
Conclusions: Adapting psychological tailoring to IPC teams in non-university hospitals to promote SSI-preventive compliance showed mixed results partly due to lower baselines in the "tailoring"-arm. Also, hand hygiene tended to be emphasized in the interventions and by their addressees, possibly explaining the positive result regarding this parameter. Overall, this suggests stricter use of bundles within multimodal strategies.
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Titanium dioxide-based UV-activated prevention of germ adhesion and biofilm formation in siphons

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Introduction: Nosocomial infections are along with antibiotic-resistant germs one of the most critical problems in hospitals. Potential sources of such infections are siphons under clinical sanitary facilities. They are permanently water-bearing systems with an average of 10^5-10^{10} CFU/ml in the barrier fluid and a pronounced multispecies biofilm, generally posing a high risk for infections. Water inflow into the barrier fluid of the siphon releases the pathogens by forming splash water and aerosols, resulting in germ transmission to patients and nursing staff. Even if the siphon is cleaned regularly and intensively, it is impossible to prevent the formation of biofilms [1]. Therefore, a new type of siphon should be equipped with a TiO_2-based surface functionalization that produces a germ-reducing effect through UV-induced superhydrophilicity (Fig. 1), i. e. prevents the adhesion of pathogens on the surface [2].

Fig. 1: Functionalized siphon prototype with integrated UVA-LED

Material/Methods: The deposition of TiO_2 of different layer thicknesses was carried out in different combinations (interlayer, substrate) using pulsed magnetron sputtering. The layer composition was examined by SEM. After conditioning the samples, the wetting behavior of the coating was measured by contact angle measurements after activation (365 nm LED) of the coating.

In an application-oriented microbiological regime on flat samples and curved geometries, the reduction of adhesion on the surface was investigated using the model organism *E. coli*. The coating was activated, incubated with 10^3 CFU/ml, the surface rinsed, followed by a second irradiation, incubation (17 °C, 22 h) and a microbial count by impedance measurement.

Statistical significance was tested using a T-test for normally distributed and a Mann-Whitney test for non-normally distributed values.

Results: The super-hydrophilic effect was observable up to 2 months in dependence on the applied substrate.

In the microbiological evaluation, after 30 min of irradiation a germ reduction up to 99 % was demonstrated on flat samples, and a complete prevention of germ adsorption was determined on curved geometries.

Conclusions: The geometry influences the size and packing density of the coating, which results in an influence on the efficiency of the effect. Even with short activation times, superhydrophilicity can prevent the accumulation of germs on the surface and thus prevent biofilm formation. The combination of this TiO_2- coating with a UV-LED should enable a permanent germ reduction in the siphon, hence preventing infection transmission.

Acknowledgement: The project was funded by the Federal Ministry for Economic Affairs and Energy.

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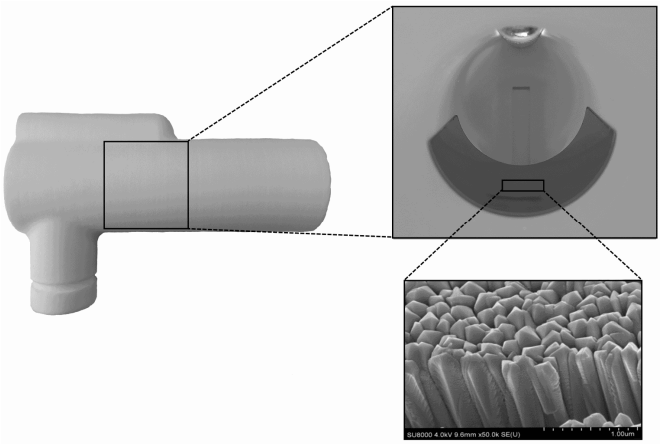
Fig. 1



146/HYPRP

Decolonisation of human skin by application of a photodynamically active hydrogel

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Introduction: Skin colonization with pathogenic, even multi-resistant bacteria is a potential source of infections. Photodynamic inactivation (PDI) of bacteria is a promising, new approach for the decolonization of the human skin. PDI needs a dye molecule (photosensitizer), which generates mainly singlet oxygen upon light exposure. Thereby bacteria are oxidative destroyed regardless their type and resistance profile. Preliminary PDI experiments already provided a decontamination efficacy on human *ex vivo* skin of 99.9% using an aqueous photosensitizer solution. In a follow up study, we aimed to improve the decolonization efficacy by applying a photodynamically active hydrogel.

Methods: Fresh e*x vivo* human skin was inoculated with bacterial suspensions of methicillin-resistant *Staphylococcus aureus* (MRSA). After visible drying of bacteria, different hydrogels containing different photosensitizers were added to the inoculated skin surface. Subsequently, the skin surface were irradiated with visible blue light at different radiant exposures (J/cm²). Then, bacteria were recovered from skin with a swap and plated via drop-plate method on Mueller Hinton Agar. Colony count unfold reduction efficacy when referenced to internal controls. After recovery, skin biopsies were taken and nitro blue tetrazolium (NBT) staining was applied to check viability of skin cells via mitochondrial activity. TUNEL stain revealed the presence of apoptotic skin cells.

Results: The results showed that PDI created an improved MRSA reduction on skin of around 99.99%. Staining of the tissue revealed no noteworthy apoptosis or lack in mitochondrial activity of the human cells due to the application of the photodynamically active hydrogel.

Conclusion: PDI should represent an easy, quick and efficient method to decolonize human skin from MRSA. The results of the study are remarkable, as substances on skin like inorganic ions or small organic molecules such as histidine are capable to inhibit the photodynamic efficacy. Since PDI is known to eradicate a large set of pathogens independent of their antibiotic resistance profiles, this new technology should be tested in clinical studies in the near future.

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Serratia marcescens outbreak in a neonatal intensive care unit due to contaminated donor milk

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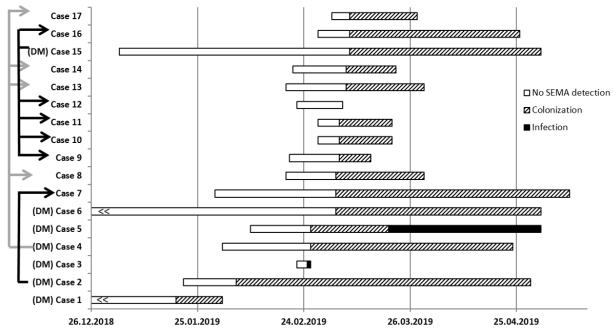
Introduction: Here we describe an outbreak of *Serratia marcescens (SEMA)* in the perinatal center of a tertiary care hospital caused by contaminated raw milk from a single breast milk donor. The aim of this study is to describe the course of the outbreak, the countermeasures taken and the implications of this incident for the use of raw donor breast milk for feeding preterm infants in our hospital.
Methods: After recognition of the outbreak situation an intensified screening program was implemented. Inpatients of the neonatal intensive care unit were screened every other day. In addition, frequent hand contact surfaces, medical equipment, shared care utensils, and surfaces in the milk kitchen were monitored by cultures. We conducted a case-control analysis to identify potential risk factors for *Serratia marcescens* infection or colonization. To test whether the isolates were genetically linked, pulsed-field gel electrophoresis and next-generation sequencing was performed on isolates of the outbreak strain and from isolates of the suspected donated breast milk.
Results: In a retrospective case-control analysis, we found a significant correlation between *Serratia marcensens* infection or colonization and the consumption of raw milk from a single female milk donor ($p < 0.0001$). Pulse field gel electrophoresis confirmed the suspected donor milk as the origin of the first part of the outbreak. The first six colonized/infected children, received unpasteurized donor milk from that single donor that was previously screened negative for the presence of SEMA. The culture system used in the microbiology laboratory had a detection limit of 100 colony forming units/ per ml. The results of the case control study indicate, that the residual *Serratia marcensens* of negatively tested donor milk samples was sufficient for colonization/ infection of infants who received raw donor milk. In the subsequent second outbreak phase, the pathogen was likely transmitted from the seven index patients to other ten preterm infants that were treated by the same nursing staff as the index patients. The outbreak was finally controlled by the implementation of an infection control bundle including a multidisciplinary infection control team, temporary nutrition of infants with formula only, repeated screening of all inpatients, strict coat and glove care, process observation, retraining of hand hygiene and continuous monitoring of environmental cleaning procedures.
Conclusions: Unpasteurized donor milk is a source of infection and colonization of preterm infants, even if the microbial load is very low.
Table 1: Risk factors for initial colonization/infection with SEMA
Figure 1: SEMA colonization and infection cases. Each bar represents the length of the hospital stay of individual patients. Open bars indicate SEMA-negative, dashed bars indicate colonization with SEMA and solid bars indicate infection with SEMA. DM=Received suspected donated breast milk

Fig. 1

Group	p
Male sex	0.31
Birthweigt < 1.000g	0.35
Birthweigt < 2.000g	0.11
Parenteral nutrition	0.39
Ventilation	0.79
Room 3116	0.77
Room 3118	0.23
Raw donor milk from suspected donor	< 0.0001
Tea	0.86
FMS	0.11
Nystatin oral	0.79
Vitamin D oral	0.52

Fig. 2



148/HYPRP

Costs of surgical site infections in orthopedic and trauma surgery: first results of a systematic review

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Introduction: Surgical site infections (SSIs) are one of the most common hospital-acquired infections (HAIs) in Europe and in the USA. The occurrence of an SSI causes a substantial increase in the economic burden of surgeries through prolonged hospital stays, additional treatments and reoperations (1, 2). Even though occurring in all surgical fields, SSIs are especially critical in orthopedic and trauma surgery due to significant long-term consequences (3). This systematic review aims to assess and evaluate the recent evidence for the additional costs of SSIs for orthopedic and trauma surgery, by including only studies using a method of adjustment for factors other than SSIs affecting costs.
Methods: A literature search was conducted in PubMed, Web of Science and Embase in April 2020. Two reviewers independently screened the records. To assess study quality a checklist focusing the assessment of transparency of cost estimates and costing methods was developed, mainly based on the CHEERS statement (4). Corrections for different currencies (and purchasing power) as well as inflation were carried out, to make the study results from different countries and study years comparable.
Results: 23 studies were included, of whom 12 used matching, 9 used regression and 2 used matching and regression to adjust for factors other than SSI affecting costs. Almost all studies adjusted for age (N=21). The next common factors used were gender (N=19), surgical procedure (N=16) and comorbidities (N=13). On average, each study adjusted for 5.8 factors (range: 1-10). In all but

one study costs for patients with SSIs were significantly higher than costs for patients without SSIs. First analyses showed great differences in additional costs for SSIs ranging from $2251 to $100,170 or 34% - 713%, respectively.

Discussion: In view of the fact that around 50% of all SSIs could be avoided using evidence-based strategies (5), it is particularly important to emphasize the costs that could be saved by preventing SSIs in order to justify the costs incurred for the implementation of prevention strategies. The results of this review can be used to inform decision-makers in regard to the allocation of financial resources.

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149/HYPRP

Reducing urinary tract catheter use in geriatric patients: Results of a pilot intervention

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Background: Indwelling urinary tract (UT) catheters (UTC) are a well-known risk factor for UT infection (UTI). Since geriatric patients are at high risk for infections an intervention with a focus on appropriate and minimal UTC use was initialized in 4 geriatric wards.

Methods: Between 11/2018 and 1/2020 unit-based data on UTC use and nosocomial UTI were collected according to methods of the German national surveillance system KISS. Wards received data feedback in 5/2019. From 7/2019 to 1/2020 the intervention focusing on i.) education and training on UTC insertion and care, ii.) daily assessment of UTC need with so called "catheter indication sheets (CS)" in patients with UTC ≥ 2 days and iii.) timely removal of unnecessary UTCs was realized.

UTC use and incidence densities for UTC-associated (ass.) UTI were calculated before and during the intervention and differences were tested. Additionally, the use of CS in patients with UTC ≥ 2 days was analyzed.

Results: 3,564 patients with 53,954 patient days (pd) and 9,208 UTC days were included.

Surveillance data showed a decrease of i.) the pooled UTC use rate from 19.1/100 pd to 15.2/100 pd ($p<0.001$) and ii.) the incidence density of UTC-ass. UTI from 1.34 to 0.95 UTC-ass. UTI/1000 pd ($p=0.18$).

During the intervention 186 from 351 patients with a UTC ≥ 2 days received a CS for daily medical UTC assessment (53 %). Median UTC days and UTC-ass.UTI/1000 UTC days were lowest in the group of patients with a CS with documented UTC indication and with at least 80 percent proof of the daily medical assessment (table 1).

Table 1: Device use und infection rates during the intervention (n=351 patients)

	All (n=351)	Patients without a CS (n=165)	Patients with a CS (n=186)	CS with documented UTC indication and with at least 80 percent proof of the daily assessment (n=26)	CS without documented UTC indication and / or without at least 80 percent proof of the daily assessment (n=160)
UTC days in Median (IQR)	10.0 (6.0, 16.0)	10.0 (7.0, 16.0)	9.0 (5.0, 15.0)	7.5 (5.2, 11.8)	10.0 (5.0, 16.0)
UTC-ass.UTI per 1000 UTC days	6.45	5.76	7.12	4.12	7.55

CS, catheter indication sheet; UTC, urinary tract catheter; UTI, urinary tract infection, IQR, interquartile range

Discussion: Data show that a surveillance-based prevention intervention reduces UTC use and UTC-ass. UTI in geriatric patients. Further studies are needed to show an effect in larger populations. To what extent the use of checklists for daily medical UTC assessment affects UTI prevention has to be further investigated. The in-depth use of daily assessment checklists seems to have a benefit for geriatric patients with UTC.

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Perioperative antibiotic prophylaxis in surgery: Results of the cluster-randomised controlled WACH-trial based on a new comprehensive compliance index in six non-university hospitals

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Background: As part of the BMG-funded WACH-trial ("Wundinfektionen und Antibiotikaverbrauch in der Chirurgie"; ANNIE2016-55-038; DRKS00015502), processes in the operating room were observed and evaluated with regard to guideline compliance. While some parameters were recognized in the participating hospitals and largely complied with, varying approaches and uncertainties emerged with regard to perioperative antibiotic prophylaxis (PAP). This is critical as almost all inpatients have an indication for PAP due to large number of risk factors. Together with an independent evaluation, a new comprehensive PAP compliance index was developed.

Methods: Based on KRINKO recommendations and the AWMF guideline for the prevention of surgical site infections (SSIs), a standardized observation sheet was developed. This instrument includes more than 15 patient-related variables as well as more than 20 variables that focus on the performed surgery procedure (SP) and the staff involved in the SP with regard to their infection control behaviour. From these data collected in the six participating non-university hospitals, a compliance rate was determined for each SP/patient using multiple items and/or risk factors such as the patient"s age, surgical method, duration of SP, elective vs. emergency intervention, insertion of implant, allergy, the physical status (following the American Society of Anaethesiologists classification ASA PS) and body mass index.

Results: In this study, staff within SP were observed in two phases and two study arms ("usual practice" vs. "tailoring" [adaptation of the PSYGIENE-approach [DRKS00010960] to infection prevention and control teams in non-university hospitals]). N= 905 SP were observed. Overall, a PAP compliance rate of 86.9% was determined. Although PAP was not always a key part of

tailoring in participating hospitals, an improvement of 5% was seen in the "intervention"-arm. However, this only approximated the baseline level of the "usual practice"-arm, which showed a decrease of 5% (p of differences: 0.038).
Conclusions: PAP is an important element in strategies to prevent SSIs. The newly developed comprehensive compliance index was determined retrospectively. Since PAP is administered before SP, it is important to have a tool to establish the indication preoperatively. Existing scores such as the National Nosocomial Infections Surveillance (NNIS) can estimate the surgical duration and wound contamination class during or after SP only, so that beforehand, only the ASA PS is known. A detailed case history of the patient including secondary diseases, allergies etc. is just as important as e.g. the surgical method or implants. Further studies and revision of a guideline should improve the indication for PAP. In this context, besides the reported differences between the observation phases and groups in the WACH-trial, the development of the comprehensive PAP compliance index using more than 20 items may turn out to be of relevance.

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Preventing surgical site infections in surgery: guideline compliance of the medical staff of a German university hospital

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Question: Surgical site infections (SSIs) are commonly occurring complications of surgical procedures that pose a serious threat to patient safety. Therefore, preventing SSIs by ensuring that process-oriented measures of evidence-based guidelines are complied to is of particular importance. Their implementation was investigated in a tertiary care university hospital.
Methods: Between August 2017 and February 2019, standardized compliance observations of physicians and nurses were conducted by trained personnel at Leipzig University Hospital (UKL) in the operating theatre (OT) and during ward rounds as a pilot for the WACH-trial (Wundinfektionen und Antibiotikaverbrauch in der Chirurgie; DRKS00015502). Compliance worksheets were used which were based on pre-, peri- and postoperative prevention measures from both the KRINKO and AWMF. Both perioperative measures (wearing surgical cap and surgical face masks correctly: yes/no; covering prepared sterile instruments: yes/no, time sterile instruments stood uncovered: in minutes) and postoperative compliance parameters (reappraisal of the indication for existing surgical drains: yes/no, examination postoperative wounds: yes/no, hand hygiene according to indication: yes/no, removing white coat: yes/no) were recorded.
Results: In a total of N=65 surgical procedures, N=292 physicians and nurses were observed. The compliance rate regarding the correct donning of face masks was 90.9%, whereas the correct donning of surgical caps was 58.6%. Sterile surgical instruments were covered in 2.3% of N=44 observations, with a mean time of open and therefore exposed surgical trays of 42.7 minutes. The reevaluation of existing surgical drains N=131 was performed in 71.8% of cases during the observation period (one observation = one ward round). Evaluation of the surgical site and postoperative wounds N=399 was observed in 71.2% of cases. Hand hygiene compliance in regards to the indication "before aseptic task" N=176 was 36.4% and therefore comparatively low (before patient contact N=403: 64.5%, after patient contact N=285: 87.7%, after body fluid exposure risk N=162: 87.0%, after contact with patient surroundings N=124: 79.0%). In N=608 ward observations, white coat removal before patient contact occurred in 4.6% of cases.

Discussion: Results show that guidelines to prevent SSIs are implemented to different degrees in usual practice. Generally, like in other areas of nosocomial infection prevention, standardized compliance observations are useful tools in assessing the implementation of evidence-based SSI-guidelines. Compliance rates provide a solid basis for tailored strategies that may sustainably reduce SSIs.

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Education isn`t something you can finish: results of a regional survey

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Introduction: Infection control nurses (ICN) and infection control link physicians (ICP) are both responsible for infection control issues. However, they differ in topic specific education and extent of engagement with infection control issues. To address their need for additional teachings/information/advice on infection control issues in all day work, we performed a structured survey. In addition, hospital Chief Executive Officer (CEOs) were enrolled.
Methods: All participants were interviewed in a quantitative questionnaire as a project of the regional bacterial resistance network. An informed consent was obtained; ethics approval has been obtained by the local committee (33/8/19).
Results: The response rate was as follows: of the 87 people we wrote to, 48 responded as follows with their written consent: 16/23 ICN (70%), 27/51 ICP (53%) and 5/13 CEO (38%). The main results of the study are summarized in Table 1. There seems to be a higher need of advice/additional information in ICP compared to ICN, e.g. concerning outbreaks (p=0.032), MDRO (p=0.005) and ABS (p=0.020), respectively. While ICN would prefer personal advice most often, ICP would prefer a telephone hotline most (p=0.01). Interestingly, time does not matter for ICN and CEOs as for ICPs (both p<0.001). All results were obtained by chi-square tests.
Conclusion: Our data suggest that the majority of both ICN and ICP have an unmet need for advice/information as well as for training on the topic of prevention and infection control. Information/advice should be addressed target group- specific. The improvement of the knowledge of ICNs and ICPs for the implementation of infection control could contribute to an improved prevention of the transmission of infectious diseases.

Fig. 1

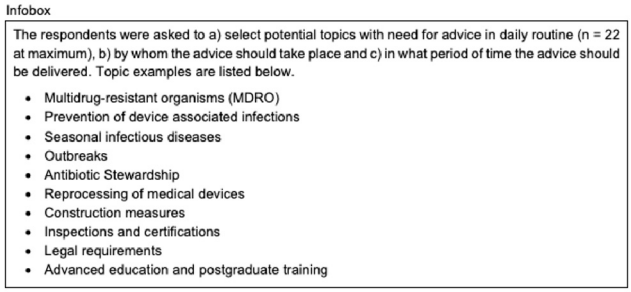

Fig. 2

Table 1. Requested topics and route of information, stratified according subgroups.

Investigated group n=48	Topics [n / (%)]					Desired route of information/advice delivery* [n / (%)]			
	Legal requirements	Construction measures	Outbreaks	MDRO	ABS	Personal advice	Telephone hotline	Via e-mail / Skype	Advice at least within 24h
ICN (n=16)	8 (50%)	9 (56%)	8 (50%)	9 (56%)	7 (44%)	11 (69%)	5 (31%)	8 (50%)	7 (44%)
ICP (n=27)	18 (67%)	15 (56%)	23 (85%)	25 (93%)	21 (78%)	12 (44%)	20 (74%)	19 (70%)	26 (96%)
CEO (n=5)	1 (20%)	3 (60%)	2 (40%)	2 (40%)	2 (40%)	4 (80%)	2 (40%)	4 (80%)	1 (20%)

ICN: infection control nurses; ICP: infection control link physicians; CEO: Chief Executive Officer; MDRO: Multidrug-resistant organism; ABS: antibiotic stewardship; * multiple response options

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Efficiency of a dry-fog based decontamination method for the reduction of bacterial contamination

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Introduction: Dry fog disinfection, which has been a topic in the food industry for a long time, has the potential to be an alternative to common disinfection methods. The possibility of the technology to decontaminate the whole room in one disinfection cycle has also led to serious exposure to this technology during the pandemic. [1] In this work, the disinfecting potential of the TBT dry fogging technology (TBT Desinfektion GmbH & Co. KG, Germany) and the corresponding disinfectant Defeat AR (Biofluid GmbH, Germany) with regard to microbiological contamination will be examined.
Methods: Defeat AR is a HOCl-based active ingredient and the principle disinfection potential of the disinfectant is tested by means of the standardized 4-field test (EN 16615) in a first step. Then TBT dry fog method for decontaminating contaminations is tested in an experimental setup. Ceramic tiles are soiled with a suspension of Staphylococcus aureus (ATCC 6538) and distributed at predetermined points in the room. An active air sampling is also performed before and after the decontamination cycle to determine the air decontamination properties.
Results: The examination of the efficacy in accordance with the EN 16615 standard consistently showed a strong efficacy of the tested disinfectant. In the airborne germ measurement, this effect was a reduction of approx. 66% of the microbiological room contamination. This germ-reducing effect could be shown at all measuring points. Similarly, decontamination of the surfaces was shown to be effective at all measuring points in the test setup we selected. Based on the calculated maximum contamination of 5x105 CFU, the fogging achieved an efficiency of >3 log on some of these surfaces. However, this decontaminating effect depends on the accessibility of the surfaces.
Discussion: Our results could confirm the general antibacterial efficacy of the fogging technology. However, there were strong differences in efficacy depending on the accessibility of the test samples. The test procedure must also be refined in order to be able to accurately determine the actual germ reduction by means of dilution levels. Further tests are needed to determine the potential of the technology more precisely.
Literature:
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154/HYPRP

Factors influencing the durability of antimicrobial coatings on different surfaces

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Introduction: Infection through contaminated surfaces is not an abstract risk, but has already been shown by several publications. [1] It has been shown that antimicrobial surfaces can significantly reduce microbiological contamination on surfaces in the long term. [2] However, there have been few studies on how surface properties and reprocessing affect the durability of these coatings. This work will therefore examine the influence of different materials and reprocessing methods on the durability of antimicrobial surface technologies.
Methods: Test samples made of different materials are subsequently provided with an TiO2Ag based antimicrobial coating (HECOSOL GmbH, Bamberg) by electrospray technique and tested for their durability and remaining activity by standard methods (ISO 22196). In a second step, abrasion tests and microbiological activity tests were done on coated wallpaper bonded to plasterboard using various cleaning and disinfecting agents and cloth systems (microfiber cloth, cotton cloth, foam cloth).
Results: While strong antimicrobial efficacy can still be demonstrated on glass surfaces even after several hundred abrasion cycles, this is no longer present on plastic surfaces after just a few cycles. The abrasion tests with various cleaning agents, disinfectants and wipe systems also showed an influence of the material and the preparation on the antimicrobial efficacy. All test samples in our experimental setup showed at least slight efficacy. However, only 5 of the 47 tested samples showed a remaining strong efficacy.
Discussion: Our results suggest that the underlying surface material and also the selection of cleaning and disinfection procedures and wipe systems appears to influence the durability of the coatings on the surfaces. In order to be able to make a statement about the long-term activity of these surface technologies, the effectiveness should be tested in the finished product and after several reprocessing cycles.
Literature:
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[2] Eichner A, Holzmann T, Eckl DB, et al. Novel photodynamic coating reduces the bioburden on near-patient surfaces thereby reducing the risk for onward pathogen transmission: a field study in two hospitals. J Hosp Infect 2020; 104: 85–91. doi:10.1016/j.jhin.2019.07.016

155/HYPRP

Antimicrobial activity in the gasphase with hypochloric acid

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Introduction: Virus-carrying aerosol particles are recognized as infection carriers in the current Corona pandemic, but their high-risk potential is often underestimated and represents the infection route that has been least systematically countered to date. As a result, aerosols currently represent an insufficiently contained mode of disease transmission at public indoor spaces (e.g.: offices, schools, gastronomy). Our study investigated if the disinfecting potential of HOCl in suspensions are transferrable to in-air cleaning applications and to what extent aerosolized HOCl solutions can deactivate indoor microbial contaminations in-air at or below legal limits.
Methods: For the liquid disinfection we used a standard suspension disinfection test protocol.
To create an atmosphere with free floating HOCl solo or in laden water particles we aerosolize HOCl solutions to create sizes below 10 µm. This allowed particles vaporize within seconds and create an HOCl laden atmosphere staying afloat for hours until HOCl

disintegrates (decays) into components like Cl2O, ClO2, Cl2, and finally HCl. Such "active" atmosphere was to interact with bacteria laden aerosol particles. For the in-air tests we conducted several experiments where aerosolized bacterial suspensions were injected into lab chambers preloaded with different HOCl gas concentrations.

Results: In suspension experiments we found sufficient deactivation efficacies for 4 bacterial organisms (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli K12 and Staphylococcus warnerii) and an enveloped virus (Vaccinia-Virus) at minimum HOCl concentrations of 200 ppm. The in-air measurement set-up allowed to follow microbe deactivation, which rate increases with the HOCl gas concentration. The deactivation is greatest for lipid enveloped gram-negative bacteria. The average microbe deactivation rate is 50%/minute.

Conclusions: We confirmed our hypothesis of the high disinfecting power of HOCl in-air at safe levels for populated indoor places. The investigated Gram-positive and -negative bacteria can be understood as a general model system for infectious particles, including enveloped viruses (to which Coronavirus belongs). No tissue irritation is to be expected. The results demonstrate applicability for real life utilization because the HOCl driven microbe deactivation has a broad operating window between effective and safe concentration levels. This approach may present a useful method in concert with existing hygiene procedures like ventilation, high class air filter systems and personal hygiene considerations

Fig. 1

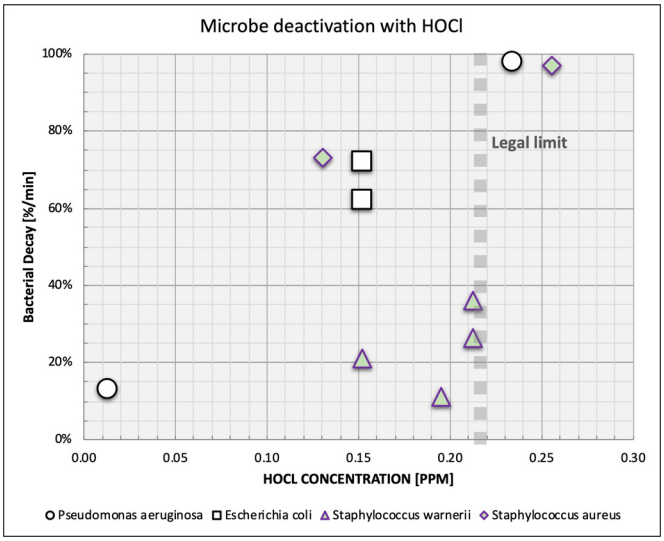

Fig. 2

#	ID	Keim	Ladung	Hülle	HOCl conc (ppm)	HOCl conc (mg/m3)	HOCl deact
1	1	Pseudomonas aeruginosa	gram -	lipid membran	0.01	0.03	13%
2	3	Pseudomonas aeruginosa	gram -	lipid membran	0.23	0.54	98%
3	27	Escherichia coli	gram -	lipid membran	0.15	0.35	62%
4	28	Escherichia coli	gram -	lipid membran	0.15	0.35	72%
5	36	Staphylococcus warnerii	gram +	Murein capsid	0.15	0.35	21%
6	37	Staphylococcus warnerii	gram +	Murein capsid	0.21	0.49	26%
7	20	Staphylococcus warnerii	gram +	Murein capsid	0.21	0.49	36%
8	21	Staphylococcus warnerii	gram +	Murein capsid	0.20	0.45	11%
9	46	Staphylococcus aureus	gram +	Murein capsid	0.13	0.30	73%
10	47	Staphylococcus aureus	gram +	Murein capsid	0.26	0.59	97%

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Assessment of approaches and methodologies for evaluation of infection preventive efficacy of ventilatition systems in Operating Theatres

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Question: Microbiological contamination of air in operating theatres (OT) is a known risk factor for surgical site infections, but it still remains an open question which methodology or standard is best suited to validate infection preventive efficacy of OT ventilation systems.

Methods: We have systematically reviewed different international ventilation standards and the current literature. Furthermore, we have analyzed various normative procedures and compared them to determine the extent to which they allow a reliable assessment of the efficacy of ventilation systems in operating theatres. Therefore, we also conducted experiments in our research OT and at selected clinical sites.

Results: Decisive for infection prevention is the reduction of airborne pathogens. Thus, the OT must be viewed as a complex thermodynamic system with several mutually influencing factors, i.e. room size, convection currents, flow obstacles such as operating lights and the design of the ventilation system. Furthermore, OT workflow must be considered. Our analysis have shown that the protected areas of TAV systems are usually too small. The actually required size of a protected area can only be determined through individual positioning and workflow analysis, unless the OT as a whole is viewed as a protected area like in the concept of temperature controlled airflow (TcAF). Comparative measurements of active and passive air sampling did show that only active sampling during live procedures allows a reliable assessment of the microbiological air burden.

Conclusions: Not only aspects of technical execution should be in focus regarding planning and qualification of OT ventilation systems. The "what is to be achieved" should be more important than the "how it is achieved" to enable optimized risk management. It might be useful to follow rather the requirements of EN ISO 14644 and the GMP guidelines than country-specific standards for OT ventilation technology. As part of the qualification (DQ, IQ, OQ, PQ) of OT ventilation systems, the PQ (Performance Qualification), i.e. the examination of the performance under real conditions for evaluating the efficacy of ventilation systems, plays a central role. The regulations in German speaking countries (DIN 1946, SWKI, ÖNORM) lack this meaningful qualification under real conditions as an "acceptance criterion". This could explain the controversial study situation on the infection-preventive efficacy of OT ventilation systems.

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Identification of microorganisms from several surfaces by MALDI-TOF MS from domestic appliances

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Introduction: New ecological trends and changes in consumer behavior are known to favor biofilm formation in household appliances, increasing the need for new antimicrobial materials and surfaces. Their development requires laboratory-cultivated biofilms, or biofilm model systems (BMS), which allow for accelerated growth and offer better understanding of the underlying formation mechanisms.

Material/Method: Here, we identified bacterial strains in wild-type biofilms from a variety of materials (glass, metal (steel), elastomers (rubber), and plastic) from domestic appliances using matrix-assisted laser desorption ionization-time of flight mass spectroscopy (MALDI-TOF-MS). Standard quantitative biofilm assays, such as crystal violet (CV) staining as well as colony-forming unit (CFU) assay, were combined with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) to characterize the biofilm formation. The washing detergent tolerance of *Pseudomonas aeruginosa* was tested by using different standard washing detergents.

Results: Staphylococci and Pseudomonads were identified by MALDI-TOF-MS as the main genera in the tested habitats and were therefore analyzed for biofilm formation by various *in vitro* methods. Water born Pseudomonads were dominantly found in compartments with water contact only, such as detergent

compartment and detergent enema. Materials in contact with the washing load, like the washing drum and sight glass are predominantly colonized with bacteria from the human skin such as Staphylococci. *Pseudomonas aeruginosa* was found to be the most dominant biofilm producer under *in vitro* conditions. Using SEM we were able to visualize the biofilm formation life cycle of *Pseudomonas aeruginosa*, from the initial attachment of planktonic bacteria to the dispersion phase of biofilm.

Discussion: The results of this study are the first critical step towards a more representative biofilm model system (BMS). This might allow the development of more effective antimicrobial materials and surfaces in domestic appliances. State-of-the-art MALDI-TOF-MS enables us to clearly identify bacterial isolates from washing machines, which is a remarkable advantage in comparison to other methods. With our used biofilm assays, we were able to analyze which bacteria are best in biofilm production. Our results suggest that Pseudomonas aeruginosa is best in producing biofilms, independently of the growth medium and substrate. In addition, SEM is superior for the analyses of all growth phases of biofilm formation. Taken together, our study clearly reveals that a combination of MALDI-TOF-MS, quantification methods (such as MTP, TM, and CFU), and the use of SEM is a powerful tool to study the formation process and evolution of biofilm production.

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A field study in two hospitals reveal that photodynamic coating reduce the bioburden on near-patient surfaces

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Introduction: Near-patient surfaces in hospitals play a role for healthcare-acquired infections. Such surfaces act as reservoirs for microbial contamination by which pathogens can be transmitted from colonized or infected patients to susceptible patients. Routine disinfection of surfaces results only in a temporal elimination of pathogens and recontamination inevitably occurs shortly between two consecutive disinfections. A novel antimicrobial coating works with the photodynamic effect, based on a dye molecule (photosensitizer). The photosensitizer is located in the thin coating that can be applied to near-patient surfaces. Upon exposure of the coating to visible light, the photosensitizer generates singlet oxygen, which escapes the coating and inactivate microorganisms on the surface. Such a new coating was tested firstly under laboratory conditions and subsequently in a field study in two hospitals under real life conditions.

Material and Methods: For laboratory experiments, *S. aureus* ATCC 6538, *S. epidermidis* (patient isolate), *E. faecium* EDCC 5271 (VRE), and *A. baumanii* ATCC 15308 were used. Cells were applied on the surface of coated samples as 50 µL spots corresponding to ~ 105 cells. The samples were kept in the dark until the suspension was visibly dried (~60 min). Then, samples were irradiated with visible light (LED) at different radiant exposures (J/cm²). After irradiation, the number of viable cells was determined by plating bacteria on agar and counting the colony forming units.

For the field study, identical surfaces in different rooms in two hospitals received a photodynamic or control coating on which the bacterial counts (CFU/cm²) were regularly assessed for 6 months. The evaluation of bacterial counts was based on EN 13697. Counted values were converted into CFU/cm² depending of the size of the respective sampled area. Throughout the study, the routine cleaning and disinfection procedures were left unchanged in both participating hospitals. Counts (mean ± SD) were compared between photodynamic and control coating (Mann-Whitney U, Kruskal-Wallis Test).

Results: The laboratory study revealed a reduction of *Staphylococcus aureus* ATCC® 25923TM of up to 4.0 log10. The field study in near-patient environments demonstrated bacterial mean values of 6.1 ± 24.7 CFU/cm² on all control coatings. Photodynamic coatings showed significantly lower mean value of 1.9 ± 2.8 CFU/cm² ($p < 0.001$). When considering benchmarks of 2.5 CFU/cm² or 5 CFU/cm², the relative risk for higher bacterial counts was reduced by 48 % (odds ratio 0.38, $p<0.001$) or 67 % (odds ratio 0.27, $p<0.001$), respectively.

Discussion: Photodynamic coatings provide a significant reduction of bacterial counts on near-patient surfaces, in particular for high bacterial loads and in addition to routine hygiene. The coating yields a persistent and autonomous antimicrobial effect independent of compliance problems and can supplement the routine hygiene measures on surfaces in hospitals.

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Implementation of the 42. BImSchV in Bavaria

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Introduction: Legionella bacteria are naturally occurring in water and can enter technical water systems from the environment. Inhalation of Legionella-containing bioaerosols can lead to Pontiac fever or Legionnaires' disease in humans. From 1998-2020, 33 documented Legionella outbreaks possibly associated with evaporative cooling systems, cooling towers, or wet scrubbers were identified internationally.

To minimize the risk of such Legionella outbreaks, the German government enacted the regulation on evaporative cooling systems, cooling towers and wet separators (42. BImSchV), which took effect in 2017. The aim of our project is to support the implementation of the 42. BImSchV in Bavaria.

Methods: In order to clarify implementation issues related to the 42. BImSchV, various materials are developed for the target groups. In addition to flyers and brochures, information exchange is supported by setting up a website and organizing information events. The knowledge gained from two systematic reviews, summarizing the methods and management strategies deployed worldwide in relation to Legionella outbreaks, was used in the preparation of German guidelines. In the event of an outbreak, potential sources of infection can be detected by culture-independent (molecular and immunological) tests in addition to routine diagnostics. These methods are used to analyze environmental water samples within the framework of the project.

Results: To date, two events on the implementation of the 42. BImSchV have been successfully carried out online. In addition to the lectures, special issues could be addressed in more detail in workshops. The online format enabled easy participation, new interaction possibilities and improved distribution of information material. The events met with great interest, with more than 150 participants. In order to inform and bring together authorities, experts and operators, further events are planned in the next two years. The steps required to prepare for Legionella outbreaks were formalized in the VDI guideline 4259-1.

Conclusions: The tight cooperation between the responsible institutions and the facile and targeted access to information help to identify problems and challenges in the implementation of the 42. BImSchV at an early stage. The implementation of the 42. BImSchV is also of great importance in the event of an outbreak. The outbreak management teams and plant operators can then quickly access the most relevant information and decide which measures are to be implemented for overarching crisis management, for damage control and decontamination. The detection of the outbreak source can be greatly accelerated by culture-independent analysis methods.

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Scientific investigation of the implementation of the hygiene concept with a focus on an open air dance event in Berlin under SARS-CoV-2 pandemic conditions

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Introduction: The events industry is particularly affected by the legal requirements to contain the corona pandemic. As one of the first measures, events were not allowed to take place in 2020. This is associated with negative consequences for the already precarious work situation of employees (e.g. technicians). In order to be able to hold events, a hygiene concept is necessary that corresponds to the current and state-specific legislation. As part of a pilot project, it is examined to what extent a hygiene concept for an open air dance event in June 2021 is implemented by the status groups employees, participants and visitors, whether ad hoc adjustments and countermeasures are taken and which optimization are there.
Method/Material: In the field Revier Südost Berlin (capacity 900 people) a total of 220 visitors (voluntary,> / = 18 years) as well as 30 employees and participants took part in a "rave". All persons had a negative rapid test or equivalent. Scientific observation (anonymously) by 10 study assistants (analog, paper-pencil) took place at 4 relevant positions. The hygiene concept was the basis for the previously piloted observation protocol and the position plan. Data was evaluated using Excel®.
Results: During the observation period, the study assistants recorded a total of 501 violations of the stipulations and requirements of the hygiene concept. Of these, 236 violations were committed by visitors. 85 of whom were warned directly by the employees and led to immediate cessation by the visitors. The obligation to wear an FFP2 mask on the dance floor was violated in only 18 cases.
265 violations were observed among employees, 129 of them during the admission time in the bar area. Overall, violations occurred predominantly in the admission, lounge, backstage and sanitary areas. Improvements were made (e. g. markings, changes to the wayfinding) and information. Dealing with artists and their accompaniments was comparatively slack.
Discussion: The hygiene concept was basically practicable.
Employees and visitors largely complied with the rules. Weak points in the implementation were the routing, keeping the distance in front of the sanitary area, especially at the beginning and at the end of the event, and the special position of the artists and their accompaniments.
This pilot study provides first insights into the degree of implementation of special requirements, organizational and subject-related features and shows concrete potential for optimization.
The results of the pilot project can be used to ensure the safety of employees in the event industry in hygiene and infection protection. Further events towards the capacity limit of this area should be evaluated. The scientific evidence or other parameters of the hygiene concept were not evaluated in this study.

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Studies on the effect of novel antimicrobial surface coatings on Bacillus endospores

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Abstract: Space stations are closed and isolated habitats with unique conditions such as cosmic radiation and microgravity. Long term exposure to this hostile space environment can affect the spacecraft as well as the human microbiota which further affects the human immune system. These conditions can also increase antibiotic resistance and virulence in bacteria. This poses a greater threat to the health of the crew making them susceptible to infectious diseases. Thus, using effective antimicrobials is crucial to avoid contamination of the spacecraft and to protect the crew from infections. AGXX is an antimicrobial surface coating consisting of micro galvanic elements of silver and ruthenium. It has been shown to kill multi-drug resistant, biofilm forming human pathogens such as Enterococcus faecalis, and methicillin-resistant Staphylococcus aureus by production of reactive oxygen species. AGXX is a long lasting, and durable antimicrobial, suitable for use on the International Space Station and in space crafts.
The conditions in the space environment can especially be problematic in case of spore-forming bacteria, such as Bacillus subtilis. As bacterial spores are the gold standard of decontamination, it is very important to investigate their response to antimicrobials. We tested the effect of AGXX on the germination of spores of B. subtilis 168. The germination profile of the spores in presence of AGXX was examined by determining the colony forming units, by photometric and fluorescence-based assays, and through microscopy. Preliminary data indicate that AGXX delays the germination of B. subtilis spores by about three hours. Thus, AGXX and related antimicrobials could present a potential novel sporicidal approach. In the SIRIUS-2021 isolation study, we will expose B. subtilis 168 spores to simulated space conditions to assess their response to novel antimicrobial surface coatings.

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Towards an intersectoral electronic hygiene report

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Introduction: When patients with infectious diseases or increased risk of infections move between different care providers, there is a need for effective exchange of information between the parties involved [1]. Standardised electronic documents are ideal for the timely and targeted exchange of information. Reference models for such transition tools in healthcare can be developed using a three-phase multi-method approach model [2]. For an electronic transfer report with hygiene-relevant information, a guideline- and literature-based data set with 75 information items and their values within 8 sections, was created in a first phase of this procedural model [3]. The aim of this study is to validate the content and structure of this data set empirically.
Material/Methods: The validation of the data set took place by means of a Delphi survey [4]. For this purpose, participants were recruited via multipliers. The relevance of the information items in the data set was assessed and commented on by the participants in each survey round, and they could also request new items to be included. The data set was modified based on these responses and sent back to the participants for re-editing until there were hardly any changes.
Results: 33 hygiene experts from the fields of medicine and nursing could be recruited for the Delphi survey. Three rounds with 19 to 28 participants were conducted, after which there were no more significant changes in the data set. A total of 348 changes were made (47 renaming, 26 deletions, 275 additions), the number of information items increased to 119, the number of sections remained at 8.
Discussion: The information items, their values, and their structure constitute an evidence- and empirically based data set. After modelling and reaching a professional and technical consensus with the hygiene societies and the HL7 Germany community, the information set can serve as a data model for a medical information object (MIO) within the German telematics infrastructure.

The Authors declare that there are no competing interests
The study was conducted as part of the ROSE project (Das lernende Gesundheitssystem in der Region Osnabrück-Emsland) funded by the Lower Saxony Ministry of Science and Culture, funding code VWZN3103

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Postersession 01
13. Sep. 2021 • 11:15–12:45

Diagnostic Procedures: Diagnostic Microbiology and Clinical Microbiology (StAG DV/FG DKM)

162/DKMP
SARS-CoV-2 confirmation of positive antigen test samples within 45 min using a fully integrated real-time PCR at the Point-of-Care

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Introduction: Due to the reduced specificity of antigen tests, in Germany every positive result must be confirmed by PCR. Usually the patient must provide a second sample for a laboratory analysis which is both time consuming and inconvenient for the patient.
This study evaluates leftover samples of positive antigen tests for confirmatory testing with a rapid PCR test.
Material/Method: Asymptomatic and symptomatic patients were tested with a commercially available SARS-CoV-2 antigen test. Positive samples were subsequently re-tested with a rapid real-time PCR based test (*test under investigation*) using 3 to 10 droplets of the leftover antigen sample. In parallel, a CE-IVD real-time PCR test was applied as a *reference test* using a second sample.
Results: A total of 54 samples were tested positively with the antigen test. 50 out of these 54 samples were confirmed positive for SARS-CoV-2 with both: the test under investigation and the reference test. 4 out of 54 samples were identified as false positive in the reference PCR.
Discussion: The test under investigation shows a 100 % positive agreement with the reference test using leftover samples of an antigen test. Thus, this study indicates that the confirmatory PCR test can be performed directly after receipt of the positive antigen test result with the same sample. Patients receive reinsurance within 45 min and infected persons can be isolated quickly.

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Application of clinical metagenomics in standard microbiological diagnostics (Dissecting ascitic microbiota composition as a model)

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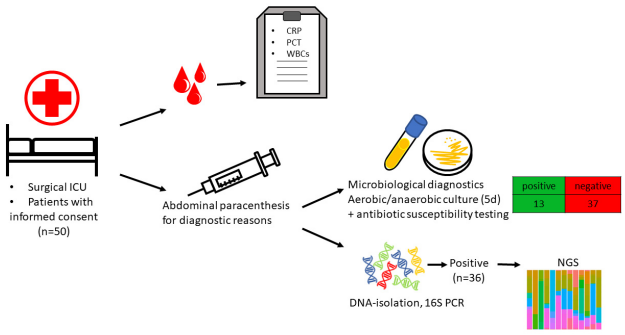
Introduction: Ascites is an unnatural accumulation of fluid in the abdominal cavity. Cirrhosis is the most common cause, but other possible causes include heart failure, tuberculosis, pancreatitis, and cancer. Infection of the ascitic fluid is a serious complication that can lead to peritonitis and sepsis and is associated with high morbidity and mortality. Around 70% of hospitalized patients remain without an identifiable infectious source. The prompt identification of the related pathogen is essential for the patients" prognosis. Here we aim to establish metagenomics next- and third-generation sequencing workflows to aid the clinical diagnosis and treatment.
Methods: From October 2019 until May 2021, informed consent was obtained from surgical intensive care unit patients that had undergone abdominal paracentesis for diagnostic reasons. Aspirate was sent for microbiological standard culture. Clinical data was obtained from medical charts as well as inflammation markers such as CRP, Leukocytes and Procalcitonin. Various high variable regions of the 16s rDNA gene have been compared for their sensitivity in ascitic materials. The V1-2 region of the 16S rDNA has been used to target bacterial communities in the ascitic fluid. The DADA2 informatic pipeline and the Genome Taxonomy Database have been used to analyze and identify bacterial taxonomy from the sequences.
Results: Over the study time, a cohort of 50 ascitic samples has been collected from the targeted intensive care patients. Clinical characteristics of the curated cohort showed a higher prevalence of male patients of 66% with a 10% death rate. From the 50 samples, 13 were culturally positive. Using V1-2 targeted PCR, 36 samples were positive including all culturally positive samples.
Furthermore, additional pathogens including E. faecalis and Klebsiella as well as anaerobic bacteria were detected. Analysis of clinical data showed correlation between elevated CRP and anaerobic bacteria in NGS-analysis.
Conclusions: Next generation sequencing has proven its potential as a fast and efficient tool in microbiological diagnostics. It offers high sensitivity compared with standard culture-based techniques. Further adjustments to sample processing and algorithms for the identification of clinical relevant pathogens are needed before its integration in daily standard microbiological diagnostics.
Figure 1: Overview of study workflow and primary results

Fig. 1



165/DKMP
Ring trial to validate a disk diffusion method for *Clostridioides difficile*

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Introduction: To identify and understand antimicrobial resistances of *Clostridioides* (*C.*) *difficile* is an essential prerequisite to follow epidemiological adaptations and to apply effective treatment options for *C. difficile* infections. Within the OHEJP IMPART project we established a disk diffusion method for the antimicrobial susceptibility testing of *C. difficile* as cost-effective alternative to the current gold standard, agar dilution (AD). Here, we conducted an international ring trial study to evaluate and validate this method in terms of applicability, robustness and interlaboratory reproducibility.
Methods: Expert laboratories were invited to participate in the ring trial and to determine inhibition zone diameters (IZDs) of eight well-characterized *C. difficile* strains for eight different antimicrobials (clindamycin, erythromycin, imipenem, moxifloxacin, metronidazole, rifampicin, tetracycline, and vancomycin) using the provided disk diffusion protocol. Finally, seven laboratories from Germany, Denmark and Portugal participated in the study.
Results: All participants were able to correctly apply the method description and analyze the eight *C. difficile* test strains for all

given antimicrobials except one strain/antimicrobial combination in one lab. Resulting IZD standard deviations (SD) were below 5 mm across all participants and antimicrobials. Metronidazole showed the highest SD independent of the investigated strain. The choice of the equipment to generate anaerobic conditions had a significant impact to the measured IZDs and SDs within the ring trial.

Conclusion: The disk diffusion method was successfully applied by seven independent laboratories to determine IZDs of eight *C. difficile* strains with different resistance profiles for eight antimicrobials of different antimicrobial classes using the provided method description. We found overall low SDs and a high reproducibility. However, these parameters were antimicrobial dependent limiting the applicability of the protocol for other antimicrobials without prior validation. Furthermore, the equipment that is used to generate anaerobic conditions has to be chosen with caution and can limit the comparability of total IZD values.

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Development of *Legionella pneumophila* serogroups classification by FT-IR spectroscopy with IR – Biotyper system

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Question: For routine purposes, the ideal method for microbial characterization requires minimum sample preparation and should be rapid, automated and inexpensive. It is well known that culture and phenotyping methods to recognize *Legionella* spp. are often inadequate, while genotypic methods are expensive and laborious.

Recently, mass spectrometry and infrared spectroscopy have proved to be rapid and successful approach for the identification of several pathogens. This technique, enables the creation of classification systems based on the intra-species identification features typical of each microorganism.

The aim of the study is the use of Fourier Transform Infrared Spectroscopy technology using the IR Biotyper system (Bruker Daltonik, Germany) for the classification of *Legionella pneumophila* (*Lp*) at serogroup (Sg) level, for diagnostic purposes as well as in outbreak events.

Lp inhabits fresh water environments and it represents a serious risk for human health, since it is the causative agent of a severe community-acquired pneumonia. It consists of 16 Sgs, most of these typed by monoclonal or polyclonal antibodies (e.g. agglutination test or indirect immunofluorescence assay).

Methods: IR Biotyper was used to classify *Lp* with an Artificial Neural Network (ANN) available in the instrument software. A dataset of well characterized isolates (n=43), belonging to Sg1-15, including both ATCC strains and environmental strains collected from different sources, was tested with a classifier implemented by the manufacturer.

All strains were cultivated onto BCYE agar at 35 ± 2°C for 48 h, with 2.5% CO2 and typed by latex agglutination test (Thermo Fisher Diagnostic, Basingstoke, UK).

All the isolates were classified with the Bruker classifier that at moment is able to discriminate between Sg1 and Sg 2-15.

Results: The preliminary results showed that IR Biotyper classifier was able to correctly discriminate Sg1 from 2-15, showing accuracy=95.9% (41/43) and error rate=4.1% (2/43). An exception was observed for the two Sg7 isolates, that were mis-classified as Sg1 (likely due to the absence of this Sg in the training set of the Bruker classifier).

Conclusions: Further studies on a larger number of isolates could be useful to implement the ANN system and to obtain a robust and reliable tool for the identification of *Lp* at the Sg level. In conclusion, the results of this study suggest that IR Biotyper could be a powerful and easy-to-use tool for characterizing *Lp* Sgs especially during cluster/outbreak investigations, to promptly undertake preventive strategies and trace the source of the infection.

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Evaluation of a novel approach to process screening cultures for multidrug resistent gram negative bacteria (MRGN)

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Introduction: In Germany screening of adult patients for multidrug resistent gram negative bacteria (MRGN) is patients is focused on bacteria resistant against 3rd generation Cephalosprines (GC) + fluorochinolone and/or carbapenems. Current concepts for culturing often consist of ESBL selective media and subsequent identification (ID) and antimicrobial susceptbility testing (AST). The objective of this study was to evaluate a culture process improving time to result by reducing the need for subsequent ID and AST.

Materials/Methods: In February 2021 random samples from hospitalised adult patients send for screening MRGN were included into this study. Samples were rectal, perineal, pharyngeal and multi-site swabs as well as tracheobronchial secretion and urine. We compared perfomance of a Brilliance ESBL plate (Thermofischer, Hennigsdorf) with applied disks (MAST Diagnostica, Reinfeld) containing Ceftazidim (10µg) and Ciprofloxacin (5µg) and a Mackonkey3 agar with disks containing Faropenem and Meropenem (10µg) to performance of a Brilliance ESBL plate with subsequent ID and AST (Vitek2; Biomerieux, Marc 1′etoile) according to EUCAST (January 2021) as a reference. Plates were incubated for at least 18 hours at 36◦C. Zone diameters were read and interpreted according to disk diffusion breakpoints from EUCAST. In case of CAT-ID disks containing Faropenem manufacturers recommendations were followed.

Results: A total of 54 samples were prospectively tested. Overall 11 samples showed groth. Those were 3 E. *coli*, 3 Klebsiella spp., 1 Citrobacter spp., 1 S. marcescens and 3 P. *aeruginosa*. Of those only 2 E. *coli* and one P. *aeruginosa* were characterized as MRGN3 and 4.

Sensitivity and specificity of the two plate-AST approach were 100% and 94% respectively (Table 1). No false negatives were detected. Overall 3 additional false positives occured. These were caused by Ciprofloxacin zone diameter being in an ATU range when compared to a susceptible reference AST. With this culture approcha potential reduction of subsequent ID and AST could be achieved in 5 cases with detected growth (46%).

Discussion: The approach for processing screening samples evaluated in this study is safe and effective in a low prevalence setting. No false negatives were identified when compared to an established process.

No carbapenamase producing bacteria were found during the evaluation. Therefore additional evaluation is necessary.

MRGN is an epidemilogical categorization used in Germany limiting the potential impact of our findings. However, reduction of ID and AST reduces time to result in negative cases and potentially could free recources.

Statistic	Value	95% CI
Sensitivity	100.00%	29.24% to 100.00%
Specificity	94.44%	84.61% to 98.84%
Positive Likelihood Ratio	18.00	5.99 to 54.06
Negative Likelihood Ratio	0.00	
Positive Predictive Value (*)	50.00%	24.98% to 75.02%
Negative Predictive Value (*)	100.00%	

Table 1: Diagnostic perfomance of a novel two plate disk diffusion screening approach for MRGN

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Can CRISPR-Cas9 re-sensitise bacteria with high-copy plasmids or with multiple resistance mechanisms?

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Introduction: In light of the ongoing emergence of antimicrobial resistance new strategies, such as reversing resistance using CRISPR-Cas9 may have a significant impact for future clinical applications. Here we evaluated the CRISPR-Cas9 potential to revert resistance when targeting the *bla*TEM gene located on a high-copy plasmid, and when targeting the *bla*KPC gene in a clinical isolate exhibiting multiple resistance mechanisms against carbapenems.

Materials and Methods: Site-specific sgRNA were designed for the *bla*TEM-1 gene harbored by *E. coli* BL21 and for the *bla*KPC gene carried by *Klebsiella michiganensis*. The *bla*TEM-1 gene was present on a high-copy plasmid (pSB1A2), which also contained the Red Fluorescent Protein (RFP). The RFP enabled us to quantify the levels of fluorescence before and after CRISPR-Cas9 treatment by a fluorescence reader, as well as quantify the percentage of RFP-positive cells via FACS. Remaining pSB1A2 plasmids were quantified via qPCR. Resistance reversal was evaluated by disk diffusion and MIC testing. The expression of the *bla*TEM–1 gene was assessed via RT-qPCR. After transformation of *K. michiganensis* the presence of the CRISPR-Cas9 plasmid and Cas9 integrity were evaluated via PCR and western blot. To identify overlapping resistance mechanisms that could impair the CRISPR-Cas9 re-sensitization, the *K. michiganensis* genome was sequenced and the expression of flagged resistance genes was evaluated via RT-qPCR.

Results: Upon CRISPR-Cas9 insertion into *E. coli* BL21 the plasmid number and the *bla*TEM–1 gene expression decreased but did not become extinct. FACS results in combination with qPCR indicated that on average 0.005% of cells were still RFP positive and carried around 48 plasmid copies/cell. Sequence alterations in *bla*TEM–1 were observed, likely resulting in a dysfunction of the gene product. Clearance of the *bla*KPC carrying plasmid was not achieved in *K. michiganensis*. However, plasmid copy number was significantly reduced ($p<0.01$) in a subpopulation of clones, with consequent resistance reduction to the intermediate level for imipenem. Interestingly, the expression of the *omp*36 gene in the treated cells was significantly reduced ($p<0.05$).

Discussion: For *E. coli* BL21 a full reversal to an antibiotic sensitive phenotype was achieved, despite *bla*TEM–1 plasmid maintenance in a subpopulation. The was not true for *K. michiganensis*. Although, the presence of the CRISPR-Cas9 plasmid could be confirmed, the Cas9 integrity was compromised, likely explaining the lack of further re-sensitization. In addition, the reduced omp36 expression, may be a responsive mechanism of the CRISPR-Cas9 challenged strains to counteract re-sensitization attempt by hampering imipenem entrance. As a conclusion, neither high plasmid copy number nor compensating resistance mechanisms can entirely prevent CRISPR-Cas9 mediated resistance reduction. Overall, the strategy needs to be optimized by accounting for overlapping resistance mechanisms.

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Biofilms in endocarditis: is there a therapeutic consequence?

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Introduction: Diagnosis of infective endocarditis is still challenging, in particular when culture remains negative. The gold standard of endocarditis diagnosis has been so far detection of the pathogen in blood cultures or from heart valve material. However, this crucial step for diagnosis may be impossible as the microorganisms evade detection by culture. One reason for this is the organization of bacteria in biofilms.

To date the antibiotic therapy is only adapted to the pathogen species and sensitivity to antibiotics according to the internationally valid ESC / EACTS guidelines. The biofilm status, activity or degradation of the pathogens that has already been caused by antibiotic therapy are so far not taken into account.

Methods: We used Fluorescence in situ Hybridization (FISH) combined with 16S rRNA-gene PCR and sequencing (FISHseq) to visualize and identify the infectious agents in heart valve tissues from endocarditis patients. The FISH signal intensity of the fluorescence labelled probes is proportional to the ribosomal content of the bacteria, which correlates with microbial metabolic activity. Thus, FISH shows not only the spatial organization of bacteria and biofilm formation, but also their metabolic activity at the time of surgery.

Results and Discussion: We found a large variability in the severity of bacterial formation and activity as detected by FISH: There were cases in which the suspected diagnosis of endocarditis was confirmed, but the microorganisms were distributed and partially degraded in the tissue, other cases with micro-colonies, or highly active, mature biofilms. Interestingly, this picture often corresponded to the clinical situation of the patient.

Consequently, we are currently developing an endocarditis scoring based on a biofilm staging by FISH to establish a risk stratification of the patients leading to treatment tailored to the individual patient. This way, FISHseq will not only enable an evidence-based decision for de-escalation therapy or escalation with biofilm-effective antibiotics, but also for or against early oralization in endocarditis patients, thus increasing safety and allowing more patients to benefit.

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Increased Teicoplanin non-susceptibility in Coagulase Negative Staphylococci: are EUCAST breakpoints valid?

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Background: Coagulase negative staphylococci (CoNS) are a major cause of nosocomial infections such as device-associated infections, bone infections, endocarditis, surgical site infections and infection in neonates. The glycopeptide antibiotic teicoplanin is an alternative therapy for the infections with CoNS. However, resistance to this antibiotic has been recently reported, thus leading to further reduction in the available treatment options. Hitherto, little is known about the strains with reduced susceptibility to teicoplanin. This study aims to gain more information about the reliability of the available antimicrobial susceptibility testing (AST) in detecting the teicoplanin-resistant CoNS.

Material/ Methods: 162 CoNS strains were collected from inpatients between August 2015 and August 2016 at the Institute of Medical Microbiology and Hospital Hygiene, Heinrich Heine University, Dusseldorf, Germany. The CoNS strains with putative clinical relevance were identified to a species level and subjected to different AST methods: Vitek-2 (bioMerieux, Marcy l"Etoile, France) for automated susceptibility, in house broth microdilution assay, gradient diffusion (Etest) and disk diffusion (MIC Test Strip and discs, Liofilchem, Italy). The results were evaluated according to European Committee on Antimicrobial Susceptibility Testing (EUCAST Clinical Breakpoints Tables v.11 (2021) and Clinical and Laboratory Standards (CLSI M100-S22, 2012) for disk diffusion method.

Results: Using Vitek-2 78 (48.1%) of the 162 strains were teicoplanin-resistant and 84 (51.9%) teicoplanin-susceptible. Broth microdilution confirmed Vitek-2 results in 40 (51.3%) of the teicoplanin-resistant strains and 71 (84.5%) of the teicoplanin-susceptible strains. Gradient diffusion confirmed the Vitek-2 results in only 3 (3.8%) of the 78 teicoplanin-resistant strains and all of the teicoplanin-susceptible strains. In disk diffusion, all but one teicoplanin-resistant (Vitek-2) strain were interpreted as susceptible.

Conclusion: The different methods used for AST of CoNS generated inconsistent and discrepant results. Considering broth microdilution as the reference method, disk diffusion did not

reliably identify any of the teicoplanin-resistant strains, therefore is no longer considered an option for routine AST of CoNS. The automated method, usually easy hands-on time for routine use, proved only in 68.5% to be accurate, with most of the cases (43.8%) correctly recognized among teicoplanin-susceptible strains. By means of all three methods, 71 strains were teicoplanin-susceptible and only three teicoplanin-resistant. Meanwhile, using CLSI breakpoints to analyse the microdilution results, only six strains were assigned to teicoplanin-intermediate and all the rest were teicoplanin-susceptible. These first results demonstrate a great degree of variability not only among different AST methods, but also among the regulatory authorities.

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Phenotypic characterisation of *Coxiella burnetii* isolates from various hosts

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Q fever is a worldwide distributed zoonotic disease and caused by the intracellular pathogen *Coxiella* (*C.*) *burnetii*. The disease outcome in humans (acute, chronic) and animals (asymptomatic, fertility disorders) can be correlated to different genomic groups, based on classical RFLP analysis. The only know virulence factor is the lipopolysaccharide and no other virulence factors are known to explain the isolate-specific virulence.
The aim of this study is to establish a model for characterizing the isolate-specific virulence using comparative proteome analysis. For this purpose, six field isolates of different host"s origin (human, sheep, goat, cattle) are characterized phenotypically. Based on cell culture infection models with three target cell lines (human monocytes - THP-1, bovine fetal placenta - F3 and udder cells - PS) the replication and invasion efficiency of the isolates will be determined. Replication rate will be calculated as the fold-increase in the number of cell-associated *Coxiella* from day 0 (24h after inoculation) compared to day 8. The invasion rate will be calculated after 1h and 4h post infection by normalizing the number of cell-associated bacteria with the inoculum. For statistical analysis the *t* test was used.
It became apparent that all isolates replicated significantly ($p < 0.05$) better in PS cells than in other cell lines with an increase of 1.8 to 2.4 log steps. The replication rates in PMA-treated, monocyte-derived macrophages THP-1 and in bovine trophoblast F3 were lower (1.5 to 2.0 and 0.8 to 1.8 fold increase). One goat isolate showed the highest replication rate in THP-1 and F3 cells with 2.0 ($p < 0.05$) and 1.6 log increase. The lowest replication efficiency in THP-1 and PS could be determined for one human isolate, with only 1.5 ($p < 0.05$) and 1.8 log increase. Whereas the sheep isolate from the same outbreak, a slightly higher increase of 2.2 log steps in udder cells (PS) could be determined. However, this isolate replicated significantly lower ($p < 0.05$) in the F3 cells compared to all other isolates. The invasion rates after 1h infection were below 10 % and after 4h, up to 23 % for all tested *Coxiella* isolates. Only one isolate from cattle showed a higher invasion rate in all cells in comparison to the other isolates (up to 10 % invasion efficiency).
Based on replication and invasion assays, we can detect phenotypic differences of the here tested isolates. In addition, gene expression analyses in the different cell models is ongoing. Isolates, which show the most significant differences, will be selected for proteome analysis. This might support the identification of potential virulence factors and adaption mechanism that could be relevant for the isolate-specific virulence.

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Implementation of a mobile application "Antiinfektiva Leitfaden" in German-speaking countries as an Antibiotic Stewardship (ABS) project

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Question: The application for mobile devices "Antiinfektiva Leitfaden" is a practical tool at the point of care ensuring appropriate antimicrobial use within the hospital. Our aim is to make this app freely accessible to both internal and external healthcare professionals to augment ABS in German-speaking countries.
Methods: At first we obtained a written consent of the intern legal department and data protection department and selected a mobile platform. The app was formatted by a hospital pharmacist without IT support using a fee-based toolbox (https://onair-appbuilder.com/de/). In a subsequent step, existing local guidelines, developed since 2013 by the multi-disciplinary ABS team (including infectious disease specialists, clinical microbiologists, clinical pharmacists, medical specialists) of a University Hospital in Germany, were transferred to the app. The homepage and the app icon were designed by the internal department of corporate communication. To implement it, training of the staff was offered.
Results: Since February 2021 the app is freely available in German language and free of charge in Apple App Store and Google Play Store. Until 26th of May 2021 the app was downloaded 1917 times. Once downloaded the app can be used without internet connection. The user interface is intuitive and the handling is self-explaining. All contents are updated regularly.
Information includes:

- evidence-based recommendations for empiric antimicrobial treatment for common infections and antimicrobial prophylaxis considering local antibiotic resistance patterns
- evidence-based recommendations for standard use of antimicrobials, e.g. dose adjustment in renal insufficiency, renal replacement therapy, liver insufficiency and obesity, Therapeutic Drug Monitoring
- links to key references, national/international guidelines, institutions
- links to training programs
- additional documents as PDF-file, e.g. for printing out or customizing
- update information regarding pharmacovigilance, critical incidents, recalls
- contact details

Conclusions: In our hospital the implementation of the mobile application "Antiinfektiva Leitfaden" was a major step towards digitisation which supports improvement of infectious disease management. Free of charge it provides a more readily access to local antimicrobial guidelines and recommendations for use of antimicrobials, for both internal and external healthcare professionals. Moreover, links to training programs are offered.

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Goats as sentinels for risk assessment of alimentary infections with the tick-borne encephalitis virus (TBEV)

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Introduction: Tick-borne encephalitis virus (TBEV) is transmitted mainly by tick bites, but also alimentary transmission is known in humans. In 2016 two human cases of tick-borne encephalitis (TBE) caused by unpasteurized infected goat milk were reported in Baden-Wuerttemberg [1]. Aim of our study was to identify hot spots of TBEV in the area of Baden-Wuerttemberg by using goats as sentinels followed by the collection an examination of ticks. Furthermore, we evaluated the risk of alimentary infection in a detected hot spot by screening goat milk samples for TBEV.
Materials and Methods: From 13 farms in Baden-Wuerttemberg, sera of goats for dairy production were tested for the presence of TBE-antibodies by ELISA. The selection included only grazing livestock farms. Based on screening results, we selected one farm for further investigation and analyzed the sera of the whole flock in 2018 and 2020 for the presence of TBE-antibodies by ELISA. The results were confirmed by serum neutralization test as "gold standard" for TBE-antibody detection. Bulk milk samples were obtained from June 2020 till November 2020. Furthermore, we collected ticks (*Ixodes ricinus*) at two times on the meadows of the farm. For detection of TBEV, RNA was extracted from the milk and ticks and TBE-PCR was performed.
Results: We detected antibodies for TBEV in several sera from four of the farms investigated. The farm selected for further investigation was located in a known hotspot [1]. The within-herd sero-prevalence for TBE on this farm was 47 % in 2018 and 36 % in 2020. The TBE-sero-incidence was 15 % (3/20) over 2 years. Nevertheless, we did not detect TBEV (RNA) in the bulk milk samples but we confirmed the presence of TBEV in collected ticks and confirmed the serological presumed hot spot.
Conclusions: Our study confirmed and increased our knowledge about the hot spot for TBEV in Baden-Wuerttemberg by using the combined approach of screening grazing goats for TBEV-specific antibodies and by testing the local tick population for TBEV virus. The results of our study shows that the hotspot is further well established over the years and a potential risk for public health still exists. The sero-incidence of TBEV in the flock and the results of milk survey lead to the hypothesis that the risk for alimentary infection is low even in a hotspot detected. Nevertheless, pasteurization of milk is still important to prevent alimentary infection.

[1] Brockmann et al., Euro Surveill. 2018 Apr;23(15):17-00336. https://doi.org/10.2807/1560-7917.ES.2018.23.15.17-00336

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Unknown mycobacterial species with a Line Probe Assay pattern of *Mycobacterium gordonae*

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Introduction: For patients with Ziehl-Neelsen smear positive specimens, but negative *M. tuberculosis* complex molecular assay, the GenoType CMdirect VER 1.0 line probe assay (Bruker HAIN Lifescience, Nehren, Germany) offers the possibility for rapid identification of nontuberculous mycobacteria (NTM) direct in patient specimens. More than 15 of the most common NTM species can be identified by specific hybridization patterns. We applied this LPA to smear positive specimens obtained from a patient with severe symptoms of pulmonary disease. However, the resulting hybridization pattern of *M. gordonae* could not be confirmed by sequence analysis.
Material and Methods: Patient specimens were analysed by smear microscopy, mycobacterial culture, TB-PCR, and some by GenoType CMdirect and direct sequencing of a part of the 16S rRNA gene. Species identification from positive cultures was done with the GenoType Mycobacterium CM VER 2.0 as well as sequence analysis of parts of the 16S rRNA and rpoB genes and the ITS sequence.
Results: Within the scope of 5 months, 11 specimens were obtained from the patient for mycobacterial diagnostics. GenoType CMdirect was conducted from all three smear positive specimens. The resulting hybridization pattern was specific for *M. gordonae*, yet subsequent 16S rRNA gene sequencing yielded an *M. kansasii* specific sequence. In total, 5 specimens were culture positive. All of these isolates were characterized by a hybridization patterns specific for *M. gordonae* and a 16srRNA gene sequence of *M. kansasii*. Sequence analysis of a part of the rpoB gene showed high similarity *M. kansasii* whereas the ITS sequence was most similar with *M. gordonae*. Sequential incubation of solid culture isolates in the dark followed by light proved photochromogenicity of the strain.
Discussion: Rapid molecular assays enable the identification of NTM species directly in patient specimens and prove as valuable tool especially in smear positive specimens with a negative TB complex molecular test. The multiple hybridization patterns for *M. gordonae* obtained from several smear positive specimens from our patient was surprising, since *M. gordonae* usually is estimated to be non-pathogenic. By sequence analysis, the strain could not unequivocally be identified and thus we estimated it to be an unknown, so far not validly described mycobacterial species. The clinical relevance of this strain so far is unclear. The patient's treatment was changed from TB therapy to a triple NTM therapy. Six follow-up specimens remained culture negative. Line probe assays for identification of mycobacterial species base upon definite sequence differences between species. However, the presence of unknown species with unknown sequences in target genes elicit the possibility of incorrect results. In case of multiple isolates from a patient with a GenoType CM pattern specific for *M. gordonae*, advanced species identification should be considered.

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In vitro* activity of temocillin, piperacillin-tazobactam, and meropenem against third generation cephalosporin-resistant *Enterobacterales

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Question: Infections caused by third generation cephalosporin-resistant *Enterobacterales* (3GCREB) pose a serious threat to public health. Previous studies have reported inferiority of piperacillin-tazobactam (PTZ) when treating infections due to 3GCREB leading to overuse of reserve antibiotics like carbapenems. Alternatively, the beta-lactam temocillin (TEM) features resistance against most beta-lactamases including AmpC- and extended spectrum beta-lactamases (ESBL) typically found in 3GCREB. In this study, we performed comparative minimal inhibitory concentration (MIC) determination of TEM, PTZ, and MER in 205 strains of *Enterobacterales*, collected from urological wards and in the majority phenotypically harboring AmpC- and ESBL. Further, we compared MIC values of PTZ and MER using different MIC methods.
Methods: TEM, PTZ, and MER MICs of 205 isolates of 3GCREB (*Escherichia* (*E.*) *coli* n=116, *Klebsiella* (*K.*) spp. n=40 [*K. pneumoniae* n=30, *K. aerogenes* n=8, *K. oxytoca* n=2], *Proteus* (*P.*) spp. n=8 [*P. mirabilis* n=7, *P. rettgeri* n=1], *Enterobacter* (*E.*) spp. n=20 [*E. cloacae* n=13 , *E. cloacae complex* n=7], *Citrobacter* (*C.*) spp. n=5 [*C. freundii* n=4, *C. braakii* n=1], *Serratia marcescens* n=2, *Morganella morganii* n=14), obtained from urine (n=197) and blood cultures (n=8) were determined by gradient diffusion method (GDM; for TEM, PTZ, MER), broth microdilution (BM; for PTZ, MER), and VITEK2® (PTZ, MER), respectively. MICs were assessed using EUCAST (version 11.0) breakpoints (where available). The isolates where phenotypically differentiated for AmpC- and/or ESBL using disc diffusion method.
Results: All isolates were sensitive to MER and the MIC distribution pattern was equal in either MIC method. In

comparison, 89.66% (104/116) *E. coli*, 96.67% (29/30) *K. pneumoniae*, and 100% (7/7) *P. mirabilis* were susceptible to TEM and 48.28% (56/116) *E. coli*, 33.33% (10/30) *K. pneumoniae*, and 100% (7/7) *P. mirabilis* sensitive to PTZ, respectively. MIC distribution of all strains showed a shift towards higher values in PTZ in GDM (MIC50 16, MIC90 >256) as compared to TEM (MIC50 8, MIC90 16). For PTZ, MICs derived from GDM were higher compared to BM (MIC50 ≤4, MIC90 >64) and VITEK2® (MIC50 8, MIC90 >128). Qualitatively, 43.41%, 62.44%, and 68.29% of all isolates were sensitive to PTZ determined by GDM, BM, and VITEK2®, respectively.

Conclusions: Our data show that, compared to PTZ, TEM could be a better carbapenem sparing alternative antibiotic for the treatment of infections due to 3GCREB. Advantageously, TEM renders a minimal risk of *Clostridium difficile* infections and has no significant inoculum effect. PTZ, however, also has good antipseudomonal activity. VITEK2® shows near concordant results with the gold standard BM. In contrast, there is a larger deviation of the MICs derived by GDM. This could be a problem as GDM is used as a confirmatory method in many routine laboratories. For comparative reasons MICs for TEM should also be determined by BM and VITEK2®.

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Emergence of cefiderocol resistance in carbapenem-resistant *Enterobacter cloacae* during cefiderocol therapy due to mutations in the catecholate siderophore receptor: *in vivo* finding and *in vitro* confirmation

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Background: Cefiderocol is a novel siderophore cephalosporine, which utilizes the bacterial iron transport system to enter the periplasmatic compartment and bind to the penicillin-binding proteins interfering with its cell-wall synthesis. Cefiderocol is a promising antimicrobial agent to treat carbapenem-resistant Gram-negatives due to its stability against metallo-betalactamases, such as the New-Delhi-Metallo-beta-lactamase (NDM). Here, we report the first case of cefiderocol resistance development during cefiderocol therapy in NDM-producing *Enterobacter cloacae*.

Methods: Antibiotic susceptibility testing was performed by the agar diffusion and broth microdilution method using iron-depleted cation-adjusted Mueller-Hinton broth and interpreted according to the EUCAST guidelines. To identify potential resistance mechanisms, cefiderocol-susceptible *E. cloacae* (n=4) from the initial and cefiderocol-resistant *E. cloacae* (n=5) were characterized by whole-genome sequencing. For *in vitro* confirmation, cefiderocol-resistant *E. cloacae* mutants were generated using serial dilution under antibiotic pressure.

Results: *E. cloacae* (n=4) isolates prior to cefiderocol therapy exhibited minimal inhibitory concentration (MIC) in the range of 2-4 mg/L. *E. cloacae* isolates (n=5) detected during therapy (re-appearance after several negative blood cultures within 21 days) exhibited a very high cefiderocol MIC of >256mg/L. Genome-wide comparison of all isolates demonstrated heterogeneous non-synonymous mutations in the catecholate siderophore receptor gene *cirA*, which were present in all resistant isolates but not in susceptible isolates. Genome sequencing of *in vitro* generated cefiderocol-resistant mutants confirmed our *in vivo* findings.

Discussion: Our findings indicate that high-level resistance can be acquired rapidly through mutations of the CirA siderophore receptor in *E. cloacae* during cefiderocol therapy. The rapid development of cefiderocol resistance during therapy is alarming and should be monitored closely.

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Development of novel antibacterial treatment strategies for *Acinetobacter baumannii* infections

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Question: *Acinetobacter baumannii* is one of the most important pathogens for which new therapeutic options are urgently needed due to its extensive antibiotic resistance. Infections with multidrug-resistant (MDR) *A. baumannii* are often considered untreatable and are associated with high mortality. Phage protein-based treatments are novel promising antibacterial treatment strategies against Gram-negative bacteria.

Methods: A novel antimicrobial protein, Artilysin® Art-Top3, consisting of an endolysin and a targeting peptide was engineered. Antibacterial activity of Art-Top3 against carbapenem-resistant Enterobacterales (CRE), *Pseudomonas aeruginosa* and *A. baumannii* clinical isolates was determined by broth microdilution, *in vitro* activity assays and time-kill kinetics. Biofilm formation assays were performed under static and dynamic conditions in order to examine the impact of Art-Top3 on mature biofilms and biofilm growth.

Results: Art-Top3 showed a three-fold higher antibacterial activity (mean MIC: 0.25 µM) against carbapenem-resistant *A. baumannii* than against other CRE such as *Enterobacter cloacae and Klebsiella pneumoniae* (mean MIC: 0.76 µM) isolates. Art-Top3 exhibited rapid killing (30 sec) of MDR *A. baumannii* isolates in time-kill kinetics. Preformed, mature *A. baumannii* biofilms treated with MIC Art-3 were strongly reduced in their biomass (≥ 50%). The simultaneous incubation of bacteria and Art-Top3 prevent biofilm formation of *A. baumannii* almost completely by penetrating the outer membrane of the bacteria as determined by 3D laser scanning microscopy.

Conclusion: The engineered protein Art-Top3 exhibited a high antibacterial activity towards carbapenem-resistant MDR *A. baumannii* in time-kill kinetics and biofilm experiments. Art- Top3 was active on several Gram-negative species and therefore these results provide a promising basis for novel therapeutic strategies of infections caused by *A. baumannii* and other Gram-negative species.

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Activity of Cefiderocol against multidrug-resistant gram-negative bacteria

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Background: Recently, WHO classified multidrug-resistant gram-negative bacteria as priority pathogens. Lately, new agents targeting these pathogens were developed such as the new Siderophore Cephalosporin Cefiderocol.

The aim of the study was to test the in vitro activity of Cefiderocol against clinical isolates of multidrug-resistant gram-negative bacteria.

Material/Methods: 275 single-patient isolates were used in this study: 191 Enterobacteriacae, 54 Pseudomonas spp. and 21 Acinetobacter spp. All of these isolates were either classified as 4MRGN according to KRINKO criteria or proven as Carbapenemase building strains. 4 Stenotrophomas maltophilia and 5 Burkholderia spp. isolates were also tested. Cefiderocol susceptibility testing was performed in cation-adjusted Mueller Hinton broth according to manufacturer instructions.

Clinical categories for all bacteria were determined using EUCAST breakpoints, except for Acinetobacter spp. and Stenotrophomonas maltophilia for which we used CLSI breakpoints. Calculated susceptibility rates include "susceptible" and "susceptible, increased exposure" for EUCAST breakpoints and "susceptible" for CLSI breakpoints.

Results: Cefiderocol showed very well performance against Acinetobacter spp. and Pseudomonas spp. MIC50/MIC90 for isolates of Acinetobacter spp. were 0,12/1 mg/L with a susceptibility rate of 100%. Isolates of Pseudomonas spp. showed MIC50/MIC90 values of 0,5/8 mg/L with a susceptibility rate of 86% for 44 Pseudomonas aeruginosa isolates (EUCAST doesn"t provide Cefiderocol breakpoints for Pseudomonas spp. other than Pseudomonas aeruginosa).
MIC50/MIC90 values for isolates of Enterobacteriacae were 1/8 mg/L with a susceptibility rate of just 62%. Amongst them were 93 KPC-producing Klebsiella pneumoniae isolates with MIC50/MIC90 values of 4/8 mg/L and a susceptibility rate of just 31%. Excluding the KPC-producing Klebsiella pneumoniae isolates, the MIC50/MIC90 values of the remaining 98 Enterobacteriacae isolates were 0,25/2 mg/L with a susceptibility rate of 91%.
The MIC values of the 4 Stenotrophomanas maltophilia isolates were in a range of <0,3-0,12 mg/L, all of them being susceptible. The 5 Burkholderia spp. isolates showed MIC values between <0,03 and >8 mg/L, only one isolate having a MIC value >0,25 mg/L.
Conclusion: Cefiderocol showed very well performance against highly problematic multidrug-resistant Acinetobacter and Pseudomonas spp. but there was a lack of activity against KPC-producing Klebsiella pneumoniae isolates.
Nevertheless, Cefiderocol shows potential utility as salvage therapy against difficult-to-treat pathogens with limited or no treatment options.

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Detection of *Salmonella spp.* from reptiles in a veterinary diagnostic institute from 2015 to 2019

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Introduction: Salmonellosis is a significant disease, affecting both humans and animals. Primarily, *Salmonella spp.* is considered to be a foodborne pathogen. However, in the USA it has been estimated that 6% of sporadic salmonellosis cases and 11% of cases in people aged under 21 are caused by reptile and amphibian contact [Mermin et al. 2004].
The finding of *Salmonella spp.* in reptile pets is not unusual, but the percentage of positive animals differs a lot in different sources and the number of animals in most of the studies is very low.
In our veterinary diagnostic institute, a total of 1080 reptiles was dissected from 2015 to 2019 to determine the cause of disease or death. Besides many other investigations, they were also examined for the presence of salmonella.
The group of reptiles examined in this study consisted of 429 snakes, 176 lizards, 347 land tortoise and 128 sweetwater turtles.
Material and Methods: From every dissected reptile organ samples were taken to follow examination routine of *Salmonella spp*. according to ISO 6579-1. Serum agglutination was carried out according to the Kauffmann-White-Scheme to determine the serovar.
Results: According to our findings, in 33 % of all investigated reptiles salmonella have been found. Snakes and lizards are often carriers of salmonella (55%, respectively 48%) whereas tortoises and turtles are rarely positive for *Salmonella spp.* (6 and 2%, respectively). Altogether 52 different salmonella serovars were found. The serovars Salmonella Enteritidis and Typhimurium, which are considered to be highly pathogenic in humans, only occurred three times in snakes or in other reptiles.
However, the exotic subspecies / serovars may also cause disease in humans.
Discussion: In contrast to former studies, only in 342 of 1080 reptiles salmonella have been found (33%) in spite of using a salmonella enrichment broth for investigating the organ tissues. According to our results, reptiles are not as often colonized with *Salmonella spp.* as expected [Rabsch 2013, Pfeifer et al. 2019]. But of course, the results reveal a zoonotic potential considering these groups of animals being kept as pets and sometimes in close contact with their owners.
In 55% of the dissected reptiles the detection of salmonella showed no correlation to the cause of disease and death in the animals. This supports the hypothesis that salmonellae are part of the normal intestinal flora of reptiles and are therefore not necessarily pathogenic in reptiles.

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Pituitary adenylate cyclase-activating polypeptide alleviates intestinal, extra-intestinal and systemic inflammatory responses during acute *Campylobacter jejuni*-induced enterocolitis in mice

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Question: Human *Campylobacter jejuni* infections are progressively rising all over the globe and constitute significant health burdens. The ubiquitously expressed pituitary adenylate cyclase-activating polypeptide (PACAP) is well-known for its cell-protective and immunomodulatory effects. In our actual intervention study, we used an acute campylobacteriosis model and assessed the potential disease-alleviating effects of exogenous PACAP.
Methods: Secondary abiotic IL-10-/- mice were perorally infected with *C. jejuni* and treated with synthetic PACAP38 intraperitoneally from day 2 until day 5 post-infection. Synthetic PACAP38 was obtained from the Department of Medical Chemistry, University of Szeged (Hungary). Once daily mice were either subjected to synthetic PACAP (1.5 mg per kg body weight, dissolved in phosphate buffered saline or to vehicle (mock) intraperitoneally from day 2 until day 5 post infection.
Results: Whereas PACAP did not interfere with the gastrointestinal colonization of the pathogen, mice from the PACAP group exhibited less severe clinical signs of *C. jejuni*-induced disease, as compared to mock controls, which were paralleled by alleviated apoptotic, but enhanced cell proliferative responses in colonic epithelia on day 6 post-infection. Furthermore, PACAP dampened the accumulation of macrophages and monocytes, but enhanced regulatory T cell responses in the colon, which were accompanied by less IFN-γ secretion in intestinal compartments in PACAP versus mock-treated mice. Remarkably, the inflammation-dampening properties of PACAP could also be observed in extra-intestinal organs, and strikingly, even the systemic circulation on day 6 post-infection.
Conclusion: For the first time, we provide evidence that synthetic PACAP might be a promising candidate to combat acute campylobacteriosis and post-infectious sequelae.

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Treatment with the probiotic compound Aviguard alleviates inflammatory responses during *Campylobacter jejuni* induced acute enterocolitis in mice

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Question: *Campylobacter jejuni* infections are progressively emerging globally. Given that probiotic feed additives such as the compound Aviguard have been shown effective in reducing enteropathogens such as *Salmonella* in vertebrates including lifestock, we here assessed potential anti-pathogenic and immune-modulatory properties of Aviguard during acute *C. jejuni* induced murine enterocolitis.
Methods: Therefore, microbiota-depleted IL10-/- mice were infected with *C. jejuni* strain 81-176 by gavage and orally treated with Aviguard or placebo from day 2 to 4 post-infection.
Results: The applied probiotic bacteria could be rescued from the intestinal tract of treated mice, but with lower obligate anaerobic bacterial counts in *C. jejuni* infected as compared to non-infected mice. Whereas comparable gastrointestinal pathogen loads could be detected in both groups until day 6 post-infection, Aviguard treatment resulted in improved clinical outcome and attenuated

apoptotic cell responses in infected large intestines during acute campylobacteriosis. Furthermore, less distinct pro-inflammatory immune responses could be observed not only in the intestinal tract, but also in extra-intestinal compartments on day 6 post-infection.
Conclusion: We here show for the first time that the probiotic compound Aviguard exerts potent disease-alleviating effects in acute *C. jejuni* induced murine enterocolitis and might be a promising probiotic treatment option for severe campylobacteriosis in humans.

287/DKMP
Direct Rapid Susceptibility Testing of Positive Blood Cultures by automated microscopic reading: comparison with standard Antimicrobial Susceptibility Testing Methods
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Introduction: Early introduction of pathogen-adapted antibiotic therapy has substantial impact on prognosis of patients with blood stream infections. Reducing the time to result for antimicrobial susceptibility testing (AST) would contribute to an optimized managment of these patients. In the recent years new AST-technologies have been developed to achieve this goal. The QMAC-dRAST system (QuantaMatrix, Seoul, Republic of Korea) is an automated system for performing AST directly from positive blood culture bottles. Based on microscopic detection of bacterial growth in antibiotic containing agarose wells results can be read in 6 to 8 hours of incubation. It was the aim of the present study to assess the performance of this new technique.
Material and Methods: Antimicrobial susceptibility testing has been performed directly from 180 positive blood culture bottles (including 15 artificial blood cultures) containing 100 Enterobacterales, 60 Staphylococcus aureus and 20 Enterococcus strains with the QMAC-dRAST system (QuantaMatrix). AST results obtained by dRAST (n=2480) have been compared with results from EUCAST disc diffusion method (AD, n=2235), broth microdilution (BMD, Micronaut, n=2020) and VITEK 2 (VI, n=1840) performed with the subcultured isolates. For antibiotics not present in the Micronaut panel and/or in the Vitek card, additional BMD by Sensititre (BMD-SEN, n=240) or Gradienttests (GT, n=360) have been performed.
Results: In Enterobacterales overall categorical agreement for dRAST with AD, BMD-MIC, VI, BMD-SEN and GT reached 97.3%, 97.8%, 96.8%, 98% and 97.5% respectively. Very major errors (VME) were observed in 9/1495 (0.6%) AD tests, 8/1200 (0.7%) BMD-MIC, 11/900 (1.2%) VI, 1/100 (1%) BMD-SEN and 2/200 (1%) GT, respectively. In Staphylococcus aureus overall categorical agreement for dRAST with AD, BMD-MIC, VI, BMD-SEN and GT reached 93.1%, 95.9%, 95.3%, 90.8% and 98.3% respectively. Very major errors (VME) were observed in 7/600 (1.1%) AD tests, 4/720 (0.5%) BMD-MIC, 6/840 (0.7%) VI, 1/120 (0.8%) BMD-SEN and 1/120 (0.8%) GT, respectively. In Entercoccus species overall categorical agreement for dRAST with AD, BMD-MIC, VI, BMD-SEN and GT reached 95.7%, 98%, 100%, 90% and 87.5% respectively. Very major errors (VME) were observed in 1/140 (0.7%) AD tests, 0/100 (0%) BMD-MIC, 0/100 (0%) VI, 2/20 (10%) BMD-SEN and 5/40 (12.5%) GT, respectively. All VME occured with Streptomycin-HLAR. Test duration were in average 7h 4‘ for testing Enterobacterales, 7h 6‘ for testing S. aureus and 7h 20‘ for testing Enterococcus species.
Conclusion: The QMAC-dRAST system is a highly accurate and robust AST technology compared to standard testing methods as EUCAST disc diffusion, BMD or Vitek for the most important sepsis pathogens.

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Performance of a SARS-CoV-2 Rapid Antigen Test (SD - Biosensor STANDARD F COVID-19 Ag FIA) compared with SARS-CoV-2 PCR in a Clinical Setting
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Background: SARS-CoV-2 rapid antigen tests (RATs) are increasingly being applied as point-of-care assays due to the fast availability of results and simplicity of use. Although high sensitivity and specificity have been reported by most manufacturers in validation trials, very few data have been published on the performance of RATs in a real-world clinical setting.
Materials and Methods: From 1 January to 30 June 2021, the nasopharyngeal swabs of 4579 patients admitted to Greifswald University Hospital were initially subjected to the fluorescence - based SD - Biosensor STANDARD F COVID-19 Ag FIA and thereafter to routine SARS-CoV-2 PCR.
Results: Of the examined nasopharyngeal swabs, 23 were found by PCR to give a positive result for SARS-CoV-2, corresponding to a positivity rate of 0.5%. Compared with the PCR gold standard, the following performance characteristics could be derived for the STANDARD F COVID-19 Ag FIA: sensitivity 69.6%, specificity 98.1%, positive predictive value 16.0% (corresponding to a total of 100 positive results of the used RAT, of which 16 could be confirmed by PCR), and negative predictive value 98.3%.
Conclusions: In the clinical setting of our maximum care hospital, about two thirds of the SARS-CoV-2 infected patients could be identified correctly, using the SD Biosensor RAT. We suggest that this is a good result in comparison with other published real-world RAT performance data. However, the performance is not good enough to replace PCR testing in a hospital, since every false-negative result leads to potentially detrimental consequences in the clinical environment, if patients are cleared for admission to the ward using only a RAT without confirmatory PCR. The prime advantage of SARS-CoV-2 RATs compared with PCR is the short time-to-result in identifying SARS-CoV-2 – positive patients. However, a real benefit for the SARS-CoV-2 hospital admission management is only gained in a high-prevalence scenario. During the SARS-CoV-2 low-prevalence scenario of our study, the low positive predictive value of only 16.0% frequently led to unnecessary isolation of patients in special COVID-19 holding areas until a negative result was obtained by subsequent PCR, hence slowing down admission to the respective treatment wards.

Eukaryotic Pathogens (FG EK)

183/EKP
Occurence and antifungal susceptibility of yeasts from the oral cavity of bats in Gabon
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Introduction: Next to bacteria bats harbour eukaryotes, especially fungi that can be disseminated by body fluids and faeces, hence becoming important sources of environmental contamination especially of migrating bat species that fly long distances. The recent emergence of fungi in wildlife (bats, amphibians, snakes) and in human populations (*Candida auris*) has highlighted the importance to analyse animal–fungus interactions. Reports on the composition and antifungal susceptibility of the yeast microbiota of bats is scarce. In this study, we systematically assessed the distribution and antifungal susceptibility of yeasts in different bat species in Gabon using individual pharyngeal swabs.
Methods: Bats were captured in Fougamou and Waka National Park in the province Ngounié, Gabon, using standard mist netting

methods. Individual pharyngeal swabs were collected from live animals, followed by culturing on Sabouraud Agar. Yeasts were identified using MALDI-TOF MS (Bruker), or by sequence analysis of the internal transcribed spacer 1 and 2 in isolates failed MALDI-TOF MS identification (scores <2.0). Antifungal susceptibility testing was performed by MICRONAUT–AM microdilution test systems (Merlin) in accordance to EUCAST.

Results: In total, pharyngeal swabs from 133 bats comprising nine different species were collected. The colonization rate was 46.6 % (n=62) with 66 yeast isolates identified. Of these, the vast majority were *Candida albicans* (71%, n=47), followed by *Rhodotorula mucilaginosa* (6%, n=4), *Candida carpophila* (3%, n=2) and one each from *Candida jaroonii*, *Candida kantuleensis*, *Candida intermedia*, *Candida pelliculosa*, *Millerozyma farinosa* and *Debaryomyces nepalensis.*

All *C. albicans* isolates were susceptible to anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, voriconazole and amphotericin B. The MICs for fluconazole of the remaining *Candida* species (n=6) ranged from 0.25 to 4mg/l, resulting in five susceptible isolates (MIC = 0.25-2mg/l) and one isolate susceptible at increased exposure (MIC = 4mg/l). MICs for anidulafungin ranged from 0.016-0.03 mg/l. The MICs of *R. mucilaginosa* for echinocandins were high (>8mg/l).

Discussion: The pharyngeal colonization rate of bats with yeasts is high (46.6%) suggesting that yeasts are common in the microbiom of bats. The dominance of one species (*C. albicans*) could indicate a selection benefit in the oral cavity of bats. The absence of resistance is most likely due to no/minimal exposure to antifungals (occuring naturally) or no transmission from reservoirs where antifungal resistances are more common (e.g. humans). High MICs of *R. mucilaginosa* for echinocandins are in line with the suggested intrinsic resistance to echinocandins in *Rhodotorula* spp..

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Myriocin affects viability and antifungal drug susceptibility of the emerging pathogen *Candida auris*

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Question: Infections with the emerging human fungal pathogen *Candida auris* are a major clinical challenge as it is one of the few yeasts which can be transmitted between patients and / or nursing staff. *C. auris* is quite resistant to several environmental stresses which is beneficial for the aforementioned nosocomial transmissions. *C. auris* isolates are normally resistant to fluconazole and can also develop additional resistance to other azoles, echinocandins or amphotericin B. Therefore, clinicians are often confronted with multidrug isolates and limited therapeutic options. This leads to a search for new compounds and alternative treatments for future therapy. Here, we examined the effects of myriocin, a well characterized inhibitor of the early steps of the sphingolipid biosynthesis, on viability and antifungal susceptibility of *C. auris*.

Methods: Isolates of *C. auris* and other *Candida* species were grown on YPD in the presence of 2.5 nM to 5 µM myriocin at 37°C. Fluorescence microscopy was used to analyze propidium iodide (PI) and fluorescein isothiocyanate (FITC) stained cells. For antifungal drug susceptibility testing, fungal cells were plated onto RPMI agar with or without myriocin prior to the application of Etests.

Results: Initially, we have screened isolates of *C. albicans, C. auris*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*, *C. parapsilosis* and *C. tropicalis* for their susceptibility to myriocin in concentrations ranging from 2.5 nM to 5 µM. A significant growth delay was observed for *C. auris* in presence of more than 250 nM myriocin. A further screening of 40 *C. auris* strains from different clades revealed that of them were highly susceptible to myriocin. As shown by life/death staining experiment, especially newly formed buds and daughter cells were killed in presence of myriocin. Interestingly, sublethal myriocin concentrations affected the resistance of *C. auris* against echinocandins and amphotericin B, leading to an increased susceptibility. This effect was however not observed for azoles.

Conclusion: Our results show that in contrast to other *Candida* species, *C. auris* is highly susceptible to the sphingolipid biosynthesis inhibitor myriocin. Treatment with myriocin affects the overall viability of *C. auris*. It also increases the susceptibility of resistant *C. auris* isolates to echinocandins and amphotericin B, indicating a crucial role of sphingolipids in antifungal drug resistance. Therefore, the sphingolipid biosynthesis pathway could be an interesting target for further development of therapy strategies to handle *C. auris* infections.

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Single- and multidrug-resistant *Candida glabrata* strains from Germany

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Question: *Candida glabrata* is a major cause of hospital acquired infections. Especially immunocompromised and critically ill patients are at risk. The first line therapy consists of echinocandins which are targeting the fungal 1,3-β-D-glucan synthase. However, in recent years, there was an increasing development of antifungal drug resistance, including multidrug resistance (MDR) against azoles and echinocandins which limits therapeutic options. Resistance is related to point mutations in the genes *FKS1* and *FKS2* encoding subunits of this enzyme.

Methods: A collection of 176 *C. glabrata* strains contributed by German hospitals and health care facilities between 2015- 2019 was analysed regarding susceptibility to echinocandins and fluconazole. Broth microdilution was performed in accordance with EUCAST and CLSI standards. Hot spot (HS) regions of the *FKS*-genes were amplified by PCR and sequenced.

Results: 39 % (n=70) of the strains were related to blood stream infection. 52 % (n=92) showed susceptibility to anidulafungin. 7 % (n=13) were initially classified as resistant to andidulafungin (EUCAST reference method), but had no *FKS* HS mutations. A broad spectrum of tests indicated that most of these strains show borderline susceptibility to echinocandins. This was underlined by conducting a national round robin test with the participation of 10 different labs. Testing for micafungin could be shown to be more precise than anidulafungin testing. Of the anidulafungin resistant isolates 40 % (n=71) harboured *FKS*-gene mutations, mostly in *FKS2* (87 %). Significant differences ($p<0.01$) in MIC- values were found, suggesting that changes at position 663 of *FKS2* can lead to high-level resistance. In particular, S663P and F659del mutations were associated with high MIC-values. 33 % of *FKS* HS mutated strains displayed concomitant fluconazole resistance, leading to MDR.

Conclusions: Phenotypic susceptibility testing can overestimate the number of resistant strains with testing for micafungin showing best predictive power for resistance. All *FKS* mutated strains showed resistance in the presence of echinocandins. Certain positions correlate with high resistance and may be connected to an adverse outcome. Genotyping might be a fast and clinically useful tool detecting resistant strains preventing therapeutic failure. MDR needs further surveillance programs.

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Development of a temporally and spatially resolved multidimensional reporter assay for host damage

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Fungi cause approx. 1.5 million deaths per year worldwide, which is comparable to diseases such as tuberculosis. Hence, the impact of fungal infections on human health is tremendous and the investigation of fungal pathogenicity and host interactions is more important than ever. Due to the complexity and the variety of factors involved, the study of infections caused by fungal pathogens often requires many different read-outs and time-consuming methods.

Thus, we generated an easy-to-use photonic high-throughput method for temporally resolved screening of host responses to diverse stressors. By lentiviral transduction, we created multiple human cell lines that report on intracellular events by activation of different fluorophores. Using these cell lines, we can for example analyse the cellular redox environment as well as the specific type of cell death induced by different fungal pathogens. Upon infection, these read-outs can be measured via microscopy and flow cytometry in real time, giving immediate insights into the kinetics of fungal infection and the host response – both to study different species and to interrogate fungal pathobiology with the use of deletion mutants.

Furthermore, this system can, in the future, be applied not only to fungal infections, but also for dissecting bacterial and viral infection processes, or even drug-induced cellular responses and damage. Collectively, this new assay will significantly expand the current selection of methods used in fungal and infection biology in general.

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The quantitative adhesion strength of single *Candida albicans* yeast cells on bovine tooth enamel

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Introduction: Caries is one of the most prevalent diseases worldwide, which is characterized by a degradation of the hard and highly mineralized tooth enamel. The opportunistic pathogen *Candida albicans* is considered as a major causal agent for caries in children (1). Semi-quantitative adhesion assays carried out on the tooth-model surface hydroxyapatite (HAP) - the main inorganic component of tooth enamel - demonstrated furthermore that *C. albicans* cell numbers were higher on saliva-treated HAP than on untreated HAP (2). The quantitative adhesion strength of this fungal pathogen on tooth enamel surfaces, however, has not been determined yet.

Method: We applied FluidFM (fluidic-force microscopy)-based single-cell force spectroscopy to determine the key adhesion parameters "adhesion force", "rupture length" and "de-adhesion work" of viable yeast cells on bovine tooth enamel in presence and absence of human saliva.

Results: We found that all three adhesion parameters were significantly enhanced on saliva-treated enamel compared to untreated enamel, when *C. albicans* yeast cells were left for 5 s on the surface. Similar observations were made for yeast cells that were preconditioned in human saliva. We found that the mean de-adhesion work (the energy needed to completely detach an adhered cell from the surface) increased for unconditioned yeast cells by a factor of ~2.2, on both, saliva-treated tooth enamel and *in situ*-generated pellicle compared to naïve enamel. Saliva-preconditioned yeast cells displayed de-adhesion work kinetics that were enhanced even by a factor of ~4.4 on both bioconditioned enamel variants, when compared to the de-adhesion work kinetics observed with saliva-preconditioned yeast cells on naïve enamel .

Conclusion: Our data indicate that *C. albicans* yeast cells are well adapted to the oral cavity and utilize host factors present in saliva to promote their tethering to the tooth enamel surface, particularly when the tooth surface is coated with a pellicle, which is known to form within seconds to minutes after each teeth brushing.

(1) Xiao J. *et al*, 2018. *Candida albicans* and Early Childhood Caries: A Systematic Review and Meta-Analysis. Caries Res. 52:102-112, doi:10.1159/000481833.

(2) Thanh Nguyen H. *et al*, 2021. *Candida albicans* Bgl2p, Ecm33p, and Als1p proteins are involved in adhesion to saliva-coated hydroxyapatite. J Oral Microbiol. 13:1879497, doi:10.1080/20002297.2021.1879497.

188/EKP

Establishing human lung organoids as a tool to study infections

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Introduction: Organoids are complex three-dimensional structures which are derived from adult or pluripotent stem cells, which can self-organize into structures that recapitulate functional and structural aspects of the tissue and organ of interest. The generation of organoids has increasingly become popular over the past decade as a new tool for researchers to study various aspects of organ development and physiology. However, the SARS-CoV-2 pandemic has shown a critical need of organoid models for infection biology. The use of lung organoids has become essential to decipher the molecular mechanisms underlying COVID-19. However, the generation of lung organoids and their use to study pathogens is still in its infancy. Several protocols for generation of lung organoids reconstituting different parts of the lung exist, but have rarely been used to comprehensively study infectious agents.

Methods: We have established human lung organoids from adult stem cells and organoid-derived monolayers at air-liquid interface. Expression of proximal lung specific cell markers as well as functional organization and physiology has been characterized using immunofluorescence, electron microscopy, transcriptomics and transepithelial electrical resistance measurement.

Results: Our organoids and organoid-derived monolayers express cell markers that are characteristic for cells found in the proximal lung, thus recapitulating and mimicking the human airway which is commonly used a gateway for various pathogens. Lung organoids show basal localization of adult stem cells and apical organization of ciliated and mucus producing cells. Organoid-derived monolayers furthermore mimic the interaction of these cells with air and can thus be used as an *in-vitro* model for infection biology by forming a monolayer that exhibits a characteristic barrier function found in the lung.

Conclusion: Here, we show that human lung organoids and lung organoid-derived monolayers can be established from human adult stem cells. Both models can further be used as a valuable tool to study various pathogenic infections. Our long-term goal is to complement our proximal human lung organoids with models that are generated from adult stem cells from different species as well as establish organoid models that mimic other parts of the lung. These can then be used as comprehensive *in-vitro* tools to study the pathogen of interest.

Food Microbiology and Food Hygiene (FG LM)

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A new MALDI Biotyper platform for same-day *Salmonella*-, *Cronobacter*- and *Campylobacter* confirmation starting from colonies

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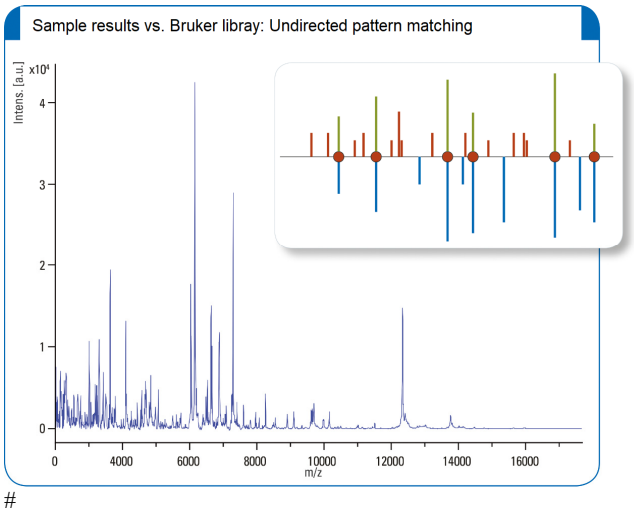
Introduction: Mass spectrometry is a useful Rapid Microbiological Method. Brukers MALDI Biotyper® is a benchtop instrument and can be used for microorganism identification, and confirmation of foodborne pathogens. Our study should show the performance of the new MALDI Biotyper® (MBT) sirius instrument platform with the positive ion mode for same-day confirmation of colonies from non-selective and selective media.
Methods: MALDI target plates were used for applying of samples, or Bacterial Test Standard, separated from each other. One part of the MBT sirius, and MBT sirius one System is a microflex mass spectrometer equipped with smartbeam laser technology. The mass spectrometer has the two purposes: Soft ionization of biological compounds and, mass spectrometric analysis of the respective ions (acquiring mass spectra). The identification software identified organisms by comparing sample results with the dedicated Bruker reference library (see picture 1).
Results: We tested different strains of *Salmonella, Campylobacter and Cronobacter* with the new MALDI Biotyper sirius platform. Different media were used for cultivation of test organisms, and results were compared for: Columbia blood agar with 5% sheep blood, CCI Agar (*Cronobacter*), mCCDA Agar (*Campylobacter*), XLD Agar (*Salmonella*). Different sample preparation procedures were applied in parallel and compared. In addition, we calculated 225 different mass spectra with the new MBT Compass HT software, and compared all data with existing standard software.
Discussion: Equivalence of MBT sirius systems to other mass spectrometry instruments was demonstrated for confirmation of *Salmonella, Campylobacter and Cronobacter* tested. Reliable and fast identification of Gram-*negative* pathogens is possible from colony material of non-selective and selective media with a significant lower workload than the workload of traditional confirmation methods and with the new software with high-speed per sample.

Fig. 1



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Tracing *Campylobacter spp.* in the environment of commercial poultry farms in Germany

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Question: *Campylobacter* (C.) *jejuni* is the most common causative agent of bacterial gastroenteritis, and *C. jejuni* is responsible for the majority of infections in humans, with chicken meat in particular being a key source of infection. In general, it is presumed that the environment surrounding poultry barns somehow serves as a reservoir for *Campylobacter* spp. However, to date, there is limited information on the environmental reservoirs of *Campylobacter* and the importance of environmental contamination. Environmental contamination is generally thought to be responsible for flock colonization, which is facilitated by inadequate biosecurity and improper hygiene practices. So far, the role of the viable but non-culturable state of *Campylobacter* (VBNC) in the environment is scarcely known, as well. To identify possible reservoirs of persistent or VBNC-*Campylobacter* and thus uncover relevant transmission routes, a longitudinal study was conducted on three broiler farms in Germany.
Methods: Three broiler farms and their adjacent environment as well as intervening cleaning and disinfection practices were semi-quantitatively analyzed for *Campylobacter* according to DIN 10272-3 towards the end of two consecutive fattening periods in summer and winter. Moreover, environmental and selected broiler house samples were treated simultaneously with propidium monoazide (PMA) and analyzed by live/dead discrimination using real-time PCR (qPCR) in the further course of the study. A systematic selection of isolates from all sampling occasions was examined by whole-genome analyses.
Results: *Campylobacter* was frequently detected in chicken houses in two out of three farms, especially in summer. In the environment, however, the pathogen was only occasionally detectable, especially in water-associated matrices, mainly in winter. In relation, Campylobacter DNA was more frequently detected in environmental samples, mainly in air samples. After cleaning and disinfection, however, *Campylobacter* could not be isolated in the broiler houses. The emission source of culturable *Campylobacter* was determined to be primarily chicken manure and with less significance work material/clothing. *C. jejuni* revealed to be the dominant species of the examined isolates. PMA-qPCR showed no evidence of VBNC *Campylobacter* in the selected barn and environmental samples.
Conclusions: The present study provides information on the relevance of *Campylobacter* in the environment in proportion to the prevalence of investigated broiler farms in Germany. The results show sporadic environmental findings in the immediate vicinity, indicating spread, persistence and possible re-entrance. Nearby water bodies harbored *C. jejuni*, which suggests ubiquity due to spread and circulation. Although the findings in nature were sporadic and no significant source of transmission has yet been identified, it should be considered that even very low levels of *Campylobacter* may colonize poultry flocks.

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Influence of RpoS and FliC on the survival of *E. coli* O104:H4 strain C227/11Φcu in soil samples

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Introduction: Several outbreaks caused by enterohemorrhagic *Escherichia coli* (EHEC) were attributed to the consumption of non-heated vegetables such as salads or sprouts. Especially, agricultural soils have been suggested as a reservoir for EHEC, and may pose a contamination source for edible plants. EHEC are able to survive for several weeks in the soil, and the influence of abiotic factors on their survival has been investigated previously. However, bacterial factors that are essential for soil survival have been determined only scarcely so far. In the current study, the survival of the EHEC O104:H4 strain C227/11Φcu should be determined in defined agricultural soil samples. In parallel, the impact of the sigma factor gene *rpoS* and the flagellin gene *fliC,* which were hypothesized to play an important role in soil survival should be investigated. Therefore, the survival of *E. coli* O104:H4 strain C227/11Φcu and its isogenic *rpoS* and *fliC* deletion mutant strains was investigated in two soil types and at two incubation temperatures.
Methods: Survival of strain C227/11Φcu and the respective *rpoS* and *fliC* deletion strains was investigated at laboratory scale using diluvial sand (DS) and alluvial loam (AL). Soil samples were

inoculated to a final inoculum of 10^8 colony forming units/g soil and incubated at either 4°C or 22°C for up to 12 weeks.
Results: Soil type and temperature influenced the survival of the *E. coli* O104:H4 strain C227/11Φcu. The survival depended more strongly on the low temperature than on the soil type, and was highest in AL and at 4°C. Incubation of strain C227/11Φcu for three months resulted in a decrease of cultivable bacteria from 10^8 to 10^6 CFU/g soil. The deletion of *rpoS* significantly decreased the survival under all cultivation conditions. For AL and 4°C, a decrease in viable counts from 10^8 to 10^1 CFU/g soil was detected within three months, which was also in this case the highest survival. The *fliC* deletion mutant did not show any difference in survival compared to the wild type strain.
Conclusions: The results of our study demonstrated that EHEC strain C227/11Φcu survive for several months in soil samples. The sigma factor RpoS was confirmed as an important determinant for the survival in soil, while no significant influence was found for FliC. To better understand, which other genetic factors may be responsible for a prolonged soil survival, global transcription patterns will be investigated under the different cultivation conditions in future experiments.

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Glycolic acid enhances the antimicrobial activity of peracetic acid
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Introduction: One in 10 people worldwide get ill after consuming contaminated food and approximately 420,000 people die each year. To overcome this challenge, researchers have been looking for new disinfection strategies for the food industry. The combination of approved biocides, with additive or synergistic activity, is an alternative to the search for new active molecules – requiring regulatory approval. This study focused on the combination of peracetic acid (PAA) and glycolic acid (GA), to control the growth of two foodborne spoilage bacteria, *Bacillus cereus* and *Pseudomonas fluorescens*.
Methods: :PAA and GA antimicrobial activity were evaluated according to the quantitative suspension test proposed in the European Standard EN 1276:2009. The influence of biocide concentration and exposure time were assessed in the antimicrobial activity. The mode of action was focused on the interaction with the cell envelope. The checkerboard microdilution analysis was performed to assess the outcomes from combinations in bacterial growth control.
Results: PAA was effective against *B. cereus* and *P. fluorescens* with minimum bactericidal concentration (MBC) of 500 and 100 µg/mL, respectively. On the other hand, GA was only effective against *P. fluorescens* (MBC = 5000 µg/mL) and promoted a partial reduction of *B. cereus* (2-log reduction at >1000 µg/mL). Both biocides interacted with both bacteria causing a drastic reduction in their culturability. The *P. fluorescens* susceptibility profile did not change over time, while cumulative damages on *B. cereus* were observed. Regarding the effects on the cell envelope, surface hydrophobicity and cell integrity of *B. cereus* were remarkably affected, and no effects were found for *P. fluorescens*. A potential additive effect between PAA and GA was detected, which was corroborated by the evaluation of their antimicrobial activity: 1 and 10 µg/mL of PAA and 100 µg/mL of GA promoted 1- and 6-log reduction of *P. fluorescens* and *B. cereus*, respectively. These combined effects were statistically similar (for *P. fluorescens*) or higher (for *B. cereus*) than the sum of isolated ones, demonstrating the potentiation of PAA by adding GA.
Conclusions: Overall, PAA was a strong oxidant and GA combines the oxidant activity with membrane effects. The combination of these biocides showed a great potential of improving their antimicrobial activity against *P. fluorescens* and *B. cereus*. The combination of PAA and GA had proven to be effective and a good promise in the food industry disinfection.

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Development and application of a phage cocktail to reduce intestinal *Campylobacter* concentrations in broiler chickens
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Introduction: Campylobacteriosis is one of the most important foodborne diseases worldwide and contaminated poultry meat is assumed to be the main source of human *Campylobacter* infections in industrialized countries. Firstly, we developed a *Campylobacter*-specific bacteriophage (phage) cocktail using a systematic *in vitro* approach. Secondly, we investigated the *in vivo* efficacy of the phage cocktail to reduce intestinal *Campylobacter* concentrations in broiler chickens.
Material and Methods: The *in vitro* lytic activity of 18 newly isolated group III phages, eight newly isolated group II phages and the NCTC phage 12673 were examined using *C. jejuni* BfR-CA-14430 as a test strain. The lytic activitiy of single phages and ten combinations thereof were investigated using a liquid culture-based assay monitored by a Tecan Spark automatic plate reader. The combination of phage NCTC 12673 (group III) and the newly isolated phage vB_CcM-LmqsCP1/1 (group II) was selected for application *in vivo*. 180 day-old Ross 308 broiler chickens were divided into two groups, a control and a treatment group. Ten days post hatch, seeders of each group were orally inoculated with the *C. jejuni* test strain. The phage cocktail was administered via drinking water at a final concentration of 10^7 PFU/ml four, three, and two days before dissection. The colonization and shedding of *Campylobacter* in broiler chickens were evaluated by semi-quantitative analysis of cloacal swabs, and cecal and colonic content. During and after phage application, phage susceptibility of *Campylobacter*-isolates recovered during the *in vivo* experiment was investigated.
Results: Combinations of group II and group III phages showed significantly higher *in vitro* lytic activities than single phages or combinations of phages of the same group. The *in vivo* efficacy could only be estimated, as the treatment group showed lower *Campylobacter* counts (4.9 $\log_{10}$ MPN/g) in cloacal swabs before phage application compared to the control group (5.7 $\log_{10}$ MPN/g). A reducing effect of the phage cocktail was indicated by the increasing distance of bacterial concentrations of the two groups during observation after phage application. 33 days post hatch, *Campylobacter* counts in colonic content were significantly reduced in the treatment group (5.7 $\log_{10}$ MPN/g) compared to the control group (7.0 $\log_{10}$ MPN/g), while there was no significant difference in cecal content. *Campylobacter* isolated from the cecum showed the largest proportion of isolates with reduced susceptibility to the phages (phage NCTC 12673: 23.6%; phage vB_CcM-LmqsCP1/1: 2.8%) compared to isolates from colonic content and cloacal swabs.
Conclusion: Combining phages of group II and III resulted in increased lytic activities *in vitro*. Further *in vivo* studies applying varying phage concentrations might be useful to increase the reduction of *Campylobacter* in broiler chickens.

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Microbial contamination in conventional electronic and manual water valve systems: installation parameters for improving drinking water quality
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Question: Water is our most important food. Drinking water is also perishable and can pose a high health risk if contaminated with pathogenic bacteria. The tap is the last link in the long chain from water supplier to consumer. Water taps have been perceived as a recognized reservoir for microorganisms and as a risk of contamination and infection [1]. In this work water samples from

different types of conventional water taps for private use were analyzed to identify parameters responsible for microbial contamination. The results will be used to design water installation systems with maximum water safety.
Methods: Four different types of water valves were installed along the same water inlet pipe. The inlet pipe was regularly flushed with fresh water to exclude possible contamination out of the valve of concern. At certain intervals water samples were taken over a period of six months and analyzed regarding their bacterial contamination (total viable count) and contamination with water pathogens – such as *Legionella pneumophilia* and *Pseudomonas aeruginosa*. All work was done according to the general requirements and guidance for microbiological examinations by culture (ÖNORM, ISO). Statistical tests (ANOVA – analysis of variance) were performed and significant differences between some of the different water installation systems could be found.
Results: No *Legionella pneumophilia* and *Pseudomonas aeruginosa* were found. The bacterial contamination in colony-forming unit per ml (CFU/ml) was within the range 0 – 1040 CFU/ml for all types of water installations. The size of the valve - correlating to a bigger volume of stagnant water within the system – seems to a have a great impact on the number of colony-forming units per ml, since we found maximum 120 CFU/ml in the smallest valve and 1040 CFU/ml in the biggest valve with a significant difference.
Conclusion: The results and decisive parameters of this work form the basis for the development of an innovative water valve construction respecting materials, valve size, intelligent action algorithms – such as automatic flushing - , which are designed to minimize or prevent the colonization and growth of bacteria and water pathogens and maintain the quality of our drinking water in a resource-efficient manner.

Ref: [1] Wingender, J., & Flemming, H. C. (2011). Biofilms in drinking water and their role as reservoir for pathogens. *International journal of hygiene and environmental health*, *214*(6), 417-423.

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Occurence of Shigatoxin producing *E. coli* in ready-to-bake dough and thermo-tolerance during baking

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Background::Shigatoxin producing *E. coli* (STEC) are a worldwide occurring cause of severe gastrointestinal disease. In the past years STEC are increasingly isolated from flour and flour-products. STEC outbreaks in North Amerika could be linked to flour and - more precisely - to raw or undercooked dough. In Germany, "ready-to-bake" and "ready-to-eat" cookie dough is sold more frequently in the last years. Here, federal food control laboratories were able to detect STEC in ready to bake cookie dough. This demonstrates that STEC can be found in this matrix in Europe too. Therefore, we aimed to determine the level of STEC contamination as well as the behavior of STEC in this matrix during baking.
Material/Methods: We analyzed ten different commercial available "ready-to-bake" cookie dough samples using standard methods (25 g) and a batch (N=21) of recalled "ready-to-bake" cookie dough with the MPN-technique using 333 g per sample. Moreover, we analyzed "at-home" baking procedures by using dough contaminated with STEC-strains of different origin.
Results: We were able to isolate two different STEC-strains from three (14%) samples of the recalled batch. These isolates were assigned to serotypes often isolated from different kinds of flour, namely O36:[H14] carrying *stx2g* and O154:[H31] with *stx1d*. Moreover, we were able to isolate different enteropathogenic *E. coli* (EPEC) strains from 14 (67%) samples of the recalled batch. The contamination level of STEC and EPEC within different samples of the recalled dough batch ranged from 0 to 0.36 MPN / 100 g and 0 to 0.92 MPN / 100 g, respectively. We further analyzed the behavior during "at-home" baking procedures. The O36:[H14] strain did not show overall increased thermo-tolerance in baking experiments (180°C or 356°F for 9 minutes) with artificially spiked dough in comparison to other STEC isolated from flour and non-flour matrices. Here, the initial STEC contamination of approx. 10^5 CFU per 10 g cookie was reduced below the limit of quantification ($10^{2.3}$ CFU by direct plating). Surprisingly, the O36:[H14] strain was sporadically detected after 9 minutes of baking and subsequent 11 minutes of cooling using cultural enrichment.
Discussion: Overall, this implies that the applied baking procedure is not able to completely inactivate STEC when highly contaminated dough is used. Thus, further characterization of the strains from dough and flour is needed to clarify transmission routes and countermeasures should be implemented to provide safety to the consumer. However, the low detection frequencies as well as low contamination levels of STEC in this matrix require a sampling and detection strategy to ensure reliable STEC- and EPEC-detection.

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Shigatoxin producing *E. coli* in pancake and waffle batter - "Dough not underestimate Pancakes"

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Background: Shigatoxin producing *E.coli* (STEC) are a worldwide occurring cause of severe gastrointestinal disease. Although animal foods represent the major vector for STEC, these bacteria have been increasingly isolated from flour and flour-products in Europe and north America. Outbreaks were traced back to flour and – more precisely – dough.
The information on the growth behaviour of STEC in dough is still scarce. So our aim is to examine liquid doughs, such as pancake or waffle batter made from commercially available premixes. An important focus is the improvement of methods for re-isolation and the behaviour of STEC in the matrix as well as during baking.
Material/Methods: Liquid dough made from "shake and bake"-pancake-premixes were spiked with different *E. coli* reference/surrogate strains using "wet" as well as "dry" inoculation technique. For the "wet"-inoculation, the bacteria were resuspended in phosphate-buffered saline (PBS) *and then mixed with the dough directly. In addition, inoculated and dried sand was used as a carrier for the "dry"-inoculation technique. Here, the sand was added to the premix during dough-preparation.*
Results: Preliminary results showed that the method of inoculation has a massive influence on the re-isolation of the bacteria. The "dry" spiking method leads to reduced cultivation of *E. coli* from the liquid dough matrix.
Discussion: Further and ongoing investigations will shed light on the growth behaviour and survival rate of STEC in liquid dough and determine whether common "home-baking" practices are sufficient to inactivate STEC or pathogenic *E. coli* in contaminated pancake and waffle dough.

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Occurence of enteropathogenic and Shigatoxin-producing *Escherichia coli* in dried small fish from local markets in Kenya

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Omena (*Rastrineobola argentea*) landed from Lake Victoria is a widely consumed small fish in Kenya and is usually sun-dried to increase shelf life. Traditionally, the process of sun drying is performed by spreading fish directly on the ground or on old fishing nets laid on the ground. During and after processing these small fish might be contaminated with bacteria. Most bacteria do

not cause illness, but some are pathogenic such as Shigatoxin-producing and enteropathogenic *E. coli* (STEC and EPEC), which can lead to severe gastro intestinal illness of consumers.
In this case study, Omena was sourced from eight Kenyan markets in Kisumu and Nairobi cities, and Bondo, Mbita, Busia, Eldoret, Kakamega and Nakuru towns, all with varying distance from Lake Victoria. At each market, Omena samples were purchased from five different small fish vending stalls and kept chilled until analysis. After rehydration of sample aliquots (11g) in buffered peptone water, the samples were analysed for the presence of *E. coli* by direct plating. Furthermore, enrichment cultures were screened for the presence of Shigatoxin-Genes (*stx1* and *stx2*) as well as *eae*-Gene (for EPEC) by PCR. Positive samples were cultured to isolate the respective pathogenic *E. coli* for further characterisation. STEC and EPEC were not detected in samples from three markets which were Bondo, Kakamega and Nakuru. STEC was detected in a total of three samples one from Kisumu and two samples from Busia markets. Whereas, the three samples that tested positive for EPEC were from Mbita, Eldoret and Nairobi markets.
This study shows that pathogenic *E. coli* can occur in dried Omena from markets in Kenya, but further research on prevalence and origin of pathogenic *E. coli* in dried Omena need to be assessed to clearly depict the risk to consumers and imply countermeasures.

Postersession 02
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Infection Immunology (FG II)

198/IIP
Using a culture-independent serology-based approach (infection array) to screen pathogen-specific antibody signatures in sepsis patients – a proof of principle study
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Introduction: The wide spread of infectious diseases, together with an increasing global threat of antibiotic resistance, necessitates an accurate pathogen identification to achieve an effective therapeutic intervention. However, pathogen detection by conventional microbiological culture and sequence-based tests still show significant shortcomings regarding sensitivity, specificity, and clinical relevance. Since patients develop an antibody response during infection that is highly specific to the invasive pathogen, we developed a culture-independent serology-based approach ("infection array") as a high-throughput screening system based on the xMAP® technology for pathogen diagnostics in patients with microbial infections.
Methods: In our current proof of principle study, we used this infection array to simultaneously quantify the plasma antibody binding against different pathogens in patients with suspected sepsis. The patient cohort comprised a total of 76 patients (51 male and 25 female; age group: 29-90 years; median: 70 years) with sepsis (23%) or septic shock (77%). Complex extracellular protein extracts from the most common bacterial sepsis pathogens were coupled to magnetic beads (MagPlex® microspheres) and merged to a master mix that was used to measure changes in pathogen-specific plasma antibody levels over the course of sepsis. A total of 632 plasma samples from sepsis patients (N=596) and healthy individuals (N=36) were examined in seven dilution steps (Bio-Plex® 200). A standard serum was added to each plate to normalize plate differences. The data were evaluated using the xMAPr App.
Results: Our results indicate that changes in patients' antibody signatures are informative. Indeed, the infection array proved to be of high sensitivity in the studied patient cohort. Pathogen-specific antibody responses were found in 64/76 patients. In 62% of the cases (40/64), the observed antibody reactions were in concordance with the microbiological finding.
Conclusions: We propose that the infection array might provide a powerful tool for the explorative profiling of the plasma antibody dynamics during infection, helping to pinpoint infection-relevant species and hence guide antimicrobial therapy, and thus complementing the established diagnostic tools. Moreover, with the identification of immunogenic proteins of frequent sepsis pathogens, this system could be optimized to allow for higher specificity and reproducibility.

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Identification of disease-associated cryptococcal proteins reactive with serum IgG from cryptococcal meningitis patients
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Introduction: *Cryptococcus neoformans*, an opportunistic fungal pathogen ubiquitously present in the environment, causes cryptococcal meningitis (CM) mainly in immunocompromised patients, such as AIDS patients. We aimed to identify disease-associated cryptococcal protein antigens targeted by the human humoral immune response.
Methods: Therefore, we analyzed sera from Colombian CM patients, with or without HIV infection, and from healthy individuals living in the same region, as well as sera from *C. neoformans*-infected mice. To identify the proteins targeted by human anti-cryptococcal IgG antibodies, we applied a quantitative 2D immunoproteome approach identifying cryptococcal protein spots preferentially recognized by sera from CM patients or healthy individuals followed by mass spectrometry analysis.
Results: Serological analysis revealed increased titers of anti-cryptococcal IgG in HIV-negative CM patients, but not HIV-positive CM patients, compared to healthy controls. In contrast, titers of anti-cryptococcal IgM were not affected by CM. Furthermore, we detected pre-existing IgG and IgM antibodies even in sera from healthy individuals. The observed induction of anti-cryptococcal IgG but not IgM during CM was supported by analysis of sera from *C. neoformans*-infected mice. Stronger increase in IgG was found in wild type mice with high lung fungal burden compared to IL-4Rα-deficient mice showing low lung fungal burden. Using immunoproteomic analysis of human sera followed by recombinant expression, we identified twenty-three cryptococcal proteins to be immunoreactive with human sera. Fourteen of them were newly described as immunoreactive proteins. Twelve proteins were classified as disease-associated antigens, based on significantly stronger immunoreactivity with sera from CM patients compared to healthy individuals.
Discussion: The proteins identified in our screen significantly expand the pool of cryptococcal proteins with potential for (i) development of novel anti-cryptococcal agents based on implications in cryptococcal virulence or survival, or (ii) development of an anti-cryptococcal vaccine, as several candidates lack homology to human proteins and are localized extracellularly. Furthermore, this study defines pre-existing anti-cryptococcal immunoreactivity in healthy individuals at a molecular level, identifying target antigens recognized by sera from healthy control persons.

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Survival of *Klebsiella pneumoniae* in porcine blood: the role of the siderophore aerobactin

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Objectives: Hypervirulent *Klebsiella pneumoniae* strains cause infections with severe symptoms in humans. Serious complications of bacteremia are endophthalmitis and liver abscesses. In piglets, *K. pneumoniae* is also an emerging pathogen causing septicemia. This study investigates the genotypical and phenotypical characteristics of different human, porcine and other veterinary *K. pneumoniae* isolates. We hypothesize that the siderophore aerobactin promotes survival of *K. pneumoniae* in porcine blood.

Material and Methods: All isolates were screened for the genes encoding the four siderophores aerobactin, enterobactin, salmochelin and yersiniabactin and the virulence marker regulator of mucoid phenotype. Siderophore production was determined semiquantitatively by cultivation on chrome azurol S agar plates. Isolates with different siderophore genotypes were compared in survival in porcine blood. Growth curves in porcine serum supplemented with different bacterial supernatants were determined to investigate a putative boost effect of siderophores.

Results: In contrast to a hypervirulent human strain the investigated veterinary strains did not carry the *rmpA* gene, nevertheless the invasive porcine strains were positive for the aerobactin gene *iucA* and proliferated in blood of 4-week-old piglets. Non-invasive *iucA*- strains were killed or showed limited survival. Cultivation on chrome azurol S agar revealed differences in siderophore generation among veterinary *K. pneumoniae* isolates. The addition of supernatants of *iucA*+ strains to porcine serum resulted in a boosted bacterial growth in the exponential phase.

Discussion: Our results suggest that aerobactin generation is important for survival of *K. pneumoniae* in porcine blood and that *iucA* is associated with invasive porcine isolates.

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High Na^+ environments block phagocyte oxidase-dependent antimicrobial activity of neutrophils

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Infection and inflammation can augment local Na^+ abundance. These increases in local Na^+ levels boost proinflammatory and antimicrobial macrophage activity and can favor a polarization of T cells to a proinflammatory Th17 phenotype. Although neutrophils play an important role in fighting intruding invaders, the impact of increased Na^+ on the antimicrobial activity of neutrophils is less clear.

Here we show that increases in Na^+ impair the ability of human and murine neutrophils to fight *Escherichia coli* and *Staphylococcus aureus*. This was paralleled by reduced cellular mobility, diminished degranulation and impaired phagocyte oxidase-dependent reactive oxygen species (ROS) production in neutrophils. High salt boosted interleukin (IL)-8 release and enhanced the activity of the osmoprotective p38 mitogen-activated protein kinase (p38/MAPK) in neutrophils, while leaving their viability unchanged.

Whereas inhibition of p38/MAPK did not result in improved neutrophil defense, pharmacological blockade of phagocyte oxidase (PHOX) or usage of PHOX-deficient neutrophils mimicked the impaired antimicrobial activity detected under high salt conditions. Stimulation of neutrophils with phorbol-12-myristate-13-acetate (PMA) overcame high salt-induced impairment in ROS production and restored antimicrobial activity of neutrophils. From these experiments, we conclude that high salt-impaired PHOX activity results in diminished antimicrobial activity.

Our findings suggest that increases in local Na^+ represent an ionic checkpoint that prevents excessive ROS production of neutrophils, which can decrease their antimicrobial potential but at the same time could reduce ROS-mediated collateral tissue damage and thereby foster the resolution of inflammation.

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Immunisation with TLR2-triggering lipidated pneumococcal antigens enhances and skews antibody responses and leads to reduced colonisation

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Introduction: Pneumococcal vaccines have important limitations, including restricted serotype coverage facilitating replacement by non-vaccine serotypes and high manufacturing costs. Therefore, one direction of research is engaging in the development of a serotype-independent and protein-based vaccine. Recently, we have shown that immunization with the non-lipidated pneumococcal lipoproteins DacB and PnrA leads to reduced pneumococcal colonization. In this study, we have investigated the effects of lipidation and vaccination route (intranasal or subcutaneous) on the level of protection and humoral as well as cellular immune responses.

Material/Method: In a mouse model of colonization, we compared the immunogenicity and protectivity of lipidated versus non-lipidated DacB or PnrA with and without additional adjuvant depending on the immunization route. Antigen-specific systemic IgG and IgG subclass levels in antisera were determined by ELISA. Cytokine profiles after intranasal pneumococcal challenge in the Nasal-associated lymphoid tissue (local) and in supernatants after spleen cell stimulation (systemic) were analyzed by flow cytometry. Triggering of TLR2 by lipidated proteins was shown by HEK-Blue™ hTLR2 cell reporter assays.

Results: Immunization of mice with TLR2-engaging lipidated proteins induced increased IgG levels independent of the application of additional adjuvant. Humoral immune responses were characterized by a Th1-skewed IgG phenotype indicated by elevated levels of IgG2 compared to non-lipidated proteins inducing an IgG1-dominated profile. In addition, mice immunized with the lipidated proteins showed reduced bacterial loads in the nasal cavity compared to the non-lipidated proteins. However, no clear cytokine profile associated with protection by lipidated proteins could be determined.

Conclusion: In principle, lipoproteins are interesting candidates for future vaccine strategies as they are conserved, abundant, and immunogenic. We showed the potential of lipidated proteins DacB and PnrA to induce protection against pneumococcal colonization. Therefore, protein lipidation might represent an attractive approach for the development of novel pneumococcal vaccines.

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C-type lectin receptors in human myeloid cells – regulation by Interleukin-4 and response to mycobacteria

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It is estimated that one third of earth"s population is latently infected with Mycobacterium (M.) tuberculosis. Epidemiological studies suggest that co-infection with helminths is associated with a more severe course of tuberculosis. Characteristic for helminth infections are high levels of the cytokine IL-4.
C-type lectin receptors (CLRs) are a family of immune receptors expressed in myeloid cells. The CLRs DECTIN-1 and the DECTIN-2 family (MINCLE, MCL, and DECTIN-2) bind ligands in the mycobacterial cell wall. To defend against tuberculosis, a type 1 immune response with the induction of pro-inflammatory cytokines is required. On the other hand, helminth infections and the expression of IL-4 are associated with type 2 immunity. We recently showed a downregulation of the DECTIN-2 family CLRs in murine APCs by IL-4. (1)
Here, we investigated the expression of DECTIN-1 and DECTIN2 family CLRs in human myeloid cells with the focus on the influence of IL-4. We isolated CD14+ monocytes from PBMCs by magnetic cell separation. Analysis of CLR mRNA and protein levels was done with qRT-PCR and FACS, resp.; cytokine expression was detected by qRT-PCR and ELISA.
We observed a dose-dependent downregulation of MINCLE, MCL and DECTIN-2 mRNA levels by IL-4 in human monocytes and GM-CSF-differentiated macrophages that was increasing over time. IL-4 also reduced DECTIN-2 family CLR mRNA in GM-CSF macrophages after stimulation with mycobacterial ligands. Flow cytometry revealed that IL-4 decreased the cell surface protein levels of MCL after 5 days of differentiation with M-CSF and GM-CSF, whereas MINCLE and DECTIN-2 were not significantly reduced. In contrast to its effect on DECTIN-2 family CLR, IL-4 did not inhibit DECTIN-1 protein levels in GM-CSF differentiated cells and upregulated it in M-CSF differentiated cells. We also compared the CLR levels in the cell membrane of granulocytes and monocytes. We could see significantly lower DECTIN-1 levels in granulocytes compared to monocytes. There was no significant difference in cell surface protein levels of the DECTIN-2 family CLRs.
We could show that IL-4 differentially regulates the expression of DECTIN-1 and the DECTIN-2 family. It will be interesting to analyze CLR expression in monocytes from patients with helminth infection to determine whether type 2 immunity impairs DECTIN-2 family expression in vivo.

1. Hupfer, T., J. Schick, K. Jozefowski, D. Voehringer, J. Ostrop, and R. Lang. 2016. Stat6-Dependent Inhibition of Mincle Expression in Mouse and Human Antigen-Presenting Cells by the Th2 Cytokine IL-4. Frontiers in immunology 7: 423.

205/IIP

Immunogenicity and protective efficacy of a *Streptococcus suis* vaccine composed of six conserved immunogens

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Introduction: The zoonotic agent *Streptococcus (S.) suis* is a major pathogen in pigs causing severe diseases worldwide. A vaccine protecting against different *S. suis* serotypes is highly needed in porcine practice to improve animal welfare and reduce the use of antibiotics. Although different vaccination approaches are under investigation, data demonstrating protection against different serotypes in pigs are very limited and no licenced vaccine is currently available in Europe. We hypothesized that immunogens prominently recognized by convalescence sera but significantly less so by sera of susceptible piglets are putative protective antigens. Accordingly, we investigated immunogenicity and protective efficacy of a multicomponent vaccine including six main conserved immunogens, namely SSU0934, SSU1869, SSU0757, SSU1950, SSU1664 and SSU0187.
Material and Methods: The recombinant vaccine was designed on the basis of quantitative immunoproteomics using a large biobank of sera from various experimental infections with different *S. suis* strains or from bacterin vaccination. Flow cytometry confirmed surface expression of the selected immunogens in three *S. suis* serotypes, frequently isolated from clinical cases. Weaning piglets were prime-booster vaccinated and infected intranasally with a *S. suis* serotype 14 strain. A bactericidal assay was conducted before challenge to determine putative killing of different *S. suis* serotypes in blood of vaccinated piglets. *S. suis*-induced oxidative burst in granulocytes was assessed as additional read out parameter by flow cytometry.
Results: Although prime-booster vaccination resulted in significantly higher IgG titers against all six immunogens compared to the placebo-treated group, no significant differences in bacterial survival in blood from either vaccinated or control animals were recorded. Furthermore, vaccinated piglets were not protected against morbidity elicited through intranasal *S. suis* serotype 14 challenge.
Induction of reactive oxygen species (ROS) in blood granulocytes was not associated with vaccination but correlated with protection as all piglets with >5% ROS survived the challenge. Further investigations suggest that antibodies elicited through natural infection with a serotype 2 strain present in the original herd might have been important for the outcome of the challenge experiment due to cross-reaction with serotype 14.
Discussion: Based on our findings we discuss that main immunogens of *S. suis* might actually not be a priori good candidates for protective antigens. On the contrary, expression of immunogens that evoke antibodies which do not mediate killing of this pathogen, might constitute an evolutionary advantage conserved in many different *S. suis* strains.

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Humoral and cellular immune response after vaccination against SARS-CoV-2 virus in hematopoietic stem cell transplantation recipients

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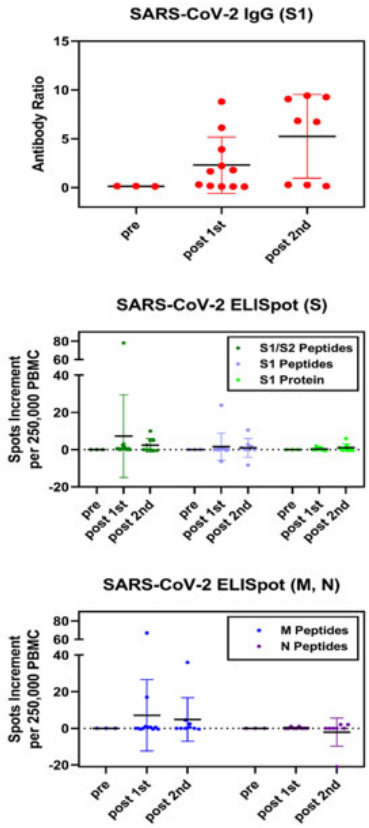
The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is causing a global pandemic, and cases continue to rise. Infection causes the corona virus disease 2019 (COVID-19), which ranges in presentation from asymptomatic to fatal. Recent reports suggested that patients with cancer, especially with hematological disorders such as leukemia, lymphoma and autologous or allogeneic hematopoietic stem cell transplantation (HSCT) had an increased risk of contracting SARS-CoV-2 than the general population, and infected, also had a higher risk of severe disease course (intensive care unit admission, invasive ventilation, or death). HSCT recipients have a high risk of developing viral infection, which reduces the quality of life after HSCT. Although SARS-CoV-2 vaccination may prevent infection, the optimal prophylaxis of COVID-19 in the HSCT setting is complex and has not been established. In the current study, HSCT recipients were vaccinated against SARS-CoV-2 using an mRNA vaccine based on mRNA molecules encoding SARS-CoV-2 spike (S) glycoprotein and vector-based vaccine carrying the full-length structural surface glycoprotein (spikes protein) of SARS-CoV-2, with a tissue plasminogen activator leader sequence. Vaccine-induced responses against spike glycoprotein antibody were assessed by enzyme-linked immunosorbent assay (ELISA). Cellular immunity against SRAS-CoV-2 was analyzed by the ELISpot method. In recipients (n=27) with SARS-CoV-2 vaccination the SARS-CoV-2 IgG antibody apparently increased (mean antibody ratio pre-vaccine 0.13, post first vaccination 2.3 and post second vaccination 5.3. In the cellular dataset, there was a slightly increased

SARS-CoV-2-specific response (spots increment, data represent median interferon gamma spots per 250,000 PBMC) after vaccination. These preliminary data indicate that vaccination with approved mRNA-based COVID-19 vaccine or vector-based COVID-19 vaccine augmented humoral and cellular immunity in stem cell transplant recipients. Follow-up examinations have to show whether this immunity is also protective.

Fig.1:



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Fungal Enolase 1 modulates responses of human naïve and memory T cells

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Introduction: *Candida albicans* and *Aspergillus fumigatus* control human immune responses by secreting immune evasion proteins like Enolase 1(Eno1) which is a moonlighting protein binding several human plasma proteins. Moreover, memory T cells with specificity for Eno1 of *A. fumigatus* (Aspf22) can be readily detected in the peripheral blood of healthy donors.

Material/Method: We used bioinformatical methods to predict and Biacore measurements to confirm predicted protein-protein interactions. Total PBMC and naïve CD4+ T cells were isolated from healthy blood donors. Proliferation of CD4+ T cells was determined by measuring CFSE dilution. Cytokine concentrations were measured by multiplex assay.

Results: Using bioinformatical methods, we predicted the interaction of Eno1 with CD4. This *in silico* prediction was confirmed in a Biacore assay where recombinant Eno1 from *A. fumigatus* bound to the extracellular domain of human CD4. To assess the relevance of this interaction for infection biology, we first investigated the impact of Eno1 from either *C. albicans* or *A. fumigatus* (73% aa homology) on cytokine secretion by purified naïve human CD4+ T cells stimulated with anti-CD3/anti-CD28 mAb-coated Dynabeads. Here, we focused on secretion of Th1 versus Th2 cytokines promoting versus hampering, respectively, antifungal immunity. Concentrations of IL-17 were below the limit of detection. *C. albicans* Eno1 induced a Th2-biased response for the majority of healthy human blood donors (n=8). For Eno1 from *A. fumigatus* we noted a Th1 or Th2 bias in 50% of donors each. We speculate that differences in the aa sequences of both Enolases are responsible for their differential biological effects. Second, memory CD4+ T cells from the majority of healthy donors responded to fungal Eno1 used as a recall antigen. In samples of donors lacking Eno1-specific memory CD4+ T cells, Eno1 blocked memory responses to other recall antigens like PPD or Td.

Discussion: Our data suggest that Eno1 is an immunomodulator of both naïve and memory CD4+ T cell responses. This study was funded by the DFG (SFB-TR124-C6) and the DAAD.

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Diversity of cell death signaling pathways in macrophages upon infection with Modified Vaccinia Virus Ankara (MVA)

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Regulated cell death frequently occurs upon infection by intracellular pathogens, and extent and regulation is often cell-type specific. We aimed to identify the cell death signaling pathways triggered in macrophages by infection with Modified Vaccinia Virus Ankara (MVA), an attenuated strain of vaccinia virus used in vaccination. While most target cells seem to be protected by anti-apoptotic proteins encoded in the MVA genome, macrophages die when infected with MVA. We targeted key signaling components of specific cell death pathways and pattern recognition pathways using genome editing and small molecule inhibitors in an in vitro murine macrophage differentiation model. Upon infection with MVA, we observed activation of mitochondrial and death-receptor-induced apoptosis pathways as well as the necroptosis pathway. Inhibition of individual pathways had little protective effect but led to compensatory death through the other pathways. In the absence of mitochondrial apoptosis, autocrine/paracrine TNF mediated apoptosis and, in the absence of caspase-activity, necroptosis. TNF induction depended on the signaling molecule STING, and MAVS and ZBP1 contributed to MVA-induced apoptosis. The mode of cell death had a substantial impact on the cytokine response of infected cells, indicating that the immunogenicity of a virus may depend not only on its PAMPs but also on its ability to modulate individual modalities of cell death. These findings provide insights into the diversity of cell death pathways that an infection can trigger in professional immune cells and advance our understanding of the intracellular mechanisms that govern the immune response to a virus.

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Monocyte progenitors give rise to multinucleated giant cells

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Introduction: Mycobacterial infections result in the formation of specialized immune cell clusters, so called granulomas. Multinucleated giant cells (MGC), large phagocytes with several nuclei, are a hallmark of the granuloma. The aim of the study was to reveal the so far undefined origin of MGC and further dissect their role in mycobacterial infections.

Methpods: Monocyte progenitors were isolated from murine bone marrow by magnetic purification and fluorescence-activated cell sorting. We analyzed the *in vitro* capacity of different progenitor cells to form MGC, produce cytokines and proliferate upon

exposure to mycobacteria. Additionally, the metabolic changes, associated with MGC formation, were evaluated. The relevance of progenitor cells in mycobacterial infections *in vivo* was demonstrated with the help of adoptive cell transfers.
Results: We found common monocyte progenitors (cMoP) to have the highest potential to form MGC. Additionally, a significant cytokine production of TNF and nitric oxide, both relevant in mycobacterial infections, was observed in cMoP. A transcriptome analysis pointed towards an increased biosynthesis of cholesterol and fatty acids in cMoP, undergoing transformation into MGC. Corresponding to this, the withdrawal of cholesterol and inhibition of the fatty acid synthase led to a distinct reduction in MGC formation. Thus, an increased lipid metabolism qualified as necessary prerequisite for the MGC transformation program.
Analyzing the role of monocyte precursors in mycobacterial infections *in vivo*, we identified a new circulating cMoP descendant, termed induced monocyte progenitor (iMoP). This c-kit^{low} CD115^{high} precursor not only showed an increased frequency in bone marrow and blood in *Mycobacterial tuberculosis* (*M.tb*) lung infection but also proved as suitable MGC progenitor *in vitro*. An intratracheal cell transfer of cMoP and iMoP into *M.tb*-infected IL-13^{tg} mice, which are susceptible to mycobacteria and show formation of mature granulomas, revealed progenitor-derived MGC formation *in vivo*.
Concluions: In conclusion, we were able to ascribe an effector function to specific monocyte progenitor cells in mycobacterial infections. Our data led to the hypothesis that cMoP give rise to circulating iMoP which then function as MGC progenitors at the site of infection. The characterization of the MGC origin and the identification of essential preconditions for their emergence, e.g. the accumulation of lipids, contribute to the understanding of their role for mycobacterial infections, which potentially opens up new opportunities for disease modulation in the future.

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Persist to resist: Characterization of persistent Klebsiella ssp in the gut microbiome and in blood of sepsis patients
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Klebsiella spp. are commonly found in the human gut microbiome but are also well known for causing nosocomial infections posing a threat to already hospitalized patients. In sepsis patients, antibiotic treatment affects negatively the diversity and viability of the gut microbiomes, paving the way for opportunistic pathogenic bacteria such as *Klebsiella.* This misbalanced state of the microbiome is in dysbiosis. A persistent *Klebsiella grimontii* strain was isolated from the feces of an sepsis patient. This particular strain was sequenced and genotyped with whole genome sequencing and compared to all *Klebsiella* the years 2017 and 2019 on the ICU. Phenotypical susceptibility and antibiotic resistance genes were compared. The phylogenetic analysis provided evidence that a nonosomical outbreak within the ICU was unlikely. Phylogenetic analysis with reference genomes for all *Klebsiella* was done. It was based on SNPs (kSNP) and average nucleotide identity (ANI) as reference independent, alignment-free methods taking small-scale and large-scale differences within the genomes into account. With affinity propagation clustering (APC) bacterial clusters could be assigned and enabled us to generate hypotheses about epidemiologic relationships between within the *Klebsiella* genus. Our data can increase our understanding of the pathogenesis and transmission of *Klebsiella* and may encourage to determine phylogenetic relationships with affinity propagation clustering.
Bühler, Sarah, Jürgen Rödel, Bettina Löffler, Michael Bauer, and Anne Busch. "Draft Genome Sequence of Persistent Klebsiella Grimontii At013-Mero-001, Isolated from Human Feces."

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https://mra.asm.org/content/ga/10/16/e00054-21.full.pdf.

211/IIP
GMCSF synergizes with LPS for IL-1β secretion by suppressing the Nrf2 antioxidant response
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Inflammasomes are multiprotein complexes that act as scaffolds for the activation of the proinflammatory cytokines IL-1β and IL-18 as well as pyroptosis, a form of regulated cell death. Interestingly, the NLRP3 inflammasome responds to a wide array of stimuli, but the actual activation mechanism is still unknown. NLRP3 activation is a two-step process: a first signal called "priming" is required for expression of pro-IL-1β and NLRP3. Then a second "activation signal" triggers the inflammasome complex formation and rapid cytokine secretion through pyroptotic pores. It is believed that NLRP3 senses a common event triggered by the second signals, possibly being K^+ efflux, Ca^{2+} influx and/or ROS production. There are however several reports showing that NLRP3 can be activated without a second classical stimulus, where a priming signal such as LPS treatment is enough to trigger a slower and prolonged IL-1β secretion in absence of cell death, amplifying the local and systemic inflammatory effects. GM-CSF treatment is one such scenario, whereby GM-CSF synergize with LPS to promote NLRP3 dependent IL-1 β secretion. Moreover, GM-CSF is expressed at high levels at sites of inflammation, as in synovial fluid from patients with rheumatoid arthritis (RA) and anti GM-CSF treatment is in clinical trial for RA and multiple sclerosis. We investigated the mechanism behind IL-1β secretion upon GM-CSF treatment. Considering the well-recognized link between oxidative stress and chronic inflammation, we looked for redox imbalance in LPS or LPS+GM-CSF treated macrophages. We show that GM-CSF synergizes with LPS for IL-1β-secretion and that it is largely blocked by addition of the ROS scavangers, MitoTEMPO and NAC, suggesting that ROS are indeed required. We further observed higher ROS production in wt macrophages comparing to Tnf$^{-/-}$ and Ripk3$^{-/-}$ cells upon LPS treatment, which correlated with loss of IL-1β secretion in the knockouts. However, GM-CSF addition had no detectable effect on ROS levels. LPS induces a biphasic upregulation of Nrf2, a master regulator of antioxidant responses, that was largely blocked or delayed by GM-CSF treatment. Loss of NRF2 upregulation correlates perfectly with the appearance of IL-1β secretion. We concluded that GM-CSF treated cells lack the capacity to inactivate the ROS that are produced though suppression of NRF2 expression, thus promoting inflammatory signaling and IL-1β secretion. In conclusion, GM-CSF represents a natural trigger for NLRP3 activation during inflammation and can contribute to the establishment of inflammation and inflammatory diseases.

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Acetate sensing by GPR43 alarms neutrophils and protects from severe sepsis
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Abstract: Bacterial sepsis is a major cause of mortality resulting from inadequate immune responses to systemic infection. Effective immunomodulatory approaches are urgently needed but it has remained elusive, which targets might be suitable for intervention. Increased expression of the G-protein coupled receptor GPR43, which is known to govern intestinal responses to acetate and other short-chain fatty acids, has been associated with sepsis patient survival. However, the mechanisms behind this observation have remained unclear. In our study, we could show that elevated acetate concentrations prime and alert neutrophils in a GPR43-dependent fashion, resulting in enhanced neutrophil chemotaxis, oxidative

burst, cytokine release and upregulation of phagocytic receptors. As a consequence, acetate priming improved the capacity of human neutrophils to eliminate methicillin-resistant Staphylococcus aureus (MRSA), which was abrogated by GPR43 inhibition. In mouse models, intra-peritoneal acetate injection transiently increased mouse serum acetate concentrations and primed blood neutrophils. Notably, it rescued wild-type mice from severe S. aureus sepsis and reduced bacterial numbers in peripheral organs by several magnitudes. Acetate treatment improved the sepsis course even when applied several hours after onset of the infection. However, in GPR43-/- mice the susceptibility to S. aureus sepsis was not influenced by acetate treatment. Thus, GPR43 could be a potential target for sepsis therapy. Our study indicates that the severity of sepsis depends critically on transient neutrophil priming by appropriate blood acetate concentrations. Preventive or therapeutic interventions based on GPR43 stimulation could become valuable strategies for reducing sepsis-associated morbidity and mortality.

Microbiota, Probiota and Host (FG PW)

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Whole genome sequencing based classification of clinical *Haemophilus spp* isolates points towards a pathogenic role of *H. haemolyticus* in mouth abscesses and lower respiratory tract infections

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Introduction: Bacteria belonging to the genus *Haemophilus* cause a wide range of diseases in humans. Of particular relevance is *H. influenzae,* which is classified by the WHO as priority pathogen due to the wide spread of ampicillin resistant strains. However, current diagnostics (e.g. mass spectrometry and PCR assays) often misclassify other *Haemophilus* spp. as *H. influenzae*, which impedes the assessment of pathogenicity, biases surveillance studies and affects therapeutic decisions. To tackle this, we developed the first whole genome sequencing (WGS) based identification algorithm tailored towards classification of all human-related *Haemophilus* species.
Materials/Methods: A gene presence/absence-based classification algorithm was developed which works directly from raw WGS reads. Our algorithm employs the open-source gene-detection tool SRST2 and a novel classification database comprising 32 marker genes. These markers were identified in a comparative genome analysis of 211 *Haemophilus* spp. strains, and evaluated among 1,186 publically available WGS datasets of diverse *Haemophilus* spp strains. Lastly, we re-classifed 262 clinical isolates from a German cohort, which were identified as *H. influenzae* by mass spectrometry between 2008 and 2013.
Results: The newly established algorithm is able to classify and discriminate between all relevant human-related *Haemophilus spp.* including *H. influenzae*, *H. haemolyticus*, *H. parainfluenzae*, *H. parahaemolyticus*, *H. sputorum*, *H. pittmaniae* and *H. ducreyi*. We could further identify putative haemin-independent *H. haemolyticus* strains and determine the serotype of typeable *H. influenzae* strains. In the German cohort, 83 out of 262 (31.7%) presumptive *H. influenzae* isolates were in fact *H. haemolyticus*, part of which associated with mouth abscesses and lower respiratory tract infections (LRT).
Discussion: Our novel classification database and algorithm has the potential to improve diagnosis and surveillance of *Haemophilus* spp. The re-classification of historical samples further points towards a potential pathogenic role of *H. haemolyticus* strains in lower respiratory tract infection and mouth abscesses, which needs to be further investigated.

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Microbial community shifts and reduction of antibiotic resistance genes over thermophilic composting of human faeces

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In times of tremendous global risks caused by the world-wide climate change, the use of sustainable agricultural practices is of primordial importance. In this context, we have been investigating the effect of 140- and 154-days thermophilic composting on the hygienisation of organic waste (consisting of human faeces and saw dust from dry toilets together with straw and green cuttings).
Compost samples from start and end of the thermophilic composting process were analyzed with regard to the bacterial community composition using 16S rRNA gene amplicon sequencing. The results show a decline from start to end of composting in terms of the two most abundant phyla *Proteobacteria* (start: 36-48 %, end: 27-30 %) and *Firmicutes* (start: 13-33 %, end: 12-16 %), whereas the abundance of *Chloroflexi*, *Gemmatimonadetes* and *Planctomycetes* rises. *Actinobacteria* is the second largest phylum in the mature compost due to reduction in the abundance of *Firmicutes*. We found a shift in the bacterial community over the course of composting with decreases in groups containing many human pathogenic bacteria, like *Pseudomonadales* (start: 5-16 %, end: 1 %) including *Pseudomonas* spp. and *Acinetobacter* spp., as well as *Bacilli* (start: 8-29 %, end: 9-12 %), or *Staphylococcaceae* and *Enterococcaceae*.
The fate of antibiotic resistance genes during the composting process was assessed by gene-specific PCR. The number of antibiotic resistance genes declined from 15 genes detected in the starting material to eight in the mature compost. Four tetracycline resistance genes (*tetB*, *tetK, tetM, tetS*), two kanamycin resistance genes (*aad*pSK41*, aph3-III*), as well as the erythromycin resistance gene *ermB,* were no longer detectable in the mature compost.
The reduction in abundance of antibiotic resistance genes indicates that thermophilic composting can help control the spread of antibiotic resistances. Along with the decline in abundance of bacterial groups comprising potential human pathogens, our results suggest that composting is a promising way to re-use human faeces in agriculture.

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Computational analysis of the gut microbiome of horses subjected to surgery revealed accumulation of bacterial resistance-associated genes during hospitalisation

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Introduction: Antimicrobial resistance is an emerging global One Health issue, affecting both human and veterinary medicine. Previous research revealed that clinics providing health care for companion animals, such as horses, are indeed "hot spots" in respect to local spread of multidrug resistant (MDR) and zoonotic bacteria. Horses receiving gentamicin/penicillin (GP) as a perioperative antibiotic prophylaxis (PAP) were frequently

colonized with MDR pathogens. Since the duration of antibiotic therapy influences the recovery time of the enteral microbiome, changes induced by two distinct GP-PAP regimens were comparatively investigated within this pilot study, particularly in regards to the emergence of genes conferring resistance to biocides and antibiotics. The results will be utilized to promote further studies on the effects of targeted stewardship programs designed to enhance the prudent use of antibiotics.

Materials and Methods: Hospitalized horses subjected to colic surgery received GP-PAP, either as a single dosage (SSG) regimen or across 5 consecutive days (5DG). Fecal samples were collected on day 0 (hospital admission), 3 and 10 (post-surgery). In total, sample sets of 12 horses (n = 36) were metagenome shotgun sequenced and computationally analysed using a robust workflow. The resistome, including genes conferring resistance to biocides and antibiotics (ARGs), was characterized and tested for statistical correlation with the study group and length of hospital stay.

Results: The results display unique gut metagenomes associated with each of the equine patients, including considerable variation between individuals at multiple taxonomic levels. Beyond the impact of hospital stay and surgery, GP-PAP caused gut metagenome perturbations which resulted in an average decrease of the α-diversity on day 3 (5DG = -2.06, SSG = -1.66) followed by an ongoing recovery process at day 10 (5DG = +2.09, SSG = +0.99). Resistome analysis further revealed a strong increase in normalized ARG abundance, particularly for the 5DG, at day 3 (5DG = +4.6 fold change (FC), SSG = +3.6 FC) and subsequent decrease on day 10 (5DG = -6.8 FC, SSG = -1.3 FC). ARG accumulation was found to be statistically significant for day 3 of the 5DG ($p = 0.032$). Furthermore, we found resistance gene abundance and α-diversity to be negatively correlated (spearman, $r = -0.61$, $p = 9.9e-05$) across all 36 samples.

Discussion: Our preliminary results help to establish an understanding of the effect of antimicrobials on the equine microbiome. While the decrease in α-diversity across treatments was to be expected, we also describe a strong increase in ARGs, especially within the 5DG. While the beneficial effect of decreased selective pressure caused by the SSG treatment is plausible, additional analyses are required to further describe the influence of the different PAP regimens on the microbiome, particularly in regards to MDR bacteria.

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SARSCOV2seq – A modification of MTBseq for the end-to-end analysis of Next Generation Sequencing data of the severe acute respiratory syndrome coronavirus 2

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Introduction: With the onset of the COVID-19 pandemic in 2020, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the first complete sequence of the virus available, Whole Genome Sequencing (WGS) soon became a valuable method for global tracking of variants. The use of genome sequencing now has become a fundamental tool to trace emergence and origin, evolution and genetic diversity as well as transmission of the virus, facilitating early decision-making to control local transmission of SARS-CoV-2. Understanding the spatial distribution and transmission dynamics is important for intervention strategies. Here reliable, quality controlled analysis of WGS data is key. We modified our validated MTBseq pipeline for next generation sequencing (NGS) data analysis of *Mycobacterium tuberculosis* complex strains to a toolbox for SARS-CoV-2 analysis.

Materials and Methods: The established MTBseq pipeline was modified with free and public available tools like iVar and pangolin and supplemented with scripted functionalities in Perl, Bash and R to be used with the ARTICv3 primer set. Analysis results were validated with sequencing data from a synthetic RNA, inter-lab evaluation of data and ring trial participation.

Results: SARS-CoV-2 NGS data can be reliably analyzed with SARSCOV2seq from raw sequence data executing the steps of alignment, quality trimming, mutation detection, consensus generation and variant type determination providing various quality statistics for mapping, genome coverage and evaluation of amplicons. Findings are summarized in a per sample report including key quality control metrics, variant type and mutations, with special emphasis on the S-gene. SARSCOV2seq outputs were evaluated against online virus classification tools and more than 1000 SARS-CoV-2 NGS datasets monitoring the emergence as well as temporal and geospatial spread of B.1.1.7 in northern Germany were analyzed so far.

Conclusion: SARSCOV2seq is an end-to-end solution for the NGS analysis of SARS-CoV-2, which can be either used for the optimization and quality control of the laboratory workflow or in a setting for variant monitoring and reporting. As exemplified by our data it was used prospectively for inferring mutations and virus variant types of the first SARS-CoV-2 cases emerging in Northern Germany (Kreis Segeberg, https://covid-monitor.de/report/de).

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Challenges and pitfalls in NGS-based genome reconstruction of SARS-CoV-2 identified by covPipe

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Introduction: For an effective containment of SARS-CoV-2 (SC2), molecular surveillance programs based on whole-genome sequencing have proved to be essential. The key is to reconstruct the viral genome reliably and accurately but in a resource-efficient manner. At the same time, specifics of various enrichment protocols need to be considered to avoid common bioinformatics pitfalls. Here, we present covPipe, a bioinformatics workflow to run reference-based genome reconstruction of SC2 genomes based on amplicon sequencing data.

Material and Methods: Preprocessing is performed using Kraken2 for taxonomic filtering, fastp for quality control and trimming, and BamClipper for primer removal. Genome reconstruction consists of mapping high-quality SC2 reads with BWA-MEM and determining variants with freebayes. The consensus sequences are built based on a reference SC2 genome and the called variants. Continuous integration and testing allowed us to extensively evaluate covPipe and carefully adjust parameter settings to address the specific pitfalls of widely used amplicon protocols for Illumina sequencing. We evaluate the pipeline's performance based on a selection of different data sets comprising multiple enrichment protocols. CovPipe is implemented using the workflow management system Snakemake and is freely accessible from tinyurl.com/covpipe.

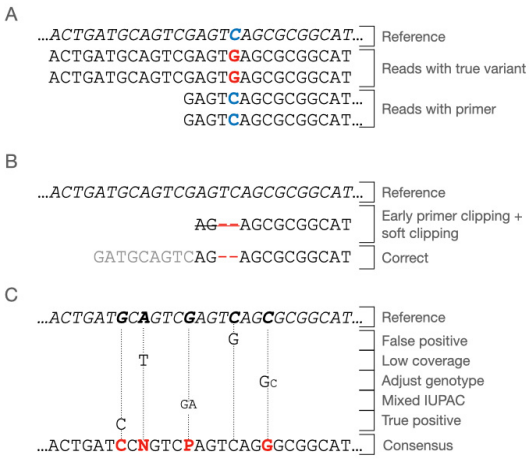
Results: We show how raw data quality control and taxonomic filtering facilitate downstream analyses. Figure 1 highlights cases of wrong consensus sequences that result from default strategies and are avoided by covPipe. For example, premature primer removal leads to amplicon-induced edge effects that can even lead to wrong lineage assignments. In addition, different variant callers and the choice of genotyping parameters influence downstream consensus reconstruction and need to be adjusted carefully (Fig. 1C). In the same context, accurate genome reconstruction can be compromised by incorrectly masked deletions and frameshifts. CovPipe's structured reporting supports both the data quality assessment and the decision-making processes of health authorities.

Discussion: CovPipe provides reference-based fully automated, flexible and reproducible SC2 genome sequence reconstruction. It is optimized for amplicon data but can also be applied to WGS data or data from hybrid capture enrichments. It is currently tested for other viruses. A wide user base and active community continuously help identify and resolve challenges with various enrichment kits for SC2 that would be hard to obtain in a single laboratory. Thus, we can continuously improve covPipe and adapt it to the needs of the users.

Figure 1. Genome reconstruction special cases. (A) Primer sequences mask true variants and need to be removed. (B) Early primer clipping may result in soft clipping and missed deletions. (C) Genotyping parameters need to be carefully set to reliably call

different variant fractions and to represent them in the final consensus.

Fig. 1



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Comparison of sorbitol-fermenting EHEC O157:H- and non sorbitol-fermenting EHEC O157:H7

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a major enteric pathogen capable of causing bloody diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome (HUS). It is linked to large outbreaks all over the world. A phenotypic feature of classic O157:H7 is the inability to rapidly ferment sorbitol, which is also used for diagnostic purposes. However, in addition to these non sorbitol-fermenting (NSF) O157 strains, a sorbitol-fermenting, nonmotile O157 variant (SF O157:H-) has emerged as an important pathogen. SF O157 infections have been linked with a higher rate of progression to HUS suggesting that this variant might be more virulent than classical NSF O157:H7 strains. SF O157 strains are nonmotile due to the lack of flagella production caused by a 12 bp deletion in the *flhC* gene, which together with *flhD* codes for the master 1 regulator of flagella synthesis. We are interested how this 12 bp-deletion affects gene transcription of virulence genes compared to strains with intact *flhC*. Other genetic differences of SF O157 strains leading to increased virulence potential have not been systematically analyzed and are of further interest in our study.
Material/Method: Curing of the 12 bp deletion in *flhC* of an SF O157:H- strain was done by transformation of functional *flhC* into its coding region. Swimming assays on soft agar were performed to analyze restoration of motility. Further, RT-PCR was used to determine gene transcription.
Long-read and short read genome sequencing of an SF O157:H- strain was performed. De novo genome assembly was accomplished and sequences were compared to the genome of a classical NSF O157:H7 strain.
Results: Curing of 12 bp deletion in *flhC* in the SF O157:H- strain did result in restored but delayed motility. The delay was due to the fact that a suppressor mutation was additionally required for motility. This mutation occurs in the spacer region between RNA polymerase and cAMP receptor protein binding sites, which changes the expression of *flhDC*. It was found, that SF O157:H- and NSF O157:H7 strains differ in this site additionally to the 12 bp deletion in the coding region of *flhC*. Motility restored strains showed the same genotype as NSF O157:H7 in these genomic regions.
De novo assembly of the sequenced SF O157:H- genome yielded two circular contigs – a chromosome of 5.5 Mb and a plasmid of 121 kb. Genome comparison showed a similar genome architecture, but a variety of genomic differences, for example in the location of prophages and mobile elements as well as further mutations leading to truncation or frame shifts in various genes, which are analyzed for their potential to increased virulence.
Discussion: The comparative analysis between the genomes and regions of flagellar genes of the here analyzed SF O157 isolate and classical NSF O157:H7 strains reveals common and differentiating features of the two variants, potentially enabling a better understanding of the underlying pathogenicity.

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The evolution and population structure of *Streptococcus parasanguinis*

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Introduction: *Streptococcus parasanguinis* is a commensal that colonizes the oral cavity, but also commonly found in the gastrointestinal tract of healthy adults. Although *S. parasanguinis* is not an established human pathogen, several studies have reported its association with native and prosthetic valve endocarditis. However, the lack of genomics-based studies of *S. parasanguinis* hamper understanding the evolutionary mechanisms underlying its within-host adaptation.
Objectives: We aimed to elucidate the population structure of *S. parasanguinis* and to determine the evolutionary pathways that allow strains of oral origin to adapt to the gut environment.
Materials and Methods: We combined mapping and pan-genome approaches on 98 genomes that were collected from various body sites and eight different countries between 1974 and 2019. Most of the genome sequences (n = 96) were retrieved from public database (NCBI), while the remaining two genomes were sequenced from strains collected 2018 from saliva and faecal samples of the same patient. Among these 98 genomes, the recombination events were detected using ClonalFrameML, while genes coding for antimicrobial resistance and virulence were detected using ABRicate.
Results: We detected 59,687 core-genome single-nucleotide polymorphisms (SNPs) and a high level of recombination events among the 98 *S. parasanguinis* genomes. The phylogenetic analysis revealed two distinct clades, clade 1 harboured 30 genomes, while clade 2 harboured the remaining 68 genomes. The genomes in clade 2 harboured novel genes, encoding for glycosyltransferases. The comparative genomics analysis revealed that the intestinal *S. parasanguinis* genomes harboured intact prophages, which were phylogenetically distinct from their oral counterparts. A large genomic element (63 genes) that encodes for the anaerobic cobalamin/vitamin B12 synthesis was detected mainly in *S. parasanguinis* genomes of intestinal and clinical origin suggesting adaptation to the anaerobic condition including the gastrointestinal tract. Another genomic fragment composes of 16 genes that encode for an accessory secretion system (aSEC) found in nine *S. parasanguinis* genomes forming a distinct phylogenetic clade.
Conclusion: Our results revealed that mobile genetic elements have played an important role in the adaptation of *S. parasanguinis* to the intestinal habitat.

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Distinct types of mycotoxin production in *Stachybotrys* (*S.*) *chartarum* based on genetic variation

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Introduction: *S. chartarum i*s a fungus frequently isolated from damp building materials or improperly stored animal forage. Human and animal exposure to the highly cytotoxic secondary metabolites (satratoxins) of this mold is linked to severe health effects (1-3). Due to either the production of satratoxins or atranones *S. chartarum* was subdivided into chemotypes A and S in the past (4). Based upon recent genetic information, three

genotypes of the fungus have been introduced: type A, which produces no satratoxins but atranones, has no satratoxin (SAT) genes but a complete set of atranone (ATR) genes; type H, which produces no satratoxins, contains a truncated cluster of SAT genes and all ATR genes; type S produces satratoxins, having the complete cluster of SAT genes but no ATR genes (5).
Materials/Methods: To characterize the satratoxin and atranone production in detail, we included four strains of *S. chartarum* for genome, transcriptome, and mycotoxin analysis. One of each genotype A and S as well as two genotype H strains were cultured under standardized conditions (potato dextrose agar, 25 °C, 0.89 aw, cultivation in the dark) and used for genome and RNAseq studies. To further confirm these results all strains were tested by mass spectrometry for their secondary metabolite profile after 21 days of culture.
Results: In contrast to the S type, no macrocyclic trichothecenes (e.g. satratoxins) were detectable by LC-MS/MS analysis for the A and H type strains (limit of detection 0.1 - 7.4 ng/g) (5). Both, the RNAseq and the genome analysis confirmed that the A strains harbor only the atranone and the S strains only the satratoxin gene clusters. The two H genotype strains analyzed lacked transcripts of the *sat11-16 genes,* which are part of the satratoxin cluster 2 (SC2) and showed significant differences in the transcription levels of the atranone genes.
Discussion: According to our results the three genotypes of *S. chartarum* show evolutionary differences in their genome, transcriptome and mycotoxin production. In addition, the genome analysis ratifies the lack of the atranone cluster in genotype S. The generated RNAseq data imply the absence of the SC2 gene cluster in both H genotypes, whereas the SC1 as well as the SC3 are still transcribed. *T*he H strain has lost parts of its SAT genes and thereby the ability to produce macrocyclic trichothecenes, but obtained the ATR genes. The LC-MS/MS analysis supports the idea of an antagonism between SC2 and the atranone cluster. Further research needs to be performed to gain more insight into the mycotoxin production of different *S. chartarum* strains.

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Exploring *Staphylococcus aureus* diversity, host adaptation and evolution by FTIR biotyping

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Introduction:
Staphylococcus aureus infections are determined by a variety of cell-envelope glycans, including the capsular polysaccharide (CP), lipo- and wall teichoic acids (LTA/WTA), glycosylated proteins, as well as poly-β(1-6)-N-acetylglucosamine (PNAG). They are known to influence bacterial interactions with the host in multiple aspects, but their adaptive capacity due to host-pathogen dynamics is still incomplete. *S. aureus* strain discrimination by Fourier-transform infrared (FTIR) spectroscopy is primarily based on the differential expression of CP and additional surface glycopolymers and turned out to be well suited to bacterial subtyping in epidemiological studies and outbreak investigations complementing current genome (WGS)-based strain typing. Here, we will provide insights into bovine *S. aureus* diversity, host adaptation and evolution using high-discriminatory FTIR spectroscopy exploring *S. aureus* glycosignatures beyond bacterial subtyping.
Methods: Whole-cell molecular fingerprinting by FTIR spectroscopy represents a user-friendly, rapid and reagent-free tool to follow *S. aureus* phenotypic responses caused by abiotic and biotic effects. We acquired FTIR spectra from bovine *S. aureus* isolates of different clonal complexes, geographic origins and followed bacterial spreading within a dairy herd and in-host evolution within individual cows. Machine learning algorithms were used to differentiate the strains based on their spectroscopic patterns.
Results: FTIR spectroscopy is suitable as a screening tool to explore bovine *S. aureus* glycosignatures leading to the definition of specific glycotypes. We determined herd-, within-herd, and within-host specific *S. aureus* glycosignatures. Furthermore, in combination with WGS, we could track the emergence of new isogenic variants implicating within-host evolution during chronic, bovine *S. aureus* mastitis.
Conclusion: Molecular fingerprinting by FTIR spectroscopy is a valuable tool for differentiating *S. aureus* strains based on their surface glycosignatures, which is particularly useful to monitor persistence, transmissibility, and host adaptive changes during infection. The observed heterogeneity of *S. aureus* glycotypes indicates the high adaptive capacity of bacterial glycopolymer expression during infection, which is relevant for host cell recognition and adhesion or immune evasion.

221/PWP

Bacterial colonisation dynamics and antibiotic resistance gene dissemination in the hospital environment after first patient occupancy: a longitudinal metagenetic study

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Background: Humans spend the bulk of their time in indoor environments. This space is shared with an indoor ecosystem of microorganisms, which are in continuous exchange with the human inhabitants. In the particular case of hospitals, the environmental microorganisms may influence patient recovery and outcome. An understanding of the bacterial community structure in the hospital environment is pivotal for the prevention of hospital-acquired infections and the dissemination of antibiotic resistance genes. In this study, we performed a longitudinal metagenetic approach in a newly opened ward at the Charité Hospital (Berlin) to characterize the dynamics of the bacterial colonization process in the hospital environment after first patient occupancy.
Results: The sequencing data showed a site-specific taxonomic succession, which led to stable community structures after only a few weeks. This data was further supported by network analysis and beta-diversity metrics. Furthermore, the fast colonization process was characterized by a significant increase of the bacterial biomass and its alpha-diversity. The compositional dynamics could be linked to the exchange with the patient microbiota. Over a time course of 30 weeks we did not detect a rise of pathogenic bacteria in the hospital environment, but a significant increase of antibiotic resistance determinants on the hospital floor.
Conclusions: The results presented in this study provide new insights into different aspects of the environmental microbiome in the clinical setting, and will help to adopt infection control strategies in hospitals and health care-related buildings.

222/PWP

Revisited role of *Helicobacter pylori cag*PAI, CagA, VacA and GGT in apoptosis during infection of gastric epithelial cells

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Introduction: *Helicobacter pylori* is a persistent gastric pathogen associated with a variety of gastric diseases ranging from peptic ulcers to stomach cancer. Various virulence factors of *H. pylori* have been identified over the years, some of which are supposed to promote the induction of apoptosis (e.g. vacuolating cytotoxin

VacA or γ-glutamyl transpeptidase GGT), while others display anti-apoptotic functions (e.g. effector protein CagA). Previous analyses gathered insights into the function of these proposed pro- and anti-apoptotic elements, however, many studies usually employed strategies such as transfection with DNA constructs expressing the factor of interest or treatment with purified proteins, which provided only limited information about how they could affect host cell death or survival in a complex scenario upon infection. Here we aimed to study the role of *H. pylori* virulence factors on apoptosis during infection of AGS gastric epithelial cells.

Material and Methods: We applied infection studies, genetic analyses, immunofluorescence microscopy and Western blotting.

Results: We generated single and double deletion mutants of the *H. pylori* wild-type strain P12, targeting genes with proposed pro-apoptotic effects (Δ*vacA*, Δ*ggt,* Δ*vacA/ggt*), anti-apoptotic effects (Δ*cagA*), or the *cag* type IV secretion system (Δ*cagPAI,* Δ*cagE*), which is required for the transport of effector molecules into the host cell. We infected AGS cells with these strains for 12 hours and determined the percentage of apoptotic cells by staining with AnnexinV conjugated with FITC and analyzing the cells under a fluorescence microscope.

Discussion: While the reduction of apoptotic cells in infections with strains lacking the pro-apoptotic factors VacA or GGT were expected, it came as a surprise that the *H. pylori* mutant lacking the anti-apoptotic factor CagA only showed a slight increase in apoptosis, compared to infections with wild-type bacteria. Interestingly, the Δ*cag*PAI and Δ*cagE* mutants exhibited a far greater rate of apoptosis than the Δ*cagA* mutant, which lead to the conclusion that the *cag*PAI itself, but not translocated CagA, is the primary driver of *H. pylori´*s anti-apoptotic properties during infection.

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Helicobacter pylori upregulate epithelial cortactin expression in a CagA- and JNK-dependent manner

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Introduction: *Helicobacter pylori* is a microbial pathogen, which can lead to various gastric disorders ranging from gastritis to gastric cancer. During infection, *H. pylori* disturbs various host cell signaling pathways, one of which may result in activation of the actin-binding protein cortactin. Cortactin is a major regulatory factor involved in the regulation of cellular cytoskeleton organization and cell movement. Moreover, cortactin has been repeatedly shown to be amplified and overexpressed in various human cancers. Here we aimed to study the overexpression of cortactin in gastric AGS and intestinal Caco-2 cancer cell lines during infection with *H. pylori*.

Material and Methods: We applied infection studies, genetic analyses, immunofluorescence microscopy and Western blotting.

Results: Cortactin protein amounts increased by 2-3 fold after 24-48 hours infection, as shown by Western blotting and immunofluorescence microscopy. Using a set of CagA-positive and CagA-negative strains as well as various isogenic mutants of *H. pylori*, we demonstrate that cortactin overexpression depends on the type IV secretion system, and in particular on the translocation of the major bacterial virulence factor CagA. Transfection experiments revealed that ectopic expression of CagA in AGS cells is sufficient to induce overexpression of cortactin. Interestingly, tyrosine phosphorylation of CagA was not required for this process since phospho-deficient CagA was capable of increasing cortactin expression. Finally, by using various kinase inhibitors, we found that the mitogen-activated protein kinase JNK (c-Jun N-terminal kinase) is essential for the pathway leading to cortactin overexpression.

Discussion: Taken together, we discovered a new CagA/JNK pathway inducing cortactin overexpression upon *H. pylori* infection, which in turn might contribute to the development of gastric disorders, including gastric cancer.

224/PWP

The pneumococcal pneumolysin destroys platelets and can be neutralised with pharmaceutical immunoglobulins

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Background: *Streptococcus pneumoniae* is a causative agent of severe diseases like meningitis, sepsis and community acquired pneumonia, which is often associated with an acute respiratory distress syndrome (ARDS). One of the main virulence factors of *S. pneumoniae* is pneumolysin (Ply), a cholesterol dependent pore forming cytolysin. Ply alters the endothelial barrier in the lung, leading to leakage of fluid into the interstitial pulmonary tissue. Platelets can seal those endothelial gaps to prevent endothelial leakage.

Earlier, Ply has been reported to activate platelets as indicated by high CD62P levels. In our study, we demonstrate that Ply induces high CD62P signals via destruction of the platelet membrane, which is accompanied by loss of platelet function. In addition, we investigated pharmaceutical IVIG as a potential treatment against the Ply induced platelet damage.

Methods: Platelets were isolated from healthy volunteers. The impact of Ply on activation and function of platelets was assessed by measuring CD62P expression, platelet aggregation, release of intracellular Ca^{2+} and platelet viability. The effect of Ply on platelet adhesion to collagen and thrombus formation was measured in a flow chamber. All experiments were performed in the presence or absence of IVIG (Privigen).

Results: Ply integrates into the platelet membrane thereby leading to formation of multiple pores. This pore formation did not result in activation of platelets. Instead, pore formation was associated with intracellular CD62P staining, Ca^{2+}-release as well as loss of platelet function and viability. Platelets remained responsive to agonists only at sublytic Ply concentrations. In the presence of the pharmaceutical IgG preparation IVIG as well as with specific antibodies against Ply, platelet function and viability was fully restored.

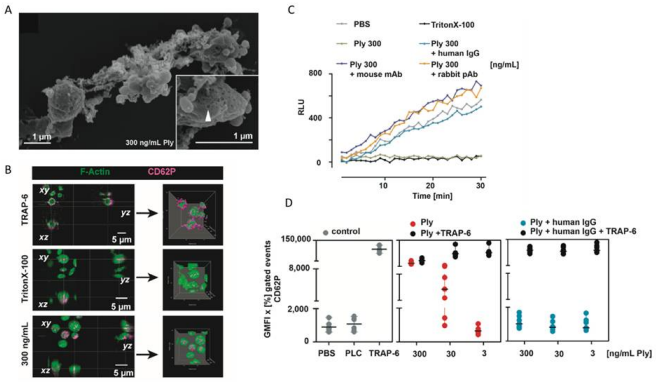
Conclusion: The pneumococcal toxin Ply destroys the platelet membrane integrity, which resultsin loss of platelet viability and function. Pharmacological immunoglobulins are able to neutralize Ply and preserve platelet function. This protective effect suggests, that neutralizing antibodies against Ply might be a rational therapeutic intervention to prevent development of ARDS in pneumococcal lung infections.

Reference:

Jahn, S. Handtke, T.P. Kohler, ...,S. Hammerschmidt, A. Greinacher (2020) ***BLOOD Advances***

Figure 1: (A) Scanning electron microscopy of platelets treated with Ply. (B) Confocal imaging and 3D reconstruction of platelets showing intracellular CD62P after Ply treatment. (C) Platelet viability as measured by increase of luminescence over time and (D) CD62P-staining of Ply treated platelets without and with subsequent TRAP-6 stimulation in the presence or absence of specific antibodies against Ply and IVIG.

Fig. 1



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Interaction between *Bartonella* Adhesin A and fibronectin gives insight into the molecular basis of bacterial adherence to human endothelial cells

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Introduction: *Bartonella henselae* is a facultative intracellular Gram-negative bacterium responsible for cat scratch disease and vascular proliferation (bacillary angiomatosis) in humans. The trimeric autotransporter adhesin (TAA) *Bartonella* adhesin A (BadA) is a major pathogenicity factor of *B. henselae,* mediating bacterial adherence to endothelial cells (ECs) presumably via binding to extracellular matrix proteins. Among the group, fibronectin (FN) is the most abundant protein in the extracellular environment and has been reported as a frequent bacterial binding protein. The identification of specific binding sites between BadA and FN might give insights into the use of anti-ligands to treat bacterial infections by a "new class" of anti-infectives. This research aimed to study and describe the interaction between BadA and FN in the bacteria-host cell adhesion process.
Methods: To evaluate the importance of the interaction between FN and BadA in *B. henselae* adhesion, FN knockout ECs were generated (LentiCRISPR) and exposed to *B. henselae* strains (wild type and BadA deficient) via *in vitro* infection models. Furthermore, to identify the specific BadA-binding sites in FN, the affinity of *B. henselae* strains against proteolytic FN fragments was evaluated using an immunosorbent assay. As a proof of concept of the importance of the specific FN regions for BadA binding, inhibition assays were performed using bacteria and heparin in competition for FN binding. Finally, the specific BadA-FN interacting peptides were further identified using mass spectrometry of cross-linked (XL-MS) BadA-FN peptides.
Results: The importance of BadA and FN interaction in the bacteria-host cell adhesion process was confirmed after the observation of reduced bacterial binding to ECs when using BadA- or FN-deficient conditions in *in vitro* infection models. Additionally, binding experiments between *B. henselae* and FN fragments showed higher BadA affinity to the heparin-binding domains within the FN protein. The importance of these specific FN sites was confirmed when bacterial binding to FN was reduced due to the competition between bacteria and heparin for FN binding. Finally, the XL-MS analysis of BadA and FN reported the exact peptide-peptide interactions occurring within the aforementioned heparin-binding regions in FN.
Conclusions: This research demonstrated that BadA and FN interaction plays a significant role in *B. henselae* adhesion to ECs. More precisely, we described the exact peptide-peptide interactions occurring between BadA and the heparin-binding domains in FN. The herein described peptides are intended to provide information for the design of BadA-targeting specific peptides to be used as "anti-ligands" to treat bacterial infections. Moreover, the experimental approach followed by this research work is suggested as a model for the study and description of the host cell interactions occurring with other TAAs from pathogenic bacteria.

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Enteral microbiome pertubations in hospitalised horses subjected to abdominal surgery

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Introduction: For hospitalized horses receiving gentamicin/penicillin (GP) as perioperative antibiotic prophylaxis (PAP), worrisome increases of zoonotic and multidrug resistant (MDR) pathogens such as extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (EC) have been reported only recently (1). However, knowledge is scarce with respect to the changes of the equine enteral microbiome associated with an increase of ESBL-EC detection rates from approximately 5% to ≥55% (1) within 10 days after abdominal surgery. Two GP-PAP regimens for horses, a novel short-term approach and a common 5-day lasting protocol, were comparatively investigated with respect to their influence on the gut microbiome.
Material and Methods: Two groups of horses subjected to colic surgery were enrolled, receiving a combination of GP for either five days (5DG) or a GP single-shot (SSG) prior to surgery. Fecal samples were collected directly at hospital admission (t_0) as well as on day 3 (t_1) and 10 (t_2) after surgery. 10 non-hospitalized horses were included as a control group. All samples were subjected to DNA extraction and 16S-rRNA V3–V4 region sequencing. Sequences were preprocessed accordingly and analyzed using kraken2 with the GreenGenes database. The resulting OTU counts were sub-sampled to a homogeneous level and used for the computation of various diversity indices. The microbial α-diversity metrics were compared between the two study groups using normal linear mixed models with time and treatment group as fixed effects and subject as random effect.
Results: We explored gut microbiota changes using samples obtained from 36 equine patients (n=15 SSG; n=11 5DG) and 10 non-hospitalized horses across three different time points (t_0, t_1, t_2). In total, 4,026,657 OTU counts (15,637–101,511 counts per sample, mean=33,555) were obtained. Inspection of the n=108 microbiomes revealed considerable inter-individual differences. Overall, microbiome samples of the hospitalized horses showed a significant decrease of α-diversity at t_1/t_2 compared to samples representing t_0 (SSG, pval=0.041; 5DG, pval=0.001). OTU-based classification at family level revealed log2 fold changes with respect to the relative abundance of *Enterobacteriaceae* (SSG=+4.57; 5DG=+6.10) from t_0 to t_1.
Discussion: The environment, disease, surgery, antibiotics and diet all have a large impact with respect to perturbations of the enteral microbiome. Here, we provide evidence that increasing ESBL-EC colonisation rates among equine patients subjected to abdominal surgery were accompanied by a decrease of α-diversity while the relative abundance of *Enterobacteriaceae* increased. Moreover, less prominent changes of gut microbiomes were recognized for horses belonging to the SSG compared to those of the 5DG. Further research is warranted to gain insights on the putative interaction of gut microbiome changes and MDR colonization in horses.

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Host-specific serum factors control the development and survival of *Schistosoma mansoni*

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Introductions: Schistosomiasis is a neglected tropical disease (NTD) caused by blood-dwelling flatworms which develop from skin-penetrating cercariae, the freely swimming water-borne infective stage into adult worms. This natural course of infection can be mimicked in experimental mouse models of schistosomiasis. However, only a maximum of 20-30% of penetrated cercariae mature into fecund adult parasites. The reasons for this are unknown but could potentially involve soluble factors of the innate immune system, such as complement factors.
Methods: Using our recently developed novel serum- and cell-free *in vitro* culture system for newly transformed schistosomula (NTS), which supports long-term parasite larval survival, we investigated the effects of mouse serum and its major soluble complement factors C1q, C3, C4 as well as sIgM *in vitro* and assessed worm development *in vivo* by infecting complement and sIgM-deficient animals.
Results: In contrast to sera from humans and a broad variety of mammalian species, serum from mice, surprisingly, killed parasites already at skin stage in vitro. Interestingly, the most efficient killing component(s) were heat-labile but did not belong to the most abundant serum complement components or consisted of unspecific immunoglobulins. Infection of complement C1q and sIgM-deficient mice with *S. mansoni* as well as *in vitro* tests with sera from mice deficient for C3 and C4 revealed no major role for these soluble factors *in vivo* in regards to parasite maturation, fecundity and associated immunopathology. Rather, the reduction of parasite maturation from cercariae to adult worms was comparable to wild type mice.
Conclusion: Taken together, our results suggest that not yet identified heat labile serum factors are major selective determinants to the host-specificity of schistosomiasis, by directly controlling schistosomal development and survival.

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Arginase 1 and the inducible nitric oxide synthase perpetuate chronic colitis due to the consumption of intraluminal L-arginine

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Introduction: The metabolism of the semi-essential amino acid L-arginine is altered in experimental colitis models and in patients with inflammatory bowel disease (IBD). Furthermore, the expression and activity of arginase 1 (Arg1) and of the inducible nitric oxide synthase (NOS2) which both compete for L-arginine as common substrate are enhanced in intestinal tissues of pre-clinical models and IBD patients. Thus, we characterized the impact of L-arginine, Arg1 and NOS2 on the induction, perpetuation and resolution of experimental colitis.
Material and Methods: We fed L-arginine-deprived and -supplemented chow to oxazolone- or DSS-treated Tie2-Cre+/- x Arg1fl/fl mice lacking Arg1 in hematopoietic and endothelial cells, NOS2-/- and Arg1 x NOS2 double knockout mice as well as respective littermate controls. Whole-genome transcriptomic patterns, the intraluminal metabolome and microbiome as well as the extent of intestinal inflammation and the physiology of in the intestinal vasculature system were assessed using immunohistochemistry, high resolution endoscopy, RNA sequence analyses, 16S rRNA sequencing, confocal laser scanning microscopy, two-photon microscopy, LC/MS and HPLC.
Results: Arginine-free chow accelerated experimental colitis while a dietary supplementation of mice with L-arginine promoted the resolution of intestinal inflammation. Unexpectedly, Tie2-Cre+/- x Arg1fl/fl, NOS2-/- and NOS2 x Arg1 double knockout mice experienced a significantly milder colitis compared to respective littermate controls. Protection from disease was associated with an accumulation of intraluminal polyamines, a decreased inflammatory cytokine production and compositional changes in the intestinal microbiota in L-arginine-supplemented wild-type litters, similar as observed in control chow fed Tie2-Cre+/- x Arg1fl/fl or NOS2-/- mice.
Fecal microbiota transfers (FMTs) from wild-type litters supplemented with L-Arginine restored the protective, anti-inflammatory phenotype in recipient mice similar as FMTs from control chow fed Tie2-Cre+/- x Arg1fl/fl or NOS2 donors, suggesting the microbiota as source for protective polyamine production. Vice versa, dietary L-arginine restriction abolished the anti-colitogenic effects of Arg1- or NOS2-deletion, suggesting that protection is related to an enhanced intraluminal availability of L-arginine, subsequent accumulation of polyamines and expansion of an anti-inflammatory microbiota.
Discussion: Together, these results strongly support the novel concept that Arg1 and NOS2 prevent the resolution of inflammation in experimental colitis and that microbiota are the predominant source of protective, anti-colitogenic polyamine production. Due to the high expression and acitivity of Arg1 and NOS2 in colitic tissues, either the blockade of both enzymes or the substitution of intraluminal L-arginine might therefore serve as novel targets for clinical intervention in IBD patients.

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Relevance of preanalytics in microbiome analysis

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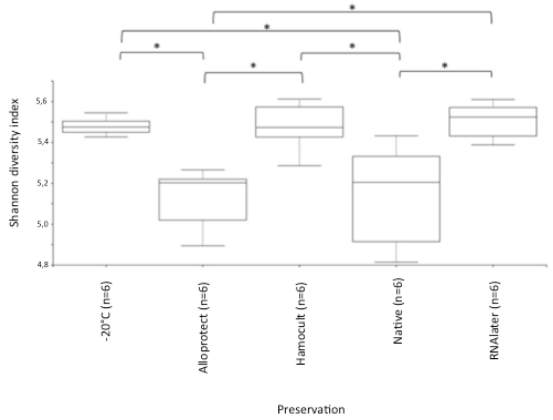
Introduction:
Microbiome analysis has evolved as a valuable tool to better understand the impact of our commensal bacteria, e.g., gut microbiota, on human health. Microbiome analysis consists of three major processes, i.e., preanalytics, sequencing, and data analysis. The diversity of applied procedures, however, limits reproducibility and comparability of data generated by microbiome analysis. 16S rDNA sequencing results, for example, can be affected by several factors such as sample collection, storage, DNA extraction and chosen primers in the variable region of 16S rDNA.
We assessed the impact of certain preanalytical factors on the results of microbiome analysis.
Materials/Methods:
A stool sample from a patient before and two days after oral administration of medically indicated 200 mg doxycycline was collected. Five different methods for sample preservation in combination with three DNA extraction methods were analysed. The preservation methods were (i) DNA extraction within 4 hours upon sampling, (ii) approx. 0,5 cm³ fecal sample supplemented immediately with 3 ml RNAlater (Qiagen) and storage for 72 hours at room temperature (20-22°C), (iii) approx. 0,5 cm³ fecal sample supplemented immediately with 3ml Alloprotect Tissue Reagent (Qiagen) and storage for 72 hours at room temperature, (iv) storage at -20 °C within 2 hours after sampling for 6 days, (v) sampling on Haemoccult (Beckmann Coulter) test cards and storage at room temperature for 6 days. Each sample was investigated in duplicate. The DNA extraction was carried out with QiaSymphony DSP Virus/ Pathogen Mini Kit (Qiagen), MagNA Pure LC DNA Isolation Kit III (Roche), and High Pure PCR Template Preparation Kit (Roche). 16S rDNA sequencing was performed according to the 16S Metagenomic Sequencing Library Preparation protocol from Illumina. Data was analysed with Qiime2 (version 2019.1).
Results:
We investigated the Shannon diversity index before and 2 days after the first intake of doxycycline. Significant differences were recognized between the preservation methods before the oral administration of antibiotics. The perservation of the stool samples at -20 °C, with RNAlater and on Haemoccult cards resulted each in a significant higher Shannon diversity index compared to the preservation with Alloprotect Tissue Buffer and the processing of

the native sample. This significant effect was not evident after the intake of doxycycline. No significant differences were recognized between the different DNA-extraction methods, neither before, nor after antibiotic intake.
Figure 1: Shannon diversity index of stool samples before intake of doxycycline, pooled by the manner of preservation. Significant differences between preservation methods are marked with an asterisks

Conclusion:
Our data reveal that the effect of preanalytics, especially stool preservation, needs specific attention in microbiome analysis

Fig. 1



292/PWP
Regulation of lugdunin biosynthesis and transport
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For bacteria, the human nasal cavity is an environment limited in nutrients, thus leading to a steady competition of members of the nasal microbiota. One well known thereof is Staphylococcus aureus, colonizing one-third of the population and causing a high risk for severe infection. Nasal screening of bacterial isolates revealed a variety of species, among which S. lugdunensis could be identified as an effective S. aureus-competing strain. StS

In recent studies we could demonstrate that this species produces a non-ribosomally produced cyclic peptide named lugdunin, which is responsible for the eradication of nasal S. aureus. The biosynthetic gene cluster and lugdunin structure as well as mode of action and producer self-resistance could be elucidated so far. However, regulation of the biosynthesis and transport of lugdunin remain ambiguous so far.

Therefore, we generated a heterologous expression system of putative promotor regions and suspected regulators of the lugdunin biosynthetic gene cluster. Whereas LugR is encoded upstream of the biosynthetic genes lugABCD, LugJ is encoded upstream of the ABC transporters lugIEFGH, indicating their respective function in the regulation of biosynthesis and transport. Analysis of reporter gene expression shows, that biosynthesis as well as transport of lugdunin are tightly regulated. Lugdunin itself leads to a strong induction of the transporter gene expression but only a minor induction of the biosynthesis. Interestingly, the lugdunin enantiomer, which is as active as lugdunin, does not induce gene expression. Binding studies of lugdunin to regulators or regulators to putative promotor regions will elucidate the interplay of lugdunin biosynthesis, transport and regulation.

Gastrointestinal Infections (FG GI)

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Giardia lamblia-induced dysregulation of bile acid homeostasis, lipid metabolism and growth following neonatal infection
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Whereas infection of adult travelers with the human enteropathogen Giardia lamblia (G. lamblia) usually causes self-limited diarrhea, exposure of the infant population in endemic areas leads to asymptomatic pathogen carriage and stunting. The mechanisms underlying the different disease outcome and the consequences of pathogen persistence have not been investigated due to the lack of a suitable animal model. Here we established a model for persistent infection by administering G. lamblia to neonate mice. Using this approach, we analysed immunological, microbiota and metabolic parameters both during the early neonatal phase of the host-parasite interaction as well as during persistent carriage in adult mice. Our results explain the enhanced susceptibility of the neonate host, identify pathogen-altered bile secretion as mechanism promoting pathogen persistence after weaning and demonstrate the influence of prolonged protozoan carriage on the infant host's lipid metabolism, reduced body weight gain and growth.

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Antibiotic resistance of *Campylobacter jejuni* isolated from diarrhoeal outpatients
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Introduction: *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis among human beings worldwide. In humans, most enteric infections caused by *Campylobacter spp.* are considered self-limited and generally do not require antimicrobial treatment. However, in severe or prolonged cases treatment can shorten illness duration. The aim of this study was to determine the antimicrobial susceptibility of *Campylobacter jejuni* isolated from

stool specimens of symptomatic outpatients from Rhine-Ruhr metropolitan region in North Rhine-Westphalia.
Materials/Methods: A total of 433 *Campylobacter spp.* were isolated from stool samples from January to December 2020. Identification of the isolates was performed by conventional methods and antimicrobial susceptibilities to ciprofloxacin, erythromycin, and tetracycline were assessed by the disk diffusion method. The results were interpreted by using EUCAST breakpoints. The patient cohort was differentiated by sex (male and female) and age (≤14, 15-29, 30-44, 45-59, 60-74, 75-89 and ≥90 years). Additionally, medical specialties of the senders were taken into account. Demographic and patients data were extracted from the Laboratory Information System MOLIS (version 4.40) and statistics program HyBASE® and included in the analysis.
Results: Considering a single *Campylobacter* isolate per patient, 60% (258) *C. jejuni* and 40% (175) *Campylobacter spp.* (not *jejuni*) isolates were identified from stool specimens. The percentage of consistency of the stool samples of the *C. jejuni* isolates were as follows: 42.2% loose-stool, 28.3% watery-stool, 28.3% soft/formed-stool, and 1.2% blood in stool specimens. The highest percentage of *C. jejuni* were found in women 52.7% (136/258) and in the age group 45-59 years 29.1% (75/248), 15-29 years 24.6% (61/248). Of 258 isolates, 242 (93.7%) antibiograms were performed, 16 (6.2%) the strains cannot be re-cultivated. Among *C. jejuni* isolates, 80.6% (195/242) were resistant to ciprofloxacin, 57.8% (140/242) to tetracycline and 1.2% (3/242) to erythromycin. The stool specimens with *C. jejuni* isolates were collected from the following medical specialties: 58.1% general medicine, 33%, internal medicine, 8.1% paediatrics and 0.8% other medical specialities.
Discussion: In our area, the highest percentage of patients infected by *C. jejuni* were female outpatients in adulthood from the general medicine and internal medicine consultation. *C. jejuni* presented a high rates of resistance to ciprofloxacin and tetracycline. These dates are similarly to other countries, these are most likely correlated to the use of these drugs in animal husbandry. It could be inferred too, in the case of Ciprofloxacin, it is a first-choice drug in the treatment of acute bacterial gastroenteritis in cases in which the identity of the causative agent has not been established is questionable. Erythromycin remains the preferred treatment option for *C. jejuni* infections.

233/GIP
Proteome profiles of *Enterococcus faecium* und *Enterococcus faecalis* under bile acid stress
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Introduction: *Enterococcus faecalis* and *Enterococcus faecium* are Gram positive coccal bacteria that inhabit the human gastrointestinal tract. Both belong to the glucose fermenting facultative anaerobes. Furthermore, in clinical environments they are known to cause nosocomial infections outside of the gastrointestinal tract, which is enhanced by their high level of antibiotic resistances. Nevertheless, growth of *E. faecalis* and *E. faecium* can be reduced by bile acids.
Among the diverse functions of bile acids is their detergent activity that can disrupt bacterial cell membranes. About 200 to 600 mg bile acids are produced by the liver per day, the human bile acid pool consists mainly of CA (~40%), CDCA (~40%), and DCA (~20%). However, the individual bile acid composition depends on several factors including the diet and diseases.
Material and Methods: In this experimental approach, *E. faecalis* and *E. faecium* were grown with different concentrations of CA, DCA and CDCA. Afterwards, the cells were disrupted, and proteins were purified. By using SWATH-MS, the proteome of all approaches was determined and further analyzed using ProteinPilot V5.0, PeakView V2.1, and SWATH quantitation microApp V2.0 to show the differences of proteomic expression of the bacteria in presence of bile acids.
Results: When incubated with different bile acids, reduced growth of *E. faecalis* and *E. faecium* can be observed after 24 hours. Both microbial species show increased optical density compared to the control grown without bile acid. As a first result after SWATH-MS, 800 proteins were identified in the approach with *E. faecalis,* 741 proteins were identified in the approach with *E. faecium* and DCA. The statistical analyses showed the upregulated components of the bile acid stress proteome.
Discussion: The stress proteome of *E. faecalis* and *E. faecium* in an environment with different bile acids was shown. Notably, many upregulated proteins are known to function as oxidative stress mechanisms, indicating that the bacteria suffer from oxidative stress when growing in the presence of bile acids.

234/GIP
Impact of *Clostridioides difficile* therapy on nosocomial acquisition of vancomycin-resistant enterococci
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Introduction: *Clostridioides difficile* infection *(CDI)* remains one of the most common healthcare-associated infections worldwide. First-line therapeutic options depend on severity of symptoms and comprise metronidazole for mild to moderate infections and oral vancomycin for initially severe cases. While the choice of drug focusses on clinical symptoms, its implications have not fully been characterized. In this context, especially the acquisition of vancomycin-resistant enterococci (VRE) has to be mentioned, which can occur due to pathogen transmission or antibiotic selection. Here, VRE acquisition after CDI treatment with metronidazole and oral vancomycin was compared.
Methods: A 2-year retrospective longitudinal study was conducted in a German university hospital. Patients with episodes of toxin positive *C. difficile* infections, metronidazole or oral vancomycin treatment and without preexisting VRE colonization/infection were included. VRE isolates from patients with preexisting colonization were collected for genetic comparison purposes. Patients" characteristics as demographic data, underlying diseases and therapies were monitored, as well as the acquisition of VRE following *C. difficile* treatment. The correlation of CDI treatment with VRE acquisition was estimated for metronidazole and oral vancomycin and compared using the qui square test. VRE isolates were subjected to whole genome sequencing for subtyping and to determine their genetic relatedness.
Results: During 2018 and 2020, 281 patients (median age 56 years, 54% of male sex) presented with toxin positive *C. difficile*, of whom 111 did not fulfill the inclusion criteria (other CDI treatments: 96; preexisting VRE colonization: 15). Of the remaining 170 patients 37 were treated with metronidazole, while 133 received oral vancomycin. In total, 11 patients acquired VRE after oral vancomycin and three after metronidazole treatment. Statistical analysis revealed no significant differences between both VRE acquisition rates. Genetic comparison of detected VRE isolates resulted in eight clusters of closely-related genotypes, of which seven comprised acquired and preexisting strains. Of all 14 acquired VRE, five isolates were not genetically related to other VRE.
Discussion: Present data suggest that both vancomycin and metronidazole potentially play a role in the acquisition of VRE. Our results suggest that CDI treatment with oral vancomycin does not increase the risk of VRE acquisition compared to metronidazole monotherapy. The undifferentiated genetic relatedness patterns of acquired and preexisting VRE isolates hint that no specific genetic features underlie the acquisition of VRE in the study population. Rather, this seems to result from both antibiotic selection and pathogen transmission, emphasizing the essential need of infection prevention bundle strategies consisting of antibiotic stewardship programs and infection control measures.

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Elucidating CagA translocation: Development and application of a split-luciferase-based CagA translocation reporter assay

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Introduction: Secretion systems are a prerequisite of many pathogenic bacteria to colonize the host and to induce severe diseases. The human gastric pathogen *Helicobacter pylori* uses the Cag type IV secretion system (CagT4SS) to inject its effector protein, the cytotoxin-associated gene A (CagA) into eukaryotic cells. Upon translocation, CagA interacts with a plethora of proteins, thus hijacking cellular signaling cascades. This aberrant cell programming is a major cause of the development of severe malignant disorders of the stomach. In the last decades, huge progress was made, deciphering the structure of the CagT4SS and characterizing CagA, but little is known about the secretion process itself. To further analyze CagA translocation, we developed a split-luciferase-based reporter assay, which is a highly sensitive method to monitor this process in end-point and real-time assays.
Method: The NanoLuc luciferase complementation system (Promega) was adapted to monitor CagT4SS activity *in vitro*. In principle, NanoLuc is split in two parts, HiBiT and LgBiT, which have a high affinity to each other and together form a functional enzyme. The LgBiT is produced by the eukaryotic host cells, the small 1.3 kDa HiBiT peptide is fused to the N-terminus of CagA. If HiBiT-CagA is injected into the host cell, HiBiT and LgBiT can interact to form an active luciferase complex. The emission of light is detected in a plate reader and directly correlates with the number of active luciferases and thus the amount of translocated HiBiT-CagA.
Results: HiBiT-tagged CagA was well-tolerated and transported, and did not interfere with downstream phenotypes such as CagA tyrosine phosphorylation. Due to the high sensitivity and time resolution, the reporter assay could be implemented to get novel insights into the kinetics and mode of action of the secretion process. Beside a fast onset of CagA translocation observed within less than 15 min after co-incubation with the host cells, delivery was soon saturated and only a minor part of the total available CagA pool was secreted. Furthermore, the assay was used to address the impact of protein unfolding on CagA translocation. For this, HiBiT-CagA was fused to various passenger domains with different folding characteristics. Our data indicated that protein unfolding is required for the translocation via the CagT4SS.
Conclusion: The advantage of the split-luciferase system used here is that the reporter tag is small and thus does not interfere with the secretion process, and that detection is very sensitive and does not require any lytic or other cumbersome post-infection treatment. This enabled monitoring CagA translocation *in vitro* in real time, giving new insights into secretion kinetics. The assay has the potential to enable advanced functional studies on CagT4SS activity, thus elucidating a central virulence principle of *H. pylori*.

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Vitamin D in acute campylobacteriosis – Results rrom an intervention study applying a clinical *Campylobacter jejuni* induced enterocolitis model

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Question: Human *Campylobacter* infections are progressively rising and of high socioeconomic impact. In the present preclinical intervention study, we investigated anti-pathogenic, immuno-modulatory, and intestinal epithelial barrier preserving properties of vitamin D applying an acute campylobacteriosis model.
Methods: Secondary abiotic IL-10$^{-/-}$ mice were perorally treated with synthetic 25-OH-cholecalciferol starting 4 days before the *Campylobacter jejuni* infection. Therefore, synthetic 25-OH-cholecalciferol was dissolved in Tween 80 (0.2% v/v) and administered to mice via the autoclaved tap water (*ad libitum*). The final concentration of the synthetic 25-OH-cholecalciferol solution was 2.5 µg/mL resulting in a daily treatment dosage of 500 µg per kg body weight. Placebo control mice received vehicle via the drinking water.
Results: Whereas 25-OH-cholecalciferol application did not affect gastrointestinal pathogen loads, 25-OH-cholecalciferol treated mice suffered less frequently from diarrhea in the midst of infection as compared to placebo control mice. Moreover, 25-OH-cholecalciferol application dampened *C. jejuni* induced apoptotic cell responses in colonic epithelia and promoted cell-regenerative measures. At day 6 post-infection, 25-OH-cholecalciferol treated mice displayed lower numbers of colonic innate and adaptive immune cell populations as compared to placebo controls that were accompanied by lower intestinal concentrations of pro-inflammatory mediators including IL-6, MCP1, and IFN-γ. Remarkably, as compared to placebo application synthetic 25-OH-cholecalciferol treatment of *C. jejuni* infected mice resulted in lower cumulative translocation rates of viable pathogens from the inflamed intestines to extra-intestinal including systemic compartments such as the kidneys and spleen, respectively, which was accompanied by less compromised colonic epithelial barrier function in the 25-OH-cholecalciferol as compared to the placebo cohort.
Conclusions: Our preclinical intervention study provides evidence that peroral synthetic 25-OH-cholecalciferol application exerts inflammation-dampening effects during acute campylobacteriosis.

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Increasing trend of 3rd generation cephalosporin-resistant clinical *Salmonella enterica* in Germany and predominance of plasmid-borne *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-14}$, and *bla*$_{CTX-M-65}$ genes

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Non-typhoidal *Salmonella enterica* (NTS) is an important gastrointestinal pathogen causing a considerable burden of disease. Resistance to 3rd generation cephalosporins poses a serious threat for treatment of severe infections of this pathogen. In this study occurrence, phylogenetic relationship, and mechanisms of 3rd generation cephalosporin resistance were investigated for clinical NTS isolates in Germany. From 2017 to 2019, 168 unique clinical *S. enterica* isolates with phenotypic resistance to 3rd generation cephalosporins were detected in a nation-wide surveillance. Compared to previous years, a significant and consistent increase in resistant isolates from 0.41% in 2005 to 1.71% in 2019 was observed. Among the resistant isolates 34 different serovars were identified, most often *S.* Infantis (24.4%), *S.* Typhimurium (16.1%) and *S.* Kentucky (12.5%). Whole genome analyses revealed extended-spectrum β-lactamase (ESBL) genes, mainly located on plasmids, as main cause for 3rd generation cephalosporin resistance: Most prevalent were *bla*$_{CTX-M-1}$ (n=55), *bla*$_{CTX-M-14}$ (n=25), and *bla*$_{CTX-M-65}$ (n=23). There was no strict correlation between serovar, phylogenetic lineage, and ESBL type but some serovar/ESBL gene combinations were detected frequently, such as *bla*$_{CTX-M-1}$ or *bla*$_{CTX-M-65}$ in *S.* Infantis. We conclude that 3rd generation cephalosporin resistance is on the rise among clinical NTS isolates in Germany, and occurrence in various *S. enterica* serovars is most probably due to multiple acquisition events of plasmids.

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Efficacy of different individually applied non-biosafety measures on *Campylobacter jejuni* colonisation in broiler chickens

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Introduction: *Campylobacter* is one of the most important causes of diarrheal disease. Campylobacteriosis in humans is caused predominantly by the two bacterial species *Campylobacter* (C.) *jejuni* and C. *coli*. The disease is strongly associated with poultry and poultry meat. In particular, broiler meat is considered a significant source of human infection. Therefore, we aimed to examine the effect of different non-biosecurity-based measures (CE-culture, carvacrol, alternative slow growing breed) on *C. jejuni* colonization in broiler chickens.
Material and Methods: Per experiment, 90 newly hatched broiler chickens of breed Ross 308 were raised in the experimental animal facility with category two biosecurity on floor housing with litter. On day 10 of age, 18 broiler chickens (seeder) were orally inoculated with 104 cfu/500 µl *C. jejuni*. Thereafter, *Campylobacter* colonization and load was determined weekly by taking cloacal swabs of 36 randomly selected untreated broilers (sentinels). At the end of each experiment (33 days post hatch), sentinels were dissected and cecum and colon content were collected for semiquantitative analysis following DIN EN ISO 10272-3. To examine the effect of (i) carvacrol broilers were fed daily with 120 mg/kg feed of carvacrol (ii) of the CE-culture, broilers were treated two times (day 1 using spray application, day 25 via drinking water application) (iii) of a different breed we obtained and fattened an alternative slower growing breed (Ranger-Gold). Broilers were provided free access to commercial broiler feed and filtered water from the municipal water supply ad libitum during the entire study. Feed was offered using commercially available poultry troughs and filtered water via nipple drinkers. The chickens" diet comprised a standard three-phase feeding program diet for broilers matching the commercial standard.
Results: Cecal count enumeration demonstrated that the *C. jejuni* load was significantly reduced for the group receiving the CE-culture (mean reduction 0.86 log10 MPN/g) compared to the control. Colon counts were significantly decreased in alle three groups (CE-culture (mean reduction 1.0 log10 MPN/g), carvacrol (mean reduction 1.25 log10 MPN/g), Ranger Gold (mean reduction 1.17 log10MPN/g)) in comparison to the control group.
Conclusion: We intend to combine measures that have already been tested to determine whether a greater reducing effect can be achieved. More specifically, we are planning to combine carvacrol with organic acids as well as bacteriophages (presented separately) with the CE-culture.

National Reference and Consulting Laboratories (StAG RK)

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NGS-based molecular surveillance of infections with Shigella sonnei, cgMLST analysis and identified clusters 2018-2020 in Germany

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Question: Shigellosis is one of the most important diarrheal infections worldwide with estimated 165 million cases per year and is responsible for approximately 60% of death in children less than 5 years of age. There are four known species of *Shigellae*: *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei*. All of these have high transmission rates, while *S. sonnei* is the predominant one. Also 75% of reported cases account for this species in Germany, with an increasing number since 2017, except for 2020 during SARS-CoV-2 pandemic (Survnet@RKI). In 2018 surveillance and outbreak detection analysis by pulsed-field gel electrophoresis and phage typing was replaced by NGS-based *cg*MLST at the NRC. Molecular characterization of *S. sonnei* isolates by *cg*MLST and performance testing for cluster detection and surveillance implementation was carried out.
Methods: In total, 226 isolates of *S. sonn*ei, received between 2018 and 2020, were characterized at the NRC. Out of these, 140 were under further investigation by Illumina MiSeq whole genome sequencing and Ridom SeqSphere+ v6.0.0 cluster analysis by the utilization of the *Escherichia/Shigella* cgMLST v1 scheme from Enterobase.
Results: By molecular characterization, 41 isolates could be connected to the European-wide outbreak strain reported by Public Health England, UK, associated with transmission amongst MSM (1-10 alleles difference). 14 further isolates, originating from a kindergarten outbreak in Berlin in September 2018, were investigated. A cluster of seven isolates was additionally detected, connected with attendance to a music festival in Poland in August 2018, and three additional clusters closely related in *cg*MLST were investigated in 2018. Nine Clusters could be identified in 2019, including one with a link to an Irish food isolate, one with travel-association to Jordan and one of nine closely related cases connected with an attendance to a festival in Saxony-Anhalt.
Conclusion: cgMLST was successfully implemented at the NRC for surveillance and outbreak detection. Our work confirms that usage of Enterobase *Escherichia/Shigella* cgMLST v1 scheme is qualified for cluster analysis of *Shigella* strains.

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Prevalence of piperacillin-tazobactam resistance in invasive *Haemophilus influenzae* in Germany

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Question: *Haemophilus influenzae* (Hi) is an important pathogen causing invasive infections. Piperacillin/Tazobactam (TZP) is a first-line agent for empirical sepsis treatment. EUCAST breakpoints for Hi and TZP are available since 2019. Tazobactam inhibits the TEM-1 β-lactamase, which is the most common resistance mechanism in Hi. However, little is known about the TZP susceptibility of Hi. Here, we provide prevalence data on TZP resistance of invasive Hi isolates in Germany 2019.
Methods: TZP resistance in Hi is defined as a minimal inhibitory concentration (MIC) of >0,25mg/l according to EUCAST. 726 strains were examined for TZP susceptibility by gradient agar diffusion (GAD). MICs higher than 0,125mg/l were verified by broth microdilution (BMD). Resistant strains were also further tested by agar dilution (AD) and piperacillin GAD performed on tazobactam-containing MH-F (MH-F/Taz) agar. Additionally, the PBP3 was analysed to detect possible β-lactam resistance mechanisms. TZP resistance was assumed if GAD results were confirmed by an additional phenotypic test.
Results: GAD resulted in 21 TZP resistant invasive Hi isolates out of 726 (2,9%). None were confirmed by BMD. Two strains were rated resistant by AD, of which one strain was additionally confirmed by piperacillin GAD performed on MH-F/Taz agar. Finally, two strains showed a MIC >0,25mg/l in at least two methods. They are both β-lactamase-producing amoxicillin-clavulanate-resistant (BLPACR) strains with PBP3 mutations characterized previously as group III-like+. Furthermore, five β-lactamase-negative ampicillin-resistant (BLNAR) and one BLPACR strain tested TZP resistant only by GAD with relevant mutations in the PBP3. Although their TZP resistance was not confirmed by a second phenotypic method, the mutations suggest a reduced affinity of β-lactam antibiotics to the PBP3 in these strains.
Conclusions: :TZP resistance of <1% is rare in invasive Hi in Germany. Our findings suggest that BMD may lead to an underestimation of the MIC of Hi. Similar observations regarding the heteroresistance to imipenem were made. GAD, in turn, may overestimate TZP MIC, since half of the strains tested resistant by GAD showed no PBP3 mutations nor phenotypic resistance by AD. Therefore, rare occurring TZP resistance detected by GAD needs confirmation by additional methods. Sequencing of *fts*I provides additional information, however, underlying resistance mechanisms including the role of PBP3 mutations require further research.

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Minor trend towards increasing penicillin resistance rates in invasive meningococcal disease over a period of 18 years in Germany

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Introduction: Neisseria meningitidis is a major cause of septicemia and meningitis and thereby leads to substantial morbidity and mortality, especially affecting children and young adults.
Antibiotics are essential for treatment of meningococcal disease. Using almost 20 years of consistent data collection at the NRL for Meningococci and Haemophilus influenzae (NRZMHi), we explored trends of penicillin susceptibility.
Methods: We explored minimal inhibitory concentrations (MICs) and resistance rates to penicillin in strains isolated from blood or cerebrospinal fluid of patients suffering from invasive meningococcal disease between 2002 to 2019 in Germany, considering the effects of potential confounders like age and sex of patients, region, and serogroup. A logistic regression generalized additive model (GAM) was applied for trend analysis of resistance rates; a linear GAM was fitted for explaining the development of logarithmic MICs. Resistance rates were compared between different subgroups by Fisher"s exact tests.
Results: In total, 5086 cases and their linked viable strains were analyzed, of which 1.67 % were classified as resistant according to EUCAST. Serogroup B was the most frequent serogroup (64.9 %) in our dataset, followed by serogroup C (23.5 %). For serogroup C a significantly higher resistance rate was detected (C: 2.6 %, B: 1.2 %, p-value: 0.0019).
Investigating descriptively molecular characteristics of meningococci, the fine types C:P1.5-1,10-8:F3-6 (p = 0.0008), C:P1.5,2:F5-8 (p = 8.71e-14) and B:P1.22,14:F5-5 (p = 0.0386) were found to be significantly associated with higher resistance rates compared to remaining strains with the same serogroup, respectively.
Overall, penicillin resistance rates increased from 0.3 % in 2007 to 6.0 % in 2019. Logarithmic MICs of penicillin increased in several waves. Strains isolated from infants and children showed higher logarithmic MICs compared to strains from adults. Additionally, serogroup was found to have an effect on logarithmic MICs.
Discussion: In conclusion, we observed a slight increase of penicillin MICs and resistance rates for invasive meningococci submitted to the NRZMHi between 2002 and 2019. Although resistance rates in contrast to the situation in gonococci are still at a very low level, this analysis emphasizes the importance of national resistance surveillance to timely identify trends.

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Report of the National Reference Centre for Multidrug-resistant Gram-negative bacteriaon Carbapenemases in Germany in 2020

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Question: Multidrug-resistance in *Enterobacterales*, *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.
Methods: The National Reference Centre 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 6001 isolates were investigated for carbapenemases in the National Reference Centre in 2020. Carbapenemases were found in 1567 *Enterobacterales* strains, 334 of *A. baumannii* and 260 of *P. aeruginosa*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 417), VIM-1 (n = 275), NDM-1 (n = 247), KPC-2 (n = 173), OXA-244 (n = 158), NDM-5 (n = 152), KPC-3 (n = 77) and OXA-181 (n = 73). In *P. aeruginosa,* VIM-2 was the most frequent carbapenemase (n = 245), followed by GIM-1 (n = 22), GES-5 (n = 21), VIM-1 (n = 14) and VIM-4 (n = 10). IMP-13, IMP-7, NDM-1 and others were found in less than 10 isolates each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (n = 203), followed by OXA-72 (n = 96) and NDM-1 (n = 17). OXA-58, GES-11 and others were found in less than 5 isolates each.
Conclusions: A variety of different carbapenemases is detected in Germany. The molecular epidemiology in Germany differs significantly from observations made in other countries like Greece, Italy or the USA with a predominance of OXA-48. Compared to previous years, variants of OXA-48, especially OXA-244, are again on the rise.

244/RKP

Implementation of molecular surveillance of invasive meningococcal disease at the National Reference Centre for Meningococci and *Haemophilus influenzae* (NRZMHi)

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Introduction: In 2014, an analysis of whole genome sequences (WGS) of meningococci based on core genome multi-locus sequence typing (cgMLST) has been established that is publically available in the PubMLST Neisseria database (1). Since 2016, meningococci are on the priority list for molecular typing integration in EU surveillance of the ECDC (2). Here we describe our experience with WGS of meningococci.
Material and Methods: Since 2019, WGS is applied to all IMD isolates. Sequences are submitted to the PubMLST Neisseria database and analysed by cgMLST. Relevant data for meningococcal typing are downloaded via RESTful Application Programming Interface to the web-based NRZMHi database.
Results: In 2019 and 2020, 228 and 105 samples, respectively, from invasive meningococcal disease were submitted to the NRZMHi of which 186 and 76, respectively, were viable meningococci. The reduction in case numbers was due to COVID-19 control measures (3).
WGS provided comprehensive data on core genome type, antigen typing, resistance gene variants and vaccine preventability. Sequence submission to the PubMLST Neisseria database supports international exchange of data. In the future, a data export to The European Surveillance System (TESSy) of the ECDC is planned promoting European surveillance.
Currently, disadvantages of WGS at the NRZMHi are only retrospective availability of typing data due to batch sequencing appr. four times per year and the restriction of genome sequences to viable isolates. Hence, conventional fine-typing needs to be re-activated in case of outbreaks and for cases, for which no viable isolates have been submitted. Furthermore, the extended costs have to be considered.
Discussion: Switching to WGS is advisable for all NRL for meningococci, however, running time, costs and typing of cases without viable isolates have to be considered.
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245/RKP

Development of an immunochromatographic assay for rapid detection of diphtheria toxin-producing corynebacteria

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Introduction: During the 1990s in Russia and neighboring countries the epidemic of diphtheria affected about 200,000 people with 5000 deaths. Outbreaks of diphtheria occurred in Bangladesh, Yemen, Venezuela in 2017-2019. Recent reports from several EU countries indicate circulation of *Corynebacterium diphtheriae* among refugees from Asia and Africa as well as children"s deaths among indigenous populations. Toxigenic *C. diphtheriae* carriers pose a serious risk for unvaccinated persons and ones with low level of immunological protection, the number of which in Europe has increased. The International system of quality control revealed the poor state of laboratory diagnosis of diphtheria in EU. This is mostly due to the absence of simple, standard and reliable method of the diphtheria toxin (DT) detection. The common method for detecting the production of DT by corynebacteria, gel immunoprecipitation or Elek test developed in 1949, is vulnerable, time consuming and laborious. PCR for DT gene detection is inaccurate as non-toxigenic *C. diphtheriae* and *C. ulcerans* strains carrying the mutated toxin gene circulate worldwide. The aim of the study was development and evaluation of rapid immunochemical test for the detection of DT.
Methods: Lateral Flow Immunoassay (LFIA) was designed using monoclonal antibodies against DT. Two hundred of toxigenic and non-toxigenic (50/50) *C. diphtheriae* and *C. ulcerans* isolated in Germany in 2019-2021 were tested for DT after 6 hours of growth on the WHO-recommended liquid nutrient medium.
Results: A complete agreement of the conventional method of DT detection - Elek test and the LFIA results for all 200 isolates of diphtheria clade corynebacteria was observed. The use of the LFIA makes it possible to significantly simplify DT detection and reduce the time of analysis from 24-48 to 6 hours. Thus, the possibility of replacing the Elek test with the LFIA appears real.
Conclusion: The availability of a simple, reliable and affordable method for DT detection will speed up and increase the accuracy of diphtheria diagnosis globally. The efficient diphtheria laboratory diagnosis will enable timely initiation of anti-diphtheritic treatment that will save lives, prevent the development of complications, as well as stop the spread of the disease through early initiation of preventive measures.
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246/RKP

Cutaneous diphtheria in refugees, Germany, 2020

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Objectives: Diphtheria is still rarely observed in industrialized countries, but migration and travelling abroad promotes imported infections. In 2020 we observed an increased number of toxigenic (*tox*+) *C. diphtheriae* strains spread in different parts of Germany. Nine refugees from different countries presented with cutaneous diphtheria. We conducted an outbreak investigation in order to detect transmission of *C diphtheriae*.
Materials and Methods: Strain identification was performed by biochemical differentiation and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Susceptibility testing was performed according to CLSI and EUCAST guidelines. Toxigenicity was verified by real-time PCR and the modified Elek-test. Next generation sequencing (NGS) analysis was carried out with MiSeq whole genome sequencing and data analysis by cgMLST. We interviewed the refugees to investigate reception camps on the escape route to Germany and vaccination status.
Results: NGS analysis revealed 2 sequence types (ST 377 and ST 698) and 3 clusters in these 9 strains. Epidemiological data indicated 3 different refugee camps in Serbia, Romania and Germany as the most likely source of strain transmission. Vaccination status was unclear in most of the cases.
Conclusions: Our outbreak investigation underlines that cutaneous diphtheria should be considered as differential diagnosis in refugees or similiar underpreviliged groups presenting wound infections. Toxigenic *C. diphtheriae* strains may be a source for secondary diphtheria cases. Adequate medical and hygienic precautions including control of vaccination status are needed to prevent human-to-human transmission especially among people living under poor hygienic standards.

247/RKP

Poliovirus containment in Germany: Update legal regulations 2021

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Background: The goal of the Global Polio Eradication Initiative (GPEI), which started in 1988, is to complete the eradication of all polioviruses (PV) by the year 2023. This should be achieved by vaccination, surveillance and containment strategies. Germany joined the GPEI in 1997 and agreed to actively support this public health goal. Laboratory containment of polioviruses and potentially infectious material (PIM) is an important measure to minimize the risk of polio PV release from laboratory stocks. Therefore, WHO developed a detailed strategy, set out in Global Action Plan III (GAPIII), including biosafety and biosecurity requirements for laboratories, vaccine producers, or any other facility that handle or store eradicated polioviruses.
Material/Method: WPV2 has been declared eradicated worldwide in 2015, and the vaccine switch from trivalent OPV to bivalent OPV1+3 occurred in 2016. Since then, activities with all PV2 (vaccine-derived viruses, OPV; vaccine-derived viruses, VDPV; and wild polioviruses, WPV) is only allowed in WHO--certified poliovirus essential facilities (PEFs). In 2019, wild poliovirus type 3 was also declared eradicated. Since PV3 remains part of the bivalent OPV vaccine, the laboratory containment of PV3 initially only includes WPV3 / VDPV3. Unlike material known to contain PV, PIM is a major challenge. It includes stool, respiratory, or wastewater samples, as well as isolates from these samples if the material was collected at a time and place where WPV was circulating and/or trivalent OPV2 was in use. Therefore, all laboratories working with enteric or respiratory pathogens (e.g. rotavirus, bacteria, parasites, influenza, etc.) but also nutritional and environmental laboratories may be at risk of PIM.
Results: In order to create a legal framework, laboratory containment was included in the Infection Protection Act (IfSG) in 2017. The §50a obliges all laboratories to inactivate PV2-containing material. From mid-2021, the destruction of PV3-containing material (except OPV3) is also expected to become mandatory by statutory order. The local health department must also be notified of any type of PV material or PIM. However, this does not necessarily require destruction of the PIM samples. Primarily, a risk analysis must be carried out.
Conclusion: The notification requirement serves to raise awareness and identify relevant laboratories. For this reason, all laboratories should assess their risk of storing potentially infectious

poliovirus material and, if necessary, comply with the notification requirement.

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The Quality Management of national reference centers and consultant laboratories of a German federal institute

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Introduction: A quality management (QM) has become constantly more essential in diagnostic laboratories during the last years. Nevertheless, the awareness of the significance of a QM in a medical laboratory did a decisive leap in 2020. Since the pandemic, quality assurance and moreover a QM have become a matter of public and political interest.
Public health microbiology laboratories hold an important role to prevent and control infectious diseases by detecting pathogens, monitoring outbreak responses and providing scientific evidence. National Reference Centers (NRC) and Consultant Laboratories (CL) improve the efficiency of infection protection.
In the following the NRCs regarded in this paper are listed:

- salmonellae and other bacterial enteritis pathogens
- measles, mumps, German measles
- staphylococci and enterococci
- poliomyelitis and enteroviruses
- influenza

In the following the CLs regarded in this paper are listed:

- listeria
- noroviruses
- rotaviruses
- cryptococcosis and rare system mycosis
- RSV, PIV and HMPV
- poxviruses
- Bacillus anthracis and CL for tularemia
- neurotoxin-producing clostridia

A QM can increase the reliability of a laboratory's diagnostic. Moreover, a good QM-System (QMS) provides the basis for legal requirements which a public health institute must meet, like the Regulation of the European Parliament and Council on in vitro diagnostic medical devices (IVDR).
This paper is supposed to introduce the QM of accredited national reference centers and consultant laboratories and outline first-hand opinions about QM from the staff. Moreover, it describes the complications and difficulties which can arise and why a QMS is nevertheless necessary.
Description of the method: This paper is based on first-hand estimations of NRC and CL staff towards QM collected by a knowledge-attitude-behavior survey and interviews. It is written by experienced quality managers, coordinating and developing the NRCs and CLs QMS according to international standards.
Results: A comprehensive QMS can support the work of NRCs and CLs significantly. It has many advantages such as clear areas of responsibility, well-defined processes and a precise, traceable path for handling clinical samples. Moreover, a QMS also facilitates the integration of new legal requirements like the IVDR.
Due to a sophisticated QMS, which includes constant self-assessments, reviews, accreditations and, above all, competent and responsible staff NRCs and CLs can maintain trust in their recommendations and above all their reliability. Finally, this can be proven by the ILAC symbol.
Discussions: Accredited diagnostic creates trust among the addressees of reports. Quality-assured methods and procedures are expected as a basis for statements from a Public Health Institute. A QM offers a competent network throughout the institute. It furthers the exchange of ideas across departments and a mutual-learning approach. A QM is a commitment to truth and correctness.

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NGS-based surveillance of Salmonella enterica ssp. enterica serovar Enteritidis

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Introduction: Among the more than 2.600 *Salmonella* Serovars, *Salmonella* (*S.*) Enteritidis is the most prevalent in industrialized countries provoking not only 50% of human *Salmonella* infections in Europe but also causing significant, often multi-national food-borne outbreaks. Due to the inherent clonality of this serovar unambiguous case identification and source attribution was often hindered since phenotypic and even molecular subtyping methods had been of insufficient discriminatory power.
Objectives: *S.* Enteritidis is one of the prioritized organisms for the intensified next generation sequencing (NGS)-based surveillance at the German NRC aiming to enhance the discrimination among certain subtypes defined by conventional subtyping methods. This would result in a more accurate determination of outbreak events and definite identification of the (food) source of infection.
Methods: In previous years, all S. Enteritidis isolates sent to the NRC underwent phage typing for pre-selection. From strains of the predominant phage types or from suspected outbreak strains genomic DNA was prepared for Illumina short read sequencing. Since 2018 a comprehensive NGS-based surveillance, covering virtually all incoming S. Enteritidis strains, has been carried out. Raw Reads were analyzed by core genome (cg)MLST (EnteroBase scheme in Ridom SeqSphere+) to determine their phylogenetic relationship and potential cluster affiliation. A cluster was defined as a group of at least three isolates differing in max. three alleles to their nearest neighbors.
Results: In the 3-year period 2018 - 2020 more than 1.700 *S.* Enteritidis genomes have been analyzed at the NRC. Phenotypically, the strains had been attributed to >30 different phage types. cgMLST revealed 73 clusters (15 of them comprising >30 isolates and 32 with <5 isolates). Although most clusters consist of only one phage type there is no strict correlation between phage type and cgMLST-based relatedness. Often isolates of the same phage type are more distant from one another than isolates of different phage types indicating that, especially for strains belonging to the predominant phage types, no conclusion can be drawn about their cluster affiliation. Still, even cgMLST sometimes reveals ambigeous results (e.g. in case of merging clusters or spatio-temporally widespread events) highlighting the need for confirmatory epidemiological data.
Conclusion: Applying the high-resolution NGS technique for depicting relationships within the *S.* Enteritidis population gives a comprehensive overview about the circulating lineages and in general reliably points out developing clusters allowing more focused outbreak investigations and subsequent control measures. Here we share our experiences from three years of NGS-based molecular surveillance for *S.* Enteritidis, illustrating the benefits but also describing the challenges that evolved with this new method.

250/RKP

Diversity of Shigatoxin-producing *Escherichia coli* in food in Germany

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Introduction: Shigatoxin-producing *Escherichia coli* (STEC) is a heterogeneous group of zoonotic bacteria also comprising the clinically relevant subgroup of enterohemorrhagic *E. coli* (EHEC). The diversity of STEC detected in food is much higher as compared to the clinical sector. This study will give an overview of STEC isolates derived from food in Germany between 2015 and 2019 and their characteristics.
Material/Methods: We received 1266 STEC isolates from the federal state laboratories on a routine basis for confirmation and typing. Those STEC were isolated from food products of animal

origin (82 %), plant origin (12 %) and production environment (4 %) or not specified (2 %). Serotype, Shiga toxin genes and the presence of additional virulence associated genes like *eae*, *ehxA* and *nleB* were determined by serology and PCR, respectively.
Results: *stx* typing resulted in 544 (43 %) *stx1*-positive STEC with subtypes *stx1a* (42 %), *stx1c* (39 %) and *stx1d* (17 %); 2 % were not further classified. Additionally, 907 isolates (72%) carried only *stx2* with *stx2b* (36 %) being predominant, followed by *stx2e*, *stx2a*, *stx2c/d*, *stx2g*, *stx2d* and *stx2f*. Furthermore, 53 strains (6%) carried two *stx2*-subtypes in varying combinations, while 187 STEC strains (15 %) had both *stx1* and *stx2*. *eae*, encoding for the virulence factor intimin was detected in 11 % of the STEC strains. Of those, 79 STEC carried *stx1* (exclusively *stx1a*) and 65 *stx2* only (*stx2a* 46 %, *stx2c/d* 34 %, others/not typed 20 %). Altogether, 1218 strains were serologically analyzed and comprised 95 different O-antigens; the remaining 185 strains (15 %) could not be typed or were rough. H-typing revealed 31 different H-types with H21 (16 %) and H28 (13 %) being most predominant. Altogether, the analyzed strains could be assigned to 163 different serotypes with the following Top-5 serotypes: O146:H28 (7 %), O146:H21 (4 %), O187:H28 (3 %), O21:H21 (3 %) and O100:H30 (3 %). In contrast, clinically more relevant serotypes O157:H7 (2 %), O26:H11 (2 %), O103:H2 (1 %) and O91:H21 (0.5 %) were less frequently or rarely (O111, O145) identified. STEC O104:H4 was not received ever since the sprout associated outbreak in 2011.

Conclusion: Overall, 65 % of the analyzed food-associated STEC strains are categorized as lowest risk for human illness, based on the concept for risk categorization published by the FAO and WHO experts (2018). However, 2.5 % of the analyzed strains are categorized with the highest risk for severe symptoms like the hemolytic uremic syndrome.
In the near future, routine characterization of STEC strains, also in the food sector, will be increasingly based on whole genome sequencing (WGS) data, thus, significantly enhancing the typing resolution. Establishing an integrated genomic surveillance capable of matching WGS data from the veterinary, food, and human sector will be of utmost importance to gain further insights on STEC sources, distribution, pathogenicity potential and transmission routes.

251/RKP

Two case reports with rare *Brucella* species - one imported and one autochthonous infection - and epidemiology of *Brucella* species from humans in Germany

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Introduction: Human brucellosis is a zoonosis that is associated primarily with sheep, goats, and cattle. It is endemic in the southern Mediterranean region, the Arabian Peninsula, Africa, Asia, and Central and South America. In Germany, brucellosis is a notifiable disease and plays a role as an imported infectious disease. The majority of infections in humans are caused by *Brucella melitensis*, followed by *Brucella abortus* and *Brucella suis*, respectively. Importation occurs either through travellers or immigrants or via contaminated food products such as unpasteurized cheese or raw meat. Autochthonous infections are very rare events.
Case reports: Patient A was diagnosed with brucellosis in November 2020, after consumption of raw cow milk during a three-month trip to Pakistan followed by persisting fever over weeks. *B. abortus* was cultured from blood culture and species was identified by PCR and whole genome sequencing. Patient B suffered from an infected hip joint endoprosthesis in March 2021. *Brucella* spp. was cultured from joint fluid. Interestingly, anamnesis revealed no history of travel but regularly hunting of wild boars in the region of Neubrandenburg. Brucella-specific PCR and whole genome sequencing revealed *B. suis* biovar 2 to be the causative species.
Conclusions: Although rare, in patients presenting with recurrent high fever brucellosis must be considered, especially when coming from endemic regions, but even in people exposed to livestock or game. National annual surveillance data (Infektionsepidemiologisches Jahrbuch) revealed that since 2001 species differentiation was performed for only 45% of brucellosis cases, and in the majority *B. melitensis* was identified (91%). Common identification systems applied in routine labs like MALDI-TOF-MS reliably detect *Brucella* spec., but do not differentiate *Brucella* down to species level. These two cases show that it is worth to perform species-specific PCR or whole genome sequencing in a specialized lab in order to identify the underlying species.

252/RKP

Identification of *Burkholderia cepacia* complex species isolated from cystic fibrosis patients by using the MALDI-TOF Mass Spectrometry System VITEK-MS

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Burkholderia cepacia complex (BCC) represent one of the most feared bacterial species in cystic fibrosis (CF) since lung infection due to BCC is associated with increased morbidity and mortality and predict a poor outcome. The BCC currently comprises at least 22 aproved and closely related species. Although the relative frequency of Burkholderia species may vary considerably from center to center, *B. multivorans* and *B. cenocepacia* are the predominant BCC species among CF patients in the UK, USA, Canada and Europe. *B. cenocepacia* has been associated with high transmissibility, significantly worse survival rate, both before and after lung transplantation and in some cases overwhelming necrotizing pneumonia. Due to the poor post-transplantation outcome B. cenocepacia patients are excluded from lung transplantation programs in several centers. Thus, accurate species identification of the *Burkholderia cepacia* complex (BCC) is a mandatory request for the adeaquate clinical and hygienic management of CF patients. In the era before MALDI-TOF technology becomes available, misidentifcation of BCC species was a major problem in CF microbiology. Regarding the high number of BCC species and ongoing database improvements the diagnostic performance of MALDI-TOF is of ongoing interest for diagnostic microbiology laboratories. We evaluated the performance of the MALDI-TOF Vitek MS system (Biomerieux, France) for the identification of a large set of *Burkholderia* species (reference as well as clinical isolates from CF patients) and several closely related nonfermentative gramnegative organism (e.g. *Pandoraea* spp., *Cupriavidus* spp.) that are typically recovered from CF sputum on the basis of current V3.0 VITEK-MS database (for comparison reference isolates were also tested by the Bruker Biotyper system). In summary, the majority of BCC isolates was correctly identified to species level, however, few misdentifications may occur suggesting that in case of CF patients, having in mind the high clinical importance of BCC in this population, definite species identification (e.g. at least in case of the first detection of a BCC species) should be performed using gene-based approaches (e.g. recA gene PCR, MLST or genome sequencing).

253/RKP

Whole genome-based population structure of *Campylobacter jejuni* and *C. coli* isolates from Germany

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Background: *C. jejuni* is the leading cause of bacterial food-borne infections of humans, which are mainly mediated by contaminated food, most often chicken. The infection is typically self-limiting and restricted to the gastrointestinal tract. In rare cases (1-5%), extra-intestinal manifestations like blood stream infections or reactive-arthritis can be observed. *C. jejuni* is also the leading cause of the rare Guillain-Barré-Syndrome, which is an acute inflammatory polyneuropathy. Since the understanding of the population structure of epidemiologically relevant Campylobacter isolates in Germany is still limited an integrated molecular surveillance of *Campylobacter* from human infections in Germany was established. The primary goal was the extended acquisition of isolates with basic epidemiological data in order to assess the representativeness of the submissions and also to be able to systematically investigate the pathogens down to the genome level. For the collection of *Campylobacter* isolates, numerous primary laboratories contributed nationwide.
Materials and Methods: *Campylobacter* spp. were cultivated under microaerophilic conditions on CCDA and species determination was carried out by PCR. Phenotypic AMR testing was performed using broth micro dilution according to EUCAST. Whole genome sequencing was performed with Illumina NextSeq, yielding paired end sequence reads.
Results: The geographical representation of the target area, in this case Germany, is an important parameter for meaningful surveillance. The obtained isolates displayed a very good coverage of central Germany with NRW, Lower Saxony, Thuringia, Berlin and Brandenburg. Especially with North Rhine-Westphalia, which recorded 21% of the *Campylobacter* enteritis reporting cases, an important federal state was well represented. On the other hand, southern Germany was only represented by Rhineland-Palatinate and Baden-Württemberg. From whole genome sequencing, an overview of the diversity of sequence types occurring in Germany (phylogeny) and the representation of potentially related infection events (cluster detection) could be obtained for 2020. Our data indicate a very diverse *Campylobacter* population. In total, the *C. jejuni* and *C. coli* isolates from 2020 represented at least 160 different MLST sequence types. Nonetheless, several prominent clusters with >20 isolates could be identified for *C. jejuni*.
Discussion: A genome-based *Campylobacter* surveillance with coverage of important federal states has been successful, but must be expanded in the future with regard to representativeness, especially from laboratories in northern and southern Germany. Several larger clusters of >10 isolates have already been successfully detected and will be further monitored.

Postersession 01
13. Sep. 2021 • 11:15–12:45

Part 1: Zoonoses (FG ZO): Infection Epidemiology and Population Genetics (FG MS)

254/MSP

Molecular epidemiologic analysis of two simultaneous outbreaks involving *Pseudomonas aeruginosa*: VIM-2 positive ST111 and blaOXA-74 positive ST235

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Objective: Water bearing systems have long been recognized to represent a high risk for *Pseudomonas aeruginosa* (PA) outbreaks in the hospital setting. However, many older buildings are still equipped with water sources (sink, shower, toilet) in high-risk departments such as ICU and Haematology/Oncology. We present 2 simultaneous prolonged MDR PA outbreaks in the ICU of a pulmonology clinic, which were resolved by Whole Genome Sequencing with Multilocus Sequence Typing (MLST) and ad-hoc core-genome (cg) MLST analysis
Methods: After the initial suspect of a PA outbreak, patients were screened weekly for 4 months for MDR gram-negative rods. All sink drains and toilets were sampled immediately, and, if positive, every 4 weeks while being disinfected twice a week. Once negative for MDR PA, the sampling was extended to every 3 months for a total of 15 months since outbreak detection. All patient and environmental samples were cultured on selective agar for MDR gram-negative bacteria. Identification with MALDI-ToF and (for clinical isolates) antimicrobial resistance testing with the agar dilution method were done. All isolates were analysed by whole-genome sequencing (WGS). The genetic relatedness and in silico antimicrobial resistance were evaluated with the Ridom SeqSphere+ sofware.
Results: From April to September 2019, 9 patients acquired a MDR PA strain during their in hospital stay. 5 of these carried a VIM-2 MBL and one patient carried the lesser known OXA-2 and OXA-74 beta-lactamase, which are also suspected to cause resistance to carbapenems. Extensive environmental investigations detected VIM-2 positive PA in 8 sink drains, 1 toilet and 1 cleaning bucket across the ICU. Concomitantly, 6 VIM-2 negative PA isolates were detected in 4 toilets, 1 sink drain and 1 shower drain. MLST showed that 10/12 VIM-2 positive isolates belonged to ST111, and a patient and 4 toilet water isolates were identified as ST235 and carried blaOXA-2 and blaOXA-74. Further 5 different ST with (2 isolate) or without (3 isolates) VIM-2 were detected: ST17, ST233, ST273, ST308 and ST446. The ad-hoc cgMLST revealed 3 ST111 clusters, one of which comprises 12/15 isolates. The ST235 isolates formed two clusters of 4 and 2 isolates, respectively. Due to the extensive contamination, all affected sites were disinfected with an oxygen releasing substance and all U-bends and cleaning buckets were exchanged. During the 15-month follow-up period, 253 environmental samples were collected, but no further MDR PA could be cultivated. Further, weekly patient screening was implemented for 4 months, but no further MDR PA acquisitions were detected.
Conclusion: While PA is widely spread in water bearing systems, WGS showed that only two main ST were involved in our hospital MDR PA outbreak. With immediate screening of patients in combination with powerful disinfection and radical exchange of contaminated devices we were able to terminate the MDR PA outbreaks in a short period of time.

255/MSP

Rising numbers of vancomycin-resistant *Enterococcus faecium* during 2020 show oligoclonal strain distribution: molecular epidemiology of VRE in a tertiary care university hospital in Germany

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Background: Vancomycin-resistant enterococci (VRE) were first described in the 1980s and have spread throughout Europe ever since. According to the yearly report of ECDC, the percentage of VRE have been rising for years and has reached 18,3% of invasive *E. faecium* (VREfm) isolates in the EU/EEA in 2019. This worrisome development is reflected in the rising numbers of VRE in clinical materials and the spread of hospital-associated clones, which belong to the clonal complex (CC) 17. We evaluated the clonality of the strains detected at a tertiary care university hospital in Germany during 2020.
Methods: We set out to analyse the first isolate of all patients carrying or infected with VREfm admitted to a tertiary care hospital during the year 2020. The presence of *vanA* or *vanB* was determined using a specific PCR. The molecular characterisation of the isolates was performed with whole genome sequencing (WGS), followed by Multilocus Sequence Typing (MLST) and core-genome (cg) MLST.
Results: A total of 310 patients with VREfm were treated at our hospital during 2020. The VREfm isolates originate from rectal

screening swabs (46.8%), urine (23.5%), bloodstream (5.8%), bile fluid (5.5%) and other clinical materials (18.1%). The *vanA* genotype was present in 43.6% of all isolates, whereas *vanB* was detected in 56.4%. Until now 97.% (303/310) of these isolates were sequenced and all isolates could be attributed to CC17. 15.2% (46/303) sequences were excluded from the current evaluation due to insufficient sequence quality or conflicting data. The WGS confirmed that clonality was high, with 8 different strain types among the isolates according to MLST (ST80, ST117, ST1299, ST78, ST275, ST262, ST721 and ST17). 3 STs predominated in this population: ST80 (33%), ST117 (30.7%) and ST1299 (28.8%). The resistance to vancomycin occurs due to *vanA* in all ST1299 isolates, whereas *vanB* is the predominating mechanism in ST80 (88.2%) and ST117 (77.2%). This pattern is also confirmed by cgMLST. One complex type (CT) in combination with one resistance gene dominates per ST: CT1065 (83.5% of ST80 with 100% *vanB*), CT71 (67.1% of ST117 with 100% *vanB*) and CT1903 (77% of ST1299 with 100% *vanA*). Finally, the number of probable VRE transmission and outbreak clusters on our ICUs differs greatly when determined by epidemiologic parameters (19 events), and cgMLST (9 events). The events confirmed by cgMLST involved a maximum of 3 isolates (defined as difference of ≤ 2 alleles) compared to 2-7 isolates according to epidemiologic data.

Conclusion: Our findings demonstrate the predominance of 3 STs within our hospital. While most isolates reflect strains detected worldwide, ST80 and ST117, about one third of the isolates represent the emerging strain ST1299, which lead to a significant rise in *vanA* at our hospital.

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Antimicrobial susceptibility and phylogenetic relations in a German cohort infected with *Mycobacterium abscessus*

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Introduction: *Mycobacterium abscessus* (Mab) is a highly antibiotic-resistant opportunistic pathogen causing clinically challenging infections in patients with preexisting lung diseases or under immunosuppression. Hence, reliable antibiotic susceptibility data are required for effective treatment. Aims of this study were to investigate (i) the congruence of genotypic and phenotypic antimicrobial susceptibility testing, (ii) the relationship between resistance profile and clinical course, and (iii) the phylogenetic relations of *M. abscessus* in a German patient cohort.

Methods: A total of 39 isolates from 29 patients infected or colonized with *M. abscessus* underwent genotypic and phenotypic drug susceptibility testing. Clinical data were correlated with susceptibility data. Phylogenetic analysis was performed by means of whole genome sequencing (WGS) and single nucleotide polymorphism (SNP) analysis.

Results: Macrolide resistance was mainly mediated by functional Erm(41) methyltransferases (T28 sequevars) in *M. abscessus* subsp. *abscessus* (n = 25) and *M. abscessus* subsp. *bolletii* (n = 2). It was significantly associated with impaired culture conversion (P = 0.02), but not with higher mortality (p=0.66). Aminoglycoside resistance due to mutations in the *rrs* gene was rare in our cohort and only found in two isolates from two different patients. According to the core SNP phylogeny, we identified three clusters of closely related isolates with SNP distances below 25. Representatives of all circulating global clones (Absc. 1, Absc. 2, and Mass. 1) were identified in our cohort. However, we could not determine evidence for in-hospital interhuman transmission from clinical data.

Conclusion: In our patient cohort, we identified three *M. abscessus* clusters with closely related isolates and representatives of the previously described international clusters but no human-to-human in-hospital transmission. Macrolide and aminoglycoside susceptibility data are critical for therapeutic decision-making in *M. abscessus* infections.

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Antimicrobial resistance of *Neisseria gonorrhoeae* in Germany 2016-2020, results from the Gonococcal Resistance Network (GORENET)

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Introduction: In Germany, *Neisseria gonorrhoeae* (NG) infections are not reportable and limited data on epidemiology and antimicrobial resistance (AMR) are available. Within the Gonococcal Resistance Network (GORENET) we monitor the NG-AMR in Germany to inform treatment guidelines (1).

Methods: Between 2016-2020, 75 laboratories sent epidemiological data and NG-isolates to the Robert Koch Institute and the German reference laboratory for NG. Isolates were tested for ceftriaxone, cefixime, azithromycin, ciprofloxacin, and penicillin susceptibility using MIC-strip test. AMR-results were interpreted according to the EUCAST breakpoints version 10.0. The proportion of resistant isolates was calculated.

Results: From 2016-2020, 2443 isolates from 75 laboratories were tested. Of these, 88.3% were from men and 10.5% from women, for 1.2% the sex was unknown. Median age was 33 (men) and 29 years (women). Most isolates from men were urethral (86.0%) or rectal (4.8%). Regarding women, most isolates were reported to originate from the cervix (48.6%) or the vagina (22.1%). Resistance to ceftriaxone was detected in one sample in 2018 and resistance to cefixime was low throughout the observation period, with a peak in 2019 with 1.9% of resistant isolates (Table 1). Isolates with minimal inhibitory concentrations above the epidemiological cut-off for azithromycin were increasing from 2016-2020. Resistance to ciprofloxacin and penicillin was high, with average proportions of resistant isolates of 60.4% (ciprofloxacin) and 17.4% (penicillin), respectively.

Discussion: Comparable to other Western countries, the majority of NG isolates originated from men (2). The first-line substance ceftriaxone remains effective in Germany, whereas resistance to the important second first-line agent azithromycin nearly doubled from 2019 to 2020. Thus, ceftriaxone monotherapy is recommended in Germany since 2019 if patient adherence is warranted (3). Cefixime-resistance remained low. Penicillin and ciprofloxacin resistance were stably high. NG susceptibility testing is crucial to monitor the AMR-situation in Germany and to inform treatment guidelines.

Table 1. Proportion of resistant isolates per year and antibiotic. For azithromycin, the isolates with minimal inhibitory concentrations above the epidemiological cut-off (>1mg/L) are shown.

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Fig. 1

Table 1. Proportion of resistant isolates per year and antibiotic. For azithromycin, the isolates with minimal inhibitory concentrations above the epidemiological cut-off (>1mg/L) are shown.

	Proportion of resistant isolates (%)				
	2016	**2017**	**2018**	**2019**	**2020**
Azithromycin	0.8	1.0	3.7	6.6	12.8
Cefixime	0.6	0.6	0.9	1.9	0.8
Ceftriaxone	0	0	0.2	0	0
Ciprofloxacin	54.3	63.8	58.5	66.2	59.5
Penicillin	16.1	15.1	17.0	21.2	17.9

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Comparison of strain typing by PFGE and cgMLST in nosocomial *P. aeruginosa* outbreaks

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Background: The worldwide spread and increase of multidrug-resistant Gram-negative bacteria represents a serious threat to public health. In particular, healthcare-associated infections in the context of hospital outbreaks are of major concern. Pulsed-field gel electrophoresis (PFGE) has been widely used for bacterial strain typing in nosocomial outbreak investigations. However, next-generation sequencing coupled with cgMLST (core genome multi-locus sequence typing) analysis has become a powerful tool to investigate the molecular epidemiology of health-care associated outbreaks.
Here, we compare typing results of PFGE and cgMLST in several nosocomial *Pseudomonas aeruginosa* outbreaks. Further, we analyze the genetic background of *bla*$_{VIM-2}$ carrying *P. aeruginosa* isolates in Germany.
Methods: Strain typing of clinical *P. aeruginosa* isolates was performed by PFGE using the SpeI restriction enzyme. Analysis of PFGE results was conducted using the BioNumerics software (Applied Maths) following the guidelines by Tenover et al., 1995. Whole-cell DNA of isolates was subjected to WGS on an Illumina MiSeq platform with 2 x 300 bp paired end reads. Genome assembly and cgMLST analysis were performed using the SeqSphere software (Ridom).
Results: Strain typing of outbreak-associated *P. aeruginosa* isolates using PFGE and cgMLST revealed similar results. Isolates assigned to the same genotype via PFGE were also identified as part of the outbreak cluster by cgMLST, albeit several isolates were assigned to different complex types. Furthermore, both methods were able to uncover profound unrelatedness between outbreak-associated strains and isolates from the same hospital setting but unrelated to the analyzed outbreak. Interestingly, most of the outbreak strains belonged to ST111 which has been previously described as high-risk clone frequently associated with nosocomial outbreaks.
Conclusions: Both PFGE and cgMLST are powerful tools to analyze healthcare-associated outbreaks. However, considering the increasing spread of multidrug-resistance among Gram-negative bacteria and the urgent need of national and international molecular surveillance programs, WGS-based typing represents the more objective and, most importantly, reproducible and thus comparable method on a global scale.

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Molecular epidemiology of vancomycin-resistant enterococci in North Rhine-Westphalia: a post-COVID surveillance update

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Introduction: Vancomycin-resistant enterococci (VRE) are multidrug-resistant organisms that pose a significant challenge in health care settings. The incidence of VRE bloodstream infections (VREBI) continues to increase globally. In Germany, the proportion of VRE among enterococci bloodstream infections increased from 10.5% to 26.3% between 2016 and 2019. Due to the COVID-19 pandemic, hospitals in North Rhine-Westphalia (NRW) reported 13% less cases in 2020 than in 2019. Here, we sought to analyze the possible effect of lower hospital admissions in the transmission of VRE, a primarily nosocomial pathogen. As opposed to colonization, subject to active screening and thus prone to underreporting, VREBI constitute a more reliable indicator of VRE incidence, since adequate diagnosis and hospital treatment are mandatory in all cases.
Methods: VRE isolates from patients with VREBI admitted to hospitals in NRW were collected by a network of 31 laboratories across the state. Only the first isolate of each patient was included in the study. Molecular analysis was performed, identifying multilocus sequence types (MLST) and vancomycin resistance determinants.
Results: In 2020, 174 VREBI cases were reported, compared to 259 in 2019. The incidence per 100,000 inhabitants dropped to 0.97 in 2020 after continuously increasing from 0.52 in 2016 to 1.44 in 2019. The most common STs were ST117 and ST80. Other STs reported in previous years (ST203, ST192, ST17 and ST78) were not detected in 2020. The proportion of ST117 decreased to 58% in 2020 from 74% in 2019, while the proportion of ST80 nearly doubled from 19% in 2019 to 36% in 2020. After a steady increase of the proportion of *vanB*-positive isolates observed between 2016 and 2019, *vanB* decreased from 81% in 2019 to 61% in 2020. In contrast, the proportion of *vanA*-positive isolates increased for the first time since the beginning of the surveillance period from 19% in 2019 to 39% in 2020.
Conclusions: Given the lack of comprehensive data on VRE colonization rates in VRE, VREBI data allows to hypothesize that a decrease in hospital admissions in 2020 in NRW resulted in a lower VRE colonization pressure and therefore less VREBI cases. The decrease of the previously most prevalent molecular features (ST117, *vanB*) could indicate that strains displaying ST80 and *vanA* have an intrinsic advantage that facilitated their transmission in an epidemiological situation less favorable for the spread of nosocomial pathogens. This findings warrant further surveillance and investigation.

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WGS-based analysis of *Campylobacter jejuni* isolated from commercial turkey farms in Germany

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Background: *Campylobacter* (*C.*) *spp.* are among the most frequent causes of bacterial gastroenteritis worldwide. In Germany, campylobacteriosis has been mainly attributed to *C. jejuni* and poultry has been reported as a main reservoir. However, there is a lack of knowledge in the epidemiology and genetic diversity of *C. jejuni* isolates from poultry particularly from turkeys. Following a Whole-Genome-Sequencing (WGS)-based approach, this study aims to understand the epidemiology and assess the genetic traits of *C. jejuni* from commercial turkey flocks in Germany.
Methodology: sixty-six *C. jejuni* were collected from epidemiologically unrelated commercial meat turkey flocks from different regions of Germany between 2010 and 2011. Phenotypic antimicrobial susceptibility testing was performed and the genomic DNA was sequenced using Illumina MiSeq. Bioinformatics

analysis comprised phylogeny and detection of antimicrobial resistant genes, virulence determinants and plasmid identification.
Results: The WGS analysis revealed a high genetic diversity indicated by 29 different sequence types and an average core-gene single nucleotide-polymorphisms distance of 14585 cgSNPs (0-26540). Resistance associated genes for at least four different antibiotic families were found in concordance to the phenotype. The genes *tet*O, *tet (O-32-O)*, *sat*4, *bla*OXA-184 and *bla*OXA-193 detected in 52%, 9%, 9%, 23% and 47%, respectively of isolated *C. jejuni*. The mutations in housekeeping genes *gyrA*_T86I and 50S were identified in 66,7% and 8%, respectively. The *cme*ABCR multidrug efflux complex was determined in all isolates. The sequenced strains carry plasmids belonging to three types including pTet plasmids (35%), pVir plasmids (5%) and small plasmids (6%). There was association between strains carrying pTet plasmids and the detection of the *tet*O gene associated with tetracyclines resistance as well as the Type IV secretion system proteins (VirB8, B9, B10, B11 and VirD4) and the virulence genes coding for flagellin protein A (*fla*A) and B (*fla*B).
Conclusion: This study demonstrates that WGS is a suitable method to study the epidemiology and genetic traits of *C. jejuni*. WGS demonstrated a high level of genetic heterogeneity of *C. jejuni* isolated from a turkey flock indicating that a single flock can be infected by multiple genotypes within one rearing cycle.
Key words: *C. jejuni*, turkey, WGS, AMR, genetic diversity, Germany

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Mammaliicoccus spp. from German dairy farms exhibit a wide range of antimicrobial resistance genes and non-wildtype phenotypes to several antibiotic classes

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Introduction: Mammaliicocci might play a major role in antimicrobial resistance (AMR) gene transmission between organisms of the family *Staphylococcaceae* such as the potential pathogenic species *S. aureus*. Regarding the One Health approach, AMR gene transmission is of high importance with respect to treatment options for serious diseases caused by staphylococci in humans and animals. The interest of our study was to analyze AMR profiles of mammaliicocci from German dairy farms to evaluate possible risks of AMR development on the farms.
Materials and Methods: On 17 German dairy farms with a history of MRSA findings, mammaliicocci were obtained from milk and swab samples of animals and from the environment. In total, 65 isolates were analyzed for AMR genes using whole genome sequencing and for phenotypic AMR determined by broth microdilution against 19 antibiotics. Non-wildtype phenotypes were interpreted according to epidemiological cut-off values of EUCAST.
Results: The genotypic and phenotypic AMR profiles of mammaliicocci from German dairy farms varied between isolates to some extent, indicating an occurrence of several strains on the farms. The isolates exhibited a non-wildtype phenotype to penicillin (58/65), cefoxitin (25/65), chloramphenicol (26/65), ciprofloxacin (25/65), clindamycin (38/65), erythromycin (17/65), fusidic acid (61/65), gentamicin (8/65), kanamycin (9/65), linezolid (1/65), mupirocin (4/64), rifampicin (1/65), sulfamethoxazol (1/65), streptomycin (20/65), quinupristin-dalfopristin (26/65), tetracycline (37/65), tiamulin (59/65) and trimethoprim (30/65). Corresponding AMR genes against different antimicrobial classes were also detected. Linezolid resistance was associated with the *cfr* gene in the respective isolate. However, discrepancies between genotypic prediction and phenotypic resistance profiles such as for fusidic acid or rifampicin were also observed.
Discussion: Mammaliicocci from dairy farms may carry a broad variety of antimicrobial resistance genes and exhibit non-wildtype phenotypes to several antimicrobial classes. It cannot be ruled out that resistance genes are transmitted from mammaliicocci to more pathogenic species of the family *Staphylococcaceae* increasing the risk of difficult-to-treat infections in humans and animals. In addition, some mismatches of AMR gene prediction and the respective phenotype illustrate the need for well-curated databases to efficiently monitor the AMR repertoire of different *Staphylococcaceae*.

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Whole genome sequencing of bacteria grown on MGIT reveals good results concerning most relevant antibiotic resistance genes

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Introduction: Worldwide, tuberculosis is considered one of the top 10 causes of death with increasing resistance to the key first line drugs isoniazid, ethambutol, pyrazinamide and rifampicin. In 2019 nearly half a million people developed rifampicin-resistant TB, of which 78 percent was multidrug resistant (1). Methods for drug resistance detection include rapid molecular tests, culture methods and sequencing technologies. Despite the advantage of Whole Genome Sequencing (WGS) of high resolution, the method is time consuming. In order to accelerate the method for drug susceptibility detection we evaluated Whole Genome Sequencing of bacteria grown in liquid (Mycobacteria growth indicator tube, MGIT) media against bacteria grown on solid (Löwenstein-Jensen) media.
Material/Methods: A total of 217 sputum specimens were cultured on solid and MGIT media. The bacterial DNA was isolated from cells, followed by library preparation and paired-end sequencing on a Miseq instrument. Genomic data was analyzed for resistance single nucleotid polymorphisms (SNPs) regarding antibiotic resistance.
Results: 177 MGIT-samples (82 percent) revealed the same genotypic resistances as samples from solid media regarding first line antibiotics isoniazid, ethambutol, pyrazinamide and rifampicin. In comparison to fluoroquinolones, aminoglycoside resistance was not always evaluable. In 39 MGIT-samples (18.1 percent) the sequencing failed.
Discussion: With a time saving of up to seven days, WGS of bacteria grown on MGIT can be considered as an optional method of drug susceptibility detection, in particular for the four first-line antibiotics isoniazid, rifampicin, ethambutol and pyrazinamide.

- (1) Global Tuberculosis Report 2020. 2020. WHO

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Insights into monitoring and prevalence studies of circulating zoonotic pathogens in German wildlife

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Rationale/Question: Information on the prevalence of zoonotic pathogens in German wildlife is scarce and long term pathogen monitoring or surveillance projects including viruses, virus-likes, bacteria and parasites did not exist previously. In 2017, the German Federal Institute for Risk Assessment (BfR) initiated a widespread monitoring project to assess presence of several zoonotic pathogens in wildlife, which can be pathogenic in humans. Here, we present data indicating presence of zoonotic pathogens from the spectrum mentioned above in an ongoing pathogen-monitoring project conducted in defined wildlife habitats in Germany (2017-2021). Animals included in the study are herbivores and omnivores that are typically hunted as game and common carnivores in Germany such as raccoon dog and fox. In longitudinal studies, environmental and ecological factors impacting pathogen prevalence are studied in conjunction. In particular, such factors comprise climate and weather conditions including implications from climate change, migratory behavior of pathogen host animals into new ecosystems or repopulation of previously common habitats and moreover pathogen population of present or emerging ecological niches. These pathogens are investigated for their zoonotic determinants and their potential to persist in their ecosystems (pathogen-host interactions). Ultimately, the data is implemented to assess pathogen occurrence including emerging and re-emerging

pathogens and prevalence in wildlife in general and to conduct or improve risk and hazard analyses with regard to human health.
Methods and Results: During the hunting seasons 2017/18, 2018/19, 2019/20 and 2020/21 samples of heart muscle, foreleg muscle, fatty tissue, fascia, diaphragm, liver, tongue, tonsils and blood were collected and tested for presence of antibodies and DNA for i) parasites: *Toxoplasma gondii*, *Cryptosporidium* spp., *Alaria alata* mesocercariae; ii) bacteria: *Yersinia* spp., *Campylobacter* spp.; iii) viruses: Hepatitis E virus, rotavirus; and proteins: SPHINX/BMMF like particles. A collection of most interesting findings from start of the project to current state will be presented.
Conclusion: Our data suggests autochthonous circulation of all investigated pathogens. Acquired data further allows us to hypothesize a direct dependency of climate conditions-pathogen-host interactions where pathogens are also associated with the environment.

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Genomic analysis of *Staphylococcus aureus* from the West African Dwarf (WAD) Goat in Nigeria

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Introduction: *Staphylococcus aureus* colonize the skin and mucous membranes of various host species, and human-animal interaction is a significant factor for cross-species transmission. However, data on *S. aureus* colonization in animals, particularly on ruminants in close contact with humans, is limited. The West African Dwarf (WAD) goat is a major livestock resource, particularly with rural dwellers and small-holder farmers, in West and Central Africa. This study aimed to investigate the population structure, antibiotic resistance, and virulence gene determinants of *S. aureus* from the WAD goat in Nigeria.
Methods: Nasal samples (n=726) were obtained from the WAD goat in five markets in Osun State, South-West Nigeria. *S. aureus* was characterized by antibiotic susceptibility testing, detection of virulence determinants, *spa* typing, and multilocus sequence typing (MLST). Representative isolates were selected for whole-genome sequencing, biofilm, and cytotoxicity assay.
Results: In this study, 90 *S. aureus* were recovered, of which 86 isolates were methicillin-susceptible, and four were *mecA*-positive (i.e., methicillin-resistant *S. aureus* [MRSA]). A diverse *S. aureus* clonal population was observed (20 sequence types [STs] and 37 *spa* types), while 35% (13/37) and 40% (8/20) were new *spa* types and STs, respectively. Eleven MLST clonal complexes (CC) were identified (CC1, CC5, CC8, CC15, CC30, CC45, CC97, CC121, CC133, CC152, CC522). The MRSA isolates were identified as t127-ST852-CC1-SCC*mec* type VII, t4690-ST152-CC152-SCC*mec* type Vc, and t8821-ST152-CC152-SCC*mec* type Vc. Phylogenetic analysis revealed that 60% (54/90) of all isolates were associated with ruminant lineages (i.e., CC133, CC522). Panton-Valentine Leukocidin (PVL)-positive *S. aureus* was identified in CC1, CC30, CC121, and CC152.
Conclusions: This is the first detailed investigation on the genomic content of *S. aureus* from the WAD goat in Nigeria. We provide evidence on the pathogenic potential of CC522 *S. aureus* by detecting the toxic shock syndrome gene and hemolysins, strong cytotoxicity, and ability to form biofilms. The *S. aureus* population of the WAD goat consists mainly of ruminant-associated lineages (e.g., CC133, CC522), interspersed with human-associated clones, including PVL-positive MRSA CC1 and CC152.
Keywords: *Staphylococcus aureus*, Ruminants, Goats, Whole genome sequencing, Nigeria

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The transmission risk of multidrug-resistant organisms between pets and humans – Preliminary results of an exploratory case control study

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Background: This project aims to assess the relevance of pet husbandry in the colonization of multidrug-resistant organisms (MDROs) of hospital patients. Currently, the potential role of pets as reservoirs of MDROs is still unclear. The project focusses on the most common MDROs in pet owners, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), 3rd generation cephalosporin-resistant Enterobacterales (3GCRE) and carbapenem-resistant Enterobacterales (CRE).
Materials/Methods: We perform an exploratory, unmatched case-control-study. Among questions about well-known risk factors, study participants are queried regarding their contact to dogs and cats. This includes information about the number of pets in the household, the closeness of contact and diseases as well as medical treatment of the pets. To assess the genetic relatedness of the human and pet MDROs, we collect nasal and rectal swabs of the participants in the hospital and their pets to test them for MDROs. Phenotypically matching MDROs in the samples of participants and their pets will be tested for genetic relatedness using whole genome sequencing (WGS). The study is currently being performed at the Charité Universitätsmedizin Berlin. The sample size will comprise about 4,000 human participants and aims at 1,000 animal samples. The study is funded by the Federal Department of Health (BMG).
Results: Among 3,285 participants so far, 25% (820/3,285) tested positive for MDROs. 54% (1,785/3,285) of participants were male and the mean age was 63 years (18-98 years). 21% of the participants (701/3,285) stated to own at least one pet dog or cat (range 1-10). Among the first 345 returned and analyzed pet samples, 12% (42/345) were positive for MDROs. In three cases MDROs of pet and owner were phenotypically matching. The matching pathogens were in two cases VRE and in the other case 3GCRE. WGS analyses revealed that two of the pairs were also genotypically matching. Further preliminary results of the first 3,500 participants will be presented at the ICPIC 2021.
Conclusions: The investigation of pet husbandry as a risk factor for colonization or infection with MDRO in this study creates an opportunity to identify patients at risk and develop potential prevention strategies. Although it is known that transmission between humans and pets basically is possible, the likelihood of this transmission is still unclear and the effect size should be investigated.

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Anti-pathogenic and immune-modulatory effects of peroral treatment with cardamom essential oil in acute murine campylobacteriosis

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Question: Human infections with enteropathogenic *Campylobacter jejuni* (*C. jejuni*) including multi-drug resistant isolates are increasing worldwide. Antibiotics-independent approaches in the combat of campylobacteriosis are therefore highly desirable. Since the health-beneficial including anti-inflammatory and anti-infectious properties of cardamom have been acknowledged for long, we here addressed potential anti-pathogenic and immune-modulatory effects of this natural compound during acute campylobacteriosis.
Methods: For this purpose, microbiota-depleted IL-10$^{-/-}$ mice were subjected to the peroral cardamom EO treatment from day 2 until

day 6 post infection, via the drinking water (*ad libitum*). Given a daily drinking volume of approximately 5 mL and a mean body weight of 20 g, the daily cardamom EO dose infected mice received was 258 mg per kg body weight.
Results: Cardamom EO treatment resulted in lower intestinal pathogen loads and improved clinical outcome of mice as early as day 3 post-infection. Furthermore, when compared to mock controls, cardamom EO treated mice displayed less distinct macroscopic and microscopic inflammatory sequelae on day 6 post-infection that were paralleled by lower colonic numbers of macrophages, monocytes, and T cells and diminished pro-inflammatory mediator secretion not only in the intestinal tract, but also in extra-intestinal and, remarkably, systemic organs.
Conclusions: Our preclinical intervention study provides the first evidence that cardamom EO comprises a promising compound for the combat of acute campylobacteriosis and presumably prevention of post-infectious morbidities.

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Garlic essential oil as promising ption for the treatment of acute campylobacteriosis – Results from a preclinical placebo-controlled intervention study
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Question: Since human infections with *Campylobacter jejuni* including antibiotic-resistant strains are rising worldwide, natural compounds might constitute promising antibiotics-independent treatment options for campylobacteriosis. Since the health-beneficial properties of garlic have been known for centuries, we here surveyed the antimicrobial and immune-modulatory effects of garlic essential oil (EO) in acute experimental campylobacteriosis.
Methods: Secondary abiotic IL-10-/- mice were orally infected with *C. jejuni* strain 81-176. Treatment with garlic-EO was initiated on day 2 post-infection. and performed until the end of the observation period (i.e., day 6 post infection). Therefore, the natural compound was dissolved in sterile phosphate buffered saline and 0.05% carboxymethyl cellulose and then applied to autoclaved tap water (final concentration of 1 g/L; daily dose of 200 mg/kg body weight). The placebo control mice received respective solution without the natural compound.
Results: Mice from the garlic-EO group displayed less severe clinical signs of acute campylobacteriosis as compared to placebo counterparts that were associated with lower ileal *C. jejuni* burdens on day 6 post infection. Furthermore, when compared to placebo application, garlic-EO treatment resulted in alleviated colonic epithelia cell apoptosis, in less pronounced C. jejuni induced immune cell responses in the large intestines, in dampened pro-inflammatory mediator secretion in intestinal and extra-intestinal compartments, and, finally, in less frequent translocation of viable pathogens from the intestines to distinct organs.
Conclusions: Given its potent immune-modulatory and disease-alleviating effects as shown in our actual preclinical placebo-controlled intervention study, we conclude that garlic-EO may be considered as promising adjunct treatment option for acute campylobacteriosis in humans.

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A BceAB-like transmembrane transporter mediates high daptomycin resistance in a livestock-associated *Mammaliicoccus sciuri* isolate
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Question: The lipopeptide antibiotic daptomycin (DAP) is commonly used to treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, resistances to DAP and other last resort antibiotics are increasingly reported. Mutations in the multiple peptide resistance factor (*mprF*) gene are common after DAP treatment and are suggested to be associated with DAP insusceptibility in *S. aureus* (Bayer et al. 2013 Ann. N.Y. Acad. Sci. 1277 139-158). However, additional resistance mechanisms seem to exist in staphylococci as well. Recently, we isolated a *Mammaliicoccus sciuri* strain from livestock which was highly DAP resistant (MIC ≥64 µg/ml) (Schoenfelder et al. 2017 Vet. Microbiol. 200: 79-87). As the *M. sciuri mprF* gene was found to be intact, the question arises how high-level DAP insusceptibility is mediated in this strain.
Methods: To reveal the underlying resistance mechanism, the isolate was pulse-exposed to high DAP concentrations, and subsequent transcriptome analyses was performed to identify upregulated genes. To verify their putative role in DAP resistance, candidate genes were then ectopically expressed under an inducible promoter in a DAP-sensitive *S. aureus* strain. Concomitantly, culture supernatants of the recombinant strains were analysed by LC/MS to monitor putative changes in daptomycin concentration and/ or its chemical integrity.
Results: *bceA* and *bceB* were identified as the most upregulated genes upon DAP treatment ($\log_2$ fold change 8.45 and 8.72 respectively). They encode an ATP transmembrane transporter originally described in *Bacillus subtilis* as the cause of bacitracin resistance (Podlesek et al. 1995 Mol. Micro. 16(5) 969-976). Ectopic expression of the *bceAB* genes in a DAP-sensitive *S. aureus* strain (MIC 1 µg/ml), rendered the strain DAP resistant (MIC 64 µg/ml). Moreover, the DAP-resistant phenotype was abolished upon introduction of early stop codons in either of the two *bce* genes. Upon transcription activation of *bceAB,* an 83 % reduction of DAP concentration in the supernatant was recorded, but LC/MS analysis revealed no degradation of the compound.
Conclusion: Although the exact functions of the membrane-associated Bce proteins are not fully understood yet, the data strongly suggest that *bce*AB is capable to mediate high-level DAP resistance in staphylococci. Investigations are currently under way to explore the genetic origin of the gene cluster and its potential for spread via horizontal gene transfer.

Postersession 02
14. Sep. 2021 • 11:15–12:45

Part 2: Zoonoses (FG ZO): Infection Epidemiology and Population Genetics (FG MS)

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Outbreak with methicillin resistant *Staphylococcus aureus* isolates lacking *mec* determinants on a neonatal ICU of a German hospital
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Question: *Staphylococcus aureus* MRLM (methicillin resistant lacking *mec*) strains are easily misclassified as methicillin susceptible (MSSA) based on the exclusive detection of *mec* genes or PBP2a. Hence, these isolates pose a threat to public health and represent a diagnostic and therapeutic challenge. Recent studies demonstrated an association of the MRLM phenotype to mutations in the phosphodiesterase GdpP. However, it is unknown how the MRLM phenotype is selected in the clinical environment. In this study, nine *S. aureus* strains (MRLM [n=6]; MSSA [n=3]), isolated from four infants during routine nasal screening at the neonatal intensive care unit (NICU) of a German hospital were investigated.
Methods: Oxacillin and cefoxitin minimal inhibitory concentrations (MIC) were determined by broth microdilution. The presence of *mec* genes was tested by PCR. *Spa*-type, MLST and cg-MLST complex-type (cgMLST CT) were deduced from whole-

genome sequences (WGS). Based on WGS, the relatedness of isolates was analyzed and visualized in a minimum spanning tree. Comparative genomics were performed using a combination of cgMLST analysis and contig alignments.
Results: All isolates were assigned to *spa*-type t3338, ST7 and CT20916. They differed from each other in a maximum of 8 of the 2249 genomic loci included in cgMLST analysis, indicating a common origin of the isolates. Six isolates displayed elevated MICs to oxacillin (MIC ≥ 2 mg/L) and cefoxitin (MIC > 4 mg/L), but lacked *mec* determinants (MRLM). Five MRLM isolates had distinct mutations in *gdpP* suggesting that these polymorphisms had evolved independently in individual infants and that the common precursor of all isolates was likely an MSSA. Beyond the mutations in *gdpP*, an unusually large number of mutations in other genomic loci were noticed. This could be due to a mutation in the DNA repair gene *mutS*, which is present in all isolates examined in comparison to reference isolates of clonal line ST7.
Conclusions: We describe an outbreak involving MRLM on a NICU. Our data indicate that initially an MSSA precursor was transmitted between neonates. This strain must have acquired resistance to β-lactams in each infant independently, under the action of a hitherto unknown selection pressure, through different mutations in *gdpP*.

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Insight into metabolic differences of porcine methicillin-resistant *Staphylococcus aureus* of CC398 and CC9 from Germany and China

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Question: Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), which are often multi-resistant to antimicrobial agents, may pose a health threat to people with frequent exposure to swine as such isolates can easily cross species barriers. Reports of infections in pigs and humans have raised awareness of the opportunistic pathogen's zoonotic potential. The global prevalence of LA-MRSA differs geographically. Contrary to the predominance of clonal complex (CC) 398 in Europe and North America, CC9 is the dominant CC in Asia. In this study, we investigated porcine LA-MRSA from Germany and China for differing metabolic properties that may explain the geographically varying success of the two CCs.
Methods: Overall, 20 porcine LA-MRSA isolates were investigated, including five dominant MRSA-CC398 from Germany and five dominant MRSA-CC9 from China as well as five rare MRSA-CC9 from Germany and five rare MRSA-CC398 from China. All isolates were tested at least twice employing Biolog Phenotype MicroArray™ technology. Metabolic responses to carbon sources, osmolytes and varying pH conditions were quantified via Area Under the Curve (AUC) of the respiration curves. By determination of 30 most informative out of 379 substances and/or conditions tested, sparse partial least squares discriminant analysis (sPLS-DA) enabled isolate differentiation based on CC and origin.
Results: The 30 selected substrates included sugars, sodium salts and amino acids among others and showed reproducible AUC values. Application of sPLS-DA revealed the possibility to cluster the isolates into four groups depending on their CC and origin. Significant variations were detected for the carbon sources acetamide, L-phenylalanine, D,L-octopamine and 2,3-butanediol. Lower AUC values indicate that within the same CC the geographically rare clone cannot metabolize the substrates as successfully as the respective dominant clone. Moreover, the Chinese MRSA-CC9 showed major differences for the carbon sources D-malic acid, m-tartaric acid and sedoheptulosan among others. Here, lower AUC values point towards a reduced usage in relation to the other three groups.
Conclusions: Some differences in the metabolic properties of porcine LA-MRSA-CC398 and -CC9 from Germany and China were detected. However, further evaluations are needed to thoroughly understand whether the variations play a role in the development of the epidemic LA-MRSA clones in swine. To confirm a possible evolutionary advantage for the dominant CCs resulting from a higher utilization rate of certain substrates, their involvement in essential metabolic pathways needs to be verified. Moreover, the discrepancy between the Chinese MRSA-CC9 and the other three groups needs to be analyzed. Due to the zoonotic potential, understanding the factors leading to the expansion of epidemic LA-MRSA clones in pigs is important and may finally contribute to a reduction of LA-MRSA colonization in humans and animals.

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Occurence of multidrug-resistant Pasteurellaceae among German cattle suffering from respiratory tract infection

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Introduction: *Mannheimia haemolytica* and *Pasteurella multocida* are important respiratory pathogens in cattle. Although multidrug-resistant (MDR) Pasteurellaceae isolates have been reported from the USA and Canada, the resistance rates for *M. haemolytica* and *P. multocida* are comperatively low in Germany [1-3]. This study analyzes two MDR Pasteurellaceae isolates of bovine origin from Germany.
Material and Methods: Lung samples taken during post-mortal examination from a severe case of pleuropneumonia of a three-month-old German calf were subjected to routine microbiology diagnostics in November 2019. The antimicrobial susceptibility of the isolates *M. haemolytica* IMT47952 and *P. multocida* IMT47951 was determined by broth microdilution according to CLSI standards. Hybrid assembly of Illumina MiSeq and MinION reads resulted in closed genomes of both isolates.
Results: *M. haemolytica* IMT47952 and *P. multocida* IMT47951 displayed MDR phenotypes. In *M. haemolytica* IMT47952 sequence analysis identified the resistance genes *sul2* (sulfonamides), *catA3* (chloramphenicol), *floR* (chloramphenicol/florfenicol), *tet*(Y) (tetracyclines), *strA-strB* (streptomycin), *mef*(C) and *mph*(G) (macrolides) in addition to a nucleotide exchange resulting in a D87Y GyrA mutation (nalidixic acid). In the sequence of *P. multocida* IMT47951 the resistance genes *sul2* and *strA-strB* were identified, too, as well as *tet*(H) (tetracyclines) and *aph(3")-Ia* (kanamycin, neomycin). Moreover, the elevated minimal inhibitory concentration values for macrolides, clindamycin and florfenicol might be explained by an A2059C transition in the 23S rRNA in this isolate. In both sequences, the resistance genes were organized in a genetic environment resembling integrative and conjugative elements (ICEs) from Pasteurellaceae [1, 2].
Conclusions: The resistance genes *tet*(Y), *mef*(C) and *mph*(G) have been identified in aquatic bacteria, but not in *M. haemolytica*, so far [4, 5]. The occurrence of MDR Pasteurellaceae isolates harbouring resistance genes within ICE-like structures among German livestock might lead to treatment failures for respiratory disease in cattle. Furthermore, as veterinary and human medicine are interlinked, ICE-like structures containing novel combinations of resistance genes might diminish treatment options in human medicine when introduced to human pathogens.

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Occurrence of human high-risk clonal lineages of Enterobacterales in a dog suffering from recurrent urinary tract infection

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Introduction: Multidrug-resistant (MDR) pathogens such as extended-spectrum β-lactamase (ESBL)-producing Enterobacterales are a threat for physicians as well as for veterinarians as they are major causes of nosocomial infections. Moreover, these MDR pathogens can be transmitted within in the community between animals and their owners in both directions. Among those, *Klebsiella pneumoniae* (*K.p.*) ST307 and *Escherichia coli* (*E.c.*) ST167 have been recently reported as emerging clonal lineages frequently associated with multidrug resistance, ESBL- or carbapenemase-production. Here, we report on the genomic characteristics and relationship of multiple *K.p.* ST307 and *E.c.* ST167 isolates from a canine case of chronic urinary tract infection (UTI).
Material and Methods: Between December 2017 and February 2019, 19 urine samples and a fecal sample were investigated. Bacterial isolates were identified using MALDI-TOF MS. Antimicrobial susceptibility testing (AST) was performed by determination of minimal inhibitory concentrations and evaluated following CLSI guidelines. Whole genome sequencing was performed using Illumina MiSeq technology; in case of *K.p.* combined with MinION technology. Genotypic characterization included the determination of ST, transferable resistance genes, and plasmids (https://cge.dtu.dk). Transconjugation experiments were performed for ESBL-*K.p.*
Results: MDR *E.c.* (n=5), ESBL- (n=6) and non-ESBL-*K.p.* (n=4) were repeatedly isolated from urine samples between December 2017 and August 2018, and ESBL-*K.p.* from a fecal sample (February 2019). AST profiles displayed no differences between strains isolated at different time points. Considering genes conferring resistance towards antibiotics, all ESBL-*K.p.* (ST307) harbored *aph*(3'')-Ib, *aac*(3)-IIa, *aac*(6')-Ib-cr, *aph*(6)-Id, *bla*TEM-1B, *bla*CTX-M-15, *bla*OXA-1, *oqx*A, *oqx*B, *qnr*B1, *fos*A, *cat*B3, *sul*2, *dfr*A14, *tet*(A), and one IncFIB plasmid. Non-ESBL-*K.p.* (ST307) carried *blaSHV-28*, *oqx*A, *oqx*B, and *fos*A. All MDR *E.c.* (ST167) were positive for *aph*(3'')-Ib, *aad*A5, *aph*(6)-Id, *bla*TEM-1B, *bla*CMY-2, *cat*A1, *sul*2, *dfr*A17, and *tet*(B). Phylogenetic analysis revealed a close relationship for the *E.c.* as well as the *K.p.* isolates, in the latter even irrespectively of the ESBL-plasmid carrier status. Transconjugation experiments confirmed transferability of ESBL-encoding genes and co-localized *sul*2, *dfr*A14, *aac*(3)-IIa, *aac*(6')-Ib-cr, and *qnr*B.
Discussion: Repeated courses of antibiotic treatments during recurrent infections of companion animals facilitate the occurrence of MDR pathogens and might compromise treatment success and animal welfare. For the animal owners, this poses the risk of zoonotic transmission.

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Characterisation of the rat gut and hair microbiota from wildlife Norway rats in German livestock farms via 16S rRNA gene sequencing focusing on zoonotic pathogens

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Introduction: Transmission of infectious diseases originating from wildlife reservoirs have become important not just due to the Corona pandemic. Norway rats (*Rattus norvegicus*), which are widely distributed on farms due to good living conditions (food, water, shelter), represent such a wildlife reservoir and can transmit various infectious diseases and parasites to humans and livestock (Heuser et al., 2016; Lovera et al. 2017). Rodent control is therefore an important hygiene measure to keep livestock healthy as well as to prevent the transmission of pathogens to humans.
Materials and Methods: In a project aiming an area-wide rodent control approach with prevention of germ spread in farms with animal husbandry, 23 farmers are cooperating with professional pest control operators for 2.5 years. Rats that died in the course of control measures were frozen and collected centrally. Subsequently, 20 rats were sectioned. Gut and hair microbiota were analyzed via 16S rRNA gene sequencing. Microbiome bioinformatics were performed with QIIME 2 2017.4 (Bolyen et al. 2019).
Results: Zoonotic pathogens were predominantly present in hair samples. Regardless of the animal species kept, *Streptobacillus moniliformis*, the causative agent of the infection commonly called "rat-bite fever", was found in more than half of the hair samples. Furthermore, we found different species of coagulase-negative staphylococci in hair samples and, in a lower frequency, other zoonotic pathogens (*Leptospira interrogans, Campylobacter jejuni, Listeria monocytogenes*) in both, hair and fecal samples.
Discussion: The findings show the transmission potential of zoonotic pathogens originating from Norway rats in German livestock farms and highlight the importance of consequent rodent control to prevent pathogen transmission to farm animals on the one hand and to humans living on the farms on the other hand.
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ESBL-producing *E. coli* – dynamics of occurrence in pig farms in North Rhine-Westphalia

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Question: Public health is increasingly threatened by the emergence of ESBL-producing Enterobacteriaceae, which are difficult to treat in nosocomial infections. These pathogens are found, among others, in persons related to pig farms[1]. The study aimed to investigate the temporally dynamic occurrence of ESBL-producing *E. coli* both between different fattening farms and within individual animal populations of a farm.
Methods: A total of 288 faecal swabs (36 animals) from 8 fattening farms were examined. The samples were taken both when the animals were delivered and shortly before slaughter. Before housing, the environment of the animals was examined by means

of boot swabs, and the respective transport vehicle (N=6) was sampled. The samples were culturally processed in the laboratory using selective and differential culture media. Results were confirmed using an e-test. Results were transferred to Excel 2016 and descriptively analyzed using IBM SPSS 25.
Results: At delivery of the animals, a total of 28% of the 144 pigs sampled were positive. At the end of fattening, ESBL-producing bacteria were found in 10%. In seven farms, at least one fecal swab tested positive for *E. coli* at the first sampling, while three farms tested positive at the end of fattening. Other *Enterobacteriaceae* were found at both the first and second sampling at three farms each, with only one farm having *Enterobacteriaceae* at both time points. In three farms, at least one fecal swab was found during the first sampling, which contained both *E. coli* and other *Enterobacteriaceae*. *E. coli* was detected in three farms after standard farm cleaning, while other *Enterobacteriaceae* were found in two farms. *E. coli* was detected in half of the transport vehicles, while both *E. coli* and other *Enterobacteriaceae* were found in one.
Conclusions: The results indicate both inter-farm differences in the ESBL status of pig populations and time-dependent variations within the same pig population. Inadequate hygiene approaches may allow further spread of pathogens. The time-dependent variation within an animal population could be due to the age-dependent change in the intestinal flora of pigs[2]. Other studies also found that there are differences between farms, so that colonization will already take place during rearing or suckling[3]. Therefore, the time of screening is essential for risk assessment for spread to other animals, humans, and the environment.

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Prevalence of *Leptospira* spp. in bank voles from a transect in North Rhine-Westphalia and Lower Saxony, Germany

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Introduction: Leptospirosis is a worldwide zoonotic disease with more than 1 million human cases annually. Infections are associated with direct contact to infected animals or indirect contact to contaminated water or soil. As not much is known about the prevalence and species diversity of *Leptospira* spp. in the reservoir host, the aim of this study was to detect *Leptospira* spp. prevalence and distribution of genomospecies and sequence types (ST).
Material and Methods: Bank voles (*Clethrionomys glareolus*), which are abundant and widely distributed in forest habitat, were collected in the years 2018 to 2020 along a transect from North Rhine-Westphalia to Lower Saxony, Germany. DNA of 1817 kidney samples was analyzed by real-time PCR targeting the *lipl32* gene. Positive samples were further analyzed by targeting the *secY* gene to determine *Leptospira* genomospecies and multilocus sequence typing (MLST) to determine the ST.
Results: The overall prevalence was 7.5% (95% confidence interval: 6.4 – 8.9). Bank voles were significantly more often infected with *L. interrogans* (83.3%)(ST24) than with *L. kirschneri* (11.5%)(ST110) and *L. borgpetersenii* (5.2%)(ST197).
Discussion: Even if case numbers of human leptospirosis in Germany are low, our study shows that pathogenic *Leptospira* spp. are present and are a persisting potential infection source for humans.

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Impact of biocides on the dissemination of antibiotic resistance in *Escherichia coli*

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Introduction: Biocides have been applied as disinfectants for decades and play a major role in the prevention of zoonotic diseases in healthcare settings and the food production chain. However, concerns have been raised that their use may contribute to the development and spread of antibiotic resistant bacteria. Therefore, we investigated the susceptibility of the indicator organism *E. coli* to frequently used biocides and clinically relevant antibiotics to identify potential associations.
Methods: Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of six biocides were determined by broth microdilution for isolates from swine (faeces and meat, n=100 each) and humans (outpatients, n=104). Biocide susceptible isolates and isolates with reduced susceptibility were distinguished by MIC95/MBC95. Antibiotic susceptibility was also determined by broth microdilution and antibiotic resistance was defined using epidemiological cut-offs according to EUCAST and Commission Implementing Decision (EU) for all human isolates and the faecal isolates from swine (n=48). The differences observed in the subpopulations were statistically verified using pairwise comparison.
Results: Reduced susceptibility to biocides and antibiotic resistance was found both in human and porcine isolates. Overall, human isolates were less susceptible to benzalkonium chloride and glutaraldehyde (MIC and MBC), octenidine dihydrochloride (MIC) and chlorocresol (MBC) than porcine isolates. However, human isolates were more susceptible to isopropanol and sodium hypochlorite. All human and porcine isolates were susceptible to amikacin and meropenem. Antibiotic resistance to ampicillin, cephalosporins (cefotaxime and ceftazidime), sulfamethoxazole and trimethoprim was most common in both groups of isolates and associated with reduced susceptibility to *glutaraldehyde,* benzalkonium chloride, octenidine dihydrochloride, isopropanol and sodium hypochlorite.
Conclusions: Biocides are extensivily used worldwide in the control of microorganisms including antibiotic-resistant bacteria. Phenotypic susceptibility testing of *E. coli* field isolates is an essential surveillance tool to get a deeper understanding of the link between biocide selection pressure and antibiotic resistance. Our results showed that the widespread use of biocides as antiseptics and disinfectants in human medicine and in food production might lead to bacterial tolerance to biocides and highlights the potential impact of biocides on the dissemination of antibiotic resistance.

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Emergence and spread of extended-spectrum beta-lactamase-producing *Escherichia coli* in hospitalised horses subjected to abdominal surgery

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Introduction: The occurrence of zoonotic and multidrug resistant (MDR) pathogens such as extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (EC) challenges both, personnel biosecurity and animal patients" health in horse clinics. Hospital stay and antibiotic treatments have only recently been identified as important risk factors for the ESBL-EC colonization of equine patients. To limit the selective pressure associated with the prolonged administration of antibiotics, current habitual patterns of perioperative antibiotic prophylaxes (PAPs) need to be revised. The aim of this study was to compare a widely accepted long-term PAP regimen to a short-term regimen, to enable more evidence-based antibiotic prescribing guidelines for horses.

Material and Methods: Two groups of horses subjected to colic surgery were enrolled in this study, both receiving a combination of gentamicin/penicillin (G/P) for either five days (5DG) or a GP single-shot (SSG) prior to surgery. Fecal samples and nostril swabs were collected directly at hospital admission (t_0) as well as on day three (t_1) and 10 (t_2) after surgery. All samples were screened for ESBL-EC by microbiological diagnostics, followed by whole genome sequencing and in-depth genomic analysis.

Results: In total, results of 98 horses with an abdominal surgery were available. Only 81 of them met the inclusion criteria for the first sample (t_0), with n=32 belonging to SSG and n=49 to the 5DG. Considering only the SSG, 1/21 (4.8%) fecal samples collected at hospital admission (t_0) were positive for ESBL-EC. The rate increased to 10/27 (37%) at t_1 and to 15/27 (55.6%) at t_2 (p=0.003). The ESBL-EC rate among samples from the 5DG was found to be 3/31 (9.7%) at t0 and increased to 17/36 (47.2%) throughout t_1 and up to 21/37 (56.8%) at t_2 (p=0.013). There was no statistically significant difference between ESBL-EC positive fecal samples of the study groups. Three dominating sequence type complexes (STC) were identified: STC10 (23%), STC86 (22.2%) and STC23 (12%). Analysis of genetic variants for each of these STC revealed close spatio-temporal relationships between the isolates of individual horses, mostly limited to a difference of four single nucleotide polymorphisms (SNPs).

Discussion: A beneficial effect of the lowered selective pressure associated with the SSG PAP regime seems likely, since ESBL-EC rates decreased in fecal samples obtained from the SSG, especially at t_1. However, probably due to the masking effects of the local ESBL-EC dynamics and spread, the differences between both PAP courses lacked statistical significance. The present study was limited to a specific selection of horse patients. Further studies not only need to increase the amount of participants, but also establish a holistically integrated antibiotic stewardship concept considering all medical indications demanding the use of antibiotics in a horse clinic to rule out confounding effects from other hospitalized horses.

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Profiling the *Coxiella burnetii* resistome with a neural network based on amino acid composition of Position-Specific Scoring Matrix (PSSM) profiles

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Coxiella burnetii is the aetiological agent of Q fever, a zoonotic disease that affects predominantly small ruminants and humans. Therapy of acute and chronic disease is based on doxycycline. Resistance problems in therapy are seldom but have been described in chronic Q fever. Anti-microbial sensitivity testing in *C. burnetii* is difficult due to the strict intracellular growth of the pathogen. Therefore, information about anti-microbial resistance is very limited. In general, discovering all antibiotic resistance genes (ARGs) of a pathogen (resistome) is an important approach to support and improve therapy of infectious diseases.

The "best hits" approach on sequence level is the most common method for resistome profiling, however this approach has not been successful with *Coxiella* genomes. To this end, we developed a machine learning (ML) approach that is based on artificial neural network, while leveraging the availability of ARGs databases to enhance analytic power, prediction quality and accuracy. Our implementation employed a feature characterization method that is based on amino acid composition of PSSM profiles to encode protein sequences. The evaluation of our model with novel and known ARG sequences showed ≈ 0.96 accuracy as well as high precision and recall.

We applied the model to predict ARGs from 61 *C. burnetii* genomes that were downloaded from RefSeq database. We observed that the most predicted ARGs in *C. burnetii* belong to the multidrug category, followed by macrolides-lincosamide streptogramin (MLS) and beta lactamase. Predicted multidrug ARGs were majorly efflux proteins with known transmembrane multidrug activity. The beta lactam ARGs were known lactamase with pseudo sequences and the predicted MLS were mainly proteins with ATP binding activities. The former could be an explanation for the well-known inefficiency of beta lactam antibiotics in Q fever therapy, whereas the latter is somehow unexpected, because macrolide antibiotics are used as second line antibiotics in acute Q fever.

Our study is the first to identify ARGs in *Coxiella* using a machine learning approach on amino acid level. This work should contribute to the body of information about antibiotic resistance properties in *C. burnetii* genomes and should be considered in future genome analyses.

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Disruption of Claudin-8 by the *Campylobacter jejuni* serine protease HtrA

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Introduction: High temperature requirement protein A (HtrA) is a serine protease expressed and secreted by the gut pathogen *Campylobacter jejuni.* HtrA is actively involved in the virulence assets of this bacterium. Recent studies with *C. jejuni* HtrA revealed that it cleaves the two proteins occludin and E-cadherin in the tight and adherens junctions of epithelial cells, respectively.

Material and Methods: We applied infection studies, genetic experiments, protein purification, immunofluorescence microscopy, Western blotting, 3D-modeling and mapping analyses.

Results: In our present study, we identified a novel target of HtrA, claudin-8, another important tight junction protein in human cells. Immunofluorescence microscopy demonstrated that claudin-8 was relocated during *in vitro* infection of Caco-2 polarized gut epithelial cells with *C. jejuni*. Infection by wild-type *C. jejuni* lead

to decreased claudin-8 signals in the tight junctions and enhanced its cytoplasmic accumulation. In contrast, this was not observed during infection by Δ*htrA* knockout or protease-inactive S197A point mutants. Western blotting analysis of uninfected vs. infected cell samples showed that the 26-kDa full-length claudin-8 protein was cleaved to produce an 18-kDa carboxy-terminal polypeptide. To corroborate these findings, we accomplished *in vitro* cleavage assays using the purified recombinant human *C. jejuni* HtrA and claudin-8 proteins. Purified claudin-8 was cleaved by recombinant HtrA *in vitro* and produced the same 18-kDa cleavage fragment. We mapped the HtrA cleavage position in the first extracellular loop of claudin-8. In agreement with these mapping analyses, 3D-modeling of the claudin-8 structure ascertained an exposed HtrA cleavage site between the amino acids alanine 58 and asparagine 59.
Discussion: Thus, *C. jejuni* HtrA operates as an important secreted virulence determinant cleaving various components both in the tight and adherens junctions. Taken together, this infection concept allows the bacterium to open cell-to-cell junctions in the gut epithelium, permitting their transmigration by a paracellular pathway leading to campylobacteriosis.

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Peptidase PepP (M24) is a novel virulence factor of *Campylobacter jejuni* contributing to murine campylobacteriosis
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Introduction: *Campylobacter jejuni* represents a major zoonotic pathogen of humans, but the underlying mechanisms of host-pathogen interactions are not yet fully clear. The availability of murine infection models in microbiota-depleted IL-10-/- mice mimic key features of campylobacteriosis and may help solving this dilemma. Aim of the present study was to determine the role of protease PepP during infection.
Material and Methods: We have used gnotobiotic IL-10-/- mice as a model system to characterize the function of a novel virulence factor of *C. jejuni*. We have applied casein zymography, RT-PCR, ELISA, immunohistochemistry and Western blotting.
Results: During a screen for proteases expressed by *C. jejuni*, we identified a peptidase of the M24 family as a potential novel virulence factor, which we named PepP. The corresponding gene is strongly conserved in various *Campylobacter* species. A constructed deletion mutant Δ*pepP* of *C. jejuni* strain 81-176 grew as efficiently as isogenic wild-type (wt) bacteria on rich media. To shed light on the potential role of this protease in mediating *C. jejuni*-induced immunopathological responses in the mammalian host, we perorally challenged microbiota-depleted IL-10-/- mice either with the wt strain or the Δ*pepP* deletion mutant. Upon infection, both strains could stably colonize the murine gastrointestinal tract with comparably high loads. Remarkably, *pepP* deficiency was associated with less severe induced malaise, associated with less distinct apoptotic and innate immune cell responses, but also with more pronounced proliferative/regenerative epithelial cell responses in the large intestines six days post-infection. Furthermore, pro-inflammatory mediators were lower in the colon, ileum and mesenteric lymph nodes of mice that had been challenged with the Δ*pepP* mutant compared to those infected with the wt strain. This also held true for extra-intestinal organs including liver, kidneys and lungs, and, strikingly, to systemic compartments.
Discussion: Taken together, our study provides first evidence that the protease PepP of the M24 family is a major virulence factor involved contributing to campylobacteriosis in the mammalian host. The finding that apoptosis in the colon is significantly diminished in mice infected with the *pepP* gene mutant highlights the epithelial layer as the first and main target of *C. jejuni* PepP in the intestines.

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The conundrum of colonisation resistance against *Campylobacter* reloaded: The gut microbiota composition in conventional mice does not prevent from *Campylobacter coli* infection
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Question: The physiological colonization resistance exerted by the murine gut microbiota prevents conventional mice from *Campylobacter jejuni* infection. In the present study we addressed whether this also held true for *Campylobacter coli.*
Methods: Three-month old female and male C57BL/6j wild type mice were perorally infected with 10^9 colony forming units of either the *C. jejuni* strain 81-176 or a *C. coli* strain that had been isolated from a patient with bloody diarrhea on two consecutive days (days 0 and 1) by gavage.
Results: Following peroral application, *C. coli* as opposed to *C. jejuni* could stably establish within the gastrointestinal tract of conventionally colonized mice until 3 weeks post-challenge. Neither before nor after either *Campylobacter* application any changes in the gut microbiota composition could be observed. *C. coli*, but not *C. jejuni* challenge was associated with pronounced regenerative, but not apoptotic responses in colonic epithelia. At day 21 following *C. coli* versus *C. jejuni* application mice exhibited higher numbers of adaptive immune cells including T-lymphocytes and regulatory T-cells in the colonic mucosa and lamina propria that were accompanied by higher large intestinal interferon-γ (IFN-γ) concentrations in the former versus the latter but comparable to naive levels. *Campylobacter* application resulted in decreased splenic IFN-γ, tumor necrosis factor-α (TNF-α), and IL-6 concentrations, whereas IL-12p70 secretion was increased in the spleens at day 21 following *C. coli* application only. In either *Campylobacter* cohort decreased IL-10 concentrations could be measured in splenic and serum samples.
Conclusions: In conclusion, the commensal gut microbiota prevents mice from *C. jejuni*, but not *C. coli* infection.

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Immune-modulatory properties of the octapeptide NAP in *Campylobacter jejuni* infected mice suffering from acute enterocolitis
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Question: Human infections with the food-borne zoonotic pathogen *Campylobacter jejuni* are progressively rising and constitute serious global public health and socioeconomic burdens. Hence, application of compounds with disease-alleviating properties are required to combat campylobacteriosis and post-infectious sequelae. In our preclinical intervention study applying an acute *C. jejuni* induced enterocolitis model, we surveyed the anti-pathogenic and immune-modulatory effects of the octapeptide NAP, which is well-known for its neuroprotective and anti-inflammatory properties.
Methods: Secondary abiotic IL-10-/- mice were perorally infected with *C. jejuni* strain 81-176. Starting on day 2 post infection and lasting until the end of the experiment mice were either treated with synthetic NAP (1.0 mg per kg body weight; dissolved in NaCl 0.9%) or received vehicle (placebo) once daily via the intraperitoneal route.
Results: NAP-treatment did not affect gastrointestinal *C. jejuni* colonization but could alleviate clinical signs of infection that was accompanied by less pronounced apoptosis of colonic epithelial cells and enhancement of cell regenerative measures on day 6 post-infection. Moreover, NAP-treatment resulted in less distinct innate and adaptive pro-inflammatory immune responses that were not

restricted to the intestinal tract but could also be observed in extra-intestinal and even systemic compartments. NAP-treatment further resulted in less frequent translocation of viable pathogens from the intestinal tract to extra-intestinal including systemic tissue sites.
Conclusions: For the first time, we here provide evidence that NAP application constitutes a promising option to combat acute campylobacteriosis.

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Toll-like receptor-4 is involved in mediating intestinal and extra-intestinal inflammation in *Campylobacter coli*-infected secondary abiotic IL-10$^{-/-}$ mice

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Question: Human *Campylobacter* infections are emerging worldwide and constitute significant health burdens. We recently showed that the immunopathological sequelae in *Campylobacter jejuni*-infected mice were due to Toll-like receptor (TLR)-4 dependent immune responses induced by bacterial lipooligosaccharide (LOS). Information regarding the molecular mechanisms underlying *Campylobacter coli*-host interactions are scarce, however. Therefore, we analyzed *C. coli*-induced campylobacteriosis in secondary abiotic IL-10$^{-/-}$ mice with and without TLR4.
Methods: On two consecutive days (i.e., days 0 and 1), sex- and age-matched secondary abiotic mice (three months of age) were perorally challenged with 10^9 colony forming units of the *C. coli* patient isolate or the murine commensal *E. coli* strain as apathogenic, non-invasive control by gavage.
Results: Independent from TLR4, *C. coli* and *E. coli* stably colonized the gastrointestinal tract, but only *C. coli* induced clinical signs of campylobacteriosis. TLR4$^{-/-}$ IL-10$^{-/-}$ mice, however, displayed less frequently fecal blood and less distinct histopathological and apoptotic sequelae in the colon versus IL-10$^{-/-}$ counterparts on day 28 following *C. coli* infection. Furthermore, *C. coli*-induced colonic immune cell responses were less pronounced in TLR4$^{-/-}$ IL-10$^{-/-}$ as compared to IL-10$^{-/-}$ mice and accompanied by lower pro-inflammatory mediator concentrations in the intestines and the liver of the former versus the latter.
Conclusions: Our study provides evidence that TLR4 is involved in mediating *C. coli*-LOS-induced immune responses in intestinal and extra-intestinal compartments during murine campylobacteriosis.

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Nitroxoline or cotrimoxazole for the therapy and prophylaxis of urinary tract infections with multidrug resistant uropathogens?

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Introduction: Urinary tract infections (UTIs) with multidrug resistant (MDR) bacteria are associated with a poor outcome, because the recommended first-line antibiotic treatment is increasingly inadequate to ensure efficient pathogen elimination. To reduce the prescription of carbapenems often used for empirical therapy of MDR uropathogens the question arises as to which oral antibiotics can still be used to manage infections by MDR uropathogens besides fosfomycin, nitrofurantoin and cotrimoxazole? Since 1967 nitroxoline is in use for the calculated therapy of uncomplicated UTIs in adults.
Material/Methods: We evaluated nitroxoline susceptibility among *Enterobacterales* isolated from 140,648 urine samples from patients with suspected UTI. Antibiotic susceptibility was determined by VITEK 2 except for nitroxoline, which was tested by disk diffusion assays. The genome sequence of an arbitrarily chosen MDR *E. coli* strain was analyzed to exemplarily describe the genomic basis for parallel resistance of *E. coli* urine isolates to many antibiotics. Oxford Nanopore MinION and Illumina MiSeq platforms were combined to close the genome sequence. Tools from the Center for Genomic Epidemiology (www.genomicepidemiology.org) were used for genome sequence analysis.
Results: MDR *Proteus mirabilis* and *Escherichia coli* isolates were consistently nitroxoline sensitive, and only 6% and <1% of the susceptible *P. mirabilis* and *E. coli* strains were nitroxoline resistant, respectively. 32% of MDR and 5% of susceptible *Klebsiella pneumoniae* isolates were nitroxoline resistant. 20% of the susceptible and 60% of the MDR *E. coli* urine isolates were resistant to cotrimoxazole.
An arbitrarily selected MDR *E. coli* isolate carried a MDR plasmid conferring resistance to seven different antibiotic classes, incl. cotrimoxazole. The AMR genes were organized in a composite structure composed of two class 1 integrons and several IS elements. To assess the extent of parallel resistance to cotrimoxazole and other drugs, we screened NCBI GenBank for co-existence of *sul*1 and *dfrA*17 alleles with other AMR genes in enterobacteria. We detected 332 class 1 integron structures associated with 108 different AMR determinants. Out of these 332 cotrimoxazole resistant cases, 20, 17 and 12 were also resistant to fosfomycin, nitrofurantoin and for both, fosfomycin and nitrofurantoin, respectively.
Discussion: Our results indicate that the treatment of symptomatic UTI according to the current guidelines may fuel the selection of MDR uropathogens due to parallel resistance. Despite increasing antibiotic resistance of uropathogens, nitroxoline, but not cotrimoxazole, remains active *in vitro* against some relevant MDR uropathogens. Particularly in *E. coli*, but also in other enterobacterial uropathogens, the frequent parallel resistance to different antibiotics due to the accumulation of AMR determinants on MGEs argues for greater consideration of nitroxoline in the treatment of uncomplicated UTIs.

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