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DKMDVV 001

Rapid antibiotic susceptibility testing of Gram-negative bacteria directly from urine samples of UTI patients using MALDI-TOF MS

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Introduction: Urinary tract infections (UTIs) are one of the most common human infections and are most often caused by Gram-negative bacteria such as *Escherichia coli* (1). In view of the increasing number of antibiotic-resistant isolates, rapidly initiating effective antibiotic therapy is essential. Therefore, a faster antibiotic susceptibility test (AST) is desirable.

Material and Methods: The MALDI-TOF MS-based phenotypic antibiotic susceptibility test (MALDI AST) has been used in blood culture diagnostics to rapidly detect antibiotic susceptibility. This study demonstrates for the first time that MALDI AST can be used to rapidly determine antibiotic susceptibility in UTIs directly from patients´ urine samples. MALDI-TOF MS enables the rapid identification and AST of Gram-negative UTIs within 4.5 hours of receiving urine samples. After preselecting urine samples with expected cultural growth six urinary tract infection antibiotics, including ciprofloxacin, cotrimoxazole, fosfomycin, meropenem, cefuroxime, and nitrofurantoin, were analyzed and compared with conventional culture-based AST methods.

Results: A total of 105 urine samples from UTI patients contained bacterial isolates for MALDI AST. An overall agreement of 94.7% was found between MALDI AST and conventional AST for the urinary tract pathogens tested. False-resistant isolates were classified as minor errors and false-susceptible isolates as major errors. Meropenem, nitrofurantoin and fosfomycin showed no misclassified isolates, while six minor errors (false-resistant) were observed for cotrimoxazole and four for ciprofloxacin. Only cefuroxime showed major and minor errors. The results are shown in the table 1 below.

Fig 1: Overview of the agreement and the major (MEs) and minor errors (MiEs) between the conventional AST and the MALDI AST for the antibiotics used in this study.

Discussion: In contrast to rapid genotypic methods MALDI AST as a phenotypic AST method can provide ASTs for basically all antibiotics regardless of resistance mechanisms. Since the MALDI-TOF MS is available in almost all microbiological laboratories, this AST method could be widely applied. As no extra equipment is required, the cost of the assay is expected to be rather low. In addition, bacterial identification can be performed simultaneously with an AST, providing important information for interpreting the results according to EUCAST guidelines. In conclusion, the MALDI AST is a suitable tool for detecting antibiotic resistance directly in patient urine samples in a much shorter time than conventional AST methods, thus providing same-day results to the physician.

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Fig. 1

	Tested isolates	Agreement	ME	%	MiE	%
Cefuroxime	79	79,8%	3	3,8%	13	16,5%
Ciprofloxacin	95	95,8%	0	0,0%	4	4,2%
Cotrimoxazole	92	93,5%	0	0,0%	6	6,5%
Fosfomycin	66	100,0%	0	0,0%	0	0,0%
Meropenem	95	100,0%	0	0,0%	0	0,0%
Nitrofurantoin	66	100,0%	0	0,0%	0	0,0%
Overall	493	94,7%	3		22	

DKMDVV 002

Nucleic acid amplification testing (NAAT) of *Staphylococcus aureus* in urine from patients with *S. aureus* bacteremia

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Introduction: *Staphylococcus aureus* bacteremia (SAB) is associated with a mortality between 15-30%. Many SAB patients (10-39%) have an asymptomatic concomitant *S. aureus* bacteriuria (SABU) which is associated with increased mortality. The early detection of *S. aureus* in urine in suspected SAB cases therefore could identify patients at risk. The objective of this study was to compare the detection of *S. aureus* in urine by nucleic acid amplification test (NAAT) vs. cultural detection.

Methods: This prospective diagnostic study was performed on urine samples (native mid-stream or catheter urine) of patients with culture-confirmed bacteremia, admitted to a tertiary care hospital between February 2020 and May 2023. Urine samples were collected ±4 days to the collection of the positive blood culture. They were cultured on standard agar and selective agar for Gram-negative and Gram-positive bacteria. NAAT detection of *S. aureus* was carried out using Xpert® test cartridges (SA Nasal Complete G3) after washing a sterile swab soaked with urine in the buffer solution of the cartridge test. Each Xpert® cartridge produces a positive or negative test result applying a threshold value (Ct-value) of 35.0.

Results: We included patients with SAB (n=100, 68% male, median age: 63.9 years) and with positive blood cultures other than *S. aureus* (Non-SAB, n=20, 75% male, median age: 61 years).

In the SAB group, 25% of urine specimens were positive for *S. aureus* by culture and 39% by NAAT (p=0.03). An additional 15 samples had a Ct-value ≥35 and were evaluated as negative based on the manufacturer's specifications. From 15 samples that were tested culture negative, but NAAT positive, one half (n=8) had a positive urine inhibitory test ("Hemmstofftest") suggesting inhibition of bacterial growth. Median Ct-values were higher in culture negative than in culture positive urine samples (32.9 vs 23.9). Only one patient with a positive urine culture had a negative NAAT test result (Ct-value=36.7).

In the Non-SAB group, 14 different species were detected in the positive blood cultures (6 *Escherichia coli*, 2 *Staphylococcus epidermidis*, 6 others). All urine samples of the Non-SAB group were culture negative for *S. aureus.* The rate of molecular detection of *S. aureus* was lower compared to the SAB group (5% vs. 39%, p=0.003). The only patient with a positive NAAT result for *S. aureus* had a Ct-value of 34.7 and we could not detect *S. aureus* in the urine culture. However, the patient was colonized with *S. aureus* in the nose.

Discussion: Direct molecular detection of *S. aureus* in urine can identify patients with SAB and detects more cases than conventional urine culture. Whether NAAT detection of *S. aureus* in urine could be used as a rapid test for SAB, should be evaluated in future studies. Urine cultures positive for *S. aureus* by NAAT in

patients without SAB or urinary tract infection might be due to contamination during the collection of the urine culture in colonised patients.

DKMDVV 003

Semi-automated antimicrobial susceptibility testing is more error-prone than EUCAST disk diffusion in detection of vancomycin resistance in *Enterococcus faecium*

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Introduction: Enterococci have become important healthcare-associated pathogens. Since hospital mortality of systemic infection with Vancomycin-resistant enterococci (VRE) is increased compared to infection with Vancomycin-susceptible enterococci, early detection of vancomycin resistance is crucial. Reliability of susceptibility testing of *Enterococcus* species varies among different methods of antimicrobial susceptibility testing (AST) (1). There is evidence that the widely used semi-automated AST device VITEK2 (bioMérieux, Nürtingen, Germany) is prone to missing vancomycin resistance in *Enterococcus faecium* (2). In this study, we evaluated the semi-automated BD Phoenix AST system (Heidelberg, Germany) and EUCAST disk diffusion in parallel regarding their ability to detect *vanA*- or *vanB*-mediated resistance in *Enterococcus faecium*.

Material/method: 306 clinical isolates from intraabdominal, urinary tract, and soft tissue specimens were tested in parallel with semi-automated AST BD Phoenix system and EUCAST disk diffusion. From the same inoculum, PCR testing for *vanA* and *vanB* genes was done. For isolates, which showed discrepant results with the different methods, phenotypic testing was repeated twice and additional AST with broth microdilution and semi-automated AST using VITEK2 was done.

Results: 40 % of included strains harbored *vanA* or *vanB* genes. 8.1 % of VRE were missed by BD Phoenix AST. In contrast, EUCAST disk diffusion identified VRE with high reliability except for one susceptible isolate harboring *vanB*. This strain was reproducibly susceptible with all phenotypic methods, consistent with vancomycin-variable *Enterococcus faecium* (3).

Discussion: Our results show that BD Phoenix AST does not detect Vancomycin resistance in *Enterococcus faecium* with satisfactory reliability. Disk diffusion results for vancomycin susceptibility showed high consistency with PCR results, provided the EUCAST recommendation was followed to not only consider the inhibition zone diameter, but also to meticulously examine the zone edge for fuzziness or colonies growing within the inhibition zone.

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DKMDVV 004

Rapid phenotypic antimicrobial susceptibility testing directly from positive blood cultures using the novel Q-linea ASTar® system: performance, time-to-result and clinical impact in laboratory routine

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Introduction: Adequate and timely antibiotic therapy is crucial for the treatment of sepsis. Various innovative systems, like the Q-linea ASTar®, have been developed to perform rapid antimicrobial susceptibility testing (AST) directly from positive blood cultures (BC).

Methods: We conducted a prospective study to evaluate ASTar® under real-life conditions with a focus on time-to-result and impact on antimicrobial therapy. Over two months, all positive BCs that showed Gram-negative rods upon microscopy, were tested with the ASTar® and our standard procedure (VITEK® 2 AST from short-term culture). Additionally, we included multidrug-resistant Gram-negative bacteria from our archive.

Results: In total, 77 bacterial strains were tested. ASTar® covered 94% of the species encountered. The categorical and essential agreement compared to broth microdilution was 96.3% and 89.3%, respectively. ASTar® caused 1.9% minor, 1.4% major and 0.5% very major errors. Categorical agreement was similar to standard procedure. The average time between BC sampling and the availability of the AST result for the attending physician was 28 h 49 min for ASTar® and 44 h 18 min for standard procedure. ASTar® correctly identified all patients who required an escalation of antimicrobial therapy and 75% of those who were eligible for a de-escalation.

Discussion: In conclusion, ASTar® provided reliable AST results and significantly shortened the time to obtain an antibiogram. However, the percentage of patients that will profit from ASTar® in a low resistance setting is limited and it is currently unclear if a change of therapy nearly 29 hours after BC sampling will have a significant impact on the patient's prognosis.

DKMDVV 005

Identification of Gram-negative bacteria and detection of bla$_{CTX\text{-}M\text{-}1}$/bla$_{CTX\text{-}M\text{-}9}$-ESBL-genes directly from positive blood cultures with the eazyplex® BloodScreen GN loop-mediated isothermal amplification assay: performance in laboratory routine and evaluation of therapeutic approaches

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Introduction: Blood-stream infections with multidrug-resistant Gram-negative bacteria are associated with high mortality. The eazyplex® BloodScreen GN loop-mediated isothermal amplification assay (BSGN-LAMP) allows identification of the most common Gram-negative sepsis pathogens (*E. coli, K. pneumoniae, K. oxytoca, P. mirabilis, Ps. aeruginosa*) and the

$bla_{CTX\text{-}M\text{-}1}$/$bla_{CTX\text{-}M\text{-}9}$ extended spectrum β-lactamase (ESBL) genes within 15 min directly from positive blood cultures (BC).

Methods: Over eight months, all positive BCs with Gram-negative rods were prospectively tested with the BSGN-LAMP (Amplex Diagnostics GmbH, Germany). Results of identification and $bla_{CTX\text{-}M\text{-}1}$/$bla_{CTX\text{-}M\text{-}9}$-detection were compared to MALDI-TOF and VITEK® 2 antimicrobial susceptibility testing (AST), respectively. Isolates with discrepant ESBL results were further tested with the double-disk synergy test. Furthermore, two treatment approaches based on the BSGN-LAMP results were theoretically evaluated. In the escalation approach, antibiotic therapy was switched to meropenem (MEM) if the BSGN-LAMP was not able to identify the pathogen or if a $bla_{CTX\text{-}M\text{-}1}$/$bla_{CTX\text{-}M\text{-}9}$ gene was detected. In the non-escalation approach, only the $bla_{CTX\text{-}M\text{-}1}$/$bla_{CTX\text{-}M\text{-}9}$ gene detection led to a switch to MEM. The impact of both approaches were also analyzed in all Gram-negative bacteria causing BSI from 2019 to 2022 (n=1640) at our institution (study part 2).

Results: 158 positive BCs were eligible for analysis. The BSGN-LAMP identified a pathogen in 75.8% of positive BCs, and the panel covered 20.8% of the 24 bacterial species encountered during routine testing. Identification was correct in 99.3% when only one pathogen was present. For mixed BCs, one or all possible pathogens were detected in 20% and 80% of cases, respectively. All phenotypic cases of ESBL (n=17) were correctly identified. However, 3rd generation cephalosporin resistance was missed in seven enterobacterales probably due to AmpC β-lactamase production. Empirical therapy at the time of BC positivity was efficient in 79.2% of patients. With the escalation approach, therapy efficiency was increased to 93.5%. However, an additional 8.4% of patients received MEM unnecessarily. With the escalation strategy, eight patients would need to be treated with MEM to avoid one ineffective therapy with piperacillin/tazobactam (TZP). By considering MEM the drug of choice in bacteria with chromosomally encoded AmpC β-lactamase the escalation strategy would lead to the correct therapy in one of six patients. The results of study part 2 confirmed these findings.

Conclusions: The BSGN-LAMP provided reliable identification and detection of 3rd generation cephalosporin resistance in the majority of patients. TZP resistance is more common in pathogens that are not included in the BSGN-LAMP panel and escalation to MEM increased the antibiotic efficacy. However, choosing the best therapeutic approach majorly depends on regional antibiotic resistance epidemiology.

DKMDVV 006

Modulation of antibiotic efficacy against *E. coli* in sequential treatment

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Question: Infections with *Escherichia coli* are frequent, especially in the urinary tract, where it is the most common pathogen. Drug resistant strains are isolated more frequently after antibiotic therapy and in recurrent infections. Due to the declining development of new antibiotics, it is imperative to optimize the use of the drugs at our disposal. One approach lies in the exploitation of cellular hysteresis, which describes changes in antibiotic efficacy after recent bacterial contact with other substances. It can be negative, resulting in increased killing of bacteria, or positive, conferring protection. Negative hysteresis treatments can also constrain drug resistance evolution. We aim to explore the potential of cellular hysteresis in the treatment of urinary tract infections (UTI) caused by *E. coli*.

Methods: We tested two reference strains of *E. coli* for hysteresis by continuous growth monitoring in minimal and artificial urine media. Antibiotics with clinical relevance for UTI treatment, including ampicillin, mecillinam, ceftriaxone, ciprofloxacin, and fosfomycin, were used in sequence. We also included a set of renally excreted non-antibiotic drugs. Bacteria were exposed to a pretreatment with one drug for an hour, followed by media exchange and a 12-hour main treatment with a different substance. Comparison of the area under the growth curve results in quantitative measurements of hysteresis. Transcriptomic analysis was performed to determine the molecular mechanism behind hysteresis patterns.

Results: Using sub-inhibitory concentrations of the main treatments (35 – 75 % MIC), we observed increases in antibiotic efficacy in up to 53% of combinations tested. Ciprofloxacin pretreatment resulted in negative hysteresis for most other drugs. Cases of protection by pretreatment were rare. The effects were similar in low concentrations of pretreatment and when using artificial urine media, but there was some discrepancy between strains. Transcriptomic analysis of a drug pair with distinct directional hysteresis (mecillinam / ciprofloxacin) revealed a strong effect of the pretreatments. Finally, we found that non-antibiotic drugs can also modulate antibiotic efficacy when in the pretreatment role.

Conclusion: Cellular hysteresis is a promising mechanism for the optimization of antibiotic therapy, including that auf *E. coli* UTI, as our results indicate. Our work establishes for the first time that hysteresis effects occur in this species, and that they can increase or decrease drug efficacy, depending on the exact sequence and order of application. Compared to previous work in *Pseudomonas aeruginosa*, we expand the scope by showing that common non-antibiotics can also induce hysteresis and may therefore affect drug efficacy. *In vitro* testing of more clinical strains for the determination of conserved patterns is required. Treatment designs with hysteresis switches may clinically improve therapy efficacy and prevent resistance evolution.

EKV 007

Virulence not required? Albumin promotes pathogenicity of (non)-damaging *Candida albicans* strains

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Research question: Virulence of the pathogenic yeast *Candida albicans* is associated with filamentation, adhesion to and invasion into host cells, and the production of the toxin candidalysin. However, clinical isolates of *C. albicans* and other *Candida* spp.,

also seem to cause infection independent of these virulence traits. Therefore, the *in vitro* pathogenicity often does not correlate with their potential to cause infection in patients. We hypothesized that specific host factors can trigger pathogenicity, but are absent in commonly used *in vitro* assays. Here, we investigated the role of albumin, the most abundant protein in the human body, but often disregarded in *in vitro* models.

Methods and results: To determine the impact of albumin, vaginal epithelial cells were infected with different *C. albicans* isolates and *Candida* species. The presence of albumin increased the damage potential, even in otherwise non-damaging and non-filamentous strains after prolonged infection (45 h). This included deletion mutants deficient in filamentation, als3 adhesin/invasin, or candidalysin production. Besides, albumin promoted the proliferation, which involved drastic metabolic and transcriptional remodeling. By screening a protein kinase deletion-mutants library we obtained insight into the pathways involved in the altered fungal behavior upon albumin supplementation. To understand how *C. albicans* processes albumin, we investigated the role of fungal and host proteases. We observed that overall protease activity is involved in the enhanced pathogenicity of *C. albicans* upon exposure to albumin, even though the increased growth is not. Intracellular host proteases are not influenced by protease inhibition and therefore still process the albumin, and its degradation products augment proliferation.

Conclusions: Our data shows that a very common host factors like albumin activates *C. albicans* pathogenic potential independent of adhesion, invasion, filamentation and toxin production. Possibly, also other host-derived factors can drive the pathogenic potential of fungi through unresolved mechanisms.

EKV 008

The histone methyltransferase SET2 regulates the expression of genes crucial for gametocyte development and parasite transmission

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Introduction: The intracellular and extracellular survival of the malaria parasite *Plasmodium falciparum* in the human and mosquito hosts is dependent on rapid morphological and physiological changes, which are accompanied by the up- and down-regulation of stage-specific transcripts. The regulation of gene expression during life cycle progression involves epigenetic control mechanisms, in particular histone post-translational modifications (PTMs). While histone PTMs are well understood for intraerythrocytic replication and immune evasion of the asexual blood stages of *P. falciparum*, their roles during sexual development need further investigation. Previous chemical loss-of-function studies of our laboratory, using the histone methyltransferase inhibitor BIX-01294, revealed significant changes in the gene expression pattern of gametocytes during maturation and gametogenesis. In this study, we aimed to investigate the role of the histone methyltransferase SET2, one of ten known SET proteins of *P. falciparum*, in the regulation of gene expression during gametocyte development.

Material: Protein expression of SET2 in the asexual and sexual blood stages of the *P. falciparum* WT NF54 was investigated by indirect immunofluorescence assay and Western blotting, using polyclonal chicken anti-SET2 antibodies as well as HA-tagged transgenic line. To functionally characterize SET2, SET2-KO parasite lines were generated with the selection-linked integration-mediated targeted gene disruption method. The generated SET2-KO lines were phenotypically evaluated via cell-based assays. Additionally, a comparative transcriptomic analysis of the ring stage of SET2-KO was conducted to unveil genes depending on SET2 in their regulation.

Results: We show that SET2 is expressed in the asexual and sexual blood stages, where it localizes to the nucleus and cytoplasm. Loss of SET2 does not affect intraerythrocytic replication, but significantly impairs gametocyte formation. Further, stage V gametocytes of the SET2-KO line display an unusual morphology, characterized by pointy-ended gametocytes. Comparative transcriptomic analysis of the ring stage of SET2-KO, confirms the role of SET2 in regulating the gene expression of the var and RIF gene family in the asexual blood stages. Down-regulated genes are correlated with microtubule and cytoskeleton-associated genes.

Conclusion: Our data point to a crucial role of the histone methyltransferase SET2 in the morphological maturation of *P. falciparum* gametocytes by regulating cytoskeleton-associated genes in addition to its previously described role in antigenic variation.

EKV 009

Heterologous production of β-carbolines in *Escherichia coli* Nissle to counter *Candida albicans* hyphae formation

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Fungal infections are a major health risk. *Candida albicans*, a common commensal of the mycobiota, is also a prominent fungal pathogen that can cause various diseases. Pathogenicity is favored by several factors, such as microbial dysbiosis caused by broad-spectrum antibiotics, immunosuppression, and compromised epithelial barrier function. Invasive candidiasis often originates from the gut, where a morphological transition from yeast to filamentous growth facilitates the fungus to invade the epithelial barrier, disseminate into the bloodstream and infect multiple organs. Slow diagnosis and challenging treatment following the dissemination contribute to a mortality rate exceeding 30%. In addition, only a few effective antifungals are available and resistances are already occurring.

An alternative to antifungals is the use of antivirulence compounds, that inhibit pathogenicity mechanisms of *C. albicans* without killing the fungus, thus promoting the commensal state. In our approach we developed a therapeutic microbe by genetically modifying the commensal bacterium *E. coli* Nissle, to produce an antivirulence compound. In particular, we expressed the enzyme NscbB that synthesizes two β-carbolines, one of them 1-acetyl-beta-carboline, a molecule found to inhibit *C. albicans* filamentation.1 Various expression systems were tested to optimize production rates, including an inducible system dependent on the presence of *C. albicans* to ensure targeted β-carboline production.

The β-carbolines were isolated by semi-preparative HPLC and used in an *in vitro* infection model with intestinal epithelial cells (IECs) to verify pathogenicity inhibiting effect. Our findings indicated that a combination of the two produced β-carbolines resulted in the strongest reduction of host damage, while treatment with either one of the β-carbolines did not significantly affect pathogenicity in our model. We are currently working on a co-infection model incorporating IECs, *C. albicans*, and 1-ABC-

producing *E. coli* Nissle to assess the direct effect of the therapeutic microbe on *C. albicans* infection.

Therapeutic microbes may represent an alternative strategy to conventional therapeutics to prevent diseases. Our engineered strain is envisioned to be administered preemptively to at-risk patients in clinical settings to avoid systemic infections with *C. albicans* by locking it in its commensal state. In addition to the β-carboline-production, the therapeutic microbe could serve as a platform for incorporating further features addressing, e.g., smart regulation and specific recognition of the pathogen in the future.

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EKV 010

Establishing a human airway organoid-derived monolayer infection model to study *Cryptococcus neoformans* and other fungal infections

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Introduction: *Cryptococcus neoformans* are environmental fungi that cause localized or disseminated infections after inhalation of infectious particles. WHO considers *C. neoformans* to have the highest need for research among mycotic pathogens due to its high incidence mostly in HIV infected, emerging antifungal resistance and One Health implications. Previous studies on pathogenicity are mainly based on animal models or isolated *ex vivo* immune cells. However, to understand the pathomechanisms of especially intracellular fungal infections there is a need for complementary models. We aim to establish an adequate human lung infection model to investigate basic parameters of early *C. neoformans* infection.

Methods: We used human airway-organoid derived monolayers (ODMs) from nasal brushings and lung tissue and infected them with *C. neoformans* at different multiplicities of infection (MOI). Barrier function of the infected monolayers was probed by measuring the transepithelial electrical resistance (TEER). After 10 days of infection ODMs were analyzed for internalization of *C. neoformans*. Fungal viability and proliferation was assessed by colony forming unit (CFU) assays and cell counting.

Results: Human airway organoid-derived monolayers could be co-cultured with *C. neoformans* for up to 10 days with different MOI´s. Probing of barrier function by measuring TEER revealed that TEER is increasing even at low MOIs, indicating a tightening of the epithelial barrier. ODMs did not collapse after prolonged exposure to *C. neoformans* and fungi were only sparsely internalized by epithelial cells. After 10 days of co-culture *C. neoformans* remained viable and could proliferate after being in contact with airway epithelial cells.

Discussion: The established co-culture model of human airway ODMs with *C. neoformans* provides a suitable *in vitro* model to study host-pathogen interactions, innate immune responses of airway epithelial cells and pathogen behavior in a system with cellular and barrier characteristics of the human lung. This model system is amenable to immunofluorescence assays, live cell imaging, cytokine assays and single cell sequencing to study colonization and uptake of different lung pathogens.

MSV 011

The Bavarian Antibiotic Resistance Database (BARDa)

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Background: Antibiotic resistance (AMR) is an increasing problem for human health worldwide. Surveillance systems are important for the observation of changes in antibiotic resistance in order to develop targeted protocols for the use of antimicrobials. In Germany, the national Antibiotic Resistance Surveillance (ARS) established and maintained by the Robert-Koch-Institute (RKI) provides data on a national level. Since 2019 the Bavarian Antibiotic Resistance Database (BARDa), administered by the Bavarian Health authority "Bayrisches Landesamt für Gesundheit und Lebensmittelsicherheit" (LGL) provides data specifically for the State of Bavaria.

Method: Bavarian laboratories transfer their anonymized data electronically to the LGL. The data is validated, analyzed, and published every 6 months. For the calendar year 2022, 485.155 data points for 11 bacterial species from 27 laboratories were analyzed. Data validation is key to receive valid aggregated data. The data set undergoes intensive external plausibility testing to ensure complete data transfer from the laboratories.In addition, plausibility testing of the aggregated data is performed to analyze influence factors such as selective testing or changes of the EUCAST norms on the data set.

Result: The resistance rates and a 95% confidence interval are reported in subsets for intensive care, regular hospital care, outpatient care, and private practice. For the 5 most common bacterial species, data from private practice is reported for the 7 districts of Bavaria.

Conclusion: BARDa is a relatively "young" surveillance project with data from 2019 – 2022. Because of the large number of participants, the amount of data analyzed, and the intensive data plausibility testing in collaboration with the participating laboratories, BARDa can now provide valid and plausible data over the course of 4 years to describe the development of AMR in the State of Bavaria. All collected and validated data is forwarded to ARS to strengthen the national surveillance program.

MSV 012

Genomic landscape of *M. avium* complex in Germany

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Introduction: The *Mycobacterium avium* complex (MAC) comprises the most frequent non-tuberculous mycobacteria (NTM) in Central Europe and currently includes twelve species. Clinically, the most relevant ones are *M. avium*, *M. intracellulare* subsp. *intracellulare* (MINT) and *M. intracellulare* subsp. *chimaera* (MCH) causing pulmonary or disseminated infections. However, the population structure and genomic landscape of MAC remain little investigated.

Methods: Illumina sequencing was performed on 328 MAC isolates from 185 German patients collected between 2006 and 2021 and set into context with publicly available MAC reference strains, as well as clinical data. Species were identified with *NTMprofiler* and sequences mapped to the according reference genomes *M. avium* ATCC 25291 or *M. intracellulare* ATCC 13950 with *MTBseq*.

Results: Overall, 229 isolates could be assigned to *M. avium*, 95 to *M. intracellulare* (46 MINT, 49 MCH), and two to *M. marseillense*. Although serial isolates showed high intrapersonal genetic stability, reinfection appeared to be a frequent event with 15/43 patients exhibiting genetically distinct MAC isolates (or even species) over the course of time. On the other hand, we identified clusters with less than 25 SNPs distance between genomes of isolates recovered from different patients, indicating possible transmission events both in *M. avium* and *M. intracellulare*.Interestingly, these clusters consisted of patients with a variety of underlying dispositions and clinical manifestations.

Discussion: In conclusion, this first comprehensive genomic study of MAC in a German cohort demonstrates that reinfection seems to be a frequent event, while clusters indicate possible person-to-person transmission or a common environmental source.

MSV 013

Vancomycin resistance transfer via membrane vesicles in enterococci

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Background: Vancomycin resistant enterococci (VRE) are multidrug resistant bacterial pathogens gaining importance in the healthcare setting. Infections with these bacteria are still associated with increased mortalities compared to infections with vancomycin susceptible enterococci (VSE). Next to a clonal transmission, the exchange of resistance determinants via horizontal gene transfer (HGT) leads to an increasing prevalence of VRE hospital inpatients. Besides transformation, conjugation and transduction, "vesiduction" via bacterial membrane vesicles (MV) has recently been identified as an HGT mechanism for bacteria to transfer resistance genes. As MV production was proven for enterococci, we here investigated whether vesiduction plays a role in the transfer of the vancomycin resistance genes *vanA* and *vanB*.

Methods: MV were isolated for VRE of the different multilocus sequencing (MLST) sequence types (ST) 80, ST117, ST192, ST203, ST721 and ST1489 harbouring either *vanA* or *vanB*. MV produced in lysogeny broth (LB) (MV/LB) and in LB supplemented with subinhibitory vancomycin concentrations (MV/VAN) were analysed for particle size and concentration using nanoparticle tracking analysis. MV/VAN released by VRE of ST80 and ST117 were additionally subjected to whole genome sequencing. Similarity between VRE isolates and MV DNA was assessed by application of core genome multilocus sequence typing (cgMLST) algorithm. Sequences of *vanA*/*vanB* were extracted and pairwise aligned. Exposure of VSE to MV was performed in LB at 1,000 and 10,000 particles per bacterium, respectively. After overnight cultivation, suspensions were plated on VRE selective agar and Colombia blood agar for the detection of phenotypic vancomycin resistance.

Results: At subinhibitory antibiotic concentrations, VRE isolates of ST80 and ST117 produced more MV than enterococci of other ST. Higher particle concentrations were detected for all ST under vancomycin stress with the most striking increase for ST80 and ST117. CgMLST analysis revealed an identity between VRE isolates and their corresponding MV in 100% of all 1423 targets for ST80 and ST117. V*anA*/*vanB* genes sequences were completely present and identical in both VRE isolates and MV-suspensions. Coincubation of VSE and MV/VAN resulted in no bacterial growth on VRE selective agar but *E. faecium* typical growth on Columbia blood agar.

Conclusion: Depending on the ST of VRE, quantitative and qualitative release of MV/VAN varied substantially. Further studies therefore need to elucidate the importance of VRE ST for resistance transfer and clinical implications. Although *van* resistance determining genes were abundant and complete in VRE-derived MV, no phenotypically effective transfer of vancomycin resistance was observed. Subsequent in-depth analyses should examine properties of enterococcal MV and their purposes during VRE infections.

MSV 014

New horizons in infection control: plasmid transmission surveillance of multidrug-resistant bacteria using long-read whole genome sequencing

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Introduction: Multidrug-resistant bacteria (MDRB) pose a significant threat to public health. Methods of genomic surveillance, such as core genome multilocus sequence typing (cgMLST), have been effective in tracking and monitoring the spread of MDRB. However, recent advancements in sequencing technology such as long-read whole genome sequencing (lrWGS) have introduced new approaches that offer the capabilities to survey plasmids encoding for antimicrobial resistance (AMR) as part of routine laboratory service. Here we compare cgMLST based clonal versus horizontal transmissions of MDRB isolates in the hospital setting using lrWGS.

Methods: As part of routine surveillance at our tertiary care hospital, we collected isolates of multidrug-resistant Enterobacterales from anorectal screening or clinical samples between January 2023 and April 2023 (n=82). Extracted DNA from bacterial isolates was sequenced using a PacBio® Sequel IIe platform. We utilized Ridom SeqSphere+ software version 9.6 beta, which features an integrated MOB-suite tool for plasmid mash distance analysis to compare plasmids across different isolates and assess their similarity. For the same time span, clonal transmission events based on cgMLST analysis and epidemiological data were analysed.

Results: In total, we analysed 82 MDRB isolates (*Escherichia coli*=55, *Klebsiella pneumoniae*=16, *Citrobacter freundii*=4, *Enterobacter cloacae* complex=3, *Serratia marcescens*=1, *Enterobacter aerogenes*=1, *Proteus mirabilis*=2). A cgMLST approach identified six clonal clusters with a high degree of genetic similarity (allelic distance ≤ 5). However, none of the clusters were confirmed as transmission events based on epidemiological data. Plasmid analysis revealed five distinct lateral transfer clusters with a high degree of similarity containg AMR genes. Among these clusters, we observed three suspected transmissions, including two that occurred within the hospital setting and one that occurred outside the hospital in the patients" retirement home. In total, these five clusters contained IncR, IncI-gamma/K1, IncFII, and IncX3 plasmids.

Conclusion: Our findings suggest that the comparative analysis of AMR plasmids can provide valuable information regarding the transmission pathways of AMR genes. In the future, this approach could become part of routine genomic surveillance of MDRB in hospitals.

MSV 015

Long-term experimental evolution of β-lactam resistance in *Haemophilus influenzae*

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Introduction: *Haemophilus influenzae* is a bacterial pathogen that is responsible for a variety of upper respiratory tract infections, yet it can also cause more severe infections such as pneumonia, meningitis, and bacteremia, particularly in young children, the elder population and immunocompromised individuals. The global rise of ampicillin-resistant *H. influenzae* strains has led to increased use of third-generation cephalosporins, but mutation-induced resistance mechanisms are still poorly understood. Therefore, this study aimed to identify β-lactam resistance pathways performing laboratory long-term evolution experiments.

Materials/Methods: Evolution of β-lactam resistance in *H. influenzae* was examined *in vitro* by passaging *H. influenzae* DSM 11121 in liquid medium containing stepwise doubled concentrations of either ampicillin, cefotaxime or ceftriaxone. Single clones were isolated from each passage and analyzed phenotypically by quantitative antimicrobial susceptibility testing and genotypically by next and third generation sequencing.

Results: Significantly reduced susceptibility to cefotaxime was associated with presence of a combination of three mutations in *ftsI*, encoding the main target of β-lactams. In addition, several candidate mutations in other genes putatively contributing to β-lactam resistance in *H. influenzae* were identified. In few clones that evolved in the presence of ceftriaxone decreased susceptibility to all three investigated β-lactams was not associated with any *ftsI* mutation but with a frameshift mutation in the *ompP2* gene.

Discussion: This study demonstrates the acquisition of β-lactam resistance by accumulation of *ftsI* mutations as well as the role of additional mechanisms in β-lactam susceptibility in *H. influenzae*. Ultimately, this PhD thesis aims to improve our understanding of the adaptive evolutionary landscape of *H. influenzae* under antibiotic therapy, and potentially provide guidance for the development of new treatment strategies.

MSV 016

Inter-genera dissemination of a VIM-1 encoding IncN plasmid in a German hospital

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Background: Mobile genetic elements are considered as the most efficient intra- and interspecies vehicles for the transfer of antimicrobial resistance determinants between bacteria. As part of our routine surveillance of carbapenem-resistant Gram-negative bacteria, we identified eight VIM-1-positive Enterobacterales species recovered from patients and the hospital environment, and characterized their mobilome.

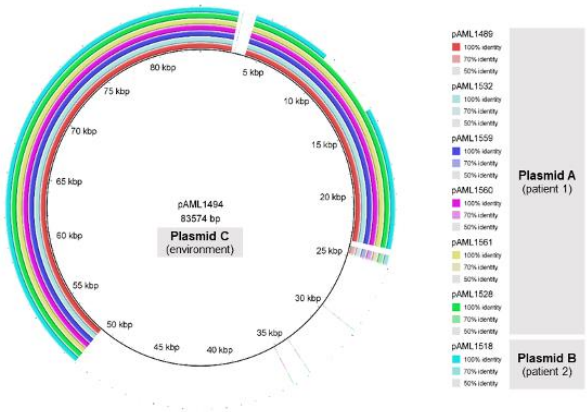
Methods: Eight carbapenemase-producing isolates, identified by MALDI-TOF as *Citrobacter amalonaticus* (AML_1561), *Escherichia coli* (AML_1559), *Enterobacter cloacae* (AML_1489), *Klebsiella oxytoca* (AML_1560), *K. pneumoniae* (AML_1528) and *Raoultella planticola* (AML_1532), recovered from a nephrology patient (patient 1), a *C. freundii* (AML_1518) from an infectious diseases patient (patient 2), and a hospital environment *Enterobacter* spp. (AML_1494) from the ICU of the internal medicine ward were collected between July and December 2022. Whole genome sequencing (WGS) was performed on the MiSeq and MinION sequencer platforms, and hybrid assemblies were generated using Unicycler. The resistome and plasmidome of the isolates was analysed using ResFinder and PlasmidFinder, respectively.

Results: WGS identified the Enterobacter isolates as *E. hormaechei* subsp. *hoffmannii* (AML_1489) and *E. roggenkampii* (AML_1494) and confirmed species identification of the other isolates. All eight isolates encoded VIM-1, which was harboured by an IncN plasmid. Comparative analysis revealed that for the six species recovered from patient 1 the *bla*$_{\text{VIM-1}}$-encoding IncN plasmids (plasmid A; 55kb) were highly similar (100% coverage, 99% identity). However, the IncN plasmid harboured by the *C. freundii* from patient 2 was 49kb (plasmid B) in size, while the IncN plasmid from the environmental isolate was 83kb (plasmid C). In addition to the VIM-1, the plasmid variants A and C harboured the antimicrobial resistance genes *aac(6')-Ib3*-like, *ant(3")-Ia*, *qnrB2*, *tet*(A) and two copies of *sul1*, while plasmid B was missing one *sul1* copy and *qnrB2*. Although plasmid C varied considerably in size from plasmid A and B, it resembled the other two plasmid variants (Figure 1). Furthermore, all three IncN plasmid variants also carried genes involved in conjugation, further supporting the hypothesis of the horizontal transfer of the plasmid between the different genera and species.

Conclusion: We identified an Enterobacterales outbreak of three variants of a plasmid encoding *bla*$_{\text{VIM-1}}$ between five genera and eight species, six of which were found in a single patient. Horizontal transfer of a multidrug-resistant plasmid was identified as the cause of the spread of *bla*$_{\text{VIM-1}}$ not only within one patient but also to another patient and the hospital environment. These findings highlight that WGS not only of bacteria but also their mobilome strengthens surveillance measures.

Figure 1. Comparative analysis of the *bla*$_{\text{VIM-1}}$-encoding plasmids. The figure was generated using BRIG v0.95.

Fig. 1



MPV 017

Through the labyrinth: how *Salmonella* penetrates the gastrointestinal mucus

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Motile enteropathogenic bacteria such as *Salmonella enterica* and *Escherichia coli* swim through aqueous environments or near solid boundaries using flagellum. The motility and virulence of pathogenic bacteria have been shown to connected by complex regulatory networks. Apart from motility, sophisticated flagellum nanomachine can also contribute to bacterial chemotaxis and evasion of host immune responses1.

For the successful host infection, pathogens need to initially penetrate the mucus layer to be able to disrupt the mucosal barrier and invade the epithelial cells in the gut. The mucus layer, secreted by goblet cells, is a biological hydrogel and play a key role in maintaining the integrity of the intestinal mucosal barrier and contribute to protection of underlying epithelial cells against gut content, microbiota and primary pathogens2. Previous studies showed the importance of the flagella driven motility in breaching of the mucosal barrier and gut colonization3. However, the exact bacterial navigation strategies and mechanisms of interaction between bacteria and mucus layer/underlying cells are unknown.

The aim of our study is to elucidate the molecular mechanism of interaction between enteric pathogen *Salmonella* Typhimurium and the gastrointestinal mucus prior to intestinal epithelium invasion with a focus on the role of bacterial flagellar apparatus. Thus, we generated a group of *Salmonella* Typhimurim ATCC 14028 mutants lacking different structural, antigenic and functional parts of flagellum such as Δ*fliB*, Δ*fliC*, Δ*flijB*/*fliC*, Δ*fliL*, Δ*motA* and Δ*cheY*. GFP-expressing pFPV25.1 plasmid introduced into these mutants enable us to track bacteria during microscopy. To this end, we use optical microscopy to follow the motion of *Salmonella* Typhimurium at interfaces and within biological hydrogels such as mucus. We will give examples, how the motility of this bacterium is affected by the properties of its environment or the *Salmonella* mutant probed.

Furthermore, by continuously recording z-stacks of samples, in which *Salmonella* interact with mucus-producing cells (HT29-MTX), we succeeded to follow the dynamics of bacterial penetration through a biologically relevant hydrogel. We will show that bacterial penetration predominantly proceeds via hydrogel voids, which are either already present or actively generated by the bacteria.

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MPV 018

Effects of single nucleotide polymorphisms in *Chlamydia pneumoniae* on the activation of human neutrophils

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Introduction: *Chlamydia pneumoniae* (*C. pneumoniae*) is an intracellular gram-negative bacterium that causes not only respiratory tract infections but is discussed being also involved in chronic non-respiratory diseases such as atherosclerotic plaque formation. Using next generation sequence, we previously demonstrated that genomes of vascular isolates derived from blood monocytes or atherosclerotic plaques were significantly different from respiratory isolates based on specific single nucleotide polymorphisms. Although *C. pneumoniae* infects a variety of tissue in the body, little is known about immunological factors that could be linked to tissue-specific pathogenicity. Human polymorphonuclear neutrophils (hPMNs) are the most common leukocytes in the circulatory system and are considered the first line of the innate immune system. Here we report that activation of hPMNs differs between vascular and respiratory isolates of *C. pneumoniae*.

Materials and methods: Vascular isolates of *C. pneumoniae* CV6 and PB2 and a respiratory isolate *C. pneumoniae* CWL029 were used in this study. hPMNswere isolated from peripheral blood from healthy adult volunteers. The sum of intra- and extracellular reactive oxygen species (ROS) was detected by the luminol-based chemiluminescence assay. Apoptosis was analysed by Annexin V/PI Staining. The activation of signalling cascades was investigated by western blot. Cytokine production was measured by the cytokine array and ELISA.

Results and conclusions: Both vascular and respiratory isolate of *C. pneumoniae* comparably inhibited apoptosis of hPMNs. However, production of ROS, cytokine release, as well as activation of AKT and MAP-kinase Erk1/2 pathways were significantly higher hPMNs infected with vascular isolates of *C. pneumoniae*. The amount of chlamydial heat shock protein 60 that plays a key role in induction of Emmprin, was significantly higher in a vascular isolate of *C. pneumoniae* compared to a respiratory isolate. In conclusions, different clinical *C. pneumoniae* isolates display diverse neutrophil responses that might be linked to tissue tropism.

MPV 019

CRISPRi based gene silencing reveals critical steps in host cell invasion by *Burkholderia thailandensis* E264

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Introduction: Members of the *Burkholderia pseudomallei* group are human-pathogenic bacteria which in part can cause severe infections such as the zoonotic diseases glanders or melioidosis. *B. thailandensis* is a nearly avirulent model organism genetically closely related to *B. pseudomallei*. Host cell invasion relies on the type 3 secretion system (T3SS) and the associated translocon BipC. Once escaped from phagocytosis, virulences factors associated with a type 6 secretion system enable *Burkholderia* intracellular motility (bim). There are four different genes located in the *bim* gene cluster. BimA is a cell surface exposed autotransporter protein which triggers actin polymerization at the bacterial cell pole. Then, actin-dependent motility contributes to intracellular persistence and subsequent dissemination by cell-to-cell spreading within the infected host.

Objectives: This work focuses on the elucidation of critical steps in *B. thailandensis* host cell invasion and intracellular motility. Cellular infection assays should be performed in combination with CRISPRi-based silencing of *Burkholderia* virulence genes.

Methods:
A previously published CRIPR gene interference system using codon-optimised dCas9 was freshly integrated into the genome of *B. thailandensis* fluorescent reporter strains. Single guide RNA molecules (sgRNA) targeting selected virulence genes were generated and *Burkholderia* CRIPRi strains generated by electroporation. Cellular infection assays were perfomed using murine macrophage-like RAW 264.7 cells or primary human macrophages.

Results and Conclusions: Statistical analyses revealed successful gene knock-down in the course of the infection assays. We could show that BipC as putative T3SS translocon is critical for the sufficient processing of *Burkholderia*-containing phagosomes involving F-actin decortion of intracellular uptake structures. Biochemical studies confirmed a weak actin-binding capability of full-length BipC as observed previously by others. *In silico* analysis of BipC protein sequences indicates the presence of additional actin-bding sites. Knock-down of *bimA* resulted in massively reduced actin polymerisation by *Burkholderia* bacterial cells 6 hours *post infectionem*, thereby confirming the suitability of the CRISPRi-based gene silencing in cellular infection assays.

MPV 020

Hot and cold – the conformational dynamics of the periplasmic chaperone SurA and their link to SurA activity

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Introduction: SurA, the main periplasmic chaperone of Gram-negative bacteria, shuttles unfolded outer membrane proteins (OMP) from the inner membrane SEC translocon to the outer membrane (OM). Due to SurA"s central role in OMP biogenesis, it is crucial for virulence and OM integrity (1-3). SurA consists of three domains: NC-core and peptidyl-prolyl-isomerase domains PPI1 and PPI2, of which NC-core and PPI1 bind client OMPs (4,5). A distinct role of PPI2 is elusive but might be linked the holdase activity for certain OMPs or an interaction with the beta-barrel assembly machinery inserting OMPs into the OM (6-8). Study of SurA conformational landscape revealed a compact but flexible domain arrangement (4,5,8-10). Hence, we aimed to investigate the link between PPI2 conformations observed *in silico* and SurA activity *in vitro*.

Methods: We created Alanine substitutions of residues involved in the PPI2:NC-core interface to weaken this interaction. Their activity was evaluated using a novel SurA activity assay. This assay uses Luciferase (Luc) as a substrate to probe SurA holdase activity. Luc is partially unfolded in the presence of SurA by gentle heating. Active SurA prevents the refolding of Luc upon lowering the temperature. This results in a reduced luminescence signal once ATP and Luciferin are added. If SurA is inactive, Luc can refold and a high signal is observed.

Results: By destabilizing the PPI2:NC-core interaction we were able to increase SurA activity *in vitro*. Preliminary analysis of SurA function with *E. coli* expressing those Alanine substitutions did not indicate loss of SurA function *in vivo*.

Conclusions: This is the first hint of the allosteric influence of PPI2 on SurA activity. We will now verify these findings using living bacteria in more detail.

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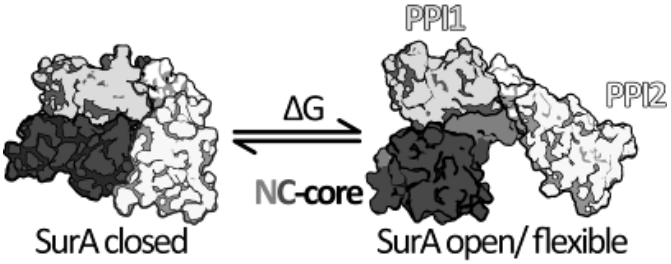
Fig. 1



MPV 021

Temperatures above 37°C lead to hypermucoviscosity in a clinical ST307 *Klebsiella pneumoniae* isolate resulting in higher virulence and mortality in *Galleria mellonella*

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Introduction: *Klebsiella (K.) pneumoniae* strains are associated with a wide range of diseases including pneumonia, wound and urinary tract infection, and sepsis. While they have traditionally been assigned to opportunistic, hypervirulent or classic types, a strict differentiation does often not apply anymore. This is mostly due to hybrid plasmids, which combine multi-drug resistance with hypervirulence traits leading to so-called convergent *K. pneumoniae* pathotypes. Hypervirulence is predominantly linked to hypermucoviscosity, which is the result of the overexpression of capsule polysaccharides presenting as highly mucoid cultures. For this study, we explored a sequence type 307 *K. pneumoniae* strain isolated from a clinical outbreak and carrying a large hybrid

plasmid regarding a temperature-dependent hypermucoviscosity switch. Studies have previously reported the impact of different temperatures on virulence features as well as the association of plasmid-encoded gene regulation with plasmid copy numbers (PCN).

Material & Methods: The hypermucoviscosity of strain PBIO1953 was studied at five different temperatures (RT, 28°C, 37°C, 40°C, 42°C) using the string and sedimentation assays as well as mucoid phenotype plates to evaluate the overproduction of capsule polysaccharides. In addition, we explored the temperature impact on bacterial virulence in an *in vivo Galleria (G.) mellonella* infection model, performed a biofilm formation assay in a 96-well-plate set-up and applied qPCR analysis for measuring the hybrid PCN upon changing temperatures. Ongoing RNA-sequencing analysis will prospectively allow the examination of the underlying mechanisms of temperature-dependent hypermucoviscosity and plasmid regulation.

Results: PBIO1953 showed hypermucoviscosity at 40°C and 42°C, which indicates a switch above 37°C from a non-hypermucoviscous to a hypermucoviscous phenotype. Interestingly, these results were associated with an increase in the PCN of the hybrid plasmid encoding not only the NDM-1 carbapenemase gene but also the mucoid regulator *rmpA*. It increased 4-fold between 37°C and 42°C, suggesting a correlation of PCN and hypermucoviscosity. Further virulence assays were performed in a temperature-dependent manner and showed an association between the attachment to plastic surfaces as well as higher mortality rates in *G. mellonella* larvae and increasing temperatures.

Discussion: The temperature-dependent hypermucoviscosity switch above 37°C is a novel development in bacterial evolution related to PCN, yet to be confirmed by RNA-sequencing but possibly contributing to the successful rapid and sophisticated adaptation to changing environments. These could include clinical conditions, such as immunological response, for example, fever. Whether these processes apply to other bacterial strains and non-pathogenic representatives remains to be investigated.

MPV 022

The mucoid phenotype of *Staphylococcus aureus* in the airways of people with cystic fibrosis is often co-isolated with small colony variants (SCVs)

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Objective: In people with cystic fibrosis (pwCF), *Staphylococcus aureus* is most commonly found in respiratory samples. Recently, we described a previously unknown mucoid phenotype (mPT) for *S. aureus* accompanied by hyperbiofilm formation in respiratory specimens of pwCF. A 5bp-deletion (5bp-del) in the intergenic region of the intercellular adhesion (*ica*) operon distinguishes these isolates from their non-mucoid predecessors. To determine the prevalence and potential impact of this adaptive mPT on lung disease in pwCF chronically infected with *S. aureus*, we conducted a prospective multicenter study.

Methods: 10 *S. aureus* colonies were picked from each respiratory specimen and characterized on Columbia blood and Congo red agar for mucoidy and size (normal/SCV). In addition, biofilm formation was examined, *spa* typing and intergenic *ica* region sequencing were performed on all isolates to verify the presence of the 5bp-del. Clinical data were documented in case report forms and included information on chronic co-infection with *Pseudomonas aeruginosa*, CFTR genotype, pulmonary function, and highly efficient modulator therapy (HEMT).

Results: Samples from 628 pwCF from 13 CF centers were analyzed. From 41 (9.1%) of 471 specimens of pwCF (71.8%) with *S. aureus* in the cross-sectional part of the study mPTs were cultured. In the subsequent longitudinal study, data from 37 pwCF with mPTs were available for comparison with data from 34 pwCF with only the normal PT of *S. aureus* (control group). Most mPTs were strong biofilm producers and almost all mPTs and normal *S. aureus* belonged to the same *spa* type. The prevalence of mPTs was dynamic throughout the study period. Significantly more isolates in the mucoid study group displayed the SCV PT (293 of 1110 isolates from 29 pwCF, 26.4%) compared to the control group (59 of 440 from 11 pwCF, 13.4%). 39.1% of mucoid isolates were SCVs, whereas 62.1% of SCVs were mucoid. Many mPTs carried the 5bp-del mutation, but there were also several mPTs with other previously unpublished mutations within the *ica* operon or unidentified mutations in other genes. The pwCFs of the two study groups did not differ by age, sex, CFTR genotypes, co-infection with *P. aeruginosa* or HEMT at baseline. However, the prevalence of SCVs, lung function (lower in the mucoid group) and HEMT at the end of the study (higher in control group) were different.

Conclusion: The prevalence of mPTs of *S. aureus* in respiratory specimens of pwCF was higher than expected. SCVs were very often detected in pwCF with mucoid mPTs of *S. aureus*. Many mPTs carried the previously described 5bp-del, but many other hitherto undescribed or unidentified mechanisms responsible for mucoidy were also detected. In summary, hyperbiofilm formation is an important adaptive mechanism for *S. aureus* survival in the hostile environment of the CF lung and is achieved by several molecular mechanisms.

Ethical vote 2017-457-f-S;ClinicalTrials.govNCT04171583Funded by DFG KA2249/7-1

PRHYV 023

Influencing factors of lethality in hospitalized patients with acute respiratory infections

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Question: Lethality of respiratory infections are influenced by a variety of individual and population-based factors. In this study, factors influencing the lethality of individuals infected with

respiratory viruses were investigated to gain a better understanding in order to develop effective preventive measures.

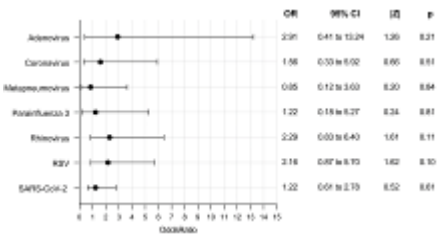
Methods: Virological as well as demographic data of adult patients hospitalized who tested positive for respiratory pathogens (Adenovirus, Coronavirus, Metapneumovirus, Parainfluenza 3, Rhinovirus, RSV, and SARS-CoV-2) was collected from July 2022 to April 2023 in a tertiary care hospital in Germany. A logistic regression analysis was performed using GraphPad Prism 9.5.1.

Results: In total, 138 out of 1,814 patients died during the hospitalization (7.61%). The most common pathogen detected was SARS-CoV-2 with 1.368 infected individuals out of which 99 (7.24%) died. Lethality was highest in individuals infected with RSV (13.13%, 13/99) and lowest in individuals infected with Influenza (5.44%, 8/147). In the logistic regression analysis, higher age manifested as a significant influencing factor on intrahospital lethality ($p<0.0001$). The Odds Ratios for the examined respiratory viruses studied compared to Influenza as reference are as follows: Metapneumovirus (0.85), SARS-CoV-2 (1.22), Parainfluenza 3 (1.22), Coronavirus (1.56), RSV (2.16), Rhinovirus (2.29) and Adenovirus (2.91) (*Figure 1*). However, none of the associations were significant.

Conclusions: In this study, age was the most significant risk factor for lethality associated with respiratory infections during hospitalization. While SARS-CoV-2 was still the predominant respiratory pathogen, intrahospital lethality was similar to other respiratory viruses, probably associated with the Omicron SARS-CoV-2 variants of concern and high vaccination rates. In comparison to Influenza, a higher risk of death was calculated for all respiratory viruses examined, except for Metapneumovirus. However, the lethality of other respiratory viruses compared to SARS-CoV-2 may be underestimated as there was a mandatory PCR entrance screening at that time only for SARS-CoV-2 during the period of data collection. This may have led to infections with other respiratory viruses potentially remaining undetected. Larger studies are needed to provide a more precise estimation of differences in lethality separated by the different viruses.

Figure 1: Odds ratio for intrahospital death of different respiratory viruses compared to Influenza

Fig. 1



PRHYV 024

Longitudinal evaluation of Influenza A seroconversion and its determinants among healthcare workers in the 2022/23 winter season

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Question: The Influenza virus is an important respiratory pathogen with high morbidity and mortality. This concerns in particular for healthcare workers (HCWs) with relatively high exposure and the risk of intrahospital transmission. A pronounced wave of Influenza A occurred in the 2022/23 winter season under generally relaxed post-COVID-19-pandemic protective measures. The rate of Influenza A seroconversion in HCWs, its determinants, and the effectiveness of the Influenza A vaccine are currently unclear, especially in the post-COVID-19 pandemic era.

Methods: As a sub-study of the CoVacSer trial, the seroconversion of Anti-Influenza-A IgG was investigated in 548 HCWs during the 2022/2023 winter season from 23 May 2022 to 11 May 2023. Subjects participated in the study before and after the Influenza wave (defined from 24 October 2022 to 8 January 2023) by providing a serum sample and completion of the study questionnaire (*Figure 1*). Anti-Influenza-A IgG levels were obtained using the SERION ELISA *classic* Influenza A IgG assay targeting Influenza A nucleoprotein and matrix proteins. Seroconversion was defined as 2-fold IgG level increase.

Results: Comparing Anti-Influenza-A IgG levels before and after the seasonal Influenza vaccination, there was no significant difference, confirming the suitability of the test methodology for infection monitoring and seroconversion evaluation (*Figure 2A*). IgG levels were significantly higher after the 2022/23 Influenza season (median Anti-Influenza-A IgG 6.0 U/ml pre vs. 7.1 post; $p<0.0001$; *Figure 2B/C*). 20.6% (83/402) showed seroconversion when comparing the study time point before and after the influenza season (*Figure 2C/D*). In the multivariate logistic regression analysis, the age category ≥ 45 years ($p=0.03$) and regular patient contact ($p=0.02$) were shown to be significantly influencing seroconversion. Male gender, BMI≥30 kg/m2, smoking, living in a household with ≥ 3 members, seasonal Influenza vaccination, and SARS-CoV-2 infection during the 2022/23 influenza season were not significantly associated with seroconversion (*Figure 2E*). Influenza vaccine effectiveness on seroconversion was 26.7%.

Conclusion: In the first Influenza season following the COVID-19 pandemic, about 20% of HCWs contracted Influenza A, which highlighting a concerning risk. This not only threatens the well-being of HCWs, but also poses a significant risk to vulnerable patients, especially in the light of the relaxation of mandatory masking protocols. Given the vulnerability of HCWs with older age and regular patient contact in the context of the moderate Influenza vaccine effectiveness on seroconversion, further action on masking protocols and other non-vaccination measures should be considered for this at-risk population.

Figure 1: Recruitment of study participants

Figure 2: Influenza A seroconversion and its influencing factors. The bold violet horizontal in 2C marks the 2-fold, the yellow horizontal the 4-fold IgG increase.

Fig. 1

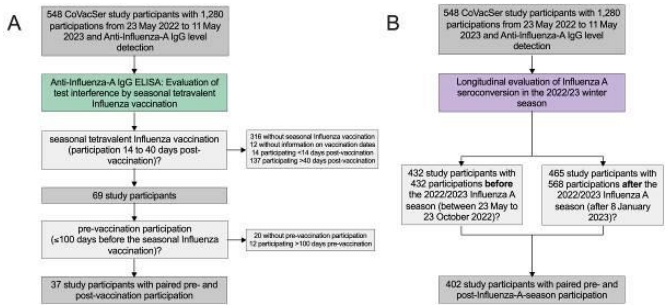
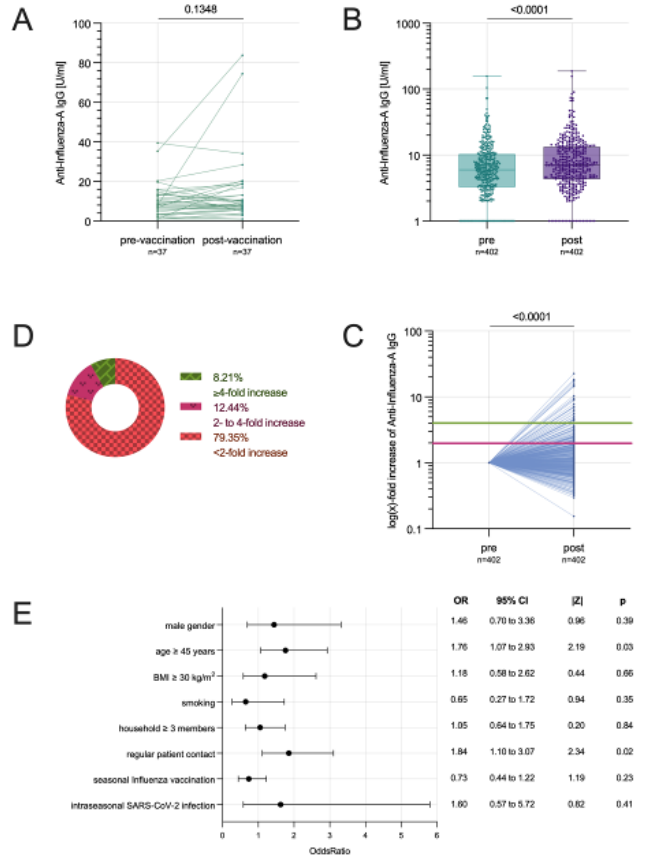


Fig. 2



PRHYV 025

Risk factors for unprotected contacts and SARS-CoV-2 infections in hospital employees.

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Introduction: At the beginning of the SARS-CoV-2 pandemic, health-care workers faced an elevated risk of unprotected exposure to the virus. In order to improve COVID-19 specific infection control measures in the hospital, risk factors for the occurrence of unprotected contacts of staff members to COVID19-infected persons and for SARS-CoV-2 infections should be identified.

Methods: A case-control study, including 3514 contacts of hospital staff members to 322 SARS-CoV-2-positive cases between January and December 2021, was performed. An unprotected contact was defined as stay in the same room as the index person without usage of a tight fitting ffp2-mask and without full SARS-CoV-2 vaccination status.

Results: Staff members without direct patient contacts had significantly more unprotected contacts among each other compared to staff members with regular close patient contacts ($P < 0.005$). That resulted in significantly more SARS-CoV-2 transmissions per contact in staff members without regular patient contacts compared to staff members with regular patient contacts ($P < 0.005$). Physicians had significant less unprotected contacts compared to the nursing staff/medical technical assistants ($P<0.005$). The risk of SARS-CoV-2 transmission, however, was not significantly different among both groups. Gender was no independent risk factor for a higher rate of unprotected contacts or SARS-CoV-2 transmission. Staff members were significantly more likely to be infected by other SARS-CoV-2-positive staff members than by SARS-CoV-2-positive patients ($P < 0.005$). Despite strict in hospital contact restrictions, SARS-CoV-2-positive index cases had an average of 10.9 contacts.

Conclusions: Despite identical contact precaution rules, staff members without regular patient contacts (e.g.: laboratory staff, administrative staff, technicians) had significantly more unprotected contacts among each other than staff members with regular close patient contacts (e.g. physicians, nurses). That paradoxically resulted in a significant higher risk of virus transmissions among staff members with overall less person to person contacts. We speculate that the higher compliance of staff working close to patients can be explained in particular by their sense of responsibility towards their vulnerable patients. Within the group of staff members working close to the patient, in 2021 physicians had significantly fewer unprotected contacts compared to nursing/medical technical staff. In contrast to nursing/medical technical staff, physicians often have their own office room, so that it is easier to spend meal and rest breaks alone than if there is only one shared recreation room on the ward for the whole team.

PRHYV 026

Outcome of hospital-acquired influenza and COVID-19 infections. A single-center retrospective case-control study

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Introduction: The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) and the influenza virus can cause nosocomial outbreaks with a high case fatality rate. In order to provide a rationale for adequate in-hospital infection control measures, we performed a head-to-head comparison of the outcome parameters monitoring requirement on intensive care unit (ICU) or intermediate care unit (IMC) and infection-related mortality of inpatients with in-hospital-acquired SARS-CoV-2 or influenza virus infection.

Methods: We performed a single-center retrospective case-control study that included 241 adult inpatients with in-hospital-acquired SARS-CoV-2-infection and 107 adult inpatients with in-hospital-acquired seasonal influenza infection. Demographic data, outcome parameters and underlying comorbidities of patients were obtained from the hospital information system. For each case of death among infected patients an assessment based on the patient records was made by three experienced physicians in order to decide whether the patient had died primarily from an underlying disease or primarily from the viral infection. In the case of differing assessments, the result chosen by two of the three raters was used. Multivariate regression analysis was performed for the assessment

of significant associations between risk factors and outcome variables.

Results: Compared to in-hospital influenza-infected inpatients, in-hospital SARS-CoV-2-infected inpatients showed significantly evaluated rates for infection-related in-house mortality and ICU or IMC monitoring requirement in the wild type/alpha and delta wave. In the Omicron BA.1/BA.2 and Omicron BA.5 waves, in-hospital SARS-CoV-2-infected inpatients did not show a significantly increased risk of infection-related in-house mortality or monitoring requirement compared to in-hospital influenza-infected inpatients. The classification, whether the patient had died primarily from the underlying disease or from the nosocomial viral infection, showed a substantial agreement between the three raters (Fleiss" kappa = 0.62 (95 % CI: 0.42–0.82)). Length of hospital stay of patients after an in-hospital-acquired SARS-CoV-2-infection decreased from 19.7 to 8.5 days what is below the average length of in-hospital stay of inpatients after an in-hospital-acquired influenza (13.0 days).

Conclusions: While the clinical course of in-hospital-acquired SARS-CoV-2 infections in the wild type, alpha and delta waves of the COVID-19 pandemic was more severe compared to in-hospital-acquired influenza infections the severity of in-hospital-acquired SARS-CoV-2-infections dropped to the level of in-hospital-acquired Influenza-infections during the Omicron waves. Considering the comparable severity of in-hospital-acquired SARS-CoV-2-Omicron and influenza infections similar levels of infection control are justified.

PRHYV 027

Quality of life and ability to work after SARS-CoV-2 infections – six-month data from a prospective, controlled cohort study

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Question: The impact of a SARS-CoV-2 infection on the quality of life and ability to work in the mid- and long-term still remains unclear because few studies follow up largely healthy, working-age individuals after an infection and compare them to a control group. We aimed to fill this gap by assessing the quality of life and ability to work for six months after SARS-CoV-2 infection in a large cohort of healthcare workers.

Methods: Our prospective, controlled cohort study assessed the quality of life and work ability of 761 healthcare workers (HCWs) from 29 September 2021 to 20 May 2023 after a PCR-confirmed SARS-CoV-2 infection ("infection group") using an electronic questionnaire. Individuals without any prior SARS-CoV-2 infection, observed after their third dose of COVID-19 vaccination, formed the control group. The World Health Organization Quality of Life (WHOQOL-BREF) index with its four dimensions of physical health, psychological health, social relationships and environment, and the Work Ability Index (WAI) were assessed before,14 days, three, and six months after the infection or third COVID-19 vaccination. Participants with a SARS-CoV-2 infection or COVID-19 vaccination during the follow-up period were excluded from the analysis.

Results: Comparing the infection (n=307) and the control group (n=454), SARS-CoV-2 infections were followed by a significantly lower work ability 14 days after infection. In addition, we found a non-significant trend towards lower physical and psychological health. Surprisingly, there was a trend towards higher environmental health scores in the infection group. For the remaining six-month observation period, both groups had similar scores in the four dimensions of the WHOQOL-BREF and the WAI (*Figure 1 and 2).* An exploratory analysis indicated that study participants ≥ 45 years had significantly lower scores in both the WAI and the WHOQOL-BREF´s physical health domain over the entire study period.

Conclusion: The ability to work and the physical health dimension of the quality of life were impaired 14 days after a SARS-CoV-2 infection. However, these impairments were mild, and we did not find a significant group difference in the ability to work or any of the WHOQOL-BREF´s dimensions after three or six months.

Interestingly, individuals over the age of 45 were had a generally lower ability to work and quality of life, potentially leaving them more vulnerable to the deteriorating impact of a SARS-CoV-2 infection. In summary, our data from this large group of HCWs indicates that in a working-age and largely healthy population, a SARS-CoV-2 infection has a temporary effect on the working ability and mostly the physical health domain of the quality of life, which lasts less than 3 months.

Figure 1: Long-term development of the WHOQOL-BREF

Figure 2: Long-term development of the WAI

For each study time point, the means of the index are shown with the standard deviations as error bars.

Fig. 1

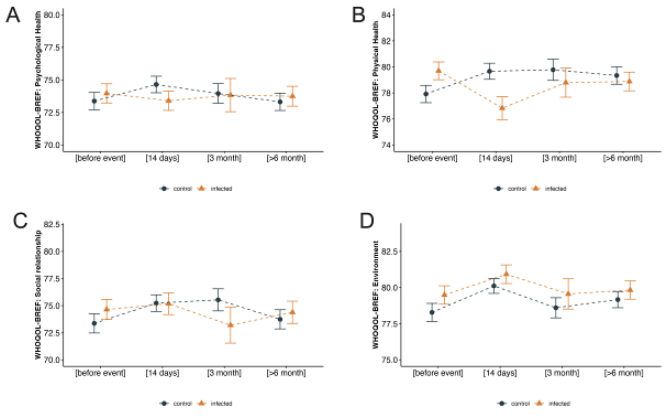
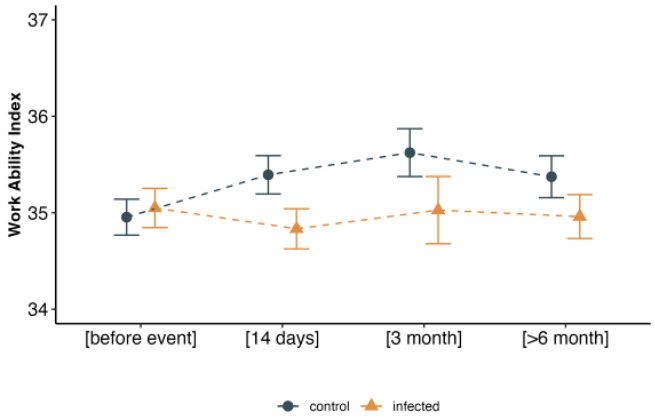


Fig. 2



PRHYV 028

Lower bounds of post-COVID syndrome incidences with persistent fatigue and ME/CFS in the cohort of hospital employees infected during the first and second COVID-19 wave

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Introduction: Post-COVID syndrome (PCS) may occur after a SARS-CoV-2 infection, leading mostly to fatigue as persisting symptom. Myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) is a systemic and chronic, often debilitating and postinfectious disease, leading to substantial fatigue and post-exertional malaise (PEM) as cardinal symptoms. The study aims to determine and characterise the incidence of PCS with ME/CFS in the cohort of SARS-CoV-2 infected hospital employees (HE).

Methods: All SARS-CoV-2 positive tested HEs between March 2020 and May 2021 were contacted between June and October 2021 for a systematic survey regarding acute and persistent symptoms. From May to July 2022, HEs with persistent fatigue were invited to participate in a structured assessment that included a screening questionnaire for PEM, classification according to the Bell-score, and the Canadian consensus criteria for the diagnosis of ME/CFS. A Schellong test was carried out to assess the HEs for orthostatic dysfunction and possible postural tachycardia syndrome (POTS). Coagulation factors and Epstein-Barr virus (EBV) antibodies were analysed. Physical examination, routine blood analysis and SARS-CoV-2 PCR were performed to rule out recent infection or other causes. Descriptive statistics and application of 95% confidence intervals (CI) were calculated with the statistical software R. Percentage values were applied to a total of 221 HEs. As only 19 HEs ran through all diagnostic tests, lower bounds were estimated.

Results: Out of 221 HEs, 48 reported fatigue in the first survey, out of which 19 (median age 51.0y, age range 25-61y, 89.4% female) reported persistent fatigue lasting from 467 to 1008 days (median 629 days) until now. None of the 19 HEs were vaccinated at the time of the first SARS-CoV-2 infection during the first two COVID-19 waves. The median Bell-Score was 60 out of 100 (40-90). 14 HEs (6.3% of 221 HEs) were positive in PEM-screening questionnaire. 7 HEs (3.2%) fulfilled all diagnostic criteria for ME/CFS. 1 HE (0.5%) was diagnosed with POTS and 4 (1.8%) with orthostatic hypotension, during the Schellong test. In the blood analysis, 8 HEs (3,6%) showed IgG against early antigens indicative for possible EBV reactivation. Acute phase reactions with either increased fibrinogen, von Willebrand factor, factor VIII or decreased antithrombin values were noted in 7 HEs (3.1%).

Conclusions: Application of diagnostic criteria of ME/CFS disease in HEs with PCS with persistent fatigue after infection during the first two SARS-CoV-2 waves revealed an incidence of at least 3%. The diagnosis of ME/CFS can offer HEs a more precise diagnosis and, thus, prevent worsening by pacing strategies. Reactivation of EBV or activation of the coagulation cascade may play a pathogenetic role.

Figure 1: HEs follow-up and selection for assessment

Table 1: Results of HEs assessment: lower bounds of incidences

Fig. 1

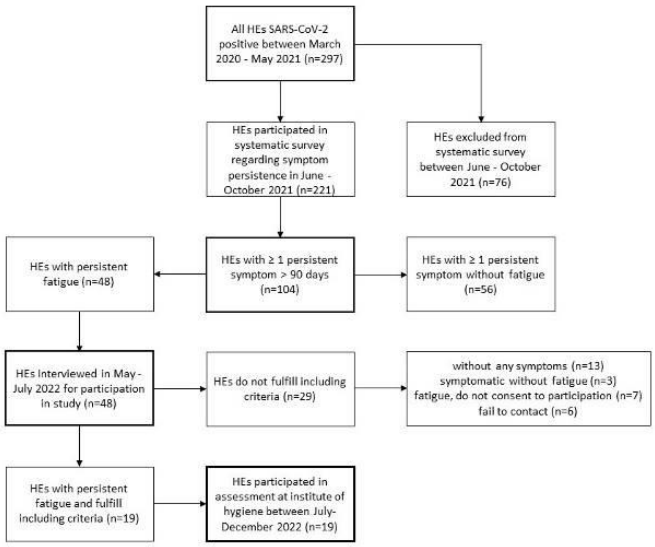

Fig. 2

	n (% of 221)	95% CI
Evaluation for ME/CFS and PEM		
Diagnosis of ME/CFS	7 (3.1)	1.2 - 6.4
Positive PEM screening	14 (6.3)	3.5 - 10.4
Schellong test:		
Orthostatic hypotension	4 (1.8)	0.4 – 4.5
Diagnosis of POTS	1 (0.5)	0.01 – 2.4
Reinfections with SARS-CoV-2		
≥ 1 reinfection with SARS-CoV-2	8 (3.6)	1.5 – 7.0
Type of SARS-CoV-2 transmission		
Definite nosocomial infection	8 (3.6)	1.5 – 7.0
Possible nosocomial infection	5 (2.2)	0.7 – 5.2
Community acquired infection	6 (2.7)	1.0 – 5.8
Blood analysis		
Complete EBV reactivation	1 (0.5)	0.01 – 2.4
Positive early antigens for EBV	8 (3.6)	1.5 – 7.0
Detection of acute phase reactants of coagulation factors	7 (3.1)	1.2 - 6.4

MSZOV 029

Detection of a transferrable daptomycin resistance mechanism in Gram-positive bacteria: A novel membrane-associated ABC transporter confers high-level daptomycin resistance in staphylococci

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Question: Daptomycin (DAP) is a reserve antibiotic effective against Gram-positive bacteria, including high priority pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE). Resistance to DAP and clinical treatment failure has been associated with the accumulation of adaptive chromosomal mutations in cell wall homeostasis genes, but so far not with horizontal acquisition of specific resistance determinants. In a study on antimicrobial resistance burden in industrialized pig farming, we previously isolated the DAP high-level resistant *Mammaliicoccus sciuri* strain TS92. Here we now set out to determine the exact mechanism of resistance in TS92, which does not seem to correspond to the previously known mechanisms.

Methods and Results: To identify the underlying resistance mechanism, we performed transcription profiling upon DAP pulse-exposure of TS92, with subsequent subcloning and mutational analysis of highly upregulated candidate genes. The experiments showed that DAP resistance is mediated by a novel two-gene operon (named *drcAB*), controlled by an adjacent, divergently transcribed two-component regulatory system (*drcRS*). Mutational analyses and heterologous expression of the distinct *drc* genes demonstrated that *drcAB* is required and sufficient to confer DAP resistance in *S. aureus* and *Bacillus subtilis*. Through high-resolution LC/MS analyses we further found that DrcAB inactivates DAP through chemical modification. The *drc* genes and proteins show homologies to membrane-associated antimicrobial peptide ABC transporters of Gram-positive bacteria, but clearly differ on nucleotide and amino acid levels from known systems present in the core genomes of Staphylococcaceae. In *M. sciuri* TS92, the *drc* genes are flanked by insertion sequences and integrated in the vicinity of a staphylococcus cassette chromosome (SCC) element, suggesting mobility and horizontal acquisition of the locus from another species.

Conclusion: With this study, we probably witnessed the first steps in the emergence of acquired DAP resistance in staphylococci. Due to its lifestyle as both soil inhabitant and animal commensal, *M. sciuri* is considered a gateway species through which genes from the resistance pool in the environment can gain access to staphylococci. Indeed, *M. sciuri* has been shown in the past to serve as a hub for the spread of numerous resistance genes into *S. aureus*. Considering the growing selection pressure due to the increasing clinical use of DAP, it is only a matter of time when *drc* genes will be transferred to pathogenic staphylococci and possibly also to other Gram-positive pathogens, arguing for vigilant future monitoring of *drc* spread.

MSZOV 030

Unraveling the success of multidrug-resistant high-risk clonal *Escherichia coli* and *Klebsiella pneumoniae* lineages: Insights from a One Health perspective

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Introduction: Antimicrobial resistance (AMR) is an important part of the One Health context, which aims to maintain the well-being of humans, animals, and the environment. The overuse of antibiotics in human and veterinary medicine has accelerated the emergence and spread of AMR with opportunistic *Enterobacterales*, particularly *Escherichia coli* and *Klebsiella pneumoniae*, becoming increasingly resistant to multiple classes of antibiotics. Furthermore, some of these strains combine multidrug-resistant (MDR) phenotypes with high-level virulence, posing a threat to both immunocompromised and healthy individuals. Consequently, the World Health Organization has ranked these MDR enterobacterial representatives as one of the most critical priority pathogens for which new antibiotics are urgently needed.

Methods: Our work focuses on understanding the driving forces and factors behind the occurrence, emergence, and adaptation of high-risk clonal lineages of MDR *E. coli* and *K. pneumoniae* in the One Health context. By integrating genomics, transcriptomics, and functional studies, the latter including a comprehensive set of *in vitro* and *in vivo* virulence and resilience as well as experimental evolution assays, we identified key factors contributing to the success of these lineages.

Results: We provide compelling evidence that high-risk clonal lineages broadly occur and circulate among clinics, wildlife, and food in international locations. In particular, globally known *E. coli* lineages such as sequence types (ST)58, ST131, ST410, and ST648, and *K. pneumoniae* ST307 play an important role in the spread and stabilization of AMR in the One Health context. Their success results from a combination of factors, including the sophisticated interplay of bacterial fitness and AMR as well as extra-chromosomal elements, e.g., hybrid resistance/virulence plasmids. In addition, our results show that bacterial hosts can rapidly mitigate fitness costs associated with AMR acquisition while MDR is maintained even in the absence of antibiotic selection pressures. This is due to compensatory at genomic and transcriptomic levels affecting major bacterial master regulators.

Discussion: In conclusion, our research sheds light on the fundamental principles that govern the interplay of AMR and bacterial virulence and advances our understanding of the factors that contribute to the emergence of successful MDR high-risk clonal lineages in the One Health context. Furthermore, our results can be leveraged as a baseline for prospective interventions and alternative therapeutic strategies to comprehensively target the global threat of AMR.

MSZOV 031

Clostridioides difficile recovered from hospital patients and livestock in Nigeria share near-identical genome sequences

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Introduction: *Clostridioides difficile* is an important One Health pathogen and a leading cause of antibiotic-associated diarrhoea commonly referred to as *C. difficile* infection (CDI). Information on the molecular epidemiology of *C. difficile* from the African continent remains scarce, despite having one of the highest burden of diarrhoea diseases globally and a widespread occurrence of known risk factors for the emergence and spread of CDI. In this study, we investigated the occurrence and molecular epidemiology of *C. difficile* among humans and animal sources from Nigeria.

Methods: A total of 2201 faecal/manure samples recovered from diarrheic human (n=1630) and animal sources (n=571) in Makurdi, North central Nigeria between January 2021 and December 2022 were cultivated for the recovery of *C. difficile*. Isolates were genome-sequenced using the Illumina technology and the sequence reads uploaded to the *Clostridioides* database on the EnteroBase platform (https://enterobase.warwick.ac.uk/species/index/clostridium). EnteroBase facilitates genome assembly, *C. difficile* PCR ribotype (RT) prediction, toxin gene detection and phylogenomic analyses (based on unique core and whole genome allelic profiles).

Results: A total of 228 isolates were recovered and sequenced, out of which 197 genomes passed the quality and assembly check and were thus available for further analyses. The *C. difficile* population structure was highly diverse. Based on hierarchial clustering (HC) of cgMLST allelic profile the isolates were distributed into 29 core-genome sequence typing complex (CC) clusters and 27 CC singletons (which correlate strongly with RTs). The *C. difficile* population was dominated by non-toxigenic strains with the toxin genes detected in only 33 isolates (human n=14; animal sources n=19): *tcdA* (32/33), *tcdB* (33/33) including three CC1(RT078) isolates harbouring the binary toxin encoding *cdt* genes. The CCs/RTs detected were among the most prevalent and clinically important *C. difficile* strains causing nosocomial infections in humans. Noteworthy is the interspecies and intraspecies sharing of extremely closely related genotypes between human and poultry (≤ 2 SNP; CC3, corresponding to RT001;), human and pigs (≤ 0-1 SNP; CC3622/RT014), human to human (≤ 0-1 SNP, CC3/RT001), pig to pig across different farms (≤ 0-1 SNP; CC19890). Interestingly, the clinically important livestock-adapted CC1/RT078 strain was detected among humans but not animals.

Conclusions: The study provides evidence for the circulation of highly diverse *C. difficile* lineages among animals and humans in Nigeria. The carriage of genetically closely related strains among humans and animals suggests the likelihood of transmission from a common reservoir or bidirectional (interspecies and intraspecies) exchange of the pathogen. Similarly, the detection of the livestock-adapted CC1/RT078 in humans infers the occurrence of a plausible zoonotic transmission.

MSZOV 032

Identifying persistent quinolone and cephalosporin resistant _Escherichia coli_ strains along the pork production chain in Thuringian pigsties

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Introduction: Rising levels of antimicrobial resistance (AMR) led the German government in 2018 to restrict veterinary usage of fluoroquinolones (FQ) and certain cephalosporins (CS), resulting in subsequent sales reductions, suggesting less usage. However, AMR determinants can be maintained in the absence of antimicrobial (AM) usage via co-selection and cross-resistance. We therefore studied the persistence of genetic elements coding for AMR and strains carrying them longitudinally within a fattening run (FR) and from one FR to another in Thuringian pigsties.

Material/method: A total of 393 ceftiofur, cefquinome- or fluoroquinolone-resistant *E. coli* strains, serving as indicator bacteria, were isolated from pooled faecal samples collected during two to three consecutive FRs of one conventional and two organic farms over a 16-month period. After assessing the resistance profiles phenotypically using a commercial system with 16 AMs (13 classes according to WHO categorization) important in veterinary medicine and characterizing isolate phylogeny by MLVA-PCR, 216 strains were selected for whole genome sequencing (WGS).

Results: The higher resolution of WGS identified strains that occurred persistently on individual farms by showing high genetic similarity during and across FRs. Many of these belonged to the clonal complex 10, but other persistent strains were also identified featuring multi-locus sequence types (ST58, ST69, ST345/410) described in the literature as typical of clones with pathogenic potential and high-risk for global expansion. The genetic basis enabling persistence in a closed-farm environment appears to be encoded in the accessory genome, but could not be narrowed done more precisely from the available data. However, association with one or several farms, phylogroup, or number of resistances to different antibiotic classes, were not found to affect strain ability to persist in this study. The persistence of the cephalosporin and fluoroquinolone resistance determinants themselves could also be demonstrated and was related to, among other things, co-selection and cross-resistance, but also to conserved localization on plasmids or successful strain-plasmid combinations.

Conclusion: The metadata on antimicrobial usage suggests it cannot be the sole factor affecting resistance prevalence or spread and maintenance of resistance determinants on a farm. A better understanding of the mechanisms underlying strain and AMR persistence will be needed to sustainably reduce AMR prevalence in the livestock sector.

MSZOV 033

Clumpy adhesion phenotype of _Escherichia coli_ gives power to tolerate high antibiotic stress

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Introduction: *E. coli* intestinal infection pathotypes are characterized by distinct adhesion patterns including the previously identified clumpy adhesion phenotype. Here we characterize

genetic factors and answer the question if this phenotype confers any advantage to clump producing *E. coli* strain 4972 . This adhesion phenotype comprised of three dimensional structures similar to that of biofilms, formed after only 4 h incubation with human epithelial cells.

Objectives: We tested the hypothesis that bacteria present in clumps exhibit distinct gene expression profiles and that some genes with altered expression are involved in clumpy adhesion. We also elucidated the contribution of *E. coli* strain 4972-encoded factors that affected clump formation and susceptibility to the antibiotic stress.

Methods: Genome of clump forming *E. coli* was sequenced and compared with already published 66 reference genomes of pathogenic and commensal *E. coli*. RNA was isolated from bacteria forming clumps and bacteria from supernatants during adhesion assays to 5637, PK-15 and Caco-2 cells and sequenced. Based on transcriptomic data, nine differentially expressed genes were targeted for deletion. Seven mutants were successfully generated, complemented and analyzed for prevalence, growth, motility, adhesion and antibiotic stress. Additionally, global proteome analysis of clumpy *E. coli* was carried out for adhered bacteria and planktonic bacteria in the supernatant.

Results: As a result of genomic comparison, clumpy *E. coli* strain was present within a clade corresponding to phylogroup A containing strains isolated from human fecal samples with diverse lifestyles. Transcriptome revealed differences among the subpopulation of sessile bacteria constituting the clumps and planktonic bacteria in the supernatant. Seven genes with successful deletions had variable distribution in different pathotypes and nonpathogenic *E. coli* with *pilV* and *spnT* genes being the least frequent or absent from most groups. Deletion of five differentially expressed genes, *flgH*, *ffp*, *pilV*, *spnT* and *yggT* affected motility, adhesion or antibiotic stress. Δ*flgH* exhibited 80 % decrease and Δ*yggT* depicted 145.5 % increase in adhesion, and upon complementation, adhesion significantly reduced to 13 %. Δ*flgH* lost motility and was regenerated when complemented whereas Δ*ffp* had significantly increased motility and reintroduction of the same gene reduced it to the wild-type level. The clumps produced by of Δ*ffp* and Δ*spnT* were more resistant and protected the bacteria with Δ*spnT* showing the best clump formation in terms of ampicillin stress protection. Δ*yggT* had the lowest tolerance to gentamicin where the antibiotic stress completely eliminated the bacteria.

Conclusion: We investigated the influence of clump formation on the cell surface adhesion and antimicrobial tolerance with the contribution of several factors conferring advantage by clump formation on susceptibility to the selected antibiotics.

Fig. 1

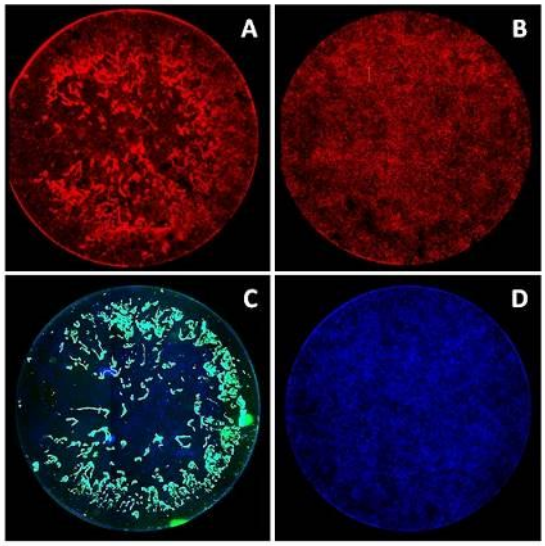
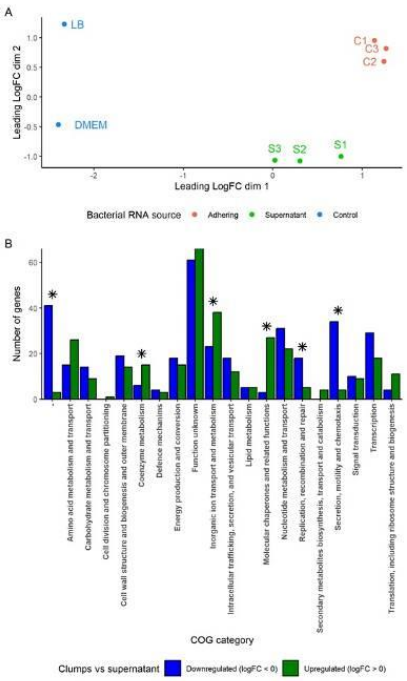


FIG. 1: Clump formation by strain 4972

(A) PI stained wild-type *E. coli* strain 4972, (B) PI stained 5637 cell line only as negative control, (C) GFP expressing 4972+pFPV25.1Kan and DAPI stained nuclei of 5637 cell line, and (D) DAPI stained nuclei of 5637 cell line only as negative control, after 4 h incubation of adhesion assay and visualized by VideoScan system.

Fig. 2



Differential gene expression of adhered and un-adhered bacteria

A: Multidimensional scaling plot of distances between the samples of RNA sequencing, leading log-fold-change is the root mean square average of the largest log2-fold changes between each pair of samples. B: Differentially expressed genes categorized based on the genes/proteins in the genome. COGs were compared between adhered and supernatant clumpy *E. coli* for all genes that had p-value lower than 0.05 in EdgeR analysis. x-axis shows the COG categories and y-axis shows the number of genes. NA (-) category corresponds to genes that were not assigned to any of the COGs.

MSZOV 034

Disease alleviating effects following prophylactic lemon and coriander essential oil treatment in mice with acute *campylobacteriosis*

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Introduction: Given the worldwide increasing prevalence of human *Campylobacter jejuni* infections and the emergence of multi-drug resistant enteropathogenic strains, antibiotic-independent approaches applying non-toxic natural compounds for the treatment and prophylaxis of campylobacteriosis appear utmost desirable. In our placebo-controlled intervention study, we surveyed potential disease-alleviating including anti-pathogenic and immune-modulatory effects upon prophylactic oral application of lemon-essential oil (LEM-EO) and coriander-essential oil (COR-EO) in acute experimental campylobacteriosis.

Methods: Therefore, secondary abiotic IL-$10^{-/-}$ mice were orally challenged with either LEM-EO or COR-EO starting seven days prior to peroral *C. jejuni* infection.

Results: Six days post-infection, slightly lower pathogen loads were assessed in the colon of mice from the LEM-EO as opposed to the COR-EO cohort if compared to placebo counterparts. Prophylactic application of both EOs improved the clinical outcome of acute campylobacteriosis, which was paralleled by less distinct pathogen-induced colonic epithelial cell apoptosis. Moreover, mice subjected to LEM-EO and COR-EO prophylaxis displayed lower colonic numbers of macrophages/monocytes and of T lymphocytes, respectively, whereas in both verum groups, basal IL-6 and IFN-γ concentrations were measured in mesenteric lymph nodes on day 6 post-infection. The oral challenge with either EOs resulted in diminished secretion of distinct pro-inflammatory mediators in the kidney as well as serum samples derived from the infected mice.

Discussion/Conclusion: The results from our preclinical in vivo study provide evidence that LEM-EO and COR-EO constitute promising prophylactic measures to prevent severe campylobacteriosis which may help to reduce the risk for development of post-infectious sequelae in *C. jejuni* infected individuals.

EKMPV 035

The *Plasmodium falciparum* CCCH zinc finger protein *Pf*md3 is a potential regulator of male gametocyte transcripts through its interaction with RNA-binding proteins

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Question: The stage transition of the malaria parasite *Plasmodium falciparum* through its complex life cycle requires a coordinated gene expression across all parasite stages and sexes. Post-transcriptional regulation of gene expression mediated by RNA-binding proteins is an important mechanism to fine tune parasite development and stage transition. In this study, we investigated the function and interactome of a *P. falciparum* putative RNA-binding CCCH-zinc finger protein, *Pf*md3. *Pf*md3 was first identified as a protein with significantly upregulated transcript expression following treatment of gametocytes with the histone deacetylase inhibitor Trichostatin A and has recently been linked to male gametogenesis.

Methods: We used loss-of-function phenotype analyses and BioID methods to study the role of *Pf*md3 in the asexual and sexual stages of *P. falciparum.*

Results: We show that *Pf*md3 is mainly expressed in the cytoplasm of immature male gametocytes. Down-regulation of *Pf*md3 reveals essential roles in asexual blood stage replication, gametocyte maturation and exflagellation. Proximal dependent labelling of *Pf*md1 interaction partners by BioID analysis showed enrichment of ribosomal proteins, other RNA-binding proteins including PABP1 and proteins involved in translational repression such Puf1 and CITH, further CCCH zinc finger proteins as well as members of the translational initiation complex eIF4G and CCR4-NOT1 complex.

Conclusions: Our data let us conclude that *Pf*md3 is a post-translationally regulated multifunctional RNA-binding protein involved in regulating the expression of male gametocyte-specific genes, while translationally repressing female gametocyte-specific genes, thereby facilitating the proper development of male gametocytes.

EKMPV 036

Parasite proliferation and host cell phenotype drive *Leishmania* exit from infected macrophages

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Introduction: *Leishmania major (L. major)* is a protozoan parasite species causing severe disease in humans. *Leishmania* promastigotes ultimately infect macrophages where parasites develop their lysis-resistant and replicative amastigote form within phagolysosomes. To progress and sustain the infection, amastigotes need to exit their primary host cell and enter yet uninfected cells, making it a critical process for *Leishmania* pathogenicity. Still, to date, the mechanism and modulators of *Leishmania* parasite exit are poorly understood especially in human primary cells. We hypothesize that the underlying mechanism of *L. major* parasite spreading is a direct cell-to-cell transfer, which is driven by the parasite proliferation and modulated by the cytokine environment of host cells.

Methods & Results: In a newly established flow cytometry analysis of *in vitro*-infected human monocyte-derived macrophages (hMDM), we were able to quantify the exit of parasites and show that it is indeed associated with the transfer of cellular material from the primary host to the receiving cell. Moreover, in accordance with results from murine *in vivo* infection experiments, axenic amastigote infected hMDM had increased Caspase-3 activity compared to uninfected cells, suggesting the occurrence of apoptosis during infection progress. By employing the proliferation reporter mKikume, expressed by *L. major* during the infection of hMDM, we could link the parasite transfer between host cells to their propagation. Egressing amastigotes detected in receiving cells had a higher proliferation status compared to those residing in primary infected cells. Interestingly, the proliferation state of parasites was also influenced by the phenotype of infected phagocytes, as we measured increased proliferation rates in pro-inflammatory and reduced rates in anti-inflammatory stimulated macrophages in comparison to non-activated hMDM.

Discussion: In summary, this data suggests that the exit ability of *Leishmania* is dependent on the parasite"s proliferation state and the phenotype of primary infected host cells, and it partially relies on the cell death induction of infected cells. We now seek to

unravel the influence of pro- and anti-inflammatory macrophage stimulation on the parasite spread to gain further insight into the dynamics of *Leishmania* exit from human host cells.

EKMPV 037

Screening for *Candida glabrata* factors involved in macrophagal escape

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Candida glabrata is a well-adapted fungal commensal of humans which can also cause superficial and systemic infections. During infection, *C. glabrata* encounters cells of the innate immune system such as macrophages, which constitute the first line of defense against such pathogens. In order to counteract these phagocytic cells, *C. glabrata* has evolved strategies to survive and replicate within macrophages for days, while causing very little damage and inflammation. *C. glabrata* has been observed to escape from macrophages after 2-3 days of infection when host cells containing high numbers of replicating yeast start bursting (1). Despite its clinical importance, *C. glabrata*"s host cell exit strategies remain poorly understood.

To further elucidate the processes and fungal factors behind *C. glabrata*"s exit, we established a *C. glabrata*-macrophage escape model. The fungal load was found to be an important factor in mediating the yeast"s exit, as a higher number of initial intracellular yeasts led to an earlier escape. We investigated whether growth in the yeast morphology *per se* is the key for the generally late exit of *C. glabrata* from host cells. However, experiments with a yeast-locked *C. albicans* strain showed that this is not the case, since even these yeast cells escaped much faster than *C. glabrata*.

Using the same escape model, we are currently screening a mutant library that represents 12% of all *C. glabrata*"s genes (2) *via* barcode sequencing. Previous studies using this library have focused on different aspects of *C. glabrata*"s persistence, biofilm formation, and antifungal properties. Our new data will provide insights into the role of *C. glabrata* factors during exit from macrophages.

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(2) Schwarzmüller, T. *et al*. (2014). Systematic phenotyping of a large-scale *Candida glabrata* deletion collection reveals novel antifungal tolerance genes. *PLoS pathogens*, *10*(6), e1004211.

EKMPV 038

Exocytosis-related mechanism is associated with the egress of *Coxiella burnetii* from host cells

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Q fever is a human disease caused by the intracellular bacteria *Coxiella burnetii (C. burnetii)*, and manifests as a cold-like illness, pneumonia, hepatitis or in rare cases as endocarditis. Infection starts with internalization into alveolar macrophages, although epithelial and endothelial cells resulted also infected. Once bacteria localize within a vacuole, it fuses with endosomes and lysosomes converting the vacuole into a replication compatible *Coxiella*-containing vacuole (CCV), becoming into a habitat for intracellular replication. Cell death is only partially responsible for *C. burnetii* egress and suggest that other pathways are also involved.

Question: To cause disease in other organs, *C. burnetii* has to egress from its host cell, but these mechanisms are not completely understood.

Methods: To track events of *C. burnetii* egress laser confocal microscopy (CLM) at fixed time points and time-lapse live cell imaging microscopy (LCIM) were used.

Results: Here, we demonstrate that also exocytosis can be mechanism of *C. burnetii* egress. Thus, CLM images showed that the CCV grew over the first 4 days of infection. Most cells contained one single vacuole filled with large amounts of bacteria. Starting from 5 days post infection, we found several small CCVs (sCCVs). Spatial distribution of these small CCVs suggested an association with the cytoplasmic membrane of the host cell. Indeed, observation of the actin cytoskeleton of infected cells revealed that sCCVs localized mainly at the apical side of the cells. Some of these sCCVs appeared open at the cell ridge releasing bacteria to the extracellular milieu. LCIM images showed that CCV suffered fission events over the time, supporting our CLM data. Further characterization also demonstrated that the ESCRT-adaptor protein ALIX decorated sCCVs at the cell ridge, indicating that *C. burnetii* might exit the host cell via exocytosis. As a matter of fact, our LCIM analysis revealed that intracellular bacteria egressed from host cells in an exocytosis-like manner without causing alterations to the host cell integrity.

Conclusions: Our observations demonstrate that *C. burnetii* might be able to escape from its host cell via fission of the CCVs and exocytosis of small single CCVs. Further work is in progress to delineate the mechanism of the exocytic escape of *C. burnetii*.

EKMPV 040

The zoonotic pathogen *Chlamydia psittaci* egresses from the host cell via formation of *Chlamydia*-containing spheres, a novel non-lytic egress pathway

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Introduction: The obligate intracellular Gram-negative bacterium *Chlamydia psittaci* acts as zoonotic pathogen. It causes the respiratory disease psittacosis which can lead to severe pneumonia. The intracellular development of *Chlamydiae* includes host cell invasion followed by intracellular growth and replication and a final host cell egress. Here, we describe and characterize a novel non-lytic egress pathway of *C. psittaci* – the formation of *Chlamydia*-containing spheres (CCS).

Methods: In this study, CCS formation was characterized in contrast to extrusion formation – the previously known non-lytic egress pathway of *Chlamydiae* – and in contrast to cellular death. We investigated the role of the host cell cytoskeleton and caspase activation using inhibitor treatments. The role of caspase activation in CCS formation was further studied using live cell fluorescence imaging and immunofluorescence staining. In addition, we examined the role of plasma membrane changes and calcium signaling in CCS formation using annexin V and SYTOX staining or chemical and genetically encoded calcium indicators, respectively. The formed CCS were further characterized using confocal laser microscopy and transmission electron microscopy.

Results: CCS are spherical, low phase contrast structures surrounded by a membrane, and contain infectious progeny and morphologically impaired cellular organelles. The formation of CCS shares several characteristics of apoptotic cell death: We found that the peptide DEVD is proteolytically cleaved in late *C. psittaci* inclusions, which continues during CCS formation. This DEVD cleavage is independent of caspase-3. CCS formation is preceded by an increase in intracellular calcium concentration in infected cells, followed by blebbing of the plasma membrane and rupture of the inclusion membrane. Finally, blebbing cells detach, thereby forming CCS. CCS are surrounded by a phosphatidylserine exposing membrane, that maintains its barrier function. Thus, the novel non-lytic egress mechanism of CCS formation is fundamentally different to extrusion formation, the previously described non-lytic egress mechanism of *Chlamydiae*.

Discussion

Host cell egress is essential for intracellular pathogens to spread inside the host and for host-to-host-transmission. Here we describe CCS formation as a novel egress pathway for the intracellular, zoonotic bacterial pathogen *C. psittaci*. This non-lytic egress pathway is fundamentally different from previously described *Chlamydiae* egress pathways. In addition, CCS formation shares several characteristics of apoptotic cell death. However, the sequence of proteolytic activity, followed by plasma membrane blebbing and the final detachment of a whole phosphatidylserine exposing former host cell is unique for *C. psittaci*, which underlines that CCS formation represents a fundamental new egress pathway for intracellular pathogens.

IIV 041: Lipidation of pneumococcal proteins induces innate and adaptive immune responses

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Introduction: Pneumococcal vaccines have important limitations, including restricted serotype coverage, which facilitates replacement by non-vaccine serotypes, and high manufacturing costs. Therefore, one research direction is to develop a serotype-independent and protein-based vaccine. Promising targets are highly conserved lipoproteins. We have previously shown that intranasal immunization of mice with lipidated proteins reduces pneumococcal colonization. Here, we investigated the impact of pneumococcal lipoproteins on the induction of an innate and adaptive immune response.

Methods: Human primary monocytes were differentiated *in vitro* into dendritic cells (moDCs) or macrophages (MDMs). Cells were infected or stimulated either with i) *lgt*-mutants compared to wild-type pneumococci, or ii) lipidated proteins DacB or MetQ compared to the non-lipidated variants. Activation profiles were assessed by expression of different surface markers and secreted cytokines. The adaptive immune response was analyzed with a T cell proliferation assay using protein-stimulated peripheral blood nuclear cells (PBMCs).

Results: At the whole-cell level, the *lgt* knockout in pneumococci had no clear and consistent differential effects on moDC stimulation. However, at the protein level, lipidation of MetQ strongly enhanced the capacity to activate moDCs and MDMs as evidenced by increased expression of costimulatory surface markers and cytokines. Interestingly, non-lipidated MetQ failed to activate innate immune cells, whereas non-lipidated DacB exhibited an intrinsic activation potential. Furthermore, lipidation mediated T cell proliferation when co-cultured with protein-stimulated PBMCs.

Discussion: Although lipidation had no clear effect on DC activation at the whole-cell level using live pneumococci, lipidation of proteins enabled innate and adaptive immune responses using human primary cells. Interestingly, we found the pneumococcal lipoprotein DacB to have an intrinsic potential to activate human antigen-presenting cells. Our study highlights the potential to lipidate promising protein candidates such as DacB and MetQ for future vaccine strategies.

IIV 042

Resolution of chronic intestinal inflammation by L-arginine supplementation

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Inducible NO synthase (NOS2) and arginase (ARG) 1 compete for the semi-essential amino acid L-arginine as common substrate. Previous studies supported the concept that ARG1 promotes tissue repair and immunosuppression, whereas NOS2 perpetuates inflammatory immune reactions and causes immunopathology.

In contrast to the widely accepted immunosuppressive function of ARG1, we have recently made the unexpected observation that ARG1 acts as anti-resolvin in mouse models of DSS-induced or oxazolone-driven colitis. In both models, we identified the enzymatic consumption of L-arginine as driver of chronic epithelial inflammation. This conclusion results from the following findings: (1) Tek2- -driven deletion of ARG1 caused resolution of epithelial inflammation in both colitis models. (2) Changes in the composition of intestinal microbiota accompanied the deletion of ARG1. Transplantation of fecal microbiota from ARG1 knockout mice into wild type (WT) recipients promoted epithelial healing, whereas transfers from WT littermates into ARG1-deficient mice prevented an accelerated recovery of epithelial lesions. (3) Most importantly, dietary L-arginine supplementation induced resolution of colitis and enhanced the recovery from epithelial damage.

Based on these data, we postulate that dietary L-arginine therapy compensates for the deficiency of this versatile amino acid, which commonly occurs during epithelial inflammation. Whether L-arginine supplementation restrains dysregulated immune responses, corrects intestinal dysbiosis and heals epithelial lesions in clinics is subject of further investigation.

IIV 043

Macrophage metabolism as a diagnostic target to identify infections

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A lot of research in the medical field aims at finding new methods to diagnose diseases. Here, we assessed the potential of the host metabolism as a diagnostic tool for infections. For that purpose, we used untargeted metabolic fingerprinting data from *Salmonella* Typhimurium-, *Klebsiella pneumoniae*- and *Escherichia coli*-infected macrophages and from macrophages which we (co)stimulated with the proinflammatory cytokines tumor necrosis

factor-alpha (TNF), TNF + interferon-gamma (INF-gamma) and interleukin (IL6). Using the fingerprinting profiles, we established a logistic zero-sum signature that identified infected cells from untreated macrophages. This signature, however, was not suited to discriminate infection from cytokine-induced sterile inflammation. In order to overcome this limitation, we built a new signature that was able to distinguish infected cells from those exposed to sterile inflammatory stimuli. This signature ("Metabolic Infection Signature") allowed for reliable discrimination of infected cells from cells exposed to sterile inflammatory stimuli and uninfected controls. From these findings, we conclude that macrophage metabolism is an interesting novel diagnostic target to identify infections.

IIV 044

Modeling immune responses of cattle to mycobacteria using magnetic bioprinted granulomas

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Tuberculosis (TB) remains a threat for human and livestock health. Mycobacteria causing TB are host-adapted pathogens which occasionally spillover to other species. *Mycobacterium bovis* causes bovine TB, a well-known zoonosis. *Mycobacterium tuberculosis* (Mtb) is adapted to humans, may trigger symptomatic infection in cattle yet these show resistance to Mtb experimental challenge. A hallmark of TB in all hosts are multicellular tissue lesions termed granulomas. Using bovine leukocytes and magnetic nanotechnologies we developed a three-dimensional granuloma model which we designated *in vitro* granuloma-like structure (IVGLS). We generated stable IVGLS resembling TB granulomas at innate stage, comprised of macrophages, or adaptive stage, comprising lymphocytes in addition. Mycobacteria replicated within IVGLS and triggered progression of macrophages towards foamy phenotypes. IVGLS, unlike conventional macrophage monolayers, released abundant phagocyte chemoattractants and Th1-associated cytokines. Magnetic bioprinted bovine granulomas facilitate studying immune responses to mycobacteria, including spatial mapping. Deciphering protective immune responses within IVGLS could contribute to vaccine development for cattle, whereas unveiling resistance mechanisms may help devise novel interventions for human TB.

IIV 045

Humanized mice are highly susceptible to *Staphylococcus aureus* infection

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Introduction: Humanized hemato-lymphoid system mice, or humanized mice, emerged in recent years as a promising model to investigate the course of infection of human-adapted or human-specific pathogens. They are highly immunodeficient mice engrafted with human hematopoietic stem cells which differentiate into various human immune cell lineages. We asked whether this model system might be advantageous for interrogating the virulence of *Staphylococcus aureus*, one of the most important human pathogens in our time.

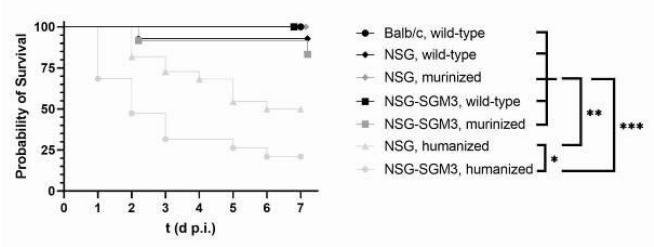
Methods: We employed humanized NSG (NOD-*scid* IL2Rgnull, huNSG) and next-generation humanized NSG-SGM3 (NOD-scid IL2Rgnull-3/GM/SF, huSGM3) mice in our infection studies. The latter ones since they show improved myeloid cell reconstitution, which might be of particular importance in studying *S. aureus* infection. In order to "humanize" the mice, we isolated CD34+ cells from human cord blood and injected them into the tail vein of ca. 6-week-old, non-lethally irradiated mice. After 12 weeks (NSG-SGM3) or 18 weeks (NSG), we infected the mice and applied additionally wild-type and "murinized" (murine stem cells instead of human ones) NSG or NSG-SGM3 mice as controls. The immune cell content was determined during the humanization period as well as during the course of infection, while histological appearance, bacterial burden and the release of cytokines/chemokines was measured at the end of the infection experiment.

Results: Humanized mice displayed a dramatically decreased survival following bacterial challenge with MRSA (see Fig. 1). Three observations were of particular interest in this regard: (I) only humanized mice developed systemic signs of disease while the infection remained restricted to the thigh muscle for all mice which lacked human immune cells, (II) the severity of disease correlated directly with the rate of humanization for both, huNSG and huSGM3 mice and (III) although huSGM3 mice harbored overall higher numbers of human immune cells, most importantly of myeloid cells (and similar numbers of murine immune cells than huNSG), they showed an even more pronounced vulnerability to *S. aureus* infection.

Discussion: Collectively, this study suggests significant differences in the efficacy of human or murine immune responses to *S. aureus* encounter *in vivo*. The human immune cell reconstitution led to a detrimental outcome in humanized mice, suggesting that native mice might not be able to reveal the full pathogenic potential of *S. aureus*, which harbors a large number of human-specific or -adapted virulence factors. We hope that humanized mice might help to sharpen the translational power of the pre-clinical evaluation of successful therapeutic approaches against *S. aureus* in the future.

Fig. 1: Survival of different mouse groups following local injection with 1x10e8 CFU *S. aureus* LAC * *lux* into the left thigh muscle.

Fig. 1



IIV 046

The role of cathepsin B in dendritic cell-mediated killing of *Histoplasma capsulatum*

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Histoplasma capsulatum (*HC*) is a human pathogenic, dimorphic fungus, capable of causing respiratory and systemic infections and particularly affecting immunocompromised individuals. While *HC* survives within macrophages (MΦ), dendritic cells (DCs) eliminate the fungus. Previous studies have indicated that amplification of HIF-1α in MΦ drives cell-mediated immunity by inactivating the pathogen-induced autophagy. In the case of DCs, a hypoxia response element in the promotor region of cathepsin B (CatB) has been identified, suggesting that HIF-1 can regulate CatB. CatB, an enzymatic agent in lysosomes has been observed to engage in inflammasome activation during an *HC* infection in DCs and demonstrated its ability of killing *Cryptococcus neoformans in vitro*. This study therefore investigated the potential synergistic effects of HIF-1α and CatB in the antifungal activity of DCs during *HC* infection.

Human monocytes were isolated from peripheral blood mononuclear cells obtained from buffy coats of healthy donors and differentiated into DCs using 114ng/ml GM-CSF and 50ng/ml IL-4 for 7 days (Peprotech, Rocky Hill, NJ, USA). DCs were subsequently infected with *HC* at a multiplicity of infection of 5:1 for 30 minutes up to 5 hours. Protein amounts of HIF-1α and CatB were measured using Western blot analysis. HIF-1α protein was enhanced using 100µM IOX2 and inhibited by 10µM CAY10585 (Cayman Chemical Company, Ann Arbor, MI, USA). Additionally, CatB protein was inhibited using 10µg/ml Ca074 (Merck Millipore, Billerica, MA, USA). The activity of CatB was assessed using an activity assay (Abcam, Cambridge, MA, USA). Recovery of yeasts was quantified counting colony-forming-units on Brain-Heart-Infusion Agar after 7 days.

During infection of DCs with *HC*, HIF-1α protein showed 1.5-fold increase compared to the uninfected control 3 hours post infection (hpi) ($p<0.05$, n=3). CatB activity was increased by 8-fold at 1 hpi ($p<0.05$, n=3). While enhanced stabilization of HIF-1α by IOX2 led to increased HIF-1α protein ($p<0.01$, n=3), there was no impact on fungal survival ($p>0.05$, n=3). Further, chemical inhibition of HIF-1α decreased HIF-1α protein by 2-fold ($p<0.05$, n=3) and promoted fungal survival compared to infected control at 5 hpi ($p\leq0.01$, n=3). The coincubation of *HC* with CatB (10µg/ml) *in vitro* for 3 hours showed a decrease in *HC* survival by 45% ($p<0.001$, n=4). Finally, inhibition of CatB reduced CatB activity by 50% in DCs ($p<0.001$, n=6) and led to a decrease in fungal killing by 40% ($p<0.05$, n=4).

The findings in this study demonstrated direct antifungal activity of CatB on *HC*. In DCs, CatB appears to be one of the key components involved in early antifungal activity, which is activated independent of HIF-1α. However, the presence of HIF-1α contributes to fungal killing mechanisms in DCs. Future research will focus on unravelling the specific molecular pathways concluding the network of HIF-1α- and CatB-mediated control of fungal infections in DCs.

PRHYV 047

The status quo of healthcare-associated infections and antimicrobials in German hospitals: results of the German point prevalence survey 2022 on healthcare-associated infections and antimicrobial use

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Question: As part of the European point prevalence survey (PPS), a national PPS on healthcare-associated infections (HAI) and antimicrobial use (AU) in acute-care hospitals was conducted in Germany in 2022 with the aim to estimate the prevalence of patients with HAI and AU.

Methods: The German national reference centre for surveillance of nosocomial infections (NRC) organized the German PPS. The PPS was performed in the months May-July 2023 following the methods and definitions established in the European Centre for Disease Prevention and Control PPS protocol. Participating countries were requested to obtain a representative sample of hospitals. In Germany, 50 acute-care hospitals were sampled by number of patient beds from the German hospital registry and invited to participate. Additionally, all other interested acute-care hospitals in Germany could participate. In all cases, participation was voluntary. Data were collected by local staff that was specifically trained in the PPS definitions and methodology by members of the NRC. Collected data were transferred to the NRC via a designated online portal. Presented results pertain to data from all participating hospitals.

Results: A total of 66,586 patients from 252 hospitals were included in the PPS. The median number of patient beds was 300. The prevalence of patients with HAI was 5.2% (95% confidence interval 5.0-5.4), and the prevalence of patients with AU was 26.9% (26.5-27.2). HAI prevalence differed by patient population and was highest for intensive care patients (15.6%) and lowest for psychiatric patients (0.5%). The most commonly recorded HAI were surgical site infections (23% of all HAI), lower respiratory tract infections (22%) and urinary tract infections (20%). For 57% (2,068 of 3,642) of HAI, causative pathogens were recorded, with *Escherichia coli* (15.4% of all pathogens), *Staphylococcus aureus* (13.1%), and *Enterococcus faecalis* (6.4) being the most common. The most frequently administered antimicrobials were penicillins plus beta-lactamase inhibitors (33% of all antimicrobials), third-generation cephalosporins (10%) and second-generation cephalosporins (9%). Around 75% (16.843 of 22.422) of all recorded antimicrobials were administered for therapy and 21% (n=4,723) for prophylaxis, with perioperative antimicrobial prophylaxis (PAP) (n=3,080) accounting for the majority of prophylactic applications. While 57% (n=1,761) of PAP was administered as single dose, 38% (n=1,161) was administered beyond the day of surgery, the remainder being multiple doses on the day of surgery.

Conclusions: HAI and AU prevalence remain at a stable level when compared to previous surveys in Germany. The frequent prescription of broad-spectrum antimicrobials and the fact that prolonged PAP still accounts for more than one third of PAP, serve as a stark reminder to reinforce antimicrobial stewardship activities in Germany.

PRHYV 048

Infection control efforts during nosocomial outbreaks in hematology-oncology

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Introduction: Due to their underlying disease and/or therapeutic regimes, patients in hematology-oncology (H/O) are often at an increased risk for nosocomial infections. Therefore, numerous nosocomial outbreaks (NO) occurred especially in these types of medical departments. Up to now, a systematic evaluation of the infection control measures in such NO settings is scarce. Aim of the present study was to determine characteristics of such NO and to evaluate the extent of the outbreak management.

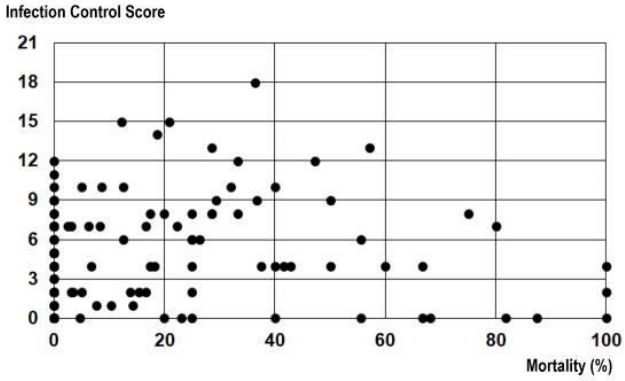
Methods: We performed a search of the Worldwide Outbreak Database (www.outbreak-database.com) for NO in H/O since the year 1960 and extracted characteristics on the setting, the patients, the clinical outcome, the type of causative agent, the source of the NO and the most likely route of transmission as well as on infection control measures as specifically described. We then evaluated the effort of the infection control management using the weighted cumulative hygiene score (WCHS) as described elsewhere (Pannewick et al. PLoS One 2021;16(4):e0249837). This score ranges from 0 (= no specific measures implemented) up to a maximum of 21. Easy to implement measures score low (e.g., education of staff = 1 point) while more challenging measures score high (e.g., closure of the entire unit = 3 points).

Results: Overall 200 NO got included in this systematic analysis. There were 203 patients with solid tumor entities, mainly mamma carcinoma (46 patients), malignancies of the central nervous system (21 patients), gut tumors (31 patients), and lung cancer (20 patients). The most common diagnoses among the 865 patients with hematologic diseases were acute myelocytic leukemia (301 patients), acute lymphocytic leukemia (136 patients), hemophilia (116 patients), and non-Hodgkin lymphoma (101 patients). The exact diagnosis was not stated in detail for 1.855 additional patients. The main pathogens in NO in H/O were vancomycin-resistant enterococci (VRE; 32 NO), *Pseudomonas spp.* (27 NO), and *Aspergillus spp.* (16 NO). The mean WCHS for all NO in H/O was 5.0 (median: 4.5). However, VRE-NO were associated with a higher WCHS result (mean: 6.5; median: 7.0). The same was observed for NO caused by *Aspergillus spp*. (mean: 6.8; median: 7.0). In contrast, the infection control effort in NO due to *Pseudomonas spp*. did not show an elevated WCHS (mean: 5.0; median: 4.0). There was no association between the mortality in NO and the corresponding WCHS as shown in the adjunct figure (n=117 NO, in which exact data on mortality was provided).

Discussion: NO in H/O remain a relevant challenge for infection control management. We recommend awareness for potential pathogen transmission especially in this highly vulnerable patient population. Implementation of appropriate infection control measures at a most early time point is crucial. Greater infection control effort may be necessary in NO caused by VRE or *Aspergillus spp*.

Figure legend: Weighted cumulative hygiene score vs. mortality.

Fig. 1



PRHYV 049

Stabilization of the hand microbiome by alcohol-based hand antisepsis

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Introduction: Importance of alcohol-based hand rub (ABHR) to prevent health care associated infections is undisputed especially in light of the spread of antimicrobial resistant bacteria as well as in epidemic and pandemic situations. Because the influence of daily frequent hand antisepsis on skin microbiome is unknown, we aimed to determine the short-term effect of repeated use of ABHR on the hand microbiome.

Method: Four nurses in a NICU were included into the prospective clinical trial (positive ethic vote, voluntary participation). A ward with a high number of hand disinfection episodes was selected. After a paid leave of 14 days (without the use of hand antisepsis) samples were taken on the first working day before the first hand rub and at the end of the shift, and continued sampled on day 1, 7 and 28. To analyze the hand microbiome composition, microbial cells were collected using the glove-juice technique and pro- and eukaryotic community profiles were created using amplicon sequencing of 16S and 18S rRNA markers.

Results: On average, hand hygiene was performed 155 times per working shift. Microbial communities were dominated by taxa typically found on human skin, e.g. Firmicutes, Proteobacteria, Actinobacteria and Fungi (such as Dothideomycetes belonging to the Ascomycota and Malasseziomycetes being classified in the Basidiomycota). Although this finding was persistent across all nurses, a clear nurse-specific microbiome signature was seen. Exposure to ABHR affected the pro- and eukaryotic communities differently. For Prokaryota, daily exposure led to the end-of-the-day microbiomes being more similar to each other across nurses (primarily Firmicutes). In contrast, longitudinal effect of 28 day-application revealed more similarity of the Eukaryotic community,

whereas no longitudinal effects were seen for the prokaryotic community.

Discussion/Conclusion: Our data shows, that regularly performed hand antisepsis with ABHR reduces the transient skin microbiota but has little effect on the composition of the resident skin microbiome. Thus, daily hand hygiene retains its significance as the most important procedure of basic hygiene.

PRHYV 050

Effect of antiseptic bathing with chlorhexidine or octenidine on the acquisition of multidrug resistant bacteria in intensive care units: a cluster-randomised controlled trial

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Objectives: We conducted a multi-center cluster-randomised controlled trial (cRCT) in 72 adult intensive care units (ICU) to investigate the effect of daily patient bathing with 2% chlorhexidine-impregnated cloths, 0.08% octenidine wash mitts or routine care with water and soap (control) on central-line associated bloodstream infection (CLABSI) rates [1; 2]. A subset of ICUs participating in this cRCT did surveillance for colonisation and infection with multidrug resistant organisms MDRO as secondary outcomes. This analysis aimed to investigate the effect of daily bathing regimes on the acquisition of MDRO in ICUs.

Material and methods: In this multi-center cRCT, colonisation / infection with MDRO was assessed by MDRO surveillance systems for methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) or multi-drug resistant gram-negative bacteria (MDRGN). These surveillance systems used routine MDRO diagnostic data, no additional MDRO screening was implemented for this trial. All colonisations / infections acquired ≥ 3 days after ICU admission were considered ICU-acquired. Secondary outcomes of this trial were incidence densities (ID) of ICU-acquired MRSA, VRE and MDRGN per 1,000 patient days. Poisson regression was applied. We used generalised estimated equation models (GEE) adjusted for potential confounders including length of stay and medical ventilation.

Results: During the intervention period, MDRO surveillance data was reported for around 50% of the full study population (n = 76,815). MRSA surveillance was conducted for 40,846; VRE surveillance for 39,761 and MDRGN surveillance for 40,402 patients. ID of ICU-acquired MRSA, VRE and MDRGN per study group are shown in table 1. GEE models did not show any significant differences of MDRO acquisition neither in the chlorhexidine nor in the octenidine group (compared with routine care).

Discussion: In this trial, antiseptic bathing with 2% chlorhexidine-impregnated cloths and 0.08% octenidine wash mitts lack a significant preventive effect on acquisition of MRSA, VRE and MDRGN in ICUs. However, there is a high likelihood that our trial was underpowered for these secondary outcomes.

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Fig. 1

Table 1: Incidence densities of ICU-acquired MRSA, VRE and MDRGN colonisations and infections

		Chlorhexidine	Octenidine	Control
MRSA	ID per 1,000 patient days (95%CI)	0.28 (0.15-0.45)	0.13 (0.05-0.27)	0.42 (0.25-0.68)
VRE	ID per 1,000 patient days (95%CI)	0.49 (0.32-0.72)	1.02 (0.76-1.35)	1.08 (0.77-1.46)
MDRGN	ID per 1,000 patient days (95%CI)	0.93 (0.69-1.22)	1.54 (1.21-1.93)	1.32 (0.99-1.73)

95%CI, 95% confidence interval. ID, incidence density. MRGN, multi-drug resistant gramnegative bacteria. MRSA, methicillin resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus faecalis / faecium.*

PRHYV 051

Strengthening knowledge and skills regarding Infection prevention and control among medical students in the practical year - Experiences and results from 4 years of the "PJ-Start" project

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Introduction: Medical students in the practical year perform supervised but also self-reliantly patient-related activities such as blood sampling or peripheral vascular catheter insertion. In doing so, the students have, for instance, contact with patients with multi-resistant bacteria (MDRB) or transmissible infectious diseases. Practical and theoretical knowledge of infection prevention and control (IPC) is therefore of central importance.

Methods: In March 2019, an interdisciplinarymodel project called "PJ-Start" [1] was founded and has since been offered to PJ-students from the fields of internal medicine, anesthesiology, pediatrics, and microbiology/hygiene. In addition to teaching clinical skills, IPC was addressed in a dedicated hygiene module. We present our concept and review our experiences and results from the past 4 years.

Results: Until May 2023, a total of 362 PJ students have been taught. In the hygiene module, which is led by an IPC nurse and a physician, hand hygiene according to the WHO-5 model, prevention of vascular catheter-associated infections, and dealing with MDRB are taught in a interactive classroom format. In addition to the indications of hand hygiene, the practical implementation of alcohol-based hand disinfection using UV boxes was trained. Regarding the prevention of vascular catheter-associated infections, the focus was on the correct hygienic skin disinfection. In terms of MDRB, the correct and appropriate use of personal protective equipment was addressed in particular. In

addition to practical-technical skills, hygiene aspects also played a role in the clinical-oriented learning modules, such as puncture of a port. During the COVID-19 pandemic, the hygiene module within "PJ Start" could largely be conducted in face-to-face format under appropriate protective measures. This was supplemented by e-learning content. Facing the COVID-19 pandemic, specific protective measures with regard to SARS-CoV-2 were also covered and practiced in detail. At the end of each hygiene module, there was the opportunity to clarify specific questions and the possibilities of contacting the IPC team during daily practice were shown. The hygiene module has been evaluated over the last 7 rounds by 85 students with an average score of 13.3 out of 15.

Discussion: The hygiene module within "PJ-Start" complements the curricular hygiene courses in the preclinical and clinical sections of the human medicine curriculum at a crucial stage of medical training. By direct contact with the IPC team and hygiene-aware clinical physicians, infection prevention is taught in an authentic and practical manner. An expansion of the "PJ Start" to include PJ-students in surgical disciplines is being considered and is currently in the concept phase.

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LMZOV 052

Microbal safety evaluation of a pea protein-based product fermented with *Lactococcus lactis* and *Yarrowia lipolytica*

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Introduction: Fermentation has been widely employed as a traditional method for food preservation, extending shelf life, and ensuring microbial stability and food safety. This study aimed to assess the microbial safety of a fermented pea protein-based product produced using a mixed starter culture of *Lactococcus lactis* and *Yarrowia lipolytica*. The safety evaluation was performed by challenge tests, wherein surrogates of typical foodborne pathogens were subjected to fermentation to determine a potential growth inhibition or inactivation by the starter cultures.

Material and Methods: Experiments were performed in 100 mL fermentation mixtures consisting of 5 % commercial pea protein isolate and 1 % glucose. The fermentation mixtures were first inoculated with the mixed starter culture or with only *L. lactis*. For the challenge tests, the fermentation mixtures were additionally inoculated with either *Escherichia coli* ATCC 11229 or *Listeria innocua* ATCC 33090. As growth control, pea protein suspensions were only inoculated with the tested surrogates. Viable counts of all strains as well as the pH-values of the mixtures were determined after 16 hours and subsequently every 24 hours throughout a 96-hour fermentation period.

Results: The challenge tests performed with *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 showed similar results. The challenge tests resulted in an increase of viable counts of the surrogates from 104 CFU/mL to 107 CFU/mL within 24 hours. After 48 h, differences to the growth controls were observed. In the fermentation with *L. lactis*, the growth of *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 were inhibited. However, after 96 h, viable counts of 102 -103 CFU/ml were still detectable. In comparison, fermentation with the mixed culture did not inhibit the growth of surrogates, even though the same pH reduction was achieved. After 48 h, the pH-values in the fermentation with *L. lactis*, and the mixed culture decreased to a final pH of about 4.3.

Discussion: The results showed that the growth of the surrogates was not sufficiently inhibited by the use of *L. lactis* and *Y. lipolytica* as a mixed culture. However, growth inhibition of *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 could be achieved with *L. lactis* as a single strain. *Y. lipolytica* probably limited the inhibition of *E. coli* ATCC 11229 and *L. innocua* ATCC 33090 during the fermentation of pea protein. To ensure process safety, further hurdles should be implemented.

LMZOV 053

Therapeutic oral Application of Carvacrol Alleviates acute *campylobacteriosis* in mice harboring a human gut microbiota

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Introduction: Human *Campylobacter jejuni* infections are rising globally. Since antibiotics are usually not indicated in acute campylobacteriosis, antibiotic-independent intervention measures are desirable. The phenolic compound carvacrol constitutes a promising candidate molecule given its antimicrobial and immune-modulatory features.

Methods: To test the disease-alleviating effects of oral carvacrol treatment in acute murine campylobacteriosis, IL-10$^{-/-}$mice harboring a human gut microbiota were perorally infected with *C. jejuni* and treated with carvacrol via the drinking water from day 2 until day 6 post-infection.

Results: Whereas *C. jejuni* stably established in the gastrointestinal tract of mice from the placebo cohort, carvacrol treatment resulted in lower pathogen loads in the small intestines on day 6 post-infection. When compared to placebo, carvacrol ameliorated pathogen-induced symptoms including bloody diarrhea that was accompanied by less distinct histopathological and apoptotic cell responses in the colon. Furthermore, innate and adaptive immune cell numbers were lower in the colon of carvacrol- versus placebo-treated mice. Notably, carvacrol application dampened *C. jejuni*-induced secretion of pro-inflammatory mediators in intestinal, extra-intestinal and systemic organs to naive levels and furthermore, resulted in distinct shifts in the fecal microbiota composition.

Discussion/Conclusion: Our preclinical placebo-controlled intervention study provides evidence that therapeutic carvacrol application constitutes a promising option to alleviate campylobacteriosis in the infected vertebrate host.

LMZOV 054

Surrogate for enterohemorrhagic *E. coli* based on the thermal resistance in different fruit beverages

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Questions: Enterohemorrhagic *Escherichia coli* (EHEC) are among the greatest microbiological challenges faced by the food industry, due to their very low infectious dose. Heat treatments such as pasteurization are applied to reduce the viable counts in the final product. At pilot plant scale, the validation of the microbiological safety is often conducted with surrogate microorganisms, which mimic the heat resistance of pathogens. As surrogates may not react identically under all conditions, there is a particular interest in identifying microorganisms to be used as surrogates for EHEC in the fruit juice industry.

Material & Methods: In the present study, the heat resistance of two foodborne EHEC strains, namely *E. coli* O157:H7 LTH 66231 and *E. coli* O113:H21 TS18/081, was determined. Furthermore, five potential surrogate bacteria were investigated. Thermal inactivation was evaluated in biological triplicate and technical duplicate at 60°C, 65°C, and 72°C. The most suitable surrogate candidate was tested in various matrices, such as phosphate buffered saline (PBS), commercial and laboratory-prepared fruit nectars with varying Brix- and pH-values. This strain was also tested for survival in strawberry nectar at 22°C and 4°C.

Results: The D- and z-values of both EHEC strains as well as potential surrogate bacteria were calculated. From five tested strains, *E. coli* ATCC 8739 is proposed as a suitable surrogate for EHEC strains, as its heat resistance at all tested temperatures in strawberry nectar is higher than that of the examined pathogens. The results also show the differences in thermal resistance of *E. coli* ATCC 8739 in the tested matrices, which indicate a clear influence of the environment on heat resistance of bacteria. *E. coli* ATCC 8739 showed also a high survival rate in strawberry nectar at 4°C, which is important for food storage.

Discussion: This study highlights the importance of the food matrix in selecting surrogate strains for technological applications. In conclusion, *E. coli* ATCC 8739 is proposed as a surrogate strain for *E. coli* O157:H7 LTH 66231 and *E. coli* O113:H21 TS18/081, that can be used in the validation of thermal as well as novel treatments in the production of fruit nectar.

LMZOV 055

Efficacy of disinfectants against biofilms of an outbreak-associated *Salmonella* Typhimurium strain

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Introduction: A prominent example of food-borne zoonotic infectious agents is *Salmonella enterica*, causing salmonellosis. Transmission of pathogens within food processing lines often occurs upon contact with contaminated surfaces. When growing on surfaces, *Salmonella* can form matrix-embedded aggregates called biofilms, in which individual cells are protected from adverse environmental conditions. Compared to planktonic cells, they show an increased tolerance towards disinfectants, antibiotics, etc. As disinfectant efficacy is evaluated against planktonic cells, recommendations could be inadequate regarding their effectiveness against biofilms, which might lead to ineffective disinfection of contaminated surfaces. Here, we analyzed two *S. enterica* isolates associated with a food-borne outbreak with regard to efficacy of disinfectants against planktonic bacteria and bacteria organized in biofilm.

Methods: *Salmonella* biofilms were grown on porous glass beads in a microtiter plate according to a previously described protocol. After two days of incubation at 20°C, biofilms were exposed to peracetic acid (PAA), glutaraldehyde (GA), H_2O_2 (1%, ready-to-use product) and an amine-based quaternary ammonium compound (QAC). Disinfectant efficacy was evaluated by assessing the number of recoverable viable colony forming units (CFU). Assessment of disinfectant efficacy against planktonic *Salmonella* was performed using the EN 1276 standard. Successful disinfection was defined as ≥ 5 $\log_{10}$ reduction in the CFU.

Results: For PAA and GA, a ≥ 5 $\log_{10}$ reduction in the CFU of *Salmonella* biofilms was achieved at a concentration of 0.1% PAA (10 min) or 0.5% GA (30 min), respectively. Using the H_2O_2 containing product, a ≥ 5 $\log_{10}$ reduction in CFU of biofilms was achieved after 30 min. In contrast, even the highest QAC concentrations tested (5%) failed to reduce CFU of *Salmonella* biofilms by ≥ 5 $\log_{10}$. In comparison, disinfection of planktonic cells was achieved with markedly lower concentrations of disinfectants (PAA: 0.002%; GA: 0.03%, QAC: 0.25%) or incubation times (H_2O_2: 1 min), thus demonstrating the higher tolerance of *Salmonella* organized in biofilms compared to planktonic cells.

Discussion: We demonstrated that markedly higher concentrations and/or contact times are necessary to accomplish inactivation of biofilm-embedded *Salmonella* as compared to planktonic *Salmonella*. Moreover, one of the substances tested in this study did not achieve the required 5 $\log_{10}$ reduction in CFU of *Salmonella* biofilms at all. Our data suggests that routinely applied disinfection programs used in the food-production may not be sufficient to effectively eradicate biofilms containing *Salmonella* – and most likely also other pathogens causing food-borne infections. Further studies are needed to understand the tolerance mechanisms of *Salmonella* biofilms and their importance in disinfectant efficacy testing.

LMZOV 056

Geno- and phenotypic characterization of *Escherichia coli* obtained from the slaughterhouse environment

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Introduction: The slaughtering process is recognized as a main source of contamination for meat. An important transmission route for microorganisms is the contact of processed meat with contaminated surfaces. Biocides have wide spread applications in food processing environments for disinfection purposes. Both, the ability to form biofilms and the survival at higher biocide concentrations may be of advantage for microorganisms to persist in meat production environments; a rationale for studying the biocide susceptibility and biofilm formation potential of *E. coli* field isolates from slaughterhouses. For this reason, in our study *E. coli* isolates were characterized using phenotypic and whole genome sequencing approaches.

Material and Methods: We analysed samples obtained from slaughterhouse surfaces in Benin City, Nigeria. *E. coli* isolates (n=64) were phenotypically recovered using standard culture followed by MALDI-TOF MS identification. Biocide and antimicrobial susceptibility were tested by broth microdilution. Isolates were further assayed for biofilm formation in microtitre plates using cristal violet staining. Expression of curli fimbriae and/or cellulose was evaluated based on the colony morphology on Congo red agar plates. Whole genome sequencing (WGS) was performed to characterize the genetic diversity of the *E. coli* strains and to portray the resistome and virulome of each isolate.

Results: Resistance to moxifloxacin (43.8%), ampicillin (31.3%), ampicillin-sulbactam (20.3%), cefotaxim (3.1%), and colistin (1.6%) was observed. Significant associations between antibiotic and biocide susceptibility were determined for triclosan and fluoroquinolones, chlorhexidine dihydrochloride and polymyxins (p<0.05). The isolates formed strong (17.2%), moderate (43.8%) or poor (39.1%) biofilms. 31% of the isolates produced curli fimbriae and/or cellulose. WGS analysis revealed a diverse phylogenetic architecture of the *E. coli* population. Among others, we identified enteropathogenic *E. coli* as well as isolates belonging to major sequence types of extraintestinal pathogenic lineages. Extended-spectrum β-lactamase (ESBL-) producing *E. coli* (n=2) were positive for *blaCTX*-M-15. Isolates carried plasmids responsible for biofilm formation and virulence promotion.

Discussion: Biocide susceptibility from our study population did not portray resistance to disinfectants since MIC and MBC values were well below in-use concentrations. Our result showed a wide-ranging phenotypic and genetic heterogeneity of *E. coli* isolates. Plasmids detected have been reported to aid antibiotic resistance dissemination via conjugation. Overall, data from our study revealed that meat-processing environments can be a reservoir of ESBL-producing and colistin resistant *E. coli* field isolates, which could be culpable in the dissemination of pathogenic clones of environmental and public health concern.

LMZOV 057

Bacteriocins for One-Health: isolation, purification and characterization of antimicrobials against foodborne zoonotic pathogens

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Introduction: Bacteriocins are a promising alternative to conventional antibiotics, as they have a targeted mechanism of action against specific bacteria, lower potential for inducing resistance and can be utilized in food preservation. The aim of this study was to develop a laboratory workflow to detect, characterize, and produce bacteriocins that can effectively combat the two most significant foodborne zoonotic pathogens worldwide, *Campylobacter* spp. and *Salmonella enterica*.

Materials and Methods: The isolation of putative bacteriocin producers involved two sampling stages. In the first sampling, a total of 1565 bacterial isolates were collected. From these isolates, cell-free supernatant (CFU) was obtained and screened for inhibitory activity against *Campylobacter* spp. and *Salmonella enterica*. In the second stage, 990 novel bacterial isolates from chicken caeca underwent the same screening process. Isolates from both sampling stages demonstrating inhibitory activity were further characterized using a MALDI-TOF biotyper and promising candidates were sequenced. Finally, we selected the top 5 candidates based on inhibitory activity, species and gene content: Additional characterization steps included enzyme, pH, and heat resistance tests, determining optimal incubation conditions for bacteriocin production, and purification by ammonium-sulfate precipitation and ethyl-acetate extraction procedures prior to mass spectrometry.

Results: The first sampling resulted in 28 isolates with inhibitory activity against *Campylobacter* and *Salmonella*. Further investigation resulted in the selection of two *Bacillus subtilis* isolates that showed reproducible inhibitory CFS that could withstand high temperature treatment up to 100 °C for 30 minutes and were resistant to digestion with proteinase K. Furthermore, their genomes were found to harbor at least four known sequences for inhibitory and well-characterized substances. Ammonium sulfate precipitated fractions indicated that the CFU of this species contained numerous inhibitory substances.

During the second stage of sampling, 250 isolates exhibiting inhibitory activity were identified and 5 of them were then selected after characterization tests. Four isolates were sequenced and interestingly, they displayed fewer than four well-known inhibitory substance sequences. The sequencing as well as the purification process continues for the remaining isolate.

Discussion: Sequencing and phenotypic analysis of isolates and CFS from the first sampling stage suggest that known antimicrobials are responsible for their antibacterial effect. In contrast, sequencing and CTS phenotype of isolates from the second sampling stage suggest that new antibacterial molecules promote their antibacterial effect. Further characterization based on the established workflows and a refined purification process will enable further identification of novel antimicrobials against foodborne pathogens.

KMV 058

A case of *Streptococcus equi* ventriculitis and endocarditis in a 66 year old man

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Question: A 66-year-old man presented to the emergency department with recurrent fever episodes > 38°C for several days, progressively impaired consciousness and signs of meningism. The neurological examination was remarkable for a Glasgow coma scale (GCS) of 9, a positive Babinski sign on the right and a pendular nystagmus. Heart auscultation revealed a newly developed grade 3 systolic murmur. Comorbidities included coronary artery disease, an insulin dependent diabetes mellitus type 2, reconstructive mitral valve surgery 20 years prior and presbyacusis. The patient was a horse breeder and had been in contact with a sick horse a few days before admission.

Methods: Noticeable laboratory results on admission included elevated systemic inflammatory markers with a white blood cell count of 15.000/πl, a C-reactive protein of 32 mg/dl and a procalcitonin of 2,2 ng/ml. An initial MRI of the head showed no signs of edema or encephalitis. Two sets of blood cultures were drawn and Cerebrospinal fluid (CSF) was collected and sent to the clinical chemistry and microbiology labs for analysis, including cultural diagnostics and molecular testing for HSV and VZV.

Results: The patient was transferred to the ICU with rapidly declining conciousness, placed on ventilation and started on ceftriaxone, ampicillin and aciclovir empirically while awaiting laboratory results. CSF analysis showed an elevated leucocyte count (55/ µl) and grampositive cocci in the gram stain. A follow-up MRI of the head revealed pyogenic ventriculitis and subdural hygromata, while transesophageal echocardiography (TEE) on day 2 after admission showed a 5mm vegetation of the mitral valve. Gentamicin was added to the therapy regimen for the treatment of both ventriculitis and endocarditis according to ESC guidelines and aciclovir was discontinued. Consistent with the gram stain results, group C *streptococci* were grown from both blood and CSF cultures subsequently and further identified by MALDI-TOF MS as *S. equi ssp. zooepidemicus*. Antimicrobioal resistance testing by Vitek2 (BioMérieux) showed full susceptibility to beta-lactams, but resistance to clindamycin. Over the next several days, the patient clinically recovered and regained full mental faculties (GCS 15) while inflammatory markers were declining. Follow-up blood cultures were negative and a TEE after four weeks of treatment showed no remaining vegetations. Therapy was switched to penicillin monotherapy on day 8 following final resistance testing results. Probably as a consequence of meningeal labyrinthitis, an anacusis remained, requiring cochlea implants on both sides before discharge from the hospital after 6 weeks.

Conclusions: While *Streptococcus equi ssp. zooepidemicus* is an opportunistic commensal in horses, it has recently been recognized as an emerging zoonotic pathogen causing rare but severe infections in humans. Epidmiologic data on transmission from horses to humans is lacking.

KMV 059

Relapsing liver abscess caused by hypervirulent *Klebsiella pneumoniae*: a clinical and microbiological case analysis

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Introduction: Liver abscess represents the prevailing manifestation of hypervirulent *Klebsiella pneumoniae* (hvKp) infection. While cases have been documented worldwide, this clinical presentation is infrequent in Germany.

Materials and Methods: In addition to describing the medical history of a patient, who was treated for liver abscess caused by *K. pneumoniae* in June 2021 and experienced a relapse after one year, all recovered *K. pneumoniae* isolates (n=5) were geno- and phenotypically characterized in detail. (Hyper)virulence and general genomic characteristics of the isolates were investigated using growth kinetics, siderophore secretion, serum resistance, survival in bile salts, hypermucoviscosity, *Galleria mellonella* mortality and whole genome sequencing (WGS) analysis.

Results: A 64-year-old male patient with a history of alcohol and nicotine abuse, type 2 diabetes mellitus, sigma diverticulosis, chronic pancreatitis, cholelithiasis, as well as multiple liver abscesses in June 2021, was admitted to the hospital in July 2022 with fever, abdominal pain and jaundice. Imaging studies revealed a septated liver abscess. The *K. pneumoniae* isolates obtained from blood culture and the liver abscess puncture during hospitalisation in July 2022, as well as the isolates recovered from blood cultures during previous admissions in June 2021, January 2022 and June 2022, were largely antibiotic-susceptible, possessing identical morphology and susceptibility profiles. The liver abscess was managed through percutaneous drainage and intravenous antibiotics. Treatment with piperacillin/tazobactam did not lead to clinical improvement, although the isolates were *in vitro* susceptible to this antibiotic. Thus, the antibiotic therapy was changed to cefotaxime, ciprofloxacin and rifampicin, which resulted in a remarkable improvement in the patient's condition. No recurring liver abscesses were observed during the follow-up. All isolates (sequence type 380) possessed capsular locus KL2, O1/O2v1 loci and other genomic features typically associated with hvKp, including the genes encoding for aerobactin (*iuc*ABCD, *iut*A), salmochelin (*iro*CDN), yersiniabactin (*ybt*AEPQSTUX, *irp*1, *irp*2, *fyu*A) and the regulator of the mucoid phenotype (*rmp*ACD). SNP-based microevolution analysis revealed only few mutations among all isolates that did not affect any typical resistance or virulence genes. All isolates caused reduced survival of *G. mellonella* compared to a classic *K. pneumoniae* control strain. Growth kinetics, siderophore secretion, serum resistance, survival in bile salts and hypermucoviscosity analysis yielded results indicative of hvKp.

Discussion: This case highlights the complexities in clinical cause and managing of liver abscesses caused by hvKp and emphasizes the demand for awareness and diagnostic tools to detect hvKp. The occurrence of liver abscess following portal spread from sigma diverticulosis raises the question of potential utility of rectal screening for hvKp.

KMV 060

A case of disseminated *Entamoeba histolytica* infection with fatal outcome

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Patient history: A 46-year old men presented initially to the emergency department with severe abdominal pain and diarrhea. The patient was working as a truck driver with employment throughout Europe, originated from Italy and resided in Germany. No pre-existing condition was known. A habitual abuse of alcohol was noted.

Clinical presentation and course of events: As hollow organ perforation was suspected, laparotomy was performed which yielded a colitis, diffuse peritonitis and perforation of the coecum and a hemicolectomy was executed. The clinical course lead to multiple re-operations, which also addressed a hepatic abscess. The patient suffered from septic shock and multiorgan failure.

Microbiological diagnostics: From intraoperative samples, *E. coli*, *B. fragilis* and *P. intermedia* were grown and *S. anginosus* and *F. mortiferum* were detected in an initially taken blood culture. *A. fumigatus* was grown from respiratory samples. The results of the pathological examination of colon biopsies taken during the first operation showed *E. histolytica* and *Aspergillus* hyphae and were available on day 9 after the first presentation. Serological testing and PCR of pleural effusion and stool for *E. histolytica* confirmed the pathological diagnosis later on. Multiple samples taken during revision operation showed *C. albicans* and *Bacteroides* spp, which was also cultured from liver abscess.

Conclusions, therapy and outcome: Due to the results of the diagnostics, a disseminated infection caused by *Entamoeba histolytica* was diagnosed and treatment with metronidazole was initiated on day 18 after first presentation, which was then continued with high dose treatment later on. Bacteria grown from blood cultures and intraabdominal samples were in line with the expected spectrum for intraabdominal infections and treated initially with piperacillin/tazobactam and then with meropenem, caspofungin, penicillin and/or vancomycin. Multiple samples growing *C. albicans* and *A. fumigatus* were interpreted as disseminated fungal infection caused by an immunocompromised state as a sequela of sepsis and the prolonged course of severe clinical infection and treated with an echinocandin and liposomal amphotericin B. Despite all efforts, the patient deceased as a result of multiorgan failure and septic shock on day 34 after the first presentation.

KMV 061

Psoas abscess due to Multidrug-resistant *Bacteroides fragilis* in Germany

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Introduction: A 50-year-old male patient was submitted to our hospital with a psoas abscess located in the area of an old gunshot wound which led to nephrectomy of the left kidney approximately 20 years ago. Therapy before submission with ampicillin/sulbactam achieved no regression in radiological imaging and inflammatory

markers. Samples revealed a monomicrobial infection with *Bacteroides fragilis* (*B. fragilis*). The strain showed resistance to all EUCAST listed antibiotics for *Bacteroides* spp.: piperacillin/tazobactam, meropenem, metronidazole and clindamycin. Additional testing indicated tigecyclin as a therapeutic option.

Among Gram-negative bacilli *B. fragilis* group are the most recovered Bacteroidaceae in clinical specimens causing mono- and polymicrobial infections. These obligate anaerobes belong to commensals of the human gut, but can also cause severe infections including bacteremia, intraabdominal infections and abscesses. In the last decades, multidrug-resistant (MDR-)*B. fragilis* has emerged in many countries around the world [1] [2] [3] [4].

During hospitalization in our clinic, the patient was treated with ampicillin/sulbactam. He refused application of tigecycline. Surgical debridements led to regression of inflammatory parameters and bacterial growth. Nevertheless, a full cure could not be achieved.

Material/methods: The isolate from intraoperative abscess material was identified with MALDI Biotyper (Bruker Daltonics GmbH). Antimicrobial susceptibility testing for piperacillin/tazobactam, meropenem, metronidazole and clindamycin was performed as a disk test (Oxoid ™) and additionally MIC strip test (Liofilchem®). An inoculum of McFarland 1.0 was plated out on FAA agar (Liofilchem®) and cultivated with antibiotic disks / MIC strips under anaerobic conditions (GENbox anaer bioMérieux®; in airtight container) at 36 ± 1°C for 16-20 h.

Results: Antimicrobial susceptibility testing revealed resistance against standardly used antibiotics with anaerobic activity - piperacillin/tazobactam, meropenem, clindamycin and metronidazole indicating a MDR-*B.fragilis* strain. Tigecyclin showed a minimal inhibitory concentration of 0.19 mg/l.

Discussion: Here we report as far as we know about the first case of an infection with a MDR-*B. fragilis* strain in Germany. In routine microbiological testing MDR-*B. fragilis* might be missed as long as antimicrobial susceptibility testing of anaerobes is not routinely performed in all laboratories. This case shows the importance of susceptibility testing especially in the context of increasing antimicrobial resistance.

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DKMZOV 062

Tellurite resistance as marker for hypervirulent *Klebsiella pneumoniae* in bloodstream infections in a German tertiary center 2021-2022: First results of a retrospective cohort study

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Introduction: Species of the *Klebsiella pneumoniae* complex (KP) are recognized as pathogens frequently causing community- and hospital-acquired (bloodstream) infections. Hypervirulent KP are responsible for severe courses of infection and tellurite resistance has been associated with hypervirulent (hv) strains [1]. By characterizing KP isolates from blood cultures at our hospital in 2021 and 2022, we aim at evaluating implications of hypervirulence and the utility of phenotypic tellurite resistance as diagnostic marker.

Materials and methods: Every first KP bloodstream isolate of a patient during a hospital stay was included. Patients with multi-species infections were excluded. Tellurite resistance was determined by agar diffusion. After Illumina-based whole genome sequencing, sequence types and a virulence score were determined using in-house pipelines, partly reusing code of Kleborate [2]. Exploratory data analysis was performed with Python 3.11, visualization with Vega-Altair v5.0.0, statistical analysis with Scipy v1.10.1 or MS Excel.

Results: We identified 96 cases of bloodstream infection with KP. 92 isolates could be phenotypically analyzed (95.8 %). In-hospital mortality was 22.8 % (21/92). To date, 71 isolates have been sequenced (74.0 %). Of those, 56/71 (78.9 %) were identified as *K. pneumoniae*, 13/71 (18.3 %) as *K. variicola* and 1/71 (1.4 %) as *K. quasipneumoniae*. 9/71 (12.7 %) isolates had a virulence score >=3, meaning at least the siderophore Aerobactin as important virulence factor is present; those were classified as hv. Of note, three hv isolates were Carbapenem-resistant. Figure 1 shows results of phenotypic and molecular characterization of isolates. Table 1 shows evaluation of Tellurite resistance as diagnostic test. In summary, tellurite resistance is associated with hv KP (DOR 67.308 [3.688 - 94.478]) with a high specificity. There was no association with death or ICU treatment.

Discussion: Tellurite resistance correlated well with hv isolates, although cutoff values for a positive test could be optimized to reach a higher sensitivity. Preliminary analysis of clinical data showed high mortality of KP bloodstream infection, but no association of tellurite resistance or hypervirulence with patient outcomes. Underlying diseases and additional endpoints are yet to be analyzed. Tellurite resistance could be an easily available quick test for hv KP before performing a molecular analysis.

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Fig. 1

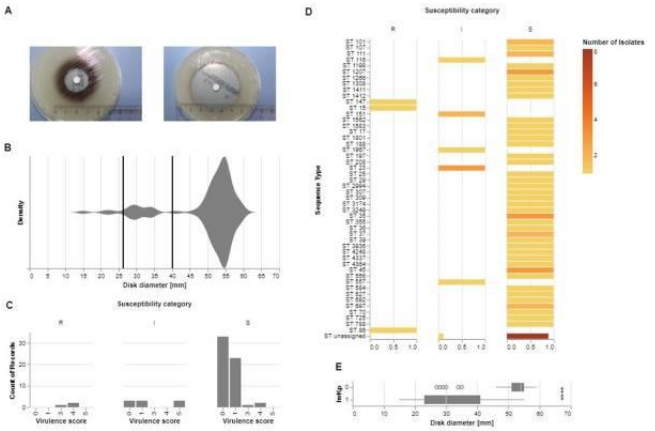

Figure 1. Overview of virulence score and multi-locus sequence types depending on tellurite disk diffusion test
A. Results of tellurite disk diffusion when resistant (left) or sensitive (right). **B.** One-dimensional kernel density estimation. Vertical lines show cutoffs for test defined during validation. **C.** Virulence score count by category. **D.** Multi locus sequence types (ST). Bars show the frequency of isolates by ST and category. **D.** Boxplots showing the disk diameter by virulence score >=3 (hvKp == 1) and <3 (hvKp == 0). (**** p < 0.001, two-sided t-test).

Fig. 2

Tellurite disk diffusion - Intermediate resistance interpreted as positive test								
		Disk diameter < 40 mm		Precision	F-measure	Accuracy	Cohen's Kappa	DOR [95 % CI]
		Pos.	Neg.					
In-hospital death	Pos.	5	16	0.357	0.286	0.709	0.112	1.944 [0.570 - 6.628]
	Neg.	9	56					
ICU stay	Pos.	7	30	0.500	0.275	0.570	0.050	1.400 [0.444 - 4.411]
	Neg.	7	42					
Virulence Score >=3	Pos.	6	3	0.500	0.571	0.873	0.499	**18.667 [3.688 - 94.478]**
	Neg.	6	56					
Tellurite disk diffusion - Intermediate resistance interpreted as negative test								
		Disk diameter <= 26 mm		Precision	F-measure	Accuracy	Cohen's Kappa	DOR [95 % CI]
		Pos.	Neg.					
In-hospital death	Pos.	2	19	0.667	0.167	0.767	0.112	6.737 [0.579 - 78.422]
	Neg.	1	64					
ICU stay	Pos.	2	35	0.667	0.100	0.581	0.038	2.742 [0.239 - 31.460]
	Neg.	1	48					
Virulence Score >=3	Pos.	3	6	1.00	0.500	0.915	0.466	**67.308 [3.120 - 1451.9158]**
	Neg.	0	62					

Table 1. Statistical evaluation of tellurite disk diffusion as diagnostic test.
Contingency table and test statistics either when intermediate resistance is interpreted as positive (upper table) test or as negative (lower table) test. (Pos., Positive; Neg., Negative; DOR, diagnostic odds ratio; CI, confidence interval; ICU, intensive care unit)

DKMZOV 063

Evaluation of FT-IR spectroscopy and machine learning for the delineation of *Escherichia coli* O-H serotypes

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Background: *Escherichia coli* is part of the colonizing enteric bacterial flora, but certain pathovars are also globally leading causes of diarrhoea, foodborne outbreaks, and various extra-intestinal infections. The lipopolysaccharide (O) and the flagellar (H) surface antigens of *E. coli* are traditionally used to delineate pathogenic clonal lineages, as the O-H combination is considered as a marker for specific serovars. Discrimination of *E. coli* serovars is traditionally performed applying serological or genomic typing of O- and H-antigens, nevertheless, both approaches present some disadvantages in terms of costs, ease-of-use and applicability in the diagnostic routine setting. In this study, we evaluated the discriminative power of Fourier Transform-Infrared (FT-IR) spectroscopy to distinguish *E. coli* isolates at O-H combination (serotype) level.

Material/Methods: A total of 103 clinical and infection control-related *E. coli* isolates, in-silico-serotyped using next generation sequencing or traditionally serotyped, were included in this study. The isolates belong to n=38 O-serogroups and n=62 different O-H serotypes, also comprising n=16 EPEC (enteropathogenic *E. coli*). Forty-four serotypes were represented by one isolate each, while 18 serotypes were represented by two or more. The isolates were analysed applying the Biotyper® system (IRBT - Bruker Daltonics, Germany). IR spectra were acquired from dried spots of bacterial suspensions in ethanol solution, in three biological replicates. Exploratory data analysis was performed by principal component analysis (PCA) and linear discriminant analysis (LDA) using the IR Biotyper® software V4.0. Machine learning was applied to create an automated classifier for the discrimination of the different serotypes, using the support vector machine (SVM) with linear kernel algorithm, included in the IRBT software. Seventy-two isolates were used to build the training set, to represent all 62 classes (serotypes). The remaining 31 isolates were used as a testing set.

Results: Exploratory data analysis showed that IRBT clustering is correlated with the O-H serotypes. For serotypes represented by more than one isolate, a different degree of relatedness between them was observed. PCA/LDA showed that the 62 serotypes are separable. The preliminary classifier showed an accuracy of 93.5% (261/279 spectra, 2/31 misclassified isolates), with the misclassification being restricted to the H-antigen level with one O145:H28 isolate misclassified as O145:H34 and one O15:H15 misclassified as O15:H16.

Conclusion: IR Biotyping proved to have the potential for the delineation of *E. coli* at serotype level, demonstrating an option for its application for clinical, infection control, public health and epidemiological purposes. An extension of the study including more strains of each serotype and the most epidemiologically relevant as well as pathovar-associated serotypes is ongoing to confirm these promising preliminary results.

DKMZOV 064

Improving the sensitivity of VRE screening by culture

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VRE screening by culture still has an only moderate sensitivity which so far were difficult to overcome. In this study we aimed to increase the sensitivity of VRE culture from clinical specimen by varying sample method, streaking and incubation temperature. Clinical sampling was performed by either perianal or intrarectal swab, inoculation was performed with a robot (WASP, Copan) in broth or on VRE agar plates at different temperatures. Finally, the sensitivity of the optimized protocol was compared to vanA/vanB PCR.
First, we evaluated the quality of sampling. We noticed that training of clinical staff substantially increased the quality of perianal swabs and that intrarectal swabs were equivalent in terms of sensitivity while being less prone to sampling mistakes. Next, we investigated the impact of incubation temperature on VRE growth and found fastest growth at 43°C. Furthermore, we analyzed whether sensitivity of VRE culture of intrarectal swabs was increased at 43°C compared to 35°C which was the case in visceral surgery patients. However, when analyzing a cohort from a spinal cord injury ward the increase of sensitivity was less evident most likely due to increased intake of antibiotics which subsequently might have increased fecal VRE load. Nevertheless,

incubation at 43°C increased growth on VRE agar after 24 h by 20% compared to 35°C. Finally, we investigated the sensitivity of the VRE culture from intrarectal swabs compared to vanA/vanB PCR which we found to be 82.5%. Taken together we increased the sensitivity of VRE screening culture by modifying sampling technique and incubation temperature.

DKMZOV 065

Rapid detection of MRSA in nasal swabs

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Questions: An early and fast MRSA detection enables a specific treatment and isolation of infected patients to avoid a spreading of the pathogen. Conventional culture methods, the current "gold standard", obtain results within 2 to 3 days, while real-time polymerase chain reaction (PCR) approaches deliver reliable results within less than two hours, thus being a potentially important part of a hospital infection-prevention program. The aim of this observational post-market performance follow-up study is to confirm the safety and performance of the Vivalytic MRSA/SA assay used according to its intended purpose within diagnostic routine compared to the current gold standard. The Vivalytic instrument is a new PCR platform using either multiplex RT PCR or microarray for detection of nucleic acids from microbes. The easy-to-use cartridge system can be used in the microbiology lab or as a point-of-care test (POCT).

Methods: 288 nasal swabs from the routine MRSA screening of a tertiary care hospital were tested in our hospital. Routine testing was performed by chromogenic MRSA/SA biplates (Becton Dickinson, Heidelberg, Germany) according to the manufacturer"s instructions. For the analysis of sensitivity, specificity and negative predictive value the Vivalytic MRSA/SA assay (Bosch Healthcare Solutions, Waiblingen, Germany) was used in parallel. This real-time PCR assay delivers results in approximately 50 minutes. To achieve comparable results both tests were performed within 72 hours.

Discrepant results were re-analysed with BD Max MRSA/SA kit, as independent third-party test.

Results: The Vivalytic MRSA/SA cartridge showed a high concordance to the reference test. For MSSA the sensitivity was 90.0% and the specificity 98.5%; the negative predictive value (NPV) was 97,6 The consistency for swabs for MRSA (n = 33) was 82.1% (sensitivity) and an excellent specificity of 98.8%. The NPV was 98,1 respectively. Time-to-result was less than one hour.

The NPV is an important marker for MRSA assays as it predicts an accurate patient management in the hospital with a minimal risk of transmission of the pathogen to other patients.

Conclusions: The Vivalytic MRSA/SA cartridge demonstrated a good concordance with a sensitive reference test and delivered accurate and rapid results. Specificity for MRSA/MSSA and sensitivity for MSSA detection was within the expected range compared to clinical performance study.

The NPV for MSSA and MRSA was excellent, predicting a minimal transmission risk for screened patients. The assay is suitable for hospital labs as well as for outpatients" settings due to its short time-to result and its easy and safe handling.

DKMZOV 066

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

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Introduction: Shiga toxigenic *E. coli* (STEC) are important pathogens causing human disease ranging from diarrhea to severe hemolytic uremic syndrome (HUS). In Germany, about 1900 STEC infections and 70 HUS cases are reported to the RKI annually and children under the age of five are mostly affected. STEC are transmitted via food and animal contact and may be responsible for large outbreaks associated with high economic costs (Frank *et al.*, New England Journal of Medicine, 2011). Timely and qualified detection of STEC, including isolate recovery in patients, animals, and food, remains of high importance. Further, isolate recovery is essential for risk profiling, infection cluster, and infection chain analysis. However, STEC diagnostics is challenging and work-intensive because STEC are a group of pathogens with high variation in marker genes. Thus, the availability of a specific rapid test for the identification of STEC isolates would be a tremendous advance. In the here presented study, we designed and evaluated a detection method based on Shiga toxin (Stx), found in all STEC, and specifically on its catalytic activity.

Methods: To detect Stx enzymatic activity, various synthetic substrates mimicking the Sarcin Ricin Loop (SRL) were designed. These SRL substrates were linked to a fluorophore and quencher that after cleavage by Stx result in a fluorescence signal. Stx enzymatic activity of the bacterial preparations was tested using a real-time cycler for fluorescence readout. The Stx activity of 65 STEC strains of distinct serogroups and harboring different Stx subtypes was analyzed. The sensitivity of the assay was determined, and specificity was examined using 29 strains of other intestinal pathogens, such as *Salmonella*.

Results: Optimal results were achieved after ~ 30 to 60 minutes. Importantly, the detection of Stx activity of the three *stx1* subtypes (*stx1a, stx1c, and stx1d*) and seven *stx2* subtypes (*stx2a – stx2g*) was possible and independent of the STEC serogroup. Stx enzymatic activity was identified either in culture supernatants or using single colonies. 5 µL of culture supernatants were sufficient for the detection of enzymatically active Stx2, while single colonies allowed the detection of both Stx1 and Stx2. For other enterobacteria not comprising *stx*, no fluorescence above baseline levels was detected.

Conclusion: In conclusion, our newly developed assay successfully detected Stx enzymatic activity in bacterial supernatants and single colonies within 30 to 60 minutes using a synthetic fluorescent SRL substrate. With this test, new possibilities for STEC detection and contamination will be possible.

DKMZOV 067

Genotypic and phenotypic analysis of *Staphylococcus borealis* isolated from clinical specimens in a tertiary hospital in Germany

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Question: *Staphylococcus borealis* is a species of coagulase-negative staphylococci (CoNS) first reported in 2020 (Pain et al. 2020). Diagnostic discrimination between *S. borealis* from *S. haemolyticus* can be challenging, since they are phenotypically similar, so that the correct species identification often fails using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The discrimination by 16S rRNA sequence seems to be unsuitable due to almost identical sequences (99.86%). The aim of this study was to evaluate methods as proposed in literature, which are suitable for correct identification of *S. borealis* that were detected from clinical specimens in a tertiary care hospital in Germany.

Methods: We included one isolate per patient from our routine diagnostics between August and November 2022, which were characterized as *S. borealis* via MALDI-TOF MS applying the Biotyper software IVD/Compass 4.2.100.19. All isolates were subjected to whole genome sequencing (WGS) to confirm the species using *in-silico* DNA-DNA hybridization (DDH) and average nucleotide identity (ANI). For comparison, the type strains of *S. borealis* (51-48^T; NZ_CUEE00000000.1) and *S. haemolyticus* (NCTC 11042^T; UHDN00000000.1) were included. For further subtyping, an *adhoc* core-genome multilocus sequencing typing (cgMLST) was employed. The production of urease was studied using slant agar and the pigmentation was assessed employing P-agar plates.

Results: In total, 34 clinical isolates, mainly isolated from the urine samples (71%, n=24), that exhibited a MALDI score of ≥1.7 (median = 2.125) were included. Using WGS and subsequent DDH, 18 isolates were confirmed as *S. borealis* and the remaining 16 isolates as *S. haemolyticus*. Pairwise analysis between *S. borealis* isolates from our hospital to the *S. borealis* reference isolate revealed an ANI of ≥99.67% and a DDH of ≥94.9%. Of the 21 isolates with a MALDI score ≥2 in total 17 isolates (81%) were identified as *S. borealis* by WGS. Analysis of the WGS data showed that a secure discrimination between both species was possible based on housekeeping genes (e.g. *rpoB*, *gyrB*). Our *ad hoc* cgMLST scheme consisting of 2,070 target genes showed no evidence of clonality (allelic distance ≥54). Phenotypic analyses showed that 16 out of 18 (89%) *S. borealis* isolates were urease positive and 100% showed yellow pigmentation on P-agar, whereas the urease test was negative and the colony pigmentation was white on P-agar for all *S. haemolyticus* isolates.

Conclusions: The majority of *S. borealis* isolates were detected in urine samples. Species identification of *S. borealis* can be difficult due to its close relationship with *S. haemolyticus*. Even MALDI scores ≥2, which indicate a "(highly) probable species identification" cannot delineate reliably *S. borealis* from *S. haemolyticus*. Hence, the use of P-agar and the examination of housekeeping genes (e.g. *rpoB*, *gyrB*) can be helpful in the accurate identification.

GIMPV 068

The role of teichoic acids on pneumococcal physiology and host-Pathogen interaction among different serotypes of *Streptococcus pneumoniae*

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Introduction: Teichoic acids (TAs) are important carbohydrate-based polymers of the cell wall of Gram-positive bacteria. They are classified into peptidoglycan-anchored wall teichoic acids (WTAs) and membrane-anchored lipoteichoic acids (LTAs). Pneumococcal WTA and LTA share the same biosynthetic pathway and differ only in their linkage to the peptidoglycan and cytoplasmic membrane (CM), respectively. The Teichoic acid ligase TacL has been identified as the enzyme responsible for the anchoring of pneumococcal LTA (Heß et al., 2017). The LytR-Cps2A-Psr-protein family (LCP) of *S. pneumoniae* is thought to be involved in anchoring WTA and capsular polysaccharide (CPS) to peptidoglycan.

Material/Methods: Isogenic mutants of *lytR*, *cps2A*, *psr* or *tacL* were generated in different pneumococcal backgrounds (serotype 2, 4, and 19F). Phenotypic characterisation, growth behavior and resistance to oxidative stress were performed in complex and chemically defined media. The surface abundance of cell wall polymers, various membrane-anchored proteins (lipoproteins) and the cytokine release from human dendritic cells after pneumococcal infection was investigated by flow cytometry. In addition, the influence of TAs on virulence (wax moth model), nasopharyngeal colonization (murine colonization model), adhesion to epithelial cells and biofilm formation was determined.

Results: Our results show the influence of TAs on lipoprotein composition, bacterial cell physiology, resistance to oxidative stress and the host immune response for several different serotypes. Furthermore, we are able to show that LTAs influence virulence, adhesion to host cells and biofilm formation.

Discussion: Our study provides further inside into the role of TAs in the formation of an intact CM and the host-pathogen interaction. In addition, our results lead to a better understanding of the mode of action of LCP-proteins.

Heß et al., Nat Commun. 2017 Dec 12;8(1):2093. doi: 10.1038/s41467-017-01720-z

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GIMPV 069

Telomerase RNA component deficiency exacerbates *S. aureus* pneumonia in mice

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Question: Telomere shortening is caused by the lack of telomerase activity and is known as a hallmark of aging, leading to cellular senescence [1]. Telomerase consists of two compartments: the telomerase TERT and the RNA template TERC [2]. Deletion of telomere components in mice is an established model for premature aging and recent studies have revealed several novel roles of TERC such as the dysregulation of inflammation in TERC negative cells via the PI3-kinase pathway [3, 4]. This is especially interesting in the context of bacterial pneumonia as a major burden of disease in the elderly, in which *Staphylococcus aureus* (*S. aureus*) is among the most frequent causative pathogens [5]. Within this study, we investigated the effect of TERC dysfunction on the innate immune response to *S. aureus*-induced pneumonia, via a TERC knockout (KO) mouse model.

Methods: An *S. aureus* pneumonia was induced in TERC-KO and wild type (WT) mice by intranasal application of *S. aureus* (USA300). The mice were sacrificed 24 hours post infection. The bacterial load was determined and the inflammatory response was measured via flow cytometry. Additionally, mRNA-sequencing of lungs from TERC-KO and WT mice was performed. To elucidate pathomechanisms, a murine *ex vivo* infection model was established.

Results: In the murine model, we could observe a higher mortality as well as an overall worse progression of the infection in the TERC-KO mice. TERC-KO mice developed a systemic infection as bacteria could be detected in the kidneys and liver of the KO but not in the WT mice. Furthermore, an increased inflammation was measured in the lungs of TERC-KO mice. RNA sequencing of the lungs revealed a dysregulation of intracellular pathways leading to excessive inflammation and severe lung damage. These findings could be confirmed by using an *ex vivo* lung model indicating TERC-associated changes in intracellular signaling.

Conclusions: Our study provides new insights into the mechanisms behind the more severe outcome of bacterial pneumonia in the elderly, as well as a novel function of TERC in the lung.

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GIMPV 070

Uncovering unique pathogenic markers of *Klebsiella pneumoniae* through multi-omics analysis to drive the development of alternative treatment strategies

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Introduction: Carbapenem-resistant and extended-spectrum beta-lactamases-producing *Enterobacterales* are considered as particular "critical" pathogens that are often multidrug-resistant (MDR). The massive use and misuse of antibiotics have resulted in the rapid distribution of such bacteria that pose a growing challenge for the treatment with conventional antibiotics, which calls for alternatives, e.g. anti-virulence strategies. MDR *Klebsiella pneumoniae (KP)* are a leading cause of nosocomial infections in immunocompromised patients, including pneumonia, bacteremia, and urinary tract infection. The diversity within the *Klebsiella* species results from a large accessory genome compared to their core genome. Understanding this diversity and common traits of pathogenic and MDR representatives might facilitate the identification of pathogen-specific markers, which might be leveraged as novel therapeutic targets.

Material and Methods: We performed a bioinformatics analysis of 5,758 genomes of pathogenic *KP* belonging to eight different sequence types (ST), as well as 982 *K. variicola* (*KV*) and *K. quasipneumoniae* (*KQ*) genomes as rather "commensal" representatives. Transcriptomic and proteomic analyses were conducted for two isolates of ST15, ST147, ST258, ST307, and *KV* each grown in urine-like medium to mid-exponential phase, mimicking infection conditions. The resulting multi-omics data were used to create a patho-core genome (PCG), proteome, and transcriptome, from which unique pathogenic markers and regulatory pathways involved in infection processes will be identified.

Results: The pangenome analysis revealed a total number of 122 genes within the PCG that were absent in commensals and present in >95% of each pathogenic ST. In-depth analysis demonstrated that those patho-core genes are mainly involved in transcription, carbohydrate- and amino acid transport, as well as in intracellular trafficking, secretion, and envelope biogenesis. Further, we observed improved growth of pathogenic *KP* strains compared to *KV* in urine-like medium, suggesting specific adaptation mechanisms of *KP*, which we are currently investigating through proteomics and transcriptomics.

Discussion: Comprehensive multi-omics analyses allow the sophisticated identification of potential new targets by comparing pathogenic *KP* to commensal *KV* and *KQ*. Among others, we identified *fimH* within the *KP* PCG, which is already a target of anti-virulence strategies. In addition, we found *dedA*, potentially involved in decreasing colistin resistance and thereby, increasing treatment efficacy. Also, rapid adaptation to different environments, e.g., the urinary tract, is an important prerequisite for colonizing various niches inside and outside the host, supporting infection progression. To gain a better understanding of these processes, the in-depth investigation through comparative genomic, transcriptomic, and proteomic analyses in infection-mimicking media is crucial.

GIMPV 071

Impact of *Neisseria meningitidis* infection on the efflux transporter P-glycoprotein in human brain endothelial cells

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Introduction: Neisseria meningitidis (*Nm*) is a commensal bacterium that colonizes the human nasopharynx and occasionally crosses the nasopharyngeal barrier, followed by systemic spread and transmigration of the meningeal blood-cerebrospinal fluid barrier (mBCSFB) causing meningitis. The mBCSFB is composed of specialized brain endothelial cells (BECs) that tightly regulate the blood-to-brain traffic due to unique properties including the presence of complex tight junction proteins and specialized transporters such as P-glycoprotein (P-gp). P-gp is a brain-to-blood efflux transporter that limits the accumulation of many substrates in the brain. Dysregulation of this transporter was recently observed during Streptococcus agalactiae infection in BECs. Here, we investigate the effect of *Nm* infection on P-gp using human in vitro BEC models.

Methods: Induced pluripotent stem cells (iPSCs) were differentiated into iPSC-derived brain endothelial cells (iBECs) according to recently described protocols. Immunofluorescence staining of adherence and tight junction markers and TEER measurements were performed to verify the quality of the iBECs.

The impact of *Nm* on P-gp was analyzed by RT-qPCR and immunoblotting. Rhodamine 123 (R123) accumulation assay was performed to assess the impact of *Nm* infection on P-gp function in BECs.

Results: *Nm* infection decreased the expression of *ABCB1* (encoding P-gp) in iBECs as well as in the brain microvascular endothelial cell line hCMED/D3 24h post-infection (p.i.). The immunoblotting analysis determined a significant decrease in P-gp abundance 24h p.i. P-gp activity in iBECs was confirmed by accumulation assay using P-gp inhibitors, namely Cyclosporin A (CsA) and Valspodar (PSC833). Infection of the cells with pathogenic *Nm* strain MC58 and non-pathogenic *N. lactamica* resulted in disruption of P-gp function in BECs. Interestingly, the addition of PSC833 to the infection condition did not increase R123 accumulation as significantly as the inhibitor alone, suggesting a competitive relationship between *Neisseria* infection and PSC833 to inhibit P-gp activity in human BECs. Moreover, infection of the cells with different *Nm* mutants exhibited similar R123 accumulation to BECs infected with the wildtype *Nm*. Interestingly, Lipooligosaccharide (LOS)-deficient *Nm* H44/76*Δlpx* in contrast to wildtype *Nm* H44/76 was not able to significantly disrupt P-gp function in BECs.

Discussion: Here we observed a significant decreased expression of P-gp in BECs on both transcript and protein levels as a result of *Nm* infection. In addition, we were able to confirm the impact of *Nm* infection on P-g activity in BECs. Moreover, our data revealed that *Nm* virulence factors, capsule, pili or OpcA alone does not contribute to P-gp inhibition during infection, however, suggest a role of meningococcal LOS in P-gp dysfunction, consistent with P-gp inhibition by lipopolysaccharide (LPS) that was observed in previous studies.

GIMPV 072

Exploring small proteins in the foodborne pathogen *Campylobacter jejuni*

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The foodborne pathogen *Campylobacter jejuni* is a microaerophilic Epsilonproteobacterium and currently the leading cause of bacterial gastroenteritis worldwide. Because its annotated genome lacks homologs of key virulence factors used by other enteric pathogens, little is known about how it causes disease. In its small genome of ~1.6 Mbp, 54 small proteins of less than 70 aa are annotated. So far almost nothing is known about their involvement in *C. jejuni* physiology and verification of translation is lacking for most of them. Furthermore, the *C. jejuni* small proteome is likely larger than what is currently annotated.

Here, we aim to globally catalog small ORFs (sORF) in *C. jejuni* and functionally characterize selected candidates. Therefore, we combined classical Ribo-seq, translation initiation site (TIS) profiling and a novel translation termination site (TTS) profiling, which revealed novel and hidden sORFs in diverse genomic contexts, as well as annotation refinements. In addition, we used mass spectrometry and epitope tagging followed by western blotting to validate sORF translation. Using this combined approach, we almost doubled the *C. jejuni* small proteome (42 novel sORFs) and validated translation of 47 annotated sORFs. The novel small protein CioY (34 aa), previously missed in the *C. jejuni* strain NCTC11168 genome annotation, is adjacently encoded to the CioAB terminal oxidase. We show that CioY is part of this terminal oxidase with potentially similar functions as the 37aa-long CydX in *E. coli*.

To gain more insights into the potential functions of small proteins, we have inspected various expression and functional genetics datasets available in our lab to identify candidates that might affect *C. jejuni* virulence. A Tn-seq screen of *C. jejuni* infections of human Caco-2 cells identified a small protein (57 aa) required for motility and colonization. Electron cryo-tomography revealed that the small lipoprotein is necessary for flagellar disk and stator assembly.

Overall, we have expanded the genome map of *C. jejuni* with high-confidence sORFs using diverse Ribo-seq approaches combined with extensive validation. We also identified a previously unknown motility factor in this bacterial pathogen. Functional characterization of this protein is revealing how small proteins might participate in the function of the complex flagella machinery.

GIMPV 073

Beyond vitaPAMPs: RNA from live and dead *Orientia tsutsugamushi* is recognised via different Toll-like receptors

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Introduction: *Orientia tsutsugamushi* (*Ot*) is an obligate intracellular bacterium that causes potentially lethal infections in humans. Despite lacking most classical bacterial PAMPs, *Ot* elicits high concentrations of cytokines in humans and mice through an unknown innate ligand that was shown to retain its stimulatory capacity also in heat-killed (hk) bacteria.

The aims of this study were to identify the unknown heat-stable *Ot* PAMP and its recognition pathways, and to study the mechanism allowing the immunological discrimination between live and dead *Ot*.

Methods: To analyse the pathways involved in *Ot* recognition, we challenged murine bone marrow-derived dendritic cells (BMDC) in the presence of endosomal acidification inhibitors, or BMDC that carried knockouts (ko) for Toll-like receptors (TLRs) and adaptor proteins, with live or hk *Ot*. In human THP1-macrophages (MO) and peripheral blood mononuclear cells (PBMC), we blocked TLR8 with Cu-CPT9a prior to challenge with *Ot*. Cytokine induction was measured by qPCR and ELISA.

The role of RNA as an *Ot* PAMP was studied by transfection of TRIzol-purified *Ot* RNA using Dotap. RNA from live and hk *Ot* was further characterized by visualization on a Bioanalyzer and dual RNA-sequencing.

Results: Endosomal acidification and the TLR adaptor protein MyD88 were crucial for cytokine induction by live and 70 °C hk *Ot*. By investigation of BMDC from mice deficient for RNA-specific endosomal TLRs, we observed a receptor switch: TLR7 was required for *tnf-α* induction by live *Ot*, while the induction by 70 °C hk *Ot* depended on TLR13. Surprisingly, the induction of *tnf-α* by transfected total RNA from live *Ot* was also TLR13-

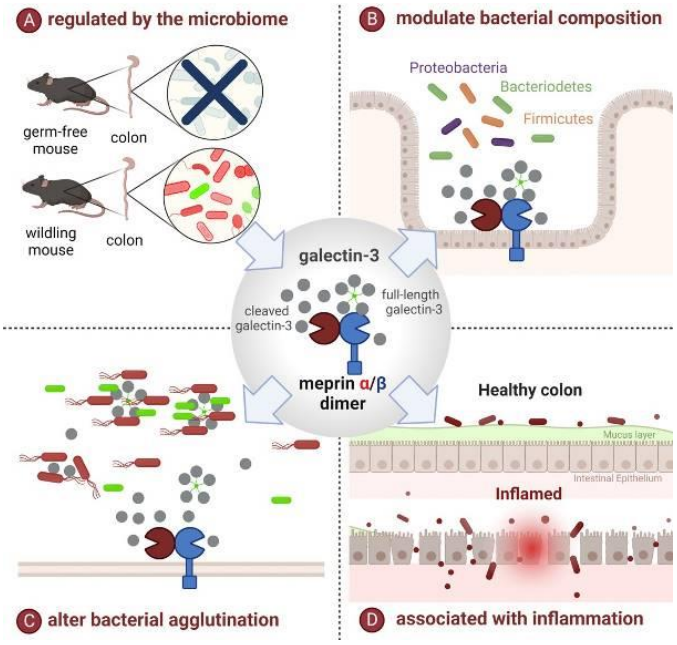
dependent, most likely related to its high content of rRNA, in which we detected a known TLR13 recognition sequence *in silico*.

As a possible RNA-specific counterpart of murine TLR13, we addressed the role of human TLR8 in recognition of *Ot*, in PBMC and THP-1MO. Interestingly, TLR8 blockage completely abrogated cytokine induction by live and 70 °C hk *Ot* or transfected *Ot* RNA, revealing a non-redundant role of TLR8 in *Ot* recognition.

We next studied the stimulatory capacity and integrity of *Ot* RNA after heat inactivation at defined temperatures. While live, 70 and 95 °C hk *Ot* strongly induced *tnf-α* and *ifn-β* in BMDC, 56 °C hk *Ot* failed to do so. Accordingly, 56 °C hk *Ot* harboured RNA of significantly lower concentrations, consisting of small fragments. While live *Ot* showed clear ribosomal bands, rRNA from 70 °C hk *Ot* was partially fragmented. Furthermore, live *Ot* contained relatively more mRNAs and tRNAs.

Discussion: Our findings identify RNA from *Ot* as a potent PAMP and highlight its role in the induction of innate inflammation. In murine phagocytes, *Ot* RNA can act as a viability associated (vita) PAMP as well as a PAMP post-mortem, but recognition is mediated by different receptors – potentially due to RNA fragmentation and availability in live vs. dead (hk) *Ot.*

Fig. 1



PWV 074

Proteolytic processing of galectin-3 by meprin metalloproteases is crucial for host-microbiome homeostasis

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The metalloproteases meprin α and meprin β are highly expressed in the healthy gut but significantly decreased in inflammatory bowel disease, implicating a protective role in mucosal homeostasis. In the colon, meprin α and meprin β form covalently linked heterodimers tethering meprin α to the plasma membrane, therefore presenting dual proteolytic activity in a unique enzyme complex. To unravel its function, we applied N-terminomics and identified galectin-3 as the major intestinal substrate for meprin α/β heterodimers. Galectin-3-deficient and meprin α/β double knockout mice show similar alterations in their microbiome in comparison to wild-type mice. We further demonstrate that meprin α/β heterodimers differentially process galectin-3 upon bacterial infection, in germ-free, conventionally housed (specific pathogen-free), or wildling mice, which in turn regulates the bacterial agglutination properties of galectin-3. Thus, the constitutive cleavage of galectin-3 by meprin α/β heterodimers may play a key role in colon host-microbiome homeostasis.

PWV 075

Progesterone-induced susceptibility to *Chlamydia muridarum* infection is dependent on the vaginal abundance of *Enterococcus faecalis* in mice

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Introduction: *Chlamydia trachomatis* is the world´s leading bacterial sexually transmitted disease. Asymptomatic and hence untreated infections with *C. trachomatis* can cause ascension of the pathogen, leading to severe sequels such as pelvic inflammatory disease and infertility. However, the complex interplay between the infection, the immune response, and particular the role of the vaginal microbiota is not completely understood yet.

Methods: We ran vaginal infection experiments using *Chlamydia muridarum* in 8 weeks old C57Bl6 mice as an established animal model of chlamydial infection. We analyzed the impact of progesterone pretreatment on the vaginal microbiome using 16S rRNA gene amplicon sequencing. After running correlation analysis between microbial taxa and the course of infection and isolation of microbes from the mouse vagina we identified potential protective microbes and performed in vivo modulation experiments to prove their role in *C. muridarum* infection. We complemented our work with tissue and immune cell analyses.

Results: Progesterone not only enhanced the susceptibility to chlamydial infections and was followed by characteristic hydrosalpinx formation but also led to significant changes in local immune responses and epithelial tissue integrity. In particular, profound alterations of the vaginal microbiota composition were observed. We further characterized the vaginal microbiota by a culturomics approach resulting in the isolation of 17 bacterial taxa with *E. faecalis* as the most abundant bacterium of the vaginal microbiota in naturally cycling mice. Following correlation analysis, direct modulation by replenishing *E. faecalis* to the vagina reduced the abundance of *C. muridarum* during the course of the infection.

Conclusions: Progesterone has been identified as a major driver of disease progression in *C. muridarum* mouse infection models. We evaluated the impact of progesterone on host immune response and vaginal microbiota and identified progesterone as a major driver of microbiota composition in the mouse vagina. Within this framework we could identify experimental modulation of the vaginal microbiome with *E. faecalis* being protective from *C. muridarum* infection in mice independent of other factors impacting on the infection. Modulation of the vaginal microbiota may, thus, become a key in understanding bacteria-bacteria and bacteria-host interactions in prevention of sexually transmitted chlamydial infections. Further in vivo studies and complementary in vitro models are undertaken to further unravel mechanistic insights into the interplay between microbiome and the pathogen.

PWV 076

Culturable lung microbiota and their interaction with the fungal lung pathogen *Aspergillus fumigatus*

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Introduction: Various microorganisms have been detected in healthy lungs by sequencing-based metagenomic studies. It has been shown, that lung microbial communities are changing upon lung disorders. However, both the presence of a residential microbiome in the lung and its potential functional role in health and disease remain unclear. Invasive aspergillosis is a fungal lung infection with high mortality rates among immunocompromised patients caused by *Aspergillus fumigatus.* A potential contribution of the lung microbiome to invasive aspergillosis is a matter of debate. We have been investigating a possible connection of culturable members of the murine lung microbiome with host cells and *A. fumigatus.*

Methods: Bacteria have been isolated from lung and trachea tissue samples of SPF BL/6 mice. Identification of bacteria was performed by 16S rDNA sequencing of pure cultures. *Ligilactobacillus murinus* isolated from the murine lower airways was fluorescently labelled by transformation with a *gfp*-bearing plasmid. Fluorescently labelled *L. murinus* has been used for investigation of interactions between the lung bacteria and host cells. Analysis of interaction between isolated bacteria and *A. fumigatus* has been performed by *in vitro* co-cultivation of the microorganisms.

Results: We could isolate bacteria belonging to 11 species from the lower respiratory tract of mice. By analysis of the microbiome of mice from different vendors, we observed that the origin of mice has an influence on the composition of the culturable microbiota. We fluorescently labelled one of the most prominently isolated bacterium *L. murinus* with GFP and showed that the GFP-labelled *L. murinus* could be internalized by murine alveolar epithelial cells. In further analyses, we have observed that *A. fumigatus* promotes growth of *L. murinus* during co-cultivation.

Conclusions: We could show that not only bacterial DNA but also living bacteria can be detected in the lungs. Our results suggest that lung bacteria can be recognized by alveolar epithelial cells, and, therefore, might play a role in the immune modulation in the lung. An immunomodulatory role of the lung microbiota might influence the host response to lung pathogens. We showed that the fungal lung pathogen *A. fumigatus* directly affects lung bacteria *in vitro* by promoting growth of *L. murinus.* These findings indicate that there is an interconnection between the host, the microbiome, and the lung pathogen *A. fumigatus*. Further studies on the lung microbiome in invasive aspergillosis could lead to the development of new methods for diagnosis and treatment of the infection.

PWV 077

Microbial metabolite dysregulation in obesity: a link between gut and alveolar sensing during influenza A virus infection

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Question: Obesity is a global health concern that affects a significant portion of the population and recent studies have shown that obesity is associated with alterations in the gut microbiome. In this context, our study aims to identify obesity-associated alterations in the gut microbiome. More precisely, we investigated the impact of altered short-chain fatty acids (SCFA) on the increased susceptibility of obese individuals to respiratory infections such as influenza.

Methods: We established a murine obesity model for viral pneumonia. In this diet-induced obesity (DIO) model we infected mice intranasally. with influenza A virus (IAV) after a high-fat diet (HFD). Shotgun metagenomic analysis was used to determine taxonomic and functional alterations of the gut microbiome. To investigate the effect of the bacterial metabolite acetate on influenza virus infection we used murine and human *ex vivo* lung slices. The underlying pathomechanism was elucidated by using lung epithelial cell lines and corresponding KOs for IP-10 and the FFAR2 receptor, generated by CRIPSR-cas9.

Results: In the DIO model, we detected significant changes in the taxonomy and metabolism of the gut microbiota. Here, especially *Firmicutes* were upregulated, and functional analysis indicated an altered carbohydrate metabolism. After infection with IAV, obese mice were characterized by increased virus titers and a prolonged course of infection. Interestingly, carbohydrate metabolism was still reduced during infection. Our findings indicate strong evidence for upregulation of cytokines and chemokines leading to antiviral effects of acetate and therefore to inhibition of influenza virus replication. Moreover, *in vitro* analysis in lung epithelial cells reveal, that the antiviral effect of acetate is dependent on FFAR2 receptor and IP10 activation.

Conclusions: Obesity is a multifactorial disease that influences the production of SCFA in the gut microbiome. Acetate, the most prominent SCFA, has a positive impact on the antiviral response in the lung. These changes in gut-lung homeostasis might be responsible for the increased susceptibility of obese individuals to respiratory viral infections such as influenza. Understanding the mechanisms underlying these effects could provide new therapeutic targets for the prevention and treatment of viral infections in obese individuals.

PWV 078

Investigating host-microbiota interactions in low microbial biomass specimens: examples from skin and lung studies

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Question: Over the last decade, numerous studies disclosed the diversity and structure of microbial species which inhabit several barrier organs. Most of this knowledge is derived from the gut which involves specimens with substantial microbial biomass.

However, investigating the microbiota in the lower respiratory tract or the skin has proven to be challenging due to lower microbial *vs.* host cell numbers, and environmental contamination. Nevertheless, as these microbial communities play an important role in maintaining a healthy functioning of these organs, it is imperative to overcome these technical challenges.

Here, we present optimized laboratory protocols and analysis pipelines to improve the characterization of host – microbiota interactions in lung and skin specimens from healthy donors and patients.

Methods: We generated bacterial profiles using high-throughput amplicon sequencing from numerous lung and skin specimens from humans and mice. The sample collections included: i) bronchoalveloar lavage fluids (BALFs) from patients of different infectious and non-infectious lung pathologies, ii) skin swabs from patients with bullous pemphigoid (BP), an autoimmune skin disorder, and iii) skin and lung biopsies from a mouse advanced inter-cross line. Protocols included digestion of extracellular DNA, quantification of bacterial load (qPCR, ddPCR), use of a mock microbial community of known composition with a wide concentration range, RNA-based profiling, and a combination of sequencing techniques. Additionally, we employed analysis pipelines incorporating statistical methods for the removal of contaminants, and defined a core microbiota.

Results: The combination of optimized laboratory protocols and analysis pipelines enabled a reliable profiling of the microbiota composition, and resulted in an improved characterization of the host-microbiota interactions. Precisely, we recovered sufficient 16S rRNA amplicon sequences from BALFs samples, uncovered distinct microbial signatures between BP patients and healthy controls, largely within the genus *Staphylococcus*, demonstrated a stronger quantitative trait mapping (QTL) signal in RNA *versus* DNA based bacterial profiling in the skin, and reported a similar improvement of genetic mapping signal in the lungs.

Conclusions: Analysis of low biomass specimens requires appropriate laboratory and analysis frameworks including profiling the active microbiota, and accounting for environmental noise, which ultimately can provide novel insights into host-microbiota interactions in lung and skin diseases.

PWV 079

Comparative metagenomic analysis of faecal and saliva samples obtained from a large variety of zoo animals

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Introduction: The One Health approach acknowledges the interconnectedness of human, animal, and environmental health and highlights the need to analyze all three components. As antimicrobial resistance (AMR) continues to increase globally, it is crucial to investigate the role of microorganisms in humans, animals, and the environment to contribute to overall homeostatic ecosystems, control diseases, and secure global health. Moreover, biosynthetic gene clusters (BGCs) are a potential target for antimicrobial compounds as the secondary metabolites they produce aid in inter-species competition. While many studies have focused on identifying novel BGCs derived from human microbiota, few have investigated the occurrence of BGCs in animal microbiota. This study aims to investigate the microbiome of captive animals from a southwestern German zoo taxonomically and functionally.

Material and Methods: We collected 55 stool and 16 saliva samples from 48 and 15 different animal species, respectively, from the zoo in Saarbrücken, Germany. These specimens were subjected to whole-genome DNA extraction and subsequent metagenomics sequencing. Computational analysis was performed using the state-of-the-art tools. We further compared our data to existing microbiome data from wildlife (Youngblut et al.)

Results: We observed differences in microbial composition of saliva and faecal samples between different diets and highlighted *Clostridium P* sp. and *Methylobacterium* sp. as differentially abundant between diet cohorts. AMR analysis revealed resistances against antibiotics that are commonly used in veterinary medicine, such as tetracyclines, macrolides, and lincosamides. However, resistances against vancomycin were found, encoded by the resistances clusters *vanD, vanG,* and *vanO.* We furthermore identified various novel bacterial species, which are an interesting target for BGCs.

Discussion: In comparison to the sampling of wildlife and subsequent microbiome analysis, our data revealed higher numbers of BGCs. We also observed considerable AMR rates. Further research is warranted to elucidate whether resistant bacteria could spread onto the zookeepers and contribute to the emergence of multi-resistant bacteria.

PRHYV 080

WGS transmission analysis based on weekly colonization screenings on neonatal wards

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Question: Periodic screening for bacterial colonization in neonates is often performed to facilitate outbreak detection.

It is sometimes assumed that bacterial species differ in their propensity for nosocomial transmission, e. g. that *Klebsiella pneumoniae* might be more prone to onward transmission than *Escherichia coli.*

In this work we linked large and complex datasets to calculate transmission rates using a meaningful denominator.

Methods: Patients on two neonatal level IV wards were screened weekly for anal and oropharyngeal colonization by multidrug-resistant as well as susceptible Gram-negative bacteria and *Staphylococcus aureus*. During a 20-month period, isolates underwent WGS and were phylogenetically analyzed by cgMLST. Microbiological data, results of typing as well as admission, discharge and transfer data of the neonates were analyzed with common Python packages (NumPy, pandas, Matplotlib, Seaborn, SciPy) in a Jupyter Notebook. The number of days, each respective roommate was exposed until their last weekly screening sample was taken, was used as denominator to calculate the transmission rate. Transmissions between siblings were excluded.

Bayesian inference was performed using PyMC and ArviZ. For analysis of transmission rates a binomial likelihood function and a Beta(0.5, 10) prior was applied, the latter putting more probability

density into lower values for the transmission rate. The project was funded by the German Innovation Fund of the Federal Joint Committee, the G-BA (01VSF16051).

Results: Data of 920 patients, of whom 11.5% had a birth weight below 1500 g, were analyzed during the study period. Transmission rates per 1000 exposure days together with 95% credible intervals were as follows: *Serratia marcescens* 5.9 (2.8 – 10.4), *Escherichia coli* 2.9 (2.0 – 4.0), *Staphylococcus aureus* 2,8 (2.0 – 3.6), *Klebsiella pneumoniae* 2.5 (1.2 – 3.9), *Enterobacter cloacae*-complex 1.4 (0.5 - 2.5), *Klebsiella oxytoca* 1.4 (0.5 – 2.6), *Citrobacter freundii* 0.0 (0 – 1.2).

Conclusions: Surprisingly, we did not observe a higher transmission rate for *K. pneumoniae* than for *E. coli* in our study. Most patients, however, were not colonized by multidrug-resistant Gram-negative bacteria or MRSA. Our results in this neonatal patient setting with strains mostly reflecting wild type strains might not reflect infection control settings in adult patients colonized with multidrug-resistant bacteria.

Of note, *S. aureus* had a transmission rate similar to *E. coli* and *K. pneumoniae*. In contrast, *S. marcescens* showed a higher transmission rate than all other species investigated in our study.

PRHYV 081

Comprehensive, prospective epidemiologic and molecular surveillance of *Klebsiella pneumoniae* supports infection control in neonatal intensive care

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Introduction: In preterm infants, colonization with *Klebsiella pneumoniae* (Kp) requires meticulous hygiene measures due to the elevated risk of invasive infections. We report about the implementation of a comprehensive, prospective, real-time surveillance concept at a NICU targeting i) basic epidemiology, ii) potential transmission and iii) virulence to support infection control.

Materials and methods: We visualized epidemiology by creating a timeline and dynamic streamgraphs complemented by prospective infection surveillance. For molecular surveillance, Illumina sequencing of *Kp* isolates was performed followed by cgMLST analysis applying Ridom"s SeqSphere+ software. Tellurite agar diffusion and String test were used for quick phenotypic virulence evaluation of *Kp* isolates found in screening samples. In addition, sequence data were used for virulence score [1] calculation with Kleborate.

Results: Of 31 infants with *Kp* isolates identified between June 2022 to April 2023 sequencing showed that 11 isolates (34.3%) actually were *Klebsiella variicola* belonging to the *Kp* complex. During the observation period one relevant epidemiologic colonization cluster occurred. Within this cluster cgMLST analysis revealed transmission of a clonal *Klebsiella variicola* isolate (sequence type 250). Cohorting and single room isolation were implemented and contributed to quick cluster control. Epidemiologic and microbiologic data were used for on-site training. All infants were only colonized, no invasive infections occurred. All isolates of the sequence type 250 were String test negative and none was Tellurite resistant (see exemplary figure 1).

In accordance with the results of the phenotypic quick tests the sequence-based virulence score calculation showed no presence of hypervirulent *Kp* strains in our cohort. Three isolates had a virulence score of 1 (Yersiniabactin present) and another three isolates had a virulence score of 2 (Yersiniabactin and Colibactin present).

Discussion: Real time evaluation of colonization epidemiology combined with molecular typing of Kp strains supported infection control in daily practice. The multimodal virulence assessment enabled evaluation of potential hypervirulent Kp strains.

References

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Fig. 1

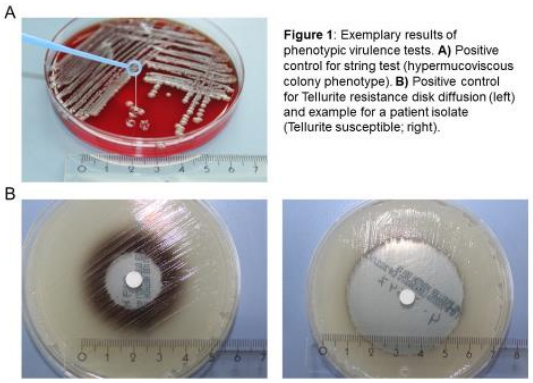

Figure 1: Exemplary results of phenotypic virulence tests. **A)** Positive control for string test (hypermucoviscous colony phenotype). **B)** Positive control for Tellurite resistance disk diffusion (left) and example for a patient isolate (Tellurite susceptible; right).

PRHYV 082

Surveillance of carbapenemase-producing *Enterobacterales* at one German tertiary care centre from 2011 to 2022

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Background: Germany is a low-prevalence country of carbapenemase-producing *Enterobacterales* (CPE). However, there is an increasing spread within the healthcare system and standardized prevalence and incidence data of CPE are scarce. A facility-wide surveillance of CPE was established at a tertiary care centre in Cologne in 2011.

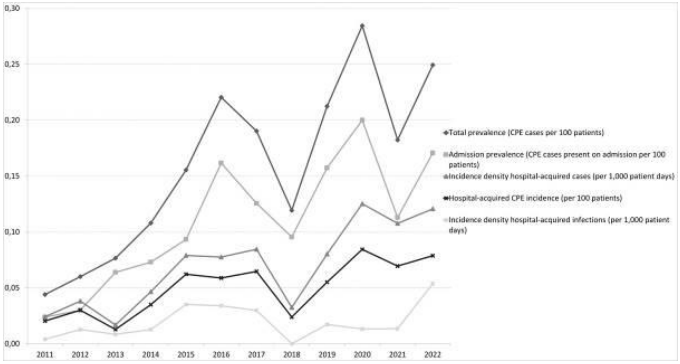
Materials/methods: We analysed all *Enterobacterales* from clinical and screening specimens based on non-susceptibility to imipenem, meropenem or ertapenem; in 2014 the EUCAST screening cut-off values for CPE were implemented. Phenotypic and genotypic carbapenemase tests were performed. Epidemiological and clinical data were collected and the mode of acquisition and hospital-acquired infections were evaluated based on the German national surveillance system KISS. Genotyping was carried out using repPCR/RAPD, PFGE or NGS.

Results: From 2011 to 2022 245 CPE from 209 patients were detected. The two most prevalent CPE were VIM-type-*E. cloacae* complex (n=71) and OXA-48-type-*K. pneumoniae* (n=42). Throughout the study period, we observed a diversification of carbapenemase types and the appearance of double-

carbapenemase-producers. Approximately 60% of the CPE were hospital-acquired, detected after a mean length of stay of 28 days. Seventeen events with at least one secondary transmission (all but one with *K. pneumoniae* or *E. cloacae* complex) were confirmed, all on high-risk units. Almost all CPE present-on-admission were associated with a hospital stay abroad or a direct transfer from another hospital. Hospital-acquired infections were observed in 40 patients (19.1 %). Eleven patients suffered from bacteraemia. We observed a steady rise in CPE-prevalence with two major dips in 2018 and 2021, the latter being probably related to the COVID-19 pandemic (Fig. 1. CPE rates at a tertiary care centre). In 2022 the incidence and prevalence were as follows: admission prevalence 0.17 (per 100 patients), incidence density of hospital-acquired cases 0.12 and incidence density of acquired infections 0.05 (both per 1000 patient days).

Conclusions: This study highlights the changing epidemiology of carbapenemases in one medical centre over a period of eleven years. The results support the need for a facility-wide CPE-surveillance system to develop unit-specific IPC strategies. Further studies should focus on a multicentre approach with an in-depth analysis of carbapenemase-production to provide benchmark rate data.

Fig. 1



PRHYV 083

Development of Mulitdrugresistant organisms (MDRO) during the COVID-19 pandemic. Results of a multicenter study in 84 hospitals

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Question: Despite the implementation of strict infection control measures in hospitals during the COVID-19 pandemic, there are numerous reports about an increased incidence rate of hospital aquired MDRO. Reasons given are the increase of antibiotic consumption, the reduced detection of MDRO with subsquent transmission, combined with the decrease of hand disinfectant (HD) consumption by health care workers. This study investigated the impact of COVID-19 on the incidence rates of MDRO, antibiotic and hand disinfectant consumption.

Methods: Retrospective, multicenter study in 84 hospitals from primary to tertiary care level in Germany. Comparison of the incidence density of MDRO per 10.000 patient days (pd) age group related, the antibiotic consumption in recommended daily doses (RDD) per 100pd and the hand disinfectant consumption per pd. MDRO refers to MRSA, VRE and multidrug resistant gram negative organisms. Baseline period was from March 2019 to February 2020, compared with the first year of COVID-19 pandemic from March 2020 to February 2021 and subsequently the second year from March 2021 to February 2022. The data were expressed as mean (standard deviation). To compare the difference between the baseline and the pandemic years, we used a two sided sign test.

Results: A significant reduction in the incidence rates of MDRO over the first 2 years of COVID-19 pandemic, compared to the baseline period was observed in all age groups ($p<0,001$). For nosocomial incidence rates of MDRO, no significant reduction was found. An increase of antibiotic consumption from 41,1RDD per 100pd (baseline period) to 45,0 and 44,5RDD per 100pd in the pandemic periods was recorded. Hand disinfection consumption increased in the first year of COVID-19 pandemic compared to the baseline period on non ICU wards from 10,3 to 12.2 HD per pd. No change on hand disinfection consumption on ICU wards.

Conclusion: The incidence density of MDRO was significantly decreased during the COVID-19 pandemic. Outbreaks or an increased import of carbapenem resistant Gram negativ organisms were not observed. The higher antibiotic consumption was due to an increased use of reserve antibiotics. The hand disinfection consumption was constant over the COVID-19 pandemic, exempt the first year with an increase on non ICU wards, hence COVID-19 wards been established and fewer elective patients been admitted.

References: Sun Jin et al.; Curr Opin Infect Dis. 2021 Aug 1;34(4):365-371; Thoma et al.; Antimicrob Resist Infect Control. 2022 Jan 21;11(1):12; Boris Augurzky et al.; Schriftenreihe zur Gesundheitsanalyse – Band 30

PRHYV 084

Clusters of NDM-1-producing *Klebsiella pneumoniae* in Germany, 2022, are associated with exposure in Ukraine or with nosocomial transmission in German hospitals

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Background: Surveillance of notifiable carbapenem-resistant Enterobacterales (CRE) in Germany revealed an increase of carbapenemase NDM-1-producing *Klebsiella pneumoniae* in 2022.

We investigated epidemiological links within genomic clusters to understand chains of transmission.

Methods: Among 200 NDM-1-producing *K. pneumoniae* isolates, received between January-September 2022 from all German regions, we applied single nucleotide variant-based cluster detection. We matched isolate information to mandatory case notification data to compare clusters regarding time and places of residence and hospitalisation, sex, age, sampled material, recent presence in Ukraine, and location in Ukraine.

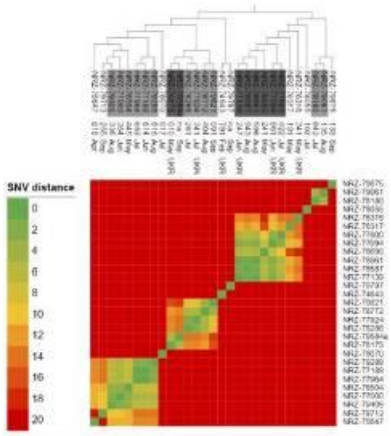
Results: We identified 24 genomic clusters (2-39 individuals/cluster). For 8 clusters there was sufficient case information for in depth investigations available. Two (2/8) cluster comprised 3 and 9 patients each from one hospital, independent of exposure in Ukraine. In six (6/8) prior hospitalisation in Ukraine predominated with diffuse covariate patterns. In the largest cluster, all 19/39 individuals with available information had been exposed to Ukraine or Russia. Individuals in this cluster were notified from 13 German districts, between 1-83 years old, with 5 females and 34 males. Data on recent locations in Ukraine were sparse only

documented for 6 individuals. Only in one cluster, hospitalisation overlapped in time and location (Dnipro) for 2/6 individuals. In 2 clusters, a patient from Ukraine was screened positive on-admission and another patient was tested positive later than 3 days post-admission in the same German hospital.

Discussion: Our analyses suggest three scenarios: Local independent NDM-1-*K. pneumoniae* outbreaks in German hospitals, imported clusters from Ukraine, and secondary transmission in hospitals. Infection prevention and control (IPC) measures thus must comprise: IPC strengthening in Ukraine, prevention of onward transmission within German hospitals, understand and stop independent infection chains. Hospital-admission screening for CRE in patients with exposure to Ukraine should be considered.

Fig. 1

Figure 1: Phylogenetic tree and heatmap based on pairwise single nucleotide variant (cgSNV) differences between isolates of *Klebsiella pneumoniae* ST307, Germany, January–September 2022 (n = 29); UKR, association with exposure to Ukraine



PRHYV 085

Epidemiology and outcomes of vancomycin-resistant enterococcus (VRE) infections in inpatients worldwide: a systematic review and meta-analysis

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Introduction: An increasing trend in VRE has been observed in many countries combined with a changing epidemiology [1]. However, many epidemiological studies are limited in scope regarding setting, patient group and/or analyzed VRE or vancomycin-susceptible (VSE) enterococcus species comparisons. We aimed to provide a comprehensive update on the epidemiology and burden of VRE infections in in-patients worldwide.

Methods: We searched MEDLINE/PubMed, the Cochrane Library, and Web of Science for observational studies which reported on VRE *faecium* and *faecalis* infections in in-patients published between January 2014 – December 2020. Risk of bias was assessed according to the Newcastle-Ottawa Scale and results were reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020). Outcomes were incidence, infection rate and mortality as well as length of stay (LOS) and healthcare costs. We conducted a meta-analysis on mortality using a random effects model.

Results: Of 681 identified publications, 57 studies were included in the analysis. Overall quality of evidence was low due to the general study design limitations of observational studies. VRE incidence was rarely and heterogeneously reported. VRE infection rate differed highly between settings and patient populations (1%-55%). The meta-analysis showed a higher mortality for VRE *faecium* bloodstream infections (BSI) compared to VSE *faecium* BSI [risk ratio, RR 1.46; 95% CI 1.17 – 1.82] (Figure 1). No difference was observable when comparing VRE *faecium* vs. VRE *faecalis* bloodstream infections or invasive infections [RR 1.00, 95% CI 0.52 – 1.93]. LOS was higher in BSIs caused by *E. faecium* vs. *E. faecalis.* Only three studies reported healthcare costs, indicating higher costs partly mediated by longer hospital stays of patients.

Discussion: This study provides a comprehensive overview of the current evidence of the epidemiology and burden associated with VRE infections in inpatients. In contrast to previous findings showing that mortality in VRE is species-related [2], our findings indicate that the vancomycin resistance may be associated with a higher mortality. We identified a lack of standardization in reporting outcomes and a lack of information regarding health care costs of VRE infections which may guide the setup and reporting of future studies.

References:

[1] Werner G et al. (VRE study group). Thirty years of VRE in Germany - "expect the unexpected": The view from the National Reference Centre for Staphylococci and Enterococci. Drug Resist Updat 2020.

[2] Kramer TS et al. The importance of adjusting for enterococcus species when assessing the burden of vancomycin resistance: a cohort study including over 1000 cases of enterococcal bloodstream infections. Antimicrob Resist Infect Control 2018.

Fig. 1

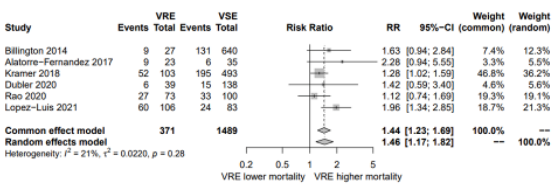

Figure 1: Meta-analysis of mortality data regarding VRE *faecium* bloodstream infections in comparison to VSE *faecium* bloodstream infections.

MSV 086

Distribution of ESBL-carrying plasmids in *Escherichia coli* and *Klebsiella pneumoniae* recovered from long-term care facility residents, staff, and linked aquatic environments: a European multi-center study

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Introduction: Antimicrobial resistance (AMR) has become an emerging threat worldwide. Breaking infection chains requires surveillance and strict infection control measures. While current routine infection control mostly focuses on clonal expansion and transmission, understanding the spread and distribution of plasmids harboring AMR is crucial for implementing successful strategies to combat the spread of multi-drug resistant bacteria.

Methods: Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* were obtained in four European centers from different sources: fecal samples of residents and staff from long-term care facilities (LTCF) and samples from differents surfaces within the corresponding LTCF. Furthermore, LTCF waste water, waste water treatment plants (WWTP), and the respective rivers downstream of the WWTP were sampled at 8 time-points. Isolates were sequenced both with Illumina short-read technology as well as Oxford Nanopore long-read technology. Hybrid-assembly and determination of the plasmid content was performed using plasmident. ESBL genes were identified using Resfinder. ESBL genes which were present in more than four isolates from different locations were selected for further analysis. In the following step, the first unique isolate of each study participant was selected, while all environmental isolates harbouring the respective ESBL gene were included.

Results: In total, 333 ESBL-producing strains (*E. coli* n=238, *K. pneumoniae* n=95) were examined for their respective AMR-content. ESBLs of different CTX-M-types were most commonly found: *bla*CTX-M-1 (n=27), *bla*CTX-M-14 (n=22), *bla*CTX-M-15 (n=207), *bla*CTX-M-27 (n=50), and *bla*CTX-M-55 (n=10). In 22% (73/333), *bla*CTX-M was non-plasmidic and could be determined on the chromosome. *bla*SHV-12 was the predominant ESBL of the non-CTX-M-type (n=31, exclusively plasmidic). In eight strains, two ESBL genes were found, each harboured by a distinct plasmid. The size of ESBL-gene-containing plasmids in median was 107.9 kb (range 6.7 – 297.1 kb).

Conclusions: We observed a large overlap of ESBL-harbouring plasmids in different reservoirs and different centers in Europe. The majority of ESBL genes were found to be plasmidic, indicating their potential for horizontal gene transfer. Considering that current outbreak detection primarily relies on species and sequence type identification, we conclude that the spread of antimicrobial resistance via horizontal gene transfer needs to be brought further into scientific focus.

MSV 087

Unveiling the diversity and characteristics of plasmids in non-tuberculous mycobacteria: insights from a comprehensive genomic study

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Question: Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment and some species are causing rising numbers of infections in humans. Yet, it is unclear if this is due to an increase in virulence or to other factors. Plasmids for instance may play a pivotal role in transmitting virulence factors and resistance genes. However, studies focusing on these genetic elements in NTM are scarce. To fill this knowledge gap, we investigated the prevalence, characteristics and diversity of NTM plasmids and evaluated the ability to reconstruct them from short read sequencing data.

Methods: A total of 187 complete genomes from NCBI belonging to 18 clinically relevant NTM species were manually screened for the presence of plasmids. All available NTM plasmid sequences (n=152) were downloaded from the curated plasmid database PLSDB. A pan-genome analysis of these plasmids was performed using Roary and virulence and resistance genes were detected with AMRfinder+. Genetic distances were calculated with mash. Lastly, platon, plasmidSPAdes and SRST2 were used to detect and reconstruct plasmids from short read sequencing data of 91 NTM isolates.

Results: Among 187 clinically relevant NTM genomes, 59 genomes from nine species harbored one to five plasmids. Plasmid-bearing species included both rapidly (*M. abscessus and M. fortuitum)* and slow growing NTM (*M. avium*, *M. intracellulare*, *M. kansasii*, *M. lentiflavum*, *M. marinum*, *M. ulcerans* and *M. gordonae)*. The 152 NTM plasmids available in PLSDB were highly diverse with regard to length (4 kbp - 864 kbp), circularity (15% linear) and genomic content. Most of the predicted genes were classified as hypothetical proteins and genes with known function were mainly related to basic plasmid functions such as segregation (e.g. parA), mobilization (e.g. relaxases) and conjugation (e.g. T4SS or T7SS). Putative resistance and virulence genes were found in 67/152 plasmids but similarity with known markers was typically low (<50% identity). Genetically closely related plasmids were found in different NTMs, indicating that these plasmids can move across species barriers. Known plasmids could be readily identified directly from short read sequencing data with SRST2. Small NTM plasmids (<50 kbp) were typically assembled *de novo* as one contig but larger plasmids were fragmented into several contigs. In addition, both plasmidSPAdes and platon falsely assembled or identified many contigs as plasmid-borne.

Conclusions: NTM plasmids are common, highly diverse and can propagate in different species, thereby contributing to horizontal gene transfer and evolution of NTM. Still, their contribution to virulence and resistance dissemination would need further experimental confirmation. Short sequencing reads can be used to detect the presence of known plasmids but de novo plasmid assembly remains a challenge. Overall, the results significantly broaden our knowledge of plasmids in NTMs and might facilitate further plasmidome studies.

MSV 088

The mysterious spread of OXA-244 producing *E.coli* ST38, insights of an genomic-epidemiological investigation in Germany, 2013-2021

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Background: OXA-244-producing *E. coli* has increased rapidly in several European countries since 2013. One large internationally spread genetic cluster of ST38 was identified by cgMLST but epidemiological links between the cases remain unknown. We aimed to increase discriminatory power and further investigate epidemiological links between cluster isolates.

Methods: Of 523 OXA-244-producing *E. coli* isolates that were received by the German national reference centre between 2013 and 2021, 224 belonged to the ST38 cgMLST cluster and were subjected to whole genome (wg)MLST to identify subclusters. Additional information regarding district of residence and hospitalisation as well as travel history was obtained by matching the isolates to mandatory case notifications. Cases were classified by possible community or nosocomial transmission, inferred from dates of detection versus hospitalisation and other variables. Semi-structured interviews were conducted with 11 cases.

Results: WgMLST revealed 23 genetic subclusters with 2-57 isolates/cluster. Interviews did not point to one single food source but revealed a travel history to Turkey, Egypt or Morocco within 24 months prior to detection in 5/11 (45%) cases compared to 6/213 (3%) cases without interview. In cases without travel history, case information hints towards nosocomial transmission in 59/213 (28%) and community transmission in 79/213 (37%) cases (and was insufficient for classification in the rest). All subclusters with >2 isolates were homogenous regarding travel to a certain country, but heterogenous regarding district of hospitalisation or residence. In both of the two largest subclusters, cases occurred between 2018 and 2021 and travel history to Turkey was present in one of the first cases. The district of residence was available in 36/56 and 11/19 cases, respectively, but different for all cases besides several from Berlin. In the largest cluster, four, three and two cases were hospitalised in three respective hospitals with the same 3-letter postal code within 12 months suggesting smaller nosocomial outbreaks.

Discussion: Travel to Northern Africa or the Middle East was frequently reported in case interviews but is underreported in routine surveillance data. Homogeneity of countries of travel within clusters might indicate elevated transmission in and importation of cases from these regions for many identified subclusters. Further transmission in Germany seems to occur frequently outside the hospital but temporo-spatial heterogeneity and missing data pose challenges to epidemiological interpretation. Travel history should be obtained and reported for carbapenemase-producing Enterobacterales (CPE) in order to facilitate cluster investigations.

MSV 089

Beyond sequential blockade: how the trimethoprim-sulfamethoxazole combination induces pleotropic cellular responses in susceptible bacteria

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Introduction: Antibiotic resistance has emerged as a critical challenge to global health, largely driven by the proliferation of multi-drug resistant bacteria. As new therapeutic strategies lag, combination antibiotic therapy presents a promising avenue for treating resistant infections through the exploitation of antibiotic synergy, where the killing power of a combination is greater than the contributions of the individual drugs. However, successful leveraging of this approach is limited by our lack of understanding of the underlying killing mechanisms involved and potential secondary targets of the antibiotics.

Trimethoprim (TMP) and sulfamethoxazole (SMX) are often advocated in combination. Both drugs target adjacent steps of the folate synthesis pathway, enhancing each other"s effects and leading to increased killing. The goal of this study was to gain a deeper molecular understanding of TMP-SUX synergistic killing.

Methods: To dissect the physiological responses induced by TMP, SMX and their combination, we conducted a range of experiments in two organisms: *E. coli* and the ESKAPE pathogen *A. baumannii*. This included a high-throughput fitness assay utilizing transposon-directed insertion site sequencing (TraDIS), to evaluate the fitness contribution of every single gene under sub-inhibitory TMP and/or SMX. We further studied the effects of these antibiotics on cell morphology and membrane permeability.

Results: Our research yielded intriguing results regarding how two different bacterial species, *E. coli* and *A. baumannii*, respond to TMP, SMX and TMP-SMX. TraDIS found that *E. coli* exhibited a reduction in respiration gene mutants when exposed to TMP-SMX, while conversely, respiration gene mutants increased in *A. baumannii.* Additionally, there was a substantial decrease in mutants related to ribosomal functionality, cell shape, and division within *A. baumannii*, while *E. coli* did not demonstrate this trend. TraDIS also identified genes (*e.g. ihfB*, *mreB*) that contribute to fitness under TMP and SMX, yet are not known drug targets or associated with resistance to either drug. Beyond these genetic findings, we also observed that SMX seemed to permeabilize the cell membrane of *E. coli* but not *A. baumannii*. Cell shape changes in response to the antibiotics also varied between organisms.

Discussion: Our research suggests that TMP-SMX elicits distinct physiological responses in *E. coli* and *A. baumannii*. While it is well documented that antibiotic combination effects can vary between organisms, this is the first detailed exploration into the underlying responses that could give rise to these differences. It emphasizes the need for a nuanced understanding of antibiotic interactions within different organisms. Additionally, our findings relating to effects outside the folate pathways underscore the need for further studying of drug mechanisms, even for well-known antibiotics, to develop more effective and tailored therapeutic strategies.

MSV 090

Pre-clinical assessment of the chlorotonil derivative WH0182 as potent novel antibacterial agent active against multidrug-resistant Gram-positive bacteria

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Introduction: Infections caused by multidrug-resistant (MDR) bacteria are an ongoing threat for our healthcare system, and new antimicrobial compounds are urgently needed to tackle this threat. A promising new class of antibiotics are chlorotonils, highly lipophilic tricyclic macrolides produced by the myxobacterium *Sorangium cellulosum*. Chlorotonil A (ChA) was shown to exert promising activities against major Gram-positive nosocomial pathogens such as *Enterococcus* spp. and *Staphylococcus aureus*. However, the applicability of this compound as antibiotic is largely hampered by its poor solubility in aqueous solutions. In order to enhance its water solubility, a number of semi-synthetic compounds were created (1). The frontrunner derivative WH0182, which possesses a nearly 1000-fold increase in water solubility when compared to ChA and maintains its high activity in the sub-µg/ml range against selected *Enterococcus* spp. or *S. aureus* isolates was tested here for its *in vivo* applicability.

Methods: Broth microdilution assays were performed to determine the MIC_{90} of WH0182 against sets of clinical *Enterococcus* spp. and *S. aureus* isolates including Vancomycin-resistant and Methicillin-resistant variants (*i.e.* VRE and MRSA/VISA). Time kill kinetics assays were carried out to identify whether WH0182 is bactericidal or bacteriostatic. Potential cytotoxic effects of WH0182 on eukaryotes were determined in Danio rerio larvae and human cell culture-based assays. The *in vivo* activity of WH0182 was tested in a *S. aureus*-based murine foreign-body infection model.

Results: Our MIC analyses revealed MIC_{90} values of 0.1 and 0.2 µg/mL for WH0182 against *Enterococcus* spp. and *S. aureus*, respectively. Time kill curves indicate a very fast-acting bactericidal effect of WH0182 with a complete killing of the bacterial cell population within <30 min when bacteria were challenged with WH0182 concentrations of 2 x MIC and higher. We could not observe signs of obvious pore formation or changes in membrane potential. When applied in human cell lines and zebrafish larvae, respectively, no signs of mitochondrial, cardio- or hepatotoxicity were observed with WH0182 up to a concentration of 8 µg/mL (*i.e.* 40 x MIC). Treatment of *S. aureus* SA113 infected catheter fragments inserted into the flanks of mice with WH0182 (25 mg/kg applied subcutaneously once per day for 6 days) allowed for a nearly 4 log_{10} reduction in bacterial loads at the catheter and in the surrounding tissue when compared to sham-treated mice. Notably, treatment efficacy of WH0182 was even superior over that of the reserve antibiotic Linezolid.

Conclusion: Our findings presented here identified the semisynthetic ChA derivative WH0182 as a highly promising candidate for drug development that might be used in the future for the treatment of infections caused by Gram-positive MDR-pathogens such as VRE and MRSA/VISA.

- (1) Hofer, W. *et al*. Angew Chem Int Ed Engl. 61(30):e202202816.

MSV 091

Development of resistance to Ceftazidime/Avibactam in *bla*$_{OXA244}$-carrying *Escherichia coli* during therapy, associated with mutations in *acrB* and *mrdA*

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Background: Here, we report an *in vivo* development of resistance to ceftazidime/avibactam during therapy in a critically ill patient with bloodstream infection caused by carbapenem-resistant, *bla*OXA-244-carrying *Escherichia coli*. Since the first isolate (ec_1) was susceptible, antimicrobial therapy with ceftazidime/avibactam was initiated. The subsequent isolates recovered from the patient in a period of 5 weeks, ec_3 to _8, had considerably higher MICs (Figure 1).

Methods: Ceftazidime/avibactam MIC was determined using the broth microdilution and the Etest method following the EUCAST standards. Comparative genomics analysis between the ceftazidime/avibactam-susceptible (ec_1 to _2) and -resistant (ec_3 to _8) isolates was performed by WGS using the Illumina MiSeq platform to identify potential resistance mechanisms. We performed a serial passage experiment using the clinical ceftazidime/avibactam-susceptible isolate (ec_1) to validate the *in vivo* finding. In this experiment, subcultures (1:50) were performed daily from an initial liquid culture while doubling the antibiotic concentration in the liquid media.

Results: Alignment of the core genome revealed the same MLST (ST405) and a close genetic relationship with SNPs ranging between 0 and 23 among the isolates, confirming a common clonal origin (Figure 2). All strains harboured OXA-244, CTX-M-15, and TEM-1B as ß-lactamases. Comparison of the draft genomes revealed missense mutations in the resistant isolates in three genes, *mrdA*, which encodes a penicillin-binding protein 2 (PBP2); *acrB*, an efflux pump gene; and *nlpl*, which encodes an outer membrane lipoprotein (Figure 1). In isolate ec_3, we detected mutations only in *acrB* and *nlpl*. There were no alterations in ß-lactamases nor in PBP3, which have been attributed to ceftazidime/avibactam resistance in *E. coli* before. In the serial passaging experiment, we observed the emergence of resistant isolates starting from the subculture step at a ceftazidime/avibactam concentration of 2 mg/L. Alignment of the core genome of the parental (ec_1) and the resistant mutant isolate (ec_1_mut) revealed alterations in *mrdA* and *acrB*, confirming the finding in clinical isolates. The resistant mutant derived from ec_1 showed mutations at different sites in these genes compared to clinical isolates.

Discussion: This is the first report demonstrating the *in vivo* emergence of resistance to ceftazidime/avibactam in *E. coli* due to alterations in *acrB* and PBP2. We are currently performing fitness cost, gene expression and site-directed mutagenesis experiments to determine the separate impact of both genes and different mutations in *acrB* on the resistant phenotype.

Figure 1 MIC of the examined isolates and mutations in the genes that altered in comparison to the initial ceftazidime/avibactam-susceptible isolate (ec_1)

Figure 2 Maximum likelihood phylogenetic tree and SNP matrix-based heatmap showing the variation in the core genome SNPs between examined isolates

Fig. 1

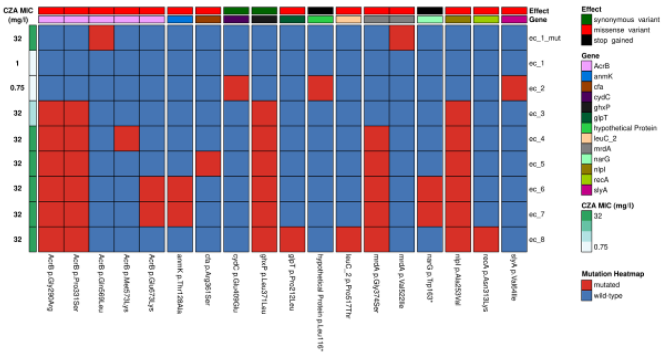
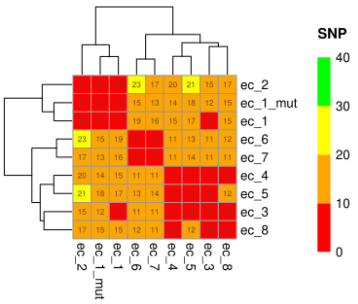


Fig. 2



MVV 092

MOI and multiple-dosage effect of a non-propagative phage-based delivery of CRISPR-Cas9 designed to re-sensitize carbapenem-resistant *E. coli*

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Introduction: The development of new strategies to target antibiotic resistant bacteria has been fostered due to the current antimicrobial resistance crisis. While conventional antimicrobial therapies select for antimicrobial resistant bacteria, CRISPR-Cas9 can be used to reduce or eliminate resistance determinants in a target microorganism, keeping the recipient bacteria alive and preventing microbiome dysbiosis. An efficient delivery of the CRISPR-Cas9 to bacteria colonizing the gut or present in biofilms, however, remains a challenge. The goal of this study was to develop a non-propagative phage-based system for delivering CRISPR-Cas9 into carbapenem-resistant *E. coli*.

Methods: A phage mutant was constructed based on homologous recombination and counter selection. The CRISPR-Cas9 plasmid was constructed using Gibson assembly and contains a sgRNA targeting carbapenem genes. Phage packaging efficiency with CRISPR-Cas9 was evaluated with dPCR. Cell survival, transduction efficiency and re-sensitization were tested *in vitro* with different multiplicity of infection (MOIs, from 10^{-1} to 10^{-4}). The effect of one, two or three consecutive administration of CRISPR-Phages was also evaluated.

Results: The CRISPR-Cas9 plasmid was packed with a ratio of ~ 1:5. The highest transduction efficiency of 10^{-3} was obtained with the MOI 10^{-1}, however it was associated with lower cell survival rates, in both targeting and non-targeting CRISPR-Phages. This indicates a killing side-effect correlated to the phage MOI and independent of the CRISPR-Cas targeting. WT bacteria outgrew the CRISPR-Cas9-carrying bacteria overtime. After 24 hours without antibiotic selection for the CRISPR-Cas9 plasmid, there was a 5-log reduction in the abundance of the CRISPR-Cas9 carrying bacteria. This was expected, as the CRISPR-Cas9 is not harboured by a conjugative plasmid. Upon CRISPR-Cas9 transduction with an MOI 10^{-1}, an initial 49% re-sensitization was achieved, which increased after 3 hours of incubation to a 90%, and reaching the highest re-sensitization rate of 99% after 7 hours of treatment. Regarding the multiple administration of phages, a significant higher number of CRISPR-Cas9 transduced cells was achieved when 3 dosages were administered. Additionally, no difference in the re-sensitization rates was observed, indicating that 3 dosages do not generate more CRISPR-Cas9 resistant cells when compared to single dosage.

Discussion: Future experiments aim at evaluating the re-sensitization in complex environments, such as the intestinal tract of animals, which represent important reservoirs of antibiotic resistance genes.

MVV 093

Heterogeneous response of MDR *Klebsiella pneumoniae* against phage LAPAZ and identification of two putative phage-encoded depolymerases

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Introduction: Recently, we have isolated a novel phage "LAPAZ" (Drexlerviridae, Webervirus) with lytic activity against several capsular types of *Klebsiella pneumoniae* (KP). Here we investigated changes in antibiotic susceptibility of multiple phage resistant clones and aimed at the identification of phage-derived depolymerases (DPOs) targeting the bacterial capsule.

Methods: Six multidrug-resistant (MDR) KP strains, encompassing four capsular types were used in this study. Antibiotic disc diffusion assays and E-tests of ampicillin (AM), ceftazidime (CAZ), ciprofloxacin (CIP), gentamycin (GM) and meropenem (MEM) were performed with phage-resistant clones. The identification of putative phage-encoded recombinant DPOs was assessed by *in silico* analysis from whole-genome sequence data. Mutations occurring in candidate genes were verified by Sanger sequencing. Recombinant phage-encoded proteins were generated by molecular cloning into an expression system.

Results: A total of 30 potentially phage-resistant clones derived from four KP strains were isolated, out of which 14 clones were verified as stable phage-resistant. Antibiotic tests showed reduced antibiotic susceptibility against CAZ, GM, and MEM for all clones except for one which displayed an increased susceptibility for CIP. The pattern of antibiotic sensitivity changes was unique for each clone. On sensitive bacteria halo appearance varied with strongest halo formation visible with capsular type 2. This aligned with the identification of two genes in the genome of LAPAZ (*orf69* and *orf72*) encoding for putative DPOs, the first of which sharing highest sequence identity of 70 % with a capsular type 2-specific DPO (YP_010115729). During lab experiments, two non-synonymous mutations occurred in *orf69*, indicating gene product activity and possible adaptation to the hosts. The second putative DPO (*orf72*) shared sequence identity with a characterized DPO of around 89 %, specific for capsular type 63 (YP_009226010). Both, *orf69* and *orf72* have been cloned into an E. coli expression system and are currently being investigated for their lysis potential, specificity, and resistance emergence followed by antibiotic sensitivity alterations.

Discussion: This work shows a versatile host response to the phage attack as all resistant clones displayed individual antibiotic sensitivity changes. This indicates that KP has an arsenal of phage-defense options, impeding phage-based approaches in clinical settings. We hypothesize that the putative DPO encoded by *orf69* is specific for capsular type 2, while the specificity of the DPO encoded by *orf72* has yet to be elucidated. Given that phage LAPAZ can infect at least four different capsular types, it is likely that additional DPOs are encoded within its genome. Since DPOs are candidates as alternative antimicrobials, further research on their lytic potential, resistance development and antibiotic interactions is promising.

MVV 094

A novel *Serratia marcescens* bacteriophage from South Africa – a tool to tackle produce associated antibiotic-resistant *Serratia marcescens*?

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Introduction: *Serratia marcescens* (family *Yersiniaceae*) is an opportunistic pathogen causing nosocomial infections in humans, potentially putting consumers at risk if present on minimally-processed fresh produce typically consumed raw. *S. marcescens* can form biofilms on surfaces, increasing its tolerance to biocides and disinfectants, rendering its control and removal in food production environments difficult. The presence of antibiotic-resistant strains of this opportunistic pathogen on fresh produce affects food safety and is a reason for concern. Therefore, this study explored a newly isolated lytic bacteriophage as a possible tool to control and eliminate this opportunistic pathogen when present on the surface of tomatoes.

Methods: Lytic phages were isolated using the double layer method and an antibiotic-resistant *Serratia marcescens* strain (Tom1) originating from tomato as the bacterial host. Water samples from the uMsunduzi River, used for irrigation by nearby vegetable farms, served as inoculum. Lytic phage isolates were obtained from plaques, and the phage morphology was characterized using transmission electron microscopy (TEM). The genome of one selected phage - *Serratia* Phage WR - was analyzed along with host range, physiochemical stability (temperature and pH), and ability to inhibit and destroy biofilms (crystal violet assay). Finally, the effectiveness of the selected phage in reducing the *S. marcescens* load on the surface of contaminated tomatoes was analyzed.

Results: Phage WR was assigned to the class *Caudoviricetes* based on its icosahedral capsid and contractile tail revealed by TEM. It showed a narrow host range, lysing only *Serratia marcescens* and no other *Enterobacterales* tested. Based on the analyzed genome, *Serratia* phage WR belongs to the class *Caudoviricetes*, with a genome size of 67.9 kb and a GC % of 49.9. *Serratia* phage WR genome exhibited the highest sequence similarity to the similarly-sized genome of phage *Serratia* MTx (GenBank: MK618717). The genome of *Serratia* phage WR lacks undesirable genes responsible for lysogeny, virulence, and antimicrobial resistance. One-step growth curves indicated a burst size of about 104 viral particles/bacterial host cell, with a latency period of about 25 minutes. The phage was stable between 8˚C-40˚C and pH 4-8. Biofilm formation over 96 h (24 h interval) was reduced significantly, and existing biofilms formed by *S. marcescens* were destructed compared to controls with inactivated or without phage particles. *S. marcescens* viable counts were reduced by about 2.2 log after treating (24 h) experimentally contaminated tomatoes with *Serratia* phage WR.

Discussion: We showed that the lytic *Serratia* phage WR (class *Caudoviricetes*) could reduce the burden of antibiotic-resistant *Serratia marcescens* on tomatoes and destructed biofilms formed. These characteristics highlight its potential as a tool to tackle *S. marcescens*, enhancing the safety of ready-to-eat produce from farm to fork.

MVV 095

Characterisation of PVL-positive *Staphylococcus argenteus* from the United Arab Emirates

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Introduction: *Staphylococcus argenteus* is a recently described staphylococcal species that is related to *S. aureus* but that lacks the staphyloxanthin operon responsible for the synthesis of the "golden" pigment in *S. aureus*. It is able to acquire both, resistance markers including SCC*mec* elements and mobile genetic elements carrying virulence-associated genes from *S. aureus*. This includes those encoding the Panton-Valentine leukocidin (PVL), which is associated mainly with severe and/or recurrent staphylococcal skin and soft tissue infections. Here, we describe the genome sequences of two PVL-positive *S. argenteus* isolates from the United Arab Emirates.

Material and methods: Two *S. argenteus* isolates were found during a study on nasal colonization and environmental contamination in academic dental clinics in the United Arab Emirates. They were identified by microarray hybridization which also allowed detection of PVL genes and assignment to a clonal complex within *S. argenteus*. Because of these results, both were sequenced using Oxford nanopore technology. This also demonstrated the presence of temperate/lysogenic phages in the staphylococcal genomes. Therefore, phages were induced by mitomycin C treatment, subjected to transmission electron microscopy and sequenced.

Results: Both isolates were assigned to *S. argenteus* ST2250. The presence of core genomic markers and genomic islands was in accordance with this assignment. They lacked SCC*mec* elements, but harboured a PVL prophage essentially identical to the published sequence of phiSa2wa_st78 (GenBank NC_055048), a PVL phage from an Australian *S. aureus* clonal complex 88 isolate. Beside the PVL prophage, one isolate carried another prophage. The second isolate carried two additional prophages, and the region between these two prophages was inverted. This "flipped" region comprised about 1,082,000 bp, or more than a third of the strain´s genome, and it included the PVL prophage.

Discussion: Temperate phages contribute to the virulence properties of their bacterial hosts, and here we describe a case in which PVL phages even crossed a species barrier transmitting PVL genes into a *S. argenteus* lineage. This observation highlights the need for a correct identification of *S. argenteus* under routine conditions. PVL-positive *S. argenteus* have previously been observed in Overseas France, Thailand, Myanmar and the US. It can be expected that any emerging pathogen will sooner or later also be identified in such a cosmopolitan setting as Dubai/UAE. Besides, we observed a one-megabase genomic inversion. Such a phenomenon has, to the best of our knowledge, been described only once, in a Russian CC8 MRSA strain (doi: 10.1371/journal.pone.0164168) where it was linked to a presence of IS256 sequences rather than to prophages.

MVV 096

New bacteriophages against Enterobacter cloacae

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Increasing antimicrobial resistance of bacteria poses a problem for human health. In 2017, the WHO published a list of 12 bacterial families that should be given special attention. Accordingly, the Enterobacterales, including *Enterobacter* (*E.*) *cloacae*, were ranked critical with the highest priority. *E. cloacae* belongs to the ESKAPE group of multidrug-resistant nosocomial pathogens and may cause pneumonia, wound, urinary tract, and blood stream infections, and also meningitis in neonates. In this project, six different *E. cloacae* bacteriophages were isolated from sewage water. Five *E. cloacae* bacteriophages were assigned to the Siphoviridae by transmission electron microscopy. In addition, one isolated bacteriophage belongs to the rare group of Podoviridae among the Enterobacter bacteriophages. The isolated bacteriophages displayed very short adsorption times of 5-15 minutes, latency times of 15-30 minutes, and high numbers of progeny phages, which is advantageous for therapeutic purposes. The host spectrum of the isolated bacteriophages against 54 different isolates of E. cloacae and E. cloacae complex was analyzed and spanned from 42 % to 68 % of the isolates. Different phages in a phage cocktail achieved the maximal effect: the mix was lytically active against 93 % of the E. cloacae isolates and 71 % of the tested E. cloacae complex strains. Moreover, the phages were tested for prevention of biofilm formation and reduction of pre-existing biofilms. Here, they were able to reduce biofilms by up to 99 %. The bacteriophages maintained their activity without significant loss over months when stored at 4 °C. These results suggest that the phages isolated may potentially serve as a therapeutic alternative for antibiotic-resistant infections.

MVV 097

Indication for a newly isolated bacteriophage genus with lytic activity against multidrug-resistant *E. coli*

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Introduction: Next generation sequencing approaches of recent years have shown a tremendous diversity of phages in nature, many of which have escaped isolation efforts so far. This study focused on the metagenome-assisted, targeted isolation and characterization of an *E. coli* phage ("Jab") which is only distantly related to known phages.

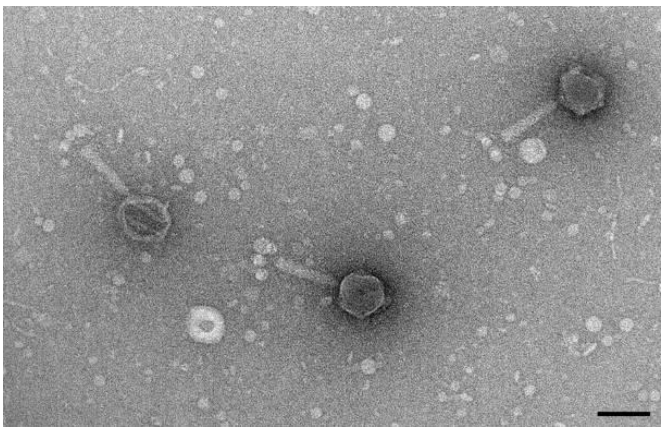
Methods: Phage Jab was initially identified by metagenome analysis in an enrichment from a slurry sample using a MDR *E. coli* strain as a host. Targeted isolation of phage Jab was then achieved by consecutive rounds of enrichment steps using an *E. coli* host previously made resistant against the more abundant phages. The complete genome sequence of phage Jab was obtained by combining Oxford Nanopore Technology long reads and short Illumina reads. Sequence similarity searches were conducted using Nucleotide BLAST searches against sequences in GenBank NCBI databases. Additionally, a genome map and a phylogenetic tree were constructed to determine the phage's evolutionary relationship to other known phages. Host range and temperature stability were evaluated, as well as phage morphology.

Results: Multiplying BLASTn query coverage by percent identity yielded less than 50%, (ICTV criterion) overall nucleotide similarity to other phage records. The best hits were 30.15%, and 5.63%, nucleotide identity with Escherichia phage UPEC06 (MW250786) and Pantoea phage vB ExiM F5M2A (OL744220), respectively, indicating that this phage may represent a novel genus. Phylogenetic tree reconstruction based on signature genes support the categorization of the phage into a single group. The genome of phage Jab consists of 142009 base pairs encoding a total of 249 protein coding genes and five tRNA genes. The majority of the 249 predicted protein coding genes encode hypothetical proteins without a known function, 71 proteins were annotated with putative functions.

The phage productively infected 30% of tested strains including MDR *E. coli*. Notably, transmission electron microscopy revealed unique spherical structures bound to the phages" tail fibers, after inoculation with some but not all bacterial strains (Fig 1).

Discussion: Utilizing uniform culture methods poses the danger of selecting genetically related phages that multiply best under prevailing laboratory conditions to the exclusion of novel, less characterized phages. The phenotypic and genome analysis of phage Jab provide valuable insights into its genetic and biological features and evolution. These findings present opportunities for further exploration of its potential for medical applications. Due to current taxonomic criteria phage Jab likely represents a new genus. We speculate, that the observed spherical structures attached to the phages tail fibers are vesicles, produced by infected bacteria as a defense strategy.

Fig. 1



MPV 098

HERC4 ubiquitin ligase attenuates oxidative stress-induced DNA damage upon bacterial and viral-bacterial infection

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Introduction: Ubiquitination is a post-translational modification that affects protein function, stability, and localization thereby balancing protein synthesis and degradation. During infection ubiquitination is crucial in regulating host cellular signalling pathways to support pathogen recognition, clearance and an efficient immune response. Respiratory infections caused by pathogens such as *Streptococcus pneumoniae* and influenza viruses represent significant threats to global health. Understanding the intricate molecular mechanisms underlying these infections is crucial for developing novel therapeutic strategies.

Method: Lung epithelial cells are the first line of defence in respiratory infections. Therefore, we analysed the ubiquitination system in the human alveolar epithelial cell line A549 and in human primary small airway epithelial cells (SAEC) upon infection with *Streptococcus pneumoniae* and Influenza A in single and viral-bacterial coinfections. Moreover, we monitored changes in the abundance of ubiquitinating enzymes on protein level via LC-MS and immunoblotting as well as on RNA level via qPCR. Detailed analysis of E3 ubiquitin ligase function during bacterial infection was performed by overexpression and CRISPR-Cas9 knockout.

Results: Analysis of host cell protein ubiquitination during *S. pneumoniae* infection revealed a significant reduction in the pool of polyubiquitinated proteins. We identified bacterial produced reactive oxygen species (ROS) as critical effectors interfering with ubiquitination. E3 ubiquitin ligases are responsible for the transfer of ubiquitin moieties to target proteins. Consistently, we observed a downregulation of several E3 ligases during infection, amongst them HERC4. By infecting A549 cells with the mutant strain *S. pneumoniae* Δ*spxB*, which is characterized by reduced ROS-production, we could confirm that the changes in HERC4 expression levels are mediated by ROS-release upon *S. pneumoniae* infection. HERC4 downregulation was even more pronounced in the viral-bacterial coinfection with influenza A virus (IAV) and was accompanied by an accumulation of phosphorylated histone H2A.X, a marker for DNA double-strand breaks. In contrast, overexpression of HERC4 prior *S. pneumoniae* infection resulted in reduced ROS-induced DNA damage and an increased histone-ubiquitination pointing to a role of HERC4 in DNA-damage-repair. Currently, ubiquitin ligase expression levels are analyzed in samples obtained from patients with *S. pneumoniae* or IAV single infection and viral-bacterial coinfection.

Discussion: In summary, we identified a mechanism, how *S. pneumoniae* interferes with the host ubiquitination system through secretion of ROS. Based on our results, we propose HERC4 as a potential marker for predicting the outcome of *S. pneumoniae* single and coinfection. Finally, tools for stabilizing HERC4 may serve to attenuate disease symptoms, in particular to dampen aggravation of IAV-infection by bacterial coinfection.

MPV 099

A general mechanism of the introduction of infection- and inflammation-induced genomic mutations

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Introduction: Some specific infections as well as chronic inflammatory processes are recognized drivers of oncogenic mutations and cancer. In some cases, bacterial or viral mutagens or oncogenes have been identified, such as the *E. coli* secondary metabolite colibactin and the proteins from human papillomavirus, E6/E7. It is conceivable that all oncogenic infectious agents possess their individual mutagenic factor. It is however also possible that a general mechanism operates in many or all cases of infection and inflammation, which would be host-derived and which would be activated in all these situations. The caspase-activated DNAse (CAD) is such a candidate. CAD is a nuclear DNAse, which is activated by sub-lethal activity of caspases in infections with all tested pathogens. CAD-activity generates DNA-single and double strand breaks in the genome.

Methods: We tested infectious agents with known oncogenic properties as well as inflammatory factors for CAD-activation and mutagenic activity. We further used a synthetic system where we can directly activate CAD in human cells, as well as pathogen-induced activation of CAD. We tested the hypothesis that CAD, despite being a non-specific DNAse, may introduce mutations that have a non-random pattern and that may correspond to mutational signatures known to occur in human cancer.

Results: TNF and IFN-b activated CAD and induced micronuclei, signs of chromosomal instability. IFN-b was able to introduce CAD-dependent genomic mutations. *Salmonella enterica*, a known oncogenic bacterium, also introduced CAD-dependent mutations, and HPV E6/E7 caused CAD-dependent signs of chromosomal instability. Repeated activation of CAD, either by direct activation or through infection with *Helicobacter pylori*, led to mutational patterns in human cell lines that were not random but that corresponded to the mutational signatures of some human cancer entities.

Conclusion: The data support the view that CAD is activated by infectious stimuli and by soluble factors commonly present in inflammation. CAD can introduce persistent genomic mutations that are not random but that conform to a characteristic mutational profile. The presence of this signature in human cancer genomes suggest that CAD-induced mutagenesis contributes to human cancer. CAD activity may be a factor that unites multiple situations of infection- and inflammation-linked oncogenicity.

MPV 100

Repurposing an anti-cancer drug for host-directed therapy of persistent staphylococcal infections

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Staphylococcus aureus is a dangerous Gram-positive pathogen that is associated with significant morbidity and mortality rates in the human population. Despite major advances in disease diagnostics and antibiotic therapy, infections caused by drug-resistant *S. aureus* remain a leading cause of death worldwide thereby requiring novel approaches against which staphylococci cannot develop resistance mechanisms. Here, we developed a unique host-directed therapeutic strategy to subvert a refined immuno-evasive maneuver of *S. aureus* by repurposing a clinical phase I and well-tolerated anti-cancer agent that blocks crucial elements of the mammalian nucleoside salvage pathway. Specifically, we demonstrate that oral administration of the anti-cancer drug at concentrations similar to those required to block tumor development in mice shields host immune cells from the cytotoxic effect of staphylococcal and host purine salvage pathway-targeting death-effector deoxyribonucleosides thereby providing significant protection from antibiotic-resistant *S. aureus* in a mouse model of bloodstream infection. Concurrently, we illustrate that the protective effect of the novel pathoblocker correlates with the accumulation of death-effector deoxyribonucleoside-resistant tissue macrophages within deep-seated abscesses, a phenomenon that promotes eradication of *S. aureus* in the infected host. Overall, our discoveries provide proof of concept that selected determinants of the mammalian nucleoside salvage pathway serve as druggable targets in the host to improve staphylococcal infection outcomes and public health.

MPV 101

Extracellular DNA plays a critical role in *Staphylococcus epidermidis* escape from human macrophages

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Background: Over the past decades, the human skin colonizing bacterium *Staphylococcus epidermidis* has been documented as a major cause of hospital-acquired infections. Despite *S. epidermidis* lacks dedicated programmes/factors for virulence and invasion it is the leading causative pathogen in central line associated blood stream infections and prosthetic joint infections, causing approximately 30% of these infections. Biofilm formation has been shown to be of key importance for immune evasion in this context.

Objectives: The main objectives of this study was to characterize the importance of specific biofilm matrix components for interactions between sessile *S. epidermidis* populations and primary human macrophages, and to unravel mechanisms supporting *S. epidermidis* evasion from phagocytic uptake.

Methods: Primary human macrophages isolated from buffy coats were infected with different *S. epidermidis* strains. Phagocytosis rates, macrophage polarization and TLR involvement were analyzed by confocal laser scanning microscopy, FACS and qPCR analysis. Furthermore transcription and protein levels of Il-1ß, TNF-a, Il-6 and Il-10 were assessed and a RNAseq experiment was carried out.

Results: The ability of professional phagocytes to uptake sessile *S. epidermidis* consortia is modulated by the biofilm matrix composition, with PIA and eDNA as key players. It could be shown that eDNA present in *S. epidermidis* biofilms can alter the macrophage polarization and pro- inflammatory activation via TLR9 recognition. After *S. epidermidis* infection TLR-mediated DNA sensing is critical for macrophage reprogramming and specific upregulation of anti-viral immune pathways.

MPV 102

Autophagy controls *Salmonella* infection via $p38^{MAPK}$/MK2 signaling pathway

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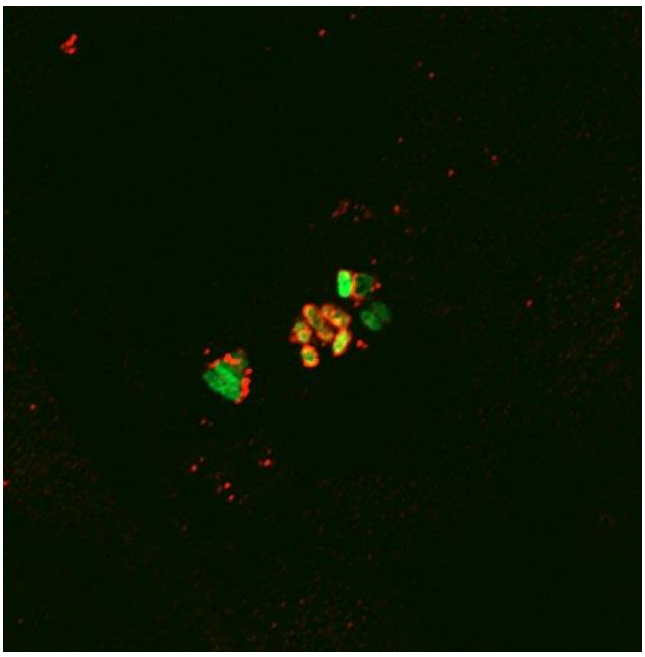
Salmonella is still a major cause of bacterial enteric illness in humans and animals throughout the world. Some antimicrobial resistant serotypes have emerged which affecting the food chain and global public health concern. Salmonellosis mostly has mild symptoms but sometimes can be life threatening depends on host factors and the serotype of *Salmonella*. Therefore, understanding the mechanisms how host defense system eliminate *Salmonella* is important in preventing and alleviating the burden of *Salmonella* infections. In this study, we investigated the role of p38MAPK/MK2 in modulating the host cell susceptibility to *Salmonella* infection. Inhibition of p38MAPK using BIRB796 or MK2 using PF-3644022 led to a significant increase of bacterial counts in *Salmonella* infected mouse embryonic fibroblasts (MEFs). We observed similar effect in MK2-deficient (MK2-/-) cells and cells harboring a kinase dead MK2-K79R mutant (MK2KR). Interestingly, western blot analysis showed that MK2-/- cells have lower level of LC3 lipidation, which is the indicator of general autophagy; lower activated TANK-binding kinase-1 (TBK1) phosphorylation on Ser172; and p62/SQTM1-Ser403 phosphorylation, which are important to promote the translocation of p62 to ubiquitinated microbes and required for efficient autophagy of bacteria, compared to MK2-rescued cells. Immunofluorescence analysis also revealed reduced co-localization of *Salmonella* with LC3 and p62 in MK2-/- MEFs. In addition, inhibition of autophagy with bafilomycin A1 (BafA1) showed increased bacterial counts in treated cells in comparison to DMSO-treated control cell. Furthermore, *Salmonella* effector *avrA* mutant showed similar bacterial counts in MK2-rescued and MK2-/- cells. Overall, these results indicate that p38MAPK/MK2-mediated protein phosphorylation modulates the host cell susceptibility to *Salmonella* infection by affecting the autophagy pathways.

Fig. 1



MPV 103

DNA hypomethylation mediates the immune response to urinary tract infection by uropathogenic *Escherichia coli*

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Introduction: Urinary tract infections (UTIs) are predominantly caused by uropathogenic *Escherichia coli* (UPEC) that can initially colonize and persist in the uroepithelial cells forming intracellular bacterial communities. UPEC can downregulate multiple innate immunity-related genes encoding cytokines and antimicrobial peptides (AMPs) following pathogen recognition by Toll-like receptors. Exactly, how UPEC can remodel host immune responses that determine disease susceptibility is poorly understood. Epigenetic mechanisms like DNA methylation play a pivotal role in the regulation of host gene expression in response to environmental stimuli independent of changes in the DNA sequence. We hypothesize that UPEC infection can change pre-existing genome-wide methylation marks to alter gene expression in the urinary tract during UTI. We used a mouse cystitis model to establish causal links between DNA methylation and gene expression including proof-of-concept evidence for a link between hypo-/hypermethylation and expression of immune gene in UPEC-infected urinary bladder.

Methods: Female C57BL/6JRj mice were infected with the UPEC strain CFT073 via transurethral catheterization, and genomic DNA and total RNA were isolated from the mouse urinary bladders after aseptic dissection. DNA methylation was examined in UPEC-infected and uninfected mice using the Illumina CpG Infinium Mouse Array which enables single-nucleotide resolution interrogation of over 285,000 methylation sites per sample. Gene expression in UPEC-infected and uninfected mice was analyzed by transcriptome sequencing (Illumina). The differential methylation of CpG sites and differential transcriptome analysis were performed using R.

Results: CpG Infinium Array and mRNA sequencing techniques allowed us to identify over 5000 differentially CpG methylated genes spread across the 22 autosomes, but only about 500 differentially expressed genes in CFT073-infected mice. We also found that differential CpG methylation is associated with only 148 out of the 500 differentially expressed genes, while 397 genes were expressed independent of CpG methylation. We observed upregulation of selected immunity-related genes related to bacterial recognition, cell signaling, apoptosis, and inflammation were associated with DNA hypomethylation at the promoter regions. On the other hand, the downregulation of gene related to tissue and muscle development in CFT073-infected mice were associated with DNA hypermethylation at the promoter regions.

Conclusions: The possibility that human pathogenic bacteria can induce methylation (hypermethylation) and demethylation (hypomethylation) of genes in mammals has been a matter of debate for decades. We provide compelling evidence that UPEC infection is accompanied by rapid changes in the genome-wide DNA methylation in mice, which determines the upregulation of selected immunity genes due to hypomethylation at the promoter regions.

GIPWV 104

Serotype-specific mutation in the virulence factor EspL identifies amino acid residue essential for EPEC-mediated RHIM protein cleavage

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Enteropathogenic *Escherichia coli* (EPEC) are Gram-negative, non-invasive gastrointestinal pathogens that cause severe watery diarrhoea in infants. EPEC use a Type III secretion system (T3SS) to translocate effector proteins into the host cell cytosol to subvert host signalling pathways. The effector protein EspL is a cysteine protease, which cleaves the host proteins RIPK1, RIPK3, and TRIF in their conserved RHIM domain, thereby inhibiting proinflammatory and cell death signalling. EspL is predicted to consist of two domains: an N-terminal protease domain with high secondary structure homology to the cysteine protease effector YopT of *Yersinia* and a C-terminal ankyrin-repeat-like domain.

In an attempt to identify regions of EspL essential target recognition, we used C-terminal deletion constructs of EspL and determined that the last 150 amino acids were not required for recognition/ cleavage of its targets RIPK1 or RIPK3, while the deletion of additional amino acids impaired their cleavage. Furthermore, random transposon insertion mutagenesis suggested that the protein structure was important for target cleavage, irrespective of the amino acid sequence. By screening a collection of clinical EPEC isolates, which adhered to target cells and formed functional T3SSs, for their ability to cleave RHIM proteins, we identified several isolates which were unable to cleave RIPK1 and RIPK3. Interestingly, these isolates belonged to the same EPEC serotype, O55:H6, and encoded a single, conserved point mutation within the protease domain of EspL. Further experiments will have to elucidate both, the regions involved in target recognition, as well as the functional consequences of the identified point mutation.

GIPWV 105

Innate activation of human neutrophils and neutrophil-like cells by the proinflammatory bacterial metabolite ADP-heptose and *Helicobacter pylori*

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Lipopolysaccharide inner core heptose metabolites, including ADP-heptose, play a substantial role in the activation of cell-autonomous innate immune responses in eukaryotic cells, via the ALPK1-TIFA signaling pathway, as demonstrated for various pathogenic bacteria [1,2]. The important role of LPS heptose

metabolites during *Helicobacter pylori* infection of the human gastric niche has been demonstrated for gastric epithelial cells and macrophages [3,4], while the role of heptose metabolites on human neutrophils has not been investigated. In this study, we aimed to gain a better understanding of the activation potential of bacterial heptose metabolites for human neutrophil cells. To do so, we used pure ADP-heptose and, as a bacterial model, *H. pylori*, which can transport heptose metabolites into the human host cell via the CagT4SS. Main questions were how bacterial heptose metabolites impact on the proinflammatory activation, alone and in the bacterial context, and how they influence maturation of human neutrophils. Results of the present study demonstrated that neutrophils respond with high sensitivity to pure heptose metabolites, and that global regulation networks and neutrophil maturation are influenced by heptose exposure. Furthermore, activation of human neutrophils by live *H. pylori* is strongly impacted by the presence of LPS heptose metabolites and the functionality of its CagT4SS. Similar activities were determined in cell culture neutrophils of different maturation states and in human primary neutrophils. In conclusion, we demonstrated that specific heptose metabolites or bacteria producing heptoses exhibit a strong activity on cell-autonomous innate responses of human neutrophils.

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GIPWV 106

High copper supplementation in piglets as a habitat filtering factor aiding colonization resistance of post-weaning microbiome

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Introduction: Copper sulphate is increasingly used as a feed additive in the pig industry since the EU-wide ban on the use of antibiotics as growth promoters. It is considered an alternative to antibiotics, as it decreases the incidence of post-weaning diarrhoea and increases the rate of feed conversion. The antimicrobial activity of copper acts as an intestinal habitat filter affecting the gut microbiota and even pathogens. We investigated the effect of high copper supplemented pig feed on the development of microbiome and metabolite production in 4–6-week-old piglets, and copper resistance in Enterobacterales isolated from pork.

Methods: Four-week-old piglets (60) were separated into two groups and fed on diet with high (therapeutic) or low (nutritional) levels of copper sulphate. Faecal samples were collected immediately prior to and on day 7 and 14 following placing piglets on low or high copper diet. Metagenome sequence of total faecal DNA was determined using short read shotgun sequencing and metabolite levels were determined by NMR. Metagenomic analyses were performed with MATAFILER pipeline. Microbiota were isolated from piglets on high and low copper diet using high throughput culturomics methods and sequenced. Analysis of copper resistance genes prevalence in pig microbiota and Enterobacterales from pork meat was performed with BacMet2.

Results: Differences between high and low copper diet were observed for the genera *Bifidobacterium*, *Clostridium*, *Escherichia*, *Holdemanella*, *Lactobacillus* and *Succinatimonas*. A high copper diet affected faecal concentrations of formate, succinate and branched chain amino acids (BCAA), which are important metabolites for intestinal colonization by multiple pathogens. Altered abundance of genes responsible for copper homeostasis, formate and BCAA metabolism were observed in the gut metagenome of pigs fed a high copper diet. 70 microbial species (including 15 new species) were isolated from piglets on high and low copper diet. Decrease in abundance of selected microbiota was associated with lower amount of copper resistance/homeostasis genes. Copper resistant Enterobacterales were identified in pork.

Discussion: Our data indicate significant influence of high copper supplementation as habitat filtering factor on piglet intestinal microbiota and their function. Widespread use of copper may have led to development of copper-resistant foodborne pathogens and decreased effectiveness of copper in the diet of piglets.

GIPWV 107

Polysaccharides as promising targets for vaccination against Clostridioides (C.) difficile infection

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Clostridioides (C.) difficile, which colonize the gut, replicate and produce colitis-mediating enterotoxins once the commensal microflora is disrupted frequently cause antibiotic-associated diarrhea (AAD). Although there is an increasing need to control the spread of this worldwide emerging infection, different vaccination strategies have failed so far to protect against C. difficile-induced AAD. Furthermore, these vaccines did not prevent the colonization of the intestinal mucosa with C. difficile. Thus, novel vaccination targets and strategies are urgently required.

The cell-surface polysaccharides PS-I, PS-II and lipoteichoic acid (LTA) are expressed by different C. difficile strains. In contrast to their enterotoxins, C. difficile are not able to switch the structure of these polysaccharides. Thus, as antibodies against these polysaccharides might inhibit the colonization of the intestinal mucosa by C. difficile, we assessed the suitability of PS-I, PS-II and LTA as biomarkers and vaccines in patients and in pre-clinical models. Thus, we first screened sera and stool samples from patients using glycan microarrays. We did not only detect antibodies against all three polysaccharides, but also an inverse correlation between antibody titers and the severity of AAD. While patients with severe or prolonged colitis had significantly lower antibody titers, patients with milder symptoms exhibited higher IgA and IgG levels against PS-I, PS-II and LTA. Some patients with low or undetectable anti-PS I and anti-PS II or anti-LTA antibody titers even suffered from recurrent infection, but none of the patients with high antibody titers. Based on our observation that IgA and IgG responses to PS-I, PS-II and LTA correlate with clinical outcomes in patients with C. difficile infections, conjugated synthetic oligosaccharide antigens derived from all three polysaccharides to CRM197, a carrier protein already used in

licensed conjugate vaccines against Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae. All semisynthetic glycoconjugates induced IgG antibodies against the respective glycan antigen. Mice vaccinated with either one of these glycoconjugates exhibited significantly less epithelial exfoliation and alterations in the intestinal architecture, edema and hemorrhage in the lamina propria, and a reduced immune cell infiltration of the epithelial layer compared to control mice. Correlating with the reduced extent of intestinal damage, we recovered also significantly less C. difficile CFUs from the intestines of glycoconjugate-vaccinated mice than from controls.

Thus, our data suggest systemic and mucosal IgA and IgG antibodies against PS-I, PS-II and LTA as clinical markers for the severity of disease in patients. As the vaccination with PS I-, PS II- and LTA- glycoconjugates protected infected mice from C. difficile-induced colitis, all three polysaccharides represent promising antigenic targets for vaccination in humans.

GIPWV 108

Development of anti-virulence agents targeting HilD, the central regulator of *Salmonella* pathogenicity

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Salmonellosis is a major food-borne illness causing approximately 153 million cases and 300,000 deaths per year. The key virulence mechanism enabling non-typhoidal *Salmonella* to cause systemic infection is its ability to invade and propagate withinthe intestinal epithelium, thus increasing the risk of life-threatening bloodstream infections. The invasion-related pathogenicity of non-typhoidal *Salmonella* is mediated by several secretion systems which are mainly under control of the positive transcriptional regulator HilD. A *hilD*-deficient *Salmonella* strain is unable to express these pathogenicity factors and is therefore avirulent.

Here we report advances in the development of a series of synthetic small molecules targeting HilD at low µM scale, subsequently blocking the invasion of *Salmonella* into human host cells. Through biochemical, biophysical, and cell-based approaches, we provide evidence on the mode of action of the compounds and their binding mode to the target HilD. Based on the structural characterization of the binding pocket and 2 rounds of structure-activity relationship analysis, structural analogues were rationally designed, among which optimized compounds with improved activity were found. Pharmacological and mouse toxicity analyses of the selected pre-lead compound demonstrated its safety and suitability for oral administration. Finally, we provide an *in vivo* proof-of-concept using a mouse model of *Salmonella* gastro-intestinal infection.

HilD inhibitors will be designed as standalone drugs to reduce the risk of an invasive infection in patients developing an enteric *Salmonella* infection, and to shorten hospitalization time while avoiding the use of direct-acting antibiotics. A combination therapy with standard-of-care antibiotics could also be considered for the treatment of invasive and antibiotic-resistant *Salmonella* infections.

RKV 109

Report of the national reference centre for multidrug-resistant Gram-negative bacteria on Carbapenemases in Germany in 2022

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Background: 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 within the next years. Among all resistance mechanisms the spread of carbapenemases is the most worrisome. However, the correct identification of carbapenemases is still challenging and molecular epidemiology of carbapenemases is required.

Materials/methods: The German 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 7.639 isolates were investigated for carbapenemases at the National Reference Centre in 2022. Carbapenemases were found in 2796 *Enterobacterales* strains, 527 of *P. aeruginosa* and 507 of *A. baumannii*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 717), NDM-1 (n = 610), VIM-1 (n = 338), KPC-2 (n = 328), NDM-5 (n = 278), OXA-244 (n = 180), OXA-181 (n = 173) and KPC-3 (n = 169) which were also found in various combinations, e.g. NDM-1/OXA-48 (n = 103). Other enzymes like OXA-232, OXA-162 or GIM-1 were found in less than 60 isolates each. In *P. aeruginosa*, VIM-2 was the most frequent carbapenemase (n = 285), followed by NDM-1 (n = 69), IMP-1 (n = 43) and GIM-1 (n = 37). Other enzymes like VIM-1 or IMP-13 were found in less than 10 isolates each. OXA-23 was again the most frequent carbapenemase in *A. baumannii* (n = 285), followed by OXA-72 (n = 180). VIM-4, GES-11, OXA-58 and others were found in less than 20 isolates each.

Conclusions: A variety of different carbapenemases is detected in Germany. The molecular epidemiology in Germany still significantly differs from observations made in other countries like Greece, Italy or the USA with a predominance of OXA-48 and VIM-1 in *Enterobacterales*. Compared to previous years, the number of OXA-48 and NDM-1 detections increased significantly since march 2022. Furthermore, the observations of NDM-5 also increased.

RKV 110

Epidemiology of *Candida auris* in Germany

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Introduction: *Candida auris* is an emerging resistant fungal pathogen with the ability to spread from patient to patient and cause nosocomial outbreaks. The COVID-19 pandemic has resulted in additional amplification of *C. auris* global spread. Several countries currently report autochthonous spreading of C. auris.

Methods: The NRZMyk is closely monitoring introductions and spreading of *C. auris* in Germany. All isolates are characterized genetically and phenotypically for antifungal drug resistance and underlying mutations. Genetic relation of isolates is analysed based on whole genome sequencing to detect patient-to-patient transmission events.

Results: Initial *C. auris* cases were linked to import of the pathogen with patients transferred into German hospitals from foreign healthcare institutions (1). While between 2015 and 2020 no more than 6 cases per year were reported to the NRZMyk, but this number increased to 12 in 2021/22. More than 50% of isolations represent colonization. The majority of isolates belonged to clade I and more than 80% are resistant to fluconazole. One primary isolate was also resistant to echinocandins and harboured a corresponding mutation of the target gene FKS. Database analyses of NRZMyk documented cases and the ARS database at the RKI show, that currently case documentation is incomplete (2). In addition, data suggest likely transmission events in Germany in addition to transmission events documented by the NRZMyk (3). Genotyping of C. auris for outbreak documentation is demanding and requires whole genome sequencing for reliable typing. The analysis of ten serial isolates from four patients of a transmission event in a Germany tertiary care revealed relevant phenotype variation even among closely related isolates.

Conclusion: The occurence of *C. auris* in Germany must be monitored closely to slow down further spread. To enable rapid action, recommendations for management of individual *C. auris* cases or potential transmission events have been published by the NRZMyk together with the NRZ for Surveillance of Nosocomial Infections (4).

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(4) Hinrichs et al., Successful control of Candida auris transmission in a German COVID-19 intensive care unit. Mycoses. 2022;65(6):643-649

RKV 111

The new consultant laboratory for human pathogenic vibrios

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Vibrionaceae comprise a large number of different species, twelve of which are known to be pathogenic to humans, including the causative agent of cholera, *Vibrio* (*V.*) *cholerae*. Cholera is caused by cholera toxin-producing *V. cholerae* of serogroups O:1 and O:139. *V. cholerae* of all other serogroups, as well as other human pathogenic species of the genus *Vibrio*, are also called non-cholera vibrios (NCV). These occur in saline waters of Germany and Europe and are becoming increasingly important as causative agents of wound infections. In Germany, infections with NCV occur more frequently during the summer months in waters with temperatures above 20 °C. The Baltic Sea is particularly affected, but also slightly saline inland waters. Starting from wounds or injuries of the skin barrier, invasive, transboundary infections may develop, which require obligatory surgical treatment. Occasionally, infections with *V. vulnificus* are even fatal in immunocompromised individuals. Eight deaths from NCV infections occurred nationwide in the years 2018 and 2019, which were characterized by long periods of hot summer weather (Brehm, 2021). Since there was no mandatory reporting of NCV in Germany until March 2020, a high number of unreported infections in recent years must be assumed. There has also been no comprehensive NCV-specific environmental monitoring to date, so that the actual risk posed by NCV to public health can currently only be inadequately assessed. Here, the newly appointed consultant laboratory for human pathogenic vibrios is presented: in the context of previous projects on vibrios and in the course of the current activities, the occurrence of NCV in the environment, as well as the characterization of virulence factors of clinical isolates in comparison to environmental isolates, will be investigated. An investigation of water and sediment samples from the Baltic Sea area in and around the area of Warnemünde in summer 2022 could thus show the presence of a variety of different NCV during the summer months at different locations. Over time, there was evidence that the composition of different *Vibrio* species varied depending on environmental factors such as temperature and salinity of the water. The isolates obtained during the studies were first identified as NCV by phenotypic analyses and species identification by sequencing of the *rpoB* gene, followed by whole-genome sequencing. The analyses of the sequences obtained are currently ongoing, but will provide valuable information on NCV naturally occurring in Germany.

Brehm TT, Berneking L, Sena Martins M, Dupke S, Jacob D, Drechsel O, Bohnert J, Becker K, Kramer A, Christner M, Aepfelbacher M, Schmiedel S, Rohde H; German Vibrio Study Group. Heatwave-associated *Vibrio* infections in Germany, 2018 and 2019. Euro Surveill. 2021 Oct;26(41):2002041

RKV 112

Genomic comparison of invasive non-typeable *Haemophilus influenzae* (NTHi) isolated in Germany ten years apart

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Background: The incidence of non-typeable Hi infections (NTHi) increased in countries with a Hib vaccine program, whereas the incidence of *H. influenzae* serotype b (Hib) infections was reduced drastically. Invasive NTHi infections are considered sporadic events. However, cases of transmission have been reported.

Aim/Methods: The aim of the study was to determine whether the population of invasive NTHi has changed during the last decade. Thereby, the Federal State of Baden-Wuerttemberg served as a model, since it shows highest incidence rates in Germany. We analyzed (I) if there were clonal clusters among usually genetic diverse NTHi; (II) profiles of surface and adherence associated virulence factors of invasive NTHI in comparison to carriage isolates; (III) the susceptibility to selected antibiotics.

Whole genome sequencing and phenotypic antibiotic susceptibility testing were performed on invasive isolates as well as carriage isolates from an earlier study for comparative genetic analysis. Genome sequences were analyzed by a previously developed cgMLST scheme. Simpson"s diversity index from sequence types (ST) was calculated. Phenotypic antibiotic resistance was correlated with genetic data.

Results: Whole genome sequences of 220 invasive NTHi isolates (50 from 2010/11 and 169 from 2019-21) were analyzed. In total, 99 STs were identified, of which 23 occurred in both time periods The most frequent STs were ST12 (n=18), ST103 (n=14), ST3 (n=7), ST145 (n=7), and ST203 (n=6). Other STs counted five or

less isolates. Ten cgMLST clusters were identified, the largest consisted of six isolates (ST103). Spatiotemporal analyses did not point at an epidemiologic link between any of the cases. Simpson diversity index showed no significant reduction of genetic diversity from 2010/11 (0.989; 95% CI [0,982-0,997]) to 2019-21 (0.977; 95% CI [0,954-0,985]).

About 23% of the isolates were ampicillin resistant, 15% were BLPAR (6% of them BLPACR) and 8% BLNAR. Cefotaxime and rifampicin resistance rates were low (<1%).

Conclusion: No reduction of genetic diversity was found in 2019-21 compared to 2010/11 in invasive NTHi from Baden-Württemberg. Transmission events could not be detected epidemiologically for any of the cases that belonged to genetic clusters.

Our results show that the significant increase of invasive NTHi infections in the years prior to the COVID19-pandemic has not led to increased risk of person-to-person transmission. NTHi infections continue to be sporadic events. Further surveillance of *H. influenzae* is important to monitor the substantial changes in the epidemiology of the disease under the influence of the COVID19-pandemic.

RKV 113

Genomic surveillance of invasive *Enterococcus faecium* isolates in Germany - a longitudinal study, 2011-2022

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Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) are increasingly detected among invasive isolates in Germany (1.4% in 2001 – 21.6% in 2021). The National Reference Centre (NRC) for Staphylococci and Enterococci at the Robert Koch Institute aims to infer trends for clonal linages of invasive *E. faecium* isolates on the basis of multilocus sequence typing (MLST) since 2011 and by means of whole genome sequencing (WGS) and corresponding core genome MLST (cgMLST) types since 2015.

Methods: We included all invasive *E. faecium* isolates sent to the NRC on a voluntary basis between 2011-2022. Invasive isolates are defined as blood culture or venous-catheter isolates. We performed antimicrobial susceptibility testing by broth microdilution and determined the vancomycin resistance genotype by PCR. We further prepared genomic libraries using the Nextera XT library preparation kit and performed WGS by means of NextSeq Illumina® sequencing technology. MLST and cgMLST types were inferred using SeqSphere+ (Ridom GmbH, Germany).

Results: Since the beginning of the survey, we observed that one sequence type (ST), ST117, has dominated (>65%, 2019). Since 2017, there has been a steady increase in ST80 isolates. Applying cgMLST typing, we detected ST117/CT71 that has been particularly dominant for many years (44%, 2019). The reasons for this overrepresentation are not yet understood. Recently, we observed an increasing trend of ST117/CT929 (32%, 2022), ST80/CT1470 and ST117/CT2505 isolates and a decline of ST117/CT71 (15%, 2022). Further, we noted a drop in *vanB*-positive VREfm (79%, 2019; 53%, 2022) and the emergence of *vanA*-type VREfm (21%, 2019; 46%, 2022). This shift very well corresponds with observed strain dynamics, e.g. decrease of solely *vanB*-type ST117/CT71 and increase of mostly *vanA*-type ST117/CT929. Our data also shows that certain clonal lineages associated with the *vanB* genotype in VREfm are not found in vancomycin-susceptible *E. faecium* (VSEfm), whereas lineages with potentially *vanA*-types are found in both, the VSEfm and VREfm populations.

Conclusion: Our longitudinal study demonstrates that the population structure of invasive VSEfm and VREfm obtained from German hospital patients are highly dynamic. What causes the rise and fall of certain VREfm clonal lineages and whether this is due to a recent VSEfm population promiscuous for *vanA*-carrying plasmids is part of ongoing investigations.

RKV 114

Insights from three years of integrated molecular surveillance of *Campylobacteriosis*

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Question: Campylobacteriosis has the highest incidence per 100,000 population among foodborne bacterial zoonoses in Germany. Thus, integrated molecular surveillance (IMS) of this disease is of central importance as part of a containment strategy. Since 2020, such surveillance has been gradually established and provides valuable information regarding the spread of dominant lineages of clinical *Campylobacter* isolates as well as the prevalence of antibiotic resistance. These data are evaluated in the context of corresponding data derived from epidemiological surveillance as well as food and farm monitoring.

Methods: *Campylobacter* spp. were cultivated under microaerophilic conditions on CCDA and species determination was carried out by PCR. Phenotypic antimicrobial resistance (AMR) testing was performed using broth microdilution according to EUCAST. Whole genome sequencing was performed with Illumina NextSeq, yielding paired end sequence reads. Cluster Analysis was achieved with the software Ridom SeqSphere+.

Results: Molecular data from more than 3400 *C. jejuni* isolates within a period of January 2020-December 2022 were evaluated. An analysis of the *C. jejuni* population shows that more than 240 different sequence types circulate in Germany. More than half of all isolates occur in a cluster, i.e. there is at least one other closely related isolate. The most common sequence types associated with large clusters are ST-19, ST-7355, ST-6175, ST-50, and ST-49. Together, they make up approximately 16% of the population. Notably, ST-7355 and ST-19 are clusters with matching isolates from food, animal and environmental samples. Nonetheless, identification of a common source is difficult due to the wide-spread nature of the cases within a cluster. In addition, chicken meat is the most likely source, which due to its very frequent consumption, offers little way to distinguish between related and unrelated cases. Apart from detection of potential outbreak clusters and dominant lineages an important part of our strategy is AMR surveillance. Our data show that almost 70% of *C. jejuni* clinical strains are resistant to ciprofloxacin. Moreover, we detected the transmissible methyltransferase gene *erm(B)* in clinical isolates, which confers resistance to macrolides, the main treatment option for campylobacteriosis. Although *erm(B)* is still rare in German *C. jejuni* and *C. coli* clinical isolates its presence increases the risk of rising macrolide resistance in these populations.

Conclusion: IMS identified dominant lineages of *C. jejuni* shared between clinical and food/animal isolates. This information could serve as a basis for targeting recurring lineages at the farm level. As our surveillance strategy focused on nationwide representation, it enabled first-time detection of the low-prevalence *erm(B)* gene in the clinical *Campylobacter* population. Since the *erm(B)* gene is easily transmitted this finding requires close monitoring.

ZOV 115

Impact of M-like Protein in phenotype switching in *Streptococcus equi* ssp. *zooepidemicus*

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Streptococcus equi ssp. *zooepidemicus* (*S. zooepidemicus*) is a zoonotic pathogen that colonises as commensal the equine tonsils and can also cause respiratory tract infections in horses. It is also associated with endocarditis, meningitis and severe systemic diseases in humans. When *S. zooepidemicus* strain MF1397 was cultivated on solid media, colonies showed apparent differences in colony morphology and appeared both, mucoid and non-mucoid. Interestingly, the non-mucoid morphology can change to a mucoid morphology upon further cultivation.

With the aim of elucidating the molecular mechanisms and possible infection-biological consequences of this culture heterogeneity, the first step was to determine the frequency of occurrence of the colony morphologies on solid media.

The electron microscopic analyses of the bacterial surface structures revealed structural similarities to the family of streptococcal M- and M-like proteins. This family of surface proteins has already been characterised as important virulence factors in some streptococcal species such as *S. pyogenes*. Among others, they assume important functions for bacterial colonisation, interaction with the several host components, and possess anti-phagocytic properties.

According to genome sequencing data, several M- and M-like proteins are encoded in the genome of *S. zooepidemicus*, of which SzM has already been identified as an M-like fibrinogen-binding protein. Following whole-genome sequencing of both morphotypes, a genome comparison also indicates a frame shift within the gene sequence for an M-like protein, leading to transcriptional termination.

In addition, results of comparative analysis of both morphotype proteomes revealed differences in the amount of M-like protein. Moreover, functional cell culture infection studies on the adherence of both morphotypes to human primary endothelial cells indicate that non-mucoid strains adhere more efficiently to host cells than mucoid strains.

In sum, our data provide first evidence for the contribution of the surface appearance of M-like proteins of *S. zooepidemicus* with the observed culture heterogeneity and assume significant implications for the bacterial adherence to host cells.

Thus, the occurrence of different morphotypes could directly play a crucial role in the efficiency of *S. zooepidemicus* colonisation in the host environment.

ZOV 116

Less pronounced immunopathological responses following oral butyrate treatment of *campylobacter jejuni* infected mice

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Introduction: Given that human *Campylobacter jejuni* infections are rising globally and antibiotic treatment is not recommended, infected patients would substantially benefit from alternative therapeutic strategies. Short-chain fatty acids such as butyrate are known for their health benefits, including anti-microbial and anti-inflammatory effects. This prompted us to investigate potential disease-alleviating properties of butyrate treatment during acute murine *C. jejuni*-induced enterocolitis.

Methods: Therefore, following gut microbiota depletion IL-$10^{-/-}$ mice were challenged with 10^9 viable *C. jejuni* cells by oral gavage and treated with butyrate via the drinking water (22 g/L) starting on day 2 post-infection.

Results: As early as day 3 post-infection, butyrate reduced diarrheal severity and frequency in treated mice, whereas on day 6 post-infection, gastrointestinal *C. jejuni* burdens and the overall clinical outcomes were comparable in butyrate- and placebo-treated cohorts. Most importantly, butyrate treatment dampened intestinal pro-inflammatory immune responses given lower colonic numbers of apoptotic cells and neutrophils, less distinct TNF-α secretion in mesenteric lymph nodes and lower IL-6 and MCP-1 concentrations in the ileum on day 6 post-infection.

Discussion/Conclusion: Results of our preclinical intervention study provide evidence that butyrate represents a promising candidate molecule for the treatment of acute campylobacteriosis.

ZOV 117

Virus-induced premature cellular senescence of the lung

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Objective: Respiratory infections of the lung are causing mild to severe diseases with organ damage and potentially long-term effects [1]. In this study, we investigated the role of influenza virus and SARS-CoV-2 infections in the establishment of premature cellular senescence of the lung.

Methods: A murine model of influenza virus pneumonia presented the appearance of senescent cells in the lungs after 21 d of infection. To investigate intracellular signaling, we established a human and murine *ex vivo* lung slice model. Findings of infections were confirmed via RNA sequencing. Additionally, we analyzed the lungs of delta and omicron infected COVID-19 patients and confirmed findings in primary lung cells via transcriptomic.

Results: We observed in our murine model a premature cellular senescence of the lung after 21 d of influenza virus infection. Here, a macrophage-associated upregulation of TNF-α, acting in a paracrine manner to induce cellular senescence in human lung fibroblasts, was detectable.

Furthermore, senescent cells in the lungs strongly influenced subsequent viral infections. Our data demonstrated a higher viral load in senescent primary lung fibroblasts, indicating an intracellular effect on viral replication. Hence, an increased

regulation of JAK/STAT signaling in senescent IAV-infected cells was detected.

Interestingly, solely omicron variants of SARS-CoV-2 influenced the expression of cell cycle genes, highlighted by an increased p21 expression in human primary lung cells and in lungs of deceased COVID-19 patients.

Conclusion: Our study provides new insights into pathomechanisms of virus-induced cellular senescence. Hence, IAV infection induces premature senescence and subsequent infections in senescent cells lead to an increased viral replication [2]. For SARS-CoV-2, the endosomal clathrin-mediated entry of omicron-variants appears to have an influence on cellular senescence. A substantially different cellular response with an upregulation of cell cycle, inflammation- and integrin-associated pathways in omicron infected cells indicates premature cellular senescence.

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ZOV 118

Induction and persistence of *Mycobacterium avium* in myeloid derived suppressor cells

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Introduction: *Mycobacterium* (*M.*) *avium* belongs to the non-tuberculous mycobacteria that comprises three major subspecies, *M. avium* subsp. *avium* (MAA), *M. avium* subsp. *hominissuis* (MAH) and *M. avium* subsp. *paratuberculosis* (MAP). *M. avium* subspecies cause chronic infections and granuloma formation in mouse infection models. We have previously shown that intraperitoneal infection of mice with MAA, but not MAH or MAP, caused severe disease due to immune suppression caused by massive splenic infiltration of monocytic (M-) myeloid derived suppressor cells (MDSC), expressing inducible nitric oxide (NO) synthase. The mechanism by which MAA induces NO-producing M-MDSC and the survival and growth of MAA in the NO-rich environment is unknown. In the present study, we determined whether the infection dose of MAA influences MDSC induction.

Materials and methods: Female C57BL/6J mice were infected intraperitoneally with MAA44156 at an infective dose of $\sim 10^7$ and $\sim 10^8$ colony-forming units (CFU) per mouse. Infected mice were monitored for 4 weeks and body weight change was measured. Mice were sacrificed on 29 day post-infection; spleen and liver were collected for bacterial plating, histopathology and flow cytometry analysis.

Results: Mice infected with 10^7 CFU (10^7 group) quickly regained body weight after an initial weight loss and overall did not show any clinical sign compared to mice infected with 10^8 CFU (10^8 group). Splenomegaly was observed in both groups with significantly lower bacterial recovery rate in 10^7 group. Hematoxylin/Eosin staining of spleen showed defined granuloma in the 10^7 group while diffused inflammation dominated by histiocytes and loss of defined lymphoid follicles were observed in the 10^8 group. Defined granuloma harboring mononuclear cells and peripheral lymphocytes were found in the liver of 10^7 group mice compared to more diffuse granuloma with higher numbers of mononuclear cells and low numbers of lymphocytes in the 10^8 group. Ziehl-Neelsen staining identified lower acid-fast bacteria loads in spleen and liver of 10^7 group mice. Flow cytometry analysis of splenic myeloid cells population showed infiltration of $CD11b^{hi}CD11c^{int}$ myeloid cells in both groups. In the 10^7 group, the numbers of $CD11b^{hi}CD11c^{int}$ cells were significantly lower suggesting the levels of MDSC in the spleen of MAA infected mice depends on the infection doses. The majority of $CD11b^{hi}CD11c^{int}$ cells were found to be M-MDSC ($CD11b^{hi}CD11c^{int}Ly\text{-}6G^{neg}Ly\text{-}6C^{hi}$).

Discussion: This study provides evidence that the presence and the immune regulatory role of MDSC in MAA infection depends on the infection dose. Low MDSC levels seem to have beneficial effects on the outcomes of the infection with overall less severe disease effects by control of bacterial burden. In contrast, high MDSC levels have detrimental effects supporting mycobacterial growth and mycobacterial disease progression.

Outlook: Identify factors that contribute to the formation of M-MDSC.

Fig. 1

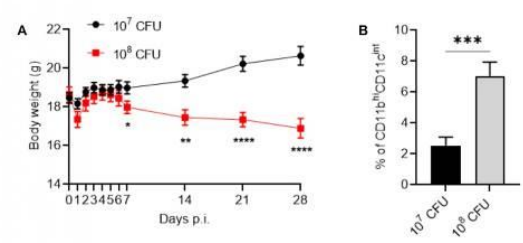

Figure 1: MAA at infection dose of $\sim 10^7$ (OD_{600nm} of 0.5) and $\sim 10^8$ (OD_{600nm} of 5) CFU caused mycobacterial disease with different degrees of severity in mice and the levels of splenic MDSC induction depends on the infection doses. **(A)** Body weight of female C57BL/6J mice infected with MAA at 10^7 CFU or 10^8 CFU. **(B)** Percentage of splenic $CD11b^{hi}CD11c^{int}$ myeloid cells of MAA infected mice (10^7 or 10^8) CFU as determined by flow cytometry.

ZOV 119

Interactions between the cytoskeleton and Orientia tsutsugamushi via Type-specific antigen 47

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Introduction: *Orientia tsutsugamushi* (OT) is a zoonotic, obligate intracellular, Gram-negative bacterium causing Scrub Typhus. The type-specific antigen 47kD of OT (TSA47)- an outer membrane protein, also secreted into the cytoplasm, having high homology to the human serine protease A1 (HtrA1), whose role in host-pathogen interactions has been ill-defined. While HtrA1 is known to degrade tubulin, interactions between TSA47 and the cytoskeleton have not been studied. Since TSA47 also has a high homology to bacterial virulence factors like DegP, we studied interactions between TSA47 and host cellular processes.

Material and Methods: To investigate the role of TSA47 in cellular interactions, we clonied a codon-optimized TSA47 construct, using different tags into the pCAGGS vector backbone & ectopically expressed TSA47 in human HuH7 hepatoma cells. Transfected cells with the dual tagged 47kD construct (FLAG-

47kD-myc), mono tagged construct (5"myc-47kD), empty vector (pCAGGS) or treated with only media for 24h, were fixed and stained for myc or FLAG tags and cellular markers. To study the cellular reaction in OT infection, HuH7 cells grown on glass coverslips were infected with cell-free *Orientia tsutsugamushi* Karp. At day 2 and 5 post infection (p.i.), infected cells were fixed, permeabilised and stained for the TSA56 surface protein of OT in combination with markers for cellular proteins (α-tubulin, HDAC6, Vimentin, Hsp70, p62 and γ tubulin) or phalloidin to stain for actin. Nocodazole treatment was performed to study the role of tubulin polymerization. Widefield (WF) and Confocal Laser Scanning Microscopy (CLSM) were used to evaluate transfected & infected cells. For Image analysis & calculation of Pearson"s correlation coefficient, ImageJ was used.

Results: The myc-TSA47 expressing cells had disrupted microtubular network. The TSA47 formed punctate aggregates of varying sizes, distributed throughout the cells and co-localized with re-distributed cellular tubulin. The actin network, however, remained intact, and did not associate with TSA47. Furthermore, compared to mock controls, TSA47 transfected cells treated with nocodazole showed a higher Pearson"s correlation coefficient with tubulin, suggesting that depolymerisation of tubulin facilitates its recruitment by TSA47. OT-infected cells had an almost completely disrupted microtubular network with OT closely associating with re-distributed alpha tubulin. In transfection, the cellular aggresome markers γ-Tubulin HDAC6 & Hsp70 showed minimal to no interaction with TSA47, but p62 was found to closely associate with TSA47.

Discussion: Our study shows that OT infection disrupts the microtubular network of OT-infected host cells. TSA47 was sufficient to mediate this disruption. TSA47 co-localized with tubulin, preferentially in its depolymerized form, but not actin, and attracted p62 as a potential autophagic marker. In sum, we showed here that OT reorganizes the micro tubular cytoskeleton via TSA47.

ZOV 120

Locking the phage trigger: An approach to attenuate enterohemorrhagic *Escherichia coli in vivo*

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) can cause life-threatening infections in humans. The use of antibiotics in EHEC infections remains controversial and a causative treatment is therefore currently not available. The cardinal virulence factor of EHEC are Shiga Toxins (Stx) that cleave an adenine from the ricin loop in the 28S rRNA and thereby irreversibly inhibit translation. Stx are are encoded in lambdoid Stx prophage and expressed late in the lytic growth cycle of the Stx phage. The expression of the clinically more relevant Stx2 is strictly dependent on phage activation, which in turn is strictly dependent on the activation of the bacterial SOS-response. The major pathway of SOS-response induction is the DNA double strand break, or replication fork collapse dependent stochastic activation of RecA via the enzyme complex RecBCD. Activation of the co-protease activity of RecA results in cleavage of the CI-like repressor of the Stx prophage, induction of the lytic growth cycle and ultimately in Stx production and bacterial lysis. We have recently constructed an improved recombination system for site-directed mutagenesis in EHEC and observed that heterologous expression of the phage λ RED functions was sufficient to repress a ciprofloxacin induced SOS-response in an *E. coli* K-12 SOS-response reporter strain. We hypothesized that a constitutive expression of *gam*, encoding for the RecBCD inhibitor Gam, might be sufficient to repress the stochastic, as well as the quinolone dependent activation of the SOS response.

Methods: We constructed a transcriptional fusion of *gam* to the *bla*CTX-M-15 promoter of pO104_90, the ESBL encoding plasmid of the hypervirulent *E. coli* O104:H4 and transferred the plasmid to our previously described EHEC O157:H7 EDL933 reporter strain. We use reporter gene assays and Western Blot to asses SOS-response induction and Stx production.

Results: We could show that the presence of the Gam expression plasmid is sufficient to completely block the ciprofloxacin induced SOS response and Stx production as judged by reporter gene measurement. Notably, we did not observe the drastic drop in optical density following ciprofloxacin treatment that was observed in the control sample, which indicated that the phage dependent bacterial lysis was blocked as well. Finally, we show that the Gam expression plasmid blocks phage induction and Stx2 expression in clinical EHEC isolates.

Conclusion: After our successful *in vitro* tests, our next goal is to elucidate the possibility to construct a donor strain that rapidly transfers a Gam expression plasmid to EHEC and the susceptible flora in the gut in order to attenuate the pathogen *in vivo*. As phage and hence Stx2 production are expected to be completely blocked in the recipients, a rapid and safe elimination of the pathogen by a subsequent antibiotic treatment may be afterwards additionally possible.

DKMDVV 121

Rapid and accurate detection of *Mycoplasmataceae* in urogenital swab and urine samples

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Questions: *M. genitalium* (MG), *M. hominis* (MH), *U. parvum* (UP) or *U. urealyticum* (UU) are bacteria without a cell wall. They are either considered as pathogenic or commensal living organisms in the urogenital tract. They belong to the category of sexually transmitted infections in men and women.

Therefore, rapid and accurate diagnostic tools are necessary for their detection in clinical specimen.

The Vivalytic instrument is a new PCR platform using either multiplex RT PCR or microarray for detection of nucleic acids from microbes. The easy-to-use cartridge system can be used in the micro lab or as a point-of-care test (POCT).

Methods: 239 urogenital samples (132 urine and 107 swabs) were tested in our hospital. The swabs were a mixture from 26 vaginal, 37 cervical, 19 urethral and 25 rectal swabs. All samples were analyzed with the Vivalytic MG, MH, UP/UU assay (Bosch Healthcare Solutions, Waiblingen, Germany) and compared to the Allplex STI Essential Assay (Seegene, Seoul, South Korea) as reference test. To achieve comparable results both tests were performed within 48 hours.

Results: In this retrospective study, the Vivalytic MG, MH, UP/UU cartridge showed a very high concordance to the reference test. For urine the concordance rate was for MG 99.2%, MH 99.2% and for UP/UU 94.6%. The consistency for swabs was 98.0%/92.8%/96.0% (MG/MH/UP&UU), respectively. Time-to-result was about one hour.

The negative percent agreement (NPA) was between 95.9% and 100.0% for all sample types andtargets. The positive percent

agreement (PPA) was 97.7% for MG, 100.0% for MH and 90.2% for UP/UU in urine samples. In swab samples the PPA for MG and UP/UU showed a very high agreement with 97.8% and 96.1% respectively and the PPA for MH was 90.0%.

Conclusions: In the clinical performance study, the Vivalytic MG, MH, UP/UU cartridge demonstrated an excellent concordance with a sensitive reference test and delivered accurate and rapid results. The assay is suitable for hospital labs as well as for outpatients" settings due to its short time-to result.

DKMDVV 122

Rapid pathogen differentiation in mycobacterial blood cultures with MALDI-TOF Sepsityper

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Introduction: In mycobacterial blood culture bottles (BC) that have been reported positive, pathogen identification to the species level requires subculturing on mycobacterial culture media and prolonged incubation. However, specific identification is of utmost importance in systemic mycobacterial infections to ensure the rapid initiation of adequate therapy and, if necessary, infection prevention measures. For aerobic, anaerobic, fungal, and pediatric BC bottles, rapid identification with the MBT Sepsityper® IVD Kit is a standard method. We adapted the protocol for the rapid identification of mycobacteria in BD BactecTM Myco/F – Lytic bottles.

Material and Methods: Mycobacterial-BC (BD BactecTM Myco/F - Lytic, Becton Dickinson, Heidelberg) were spiked with *Escherichia coli*, various non-tuberculous mycobacteria (NTM) and *Mycobacterium tuberculosis*. After a positivity alert by the BD BactecTM system, BC were processed according to the MBT Sepsityper® IVD Kit Protocol (Bruker, Bremen). The protocol was modified by the use of glass beads and heat inactivation. The time-to-report was compared to the previous identification workflow for positive mycobacterial BC.

Results: Different variations of the Sepsityper protocol were tested with regard to the individual evaluation of the most reliable Maldi-TOF scores and the correct pathogen identification. The differentiation within NTM species was found to vary, although these could always be differentiated from *M. tuberculosis* complex (MTBK). The identification of *M. gordonae* was particularly difficult depending on the test protocol. Overall, the sepsityper method was on average 6.5 days (range 1-8) faster in terms of pathogen identification compared to the standard method with identification from subcultures on solid mycobacterial media by MALDI-TOF or PCR.

Discussion: Rapid mycobacterial diagnostics are hampered by the slow growth on culture and the frequently paucibacillary character of these infections. Especially in the case of suspected systemic mycobacterial disease, which is detected by BC, rapid diagnosis is relevant. In the case of positive BC, there is currently no approved procedure for rapid detection of the pathogen. Using a modified MBT Sepsityper® protocol, we were able to show that a reliable differentiation between NTM and MTBK is possible within a few hours. Depending on the NTM species, this also includes reliable differentiation to the species level. This approach has the potential to considerably shorten the time required for identification of systemic mycobacterial infections. Further questions to be addressed are a standardized workflow for patient samples, and optimization of the protocol for reliable differentiation of NTM.

DKMDVV 123

Nanomotion technology in combination with machine learning: a new approach for a rapid antibiotic susceptibility test for *Mycobacterium tuberculosis*

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Question: Nanomotion technology is a growth-independent approach that can be used to detect and record the vibrations of bacteria attached to microcantilevers.

Methods: We have developed a nanomotion-based antibiotic susceptibility test (AST) protocol for Mycobacterium tuberculosis (MTB). The protocol was used to predict strain phenotype towards isoniazid (INH), rifampicin (RIF) and bedaquiline (BDQ) using a leave-one-out cross-validation (LOOCV) and machine learning techniques. This MTB-nanomotion protocol takes 21 hours, including cell suspension preparation, optimized bacterial attachment to functionalized cantilever, and nanomotion recording before and after antibiotic exposure.

Results: We applied this protocol to MTB isolates (n=46) and were able to discriminate between susceptible and resistant strains for INH and RIF with a maximum sensitivity of 97.4% and 100%, respectively, and a maximum specificity of 100% for both antibiotics when considering each nanomotion recording to be a distinct experiment. Grouping recordings as triplicates based on source isolate improved sensitivity and specificity to 100% for both antibiotics. For the new drug BDQ, we achieved a sensitivity of 100% and a specificity of 92.8%.

Conclusions: Nanomotion technology can potentially reduce time-to-result significantly compared to the days and weeks currently needed for current phenotypic ASTs for MTB. It can further be extended to other anti-TB drugs to help guide more effective TB treatment. This approach also represents a new tool for the study of growth-independent drug responses. Our results open the door for further method development and pre-clinical studies using nanomotion technology to examine MTB isolates, working towards the ultimate goal of developing an AST possessing high performance and thereby combining the advantages of broadly applicable phenotypic ASTs and rapid molecular diagnostic methods.

Fig. 1

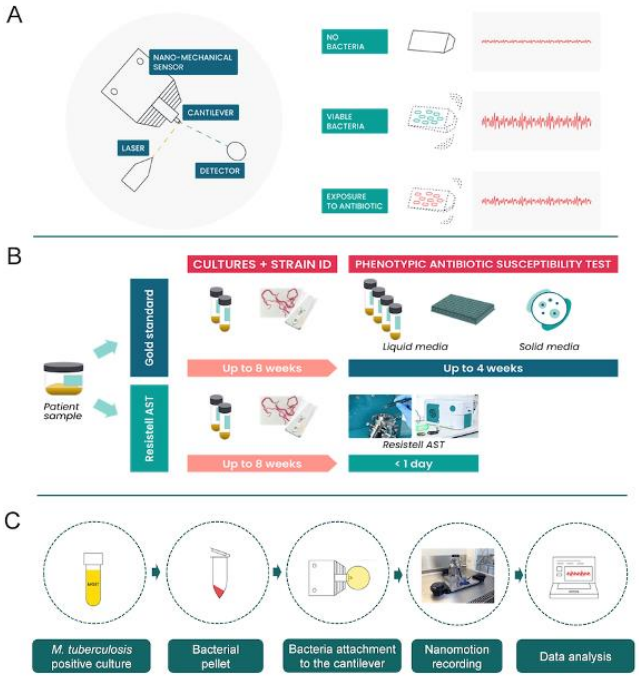
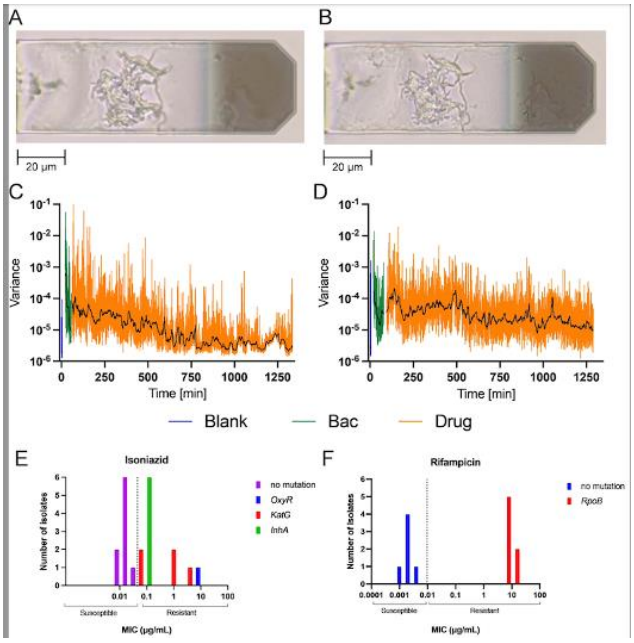


Fig. 2



DKMDVV 124

Ultra-fast identification and antibiotic susceptibility testing of bacteria via AI-enhanced single-cell analysis

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Antimicrobial resistance (AMR) is one of the top 10 global health threats with 700.000 deaths annually, according to the WHO. The current gold standard methods for antimicrobial susceptibility testing (AST) typically take up to 72 hours (including culture time), leading to ineffective treatments and high mortality rates. Improving the clinical outcome and reducing mortality greatly depends on the early identification of the pathogens and AST results. Here, we are introducing an AI-enhanced analytical method to detect the earliest signs of metabolic reprogramming in single cells and predict cell fate, enabling rapid AST and ID of bacteria in just 30 to 60 minutes. Our technology is a single-cell analysis platform that is culture free method and can analyse a patient's primary sample with no or short time culture.

The Biospex analyser implements Raman Single Laser Beam Trapping (Raman-SLBT) and artificial intelligence (AI) Predictomics. Raman-SLBT combines two Nobel prize-winning laser technologies in a single laser beam. Laser Trapping creates a force field that attracts and immobilizes single cells in the laser focus and Raman spectroscopy simultaneously records the molecular fingerprint of laser-trapped cells in a Raman spectrum. Our proprietary AI Predictomics model analyses spectral dynamics resulting from changes in macromolecule compositions of the cell over time (e.g., RNA, DNA, lipids, or proteins), enabling the detection of antibiotic response and identifying the phenotypic fingerprint of bacteria. The AI contains multiple classification sub-models trained on different Raman multi-omics data of bacteria at different incubation conditions and time frames. The AI uses the collective voting of the different classification models to infer the species ID, phenotype, and AST results.

Biospex can detect the resistance phenotype of bacteria without antibiotic treatment. The Raman results could discriminate 80 strains of multidrug-resistant gram-negative (4MRGN) klebsiella pneumonia from 40 other klebsiella species those susceptible to most antibiotics families, with 94% accuracy. Furthermore, Biospex AST of 14 strains with different resistance profiles from the 7 most clinically relevant pathogens was successfully achieved with 100% agreement with the lab routine methods. Moreover, Raman-SLBT technology can analyse bacteria directly from primary samples such as urine or from positive blood cultures.

This breakthrough technology is introducing a new concept to the clinical microbiology field, i.e., the detection of early multi-omics changes in single bacteria cells for ultrafast analysis, surpassing by far the speed of current standard procedures, which rely on measuring growth arrest or cellular disintegration of bacteria. Therefore, the total turnaround time for the AST analysis will be accelerated more than 24-fold compared to the current standard techniques.

Fig. 1

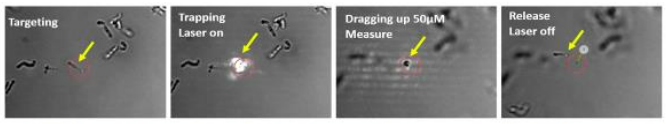

Figure 1: Bright field images using Biospex analyzer illustrating the Laser optical trapping of single bacteria cell (Bacillus cereus) in buffer solution

Fig. 2

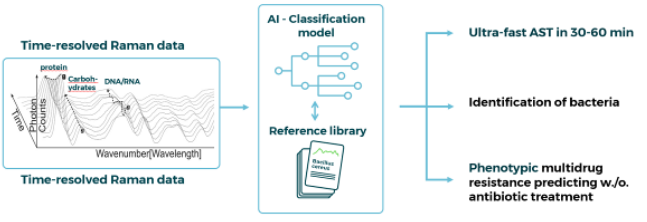

Figure 2. Schematic representation of the AI predictomics of time resolved Raman data. The spectral panel display the multi-omics changes of E.coli in 10 minutes time frame after incubation in Müller Hinton broth.

DKMDVV 125

New insight into endocarditis: Do biofilms impact therapy success?

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Question: Infectious endocarditis is a life-threatening disease associated with high mortality. Diagnosis is based on the 2023 ISCVID Duke criteria, which does for the first time take information from molecular techniques into account. However, to date, antibiotic therapy is solely adapted to the pathogen species and sensitivity according to the ESC / EACTS guidelines. Neither the biofilm status, activity nor degradation of the pathogens due to previous antibiotic therapy are taken into account so far.

Methods: We used FISHseq (Fluorescence in situ Hybridization (FISH) combined with 16S rRNA-gene PCR and sequencing) 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. We statistically correlated formation, amount, activity and therapy of these endocarditis patients.

Results: We found a large variability in the severity of biofilm presentation 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; and other cases with micro-colonies or highly active, mature biofilms. Interestingly, bacterial activity correlated significantly with biofilm formation, independent of the duration of treatment before surgery.

Conclusions: 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 may enable not only evidence-based therapy decision making, but also for or against early de-escalation of antibiotic therapy in endocarditis patients.

DKMDVV 126

Influence of symptoms, vaccination, and Omicron BA.1/2 and BA.4/5 on SARS-CoV-2 antigen rapid tests

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Question: SARS-CoV-2 antigen rapid diagnostic tests (RDT) are an established point-of-care tool for diagnosis, screening, and self-testing. In contrast, the reference standard for SARS-CoV-2 diagnostics, reverse transcription polymerase chain reaction (RT-qPCR), is limited by its diagnostic capacity and infrastructural requirements. Previous studies could not clarify which of the temporally closely correlated factors – the COVID-19 vaccination and the Omicron virus variant of concern (VOC), especially the sublineages BA.4/5 – is responsible for the reported impairment of RDT sensitivity.

Methods: The clinical point-of-care usage of RDTs (one of NADAL®, PANBIO™, or MEDsan®) in a tertiary care hospital was prospectively compared with RT-qPCR as reference standard from the 12th of November 2020 to the 30th of September 2022. A total of 54,740 RDT/RT-qPCR pairs from 38,373 study participants with an age ≥ 18 years were included. COVID-19 vaccination status and COVID-19 symptoms were recorded from the hospital information system (Figure 1).

Results: Overall, RDT sensitivity was 36.4% (433/1,189; 95% CI: 33.7%-39.2%), RDT specificity 99.7% (53,375/53,551; 95% CI: 99.6%-99.7%) compared to RT-qPCR. In the lasso regression model viral load (Odds Ratio (OR): 2.3; 95% CI: 2.0-2.6; $p<0.001$) and typical COVID-19 symptoms (OR: 2.5; 95% CI: 1.7-3.7; $p<0.001$) were identified as significant factors influencing the RDT performance. The factors age ($p=0.45$), atypical COVID-19 symptomatology ($p=0.09$), COVID-19 vaccination ($p=0.30$), infection with the Omicron BA.1/2 VOC ($p=0.06$), and Omicron BA.4/5 VOC ($p=0.36$) obtained no significant influence (Figure 2).

Conclusions: In this study, only COVID-19 typical symptomatology was shown to be a key influencing factor on RDT sensitivity, in addition to viral load as already known. In summary, RDTs provide a reliable diagnostic tool for rapid detection of symptomatic individuals with high SARS-CoV-2 viral loads. Thus, shortly after the onset of symptoms at the onset of illness and correspondingly at high viral load, a reliable RDT result can be expected. However, RDTs show a clear limitation in their use as a screening method and especially in asymptomatic SARS-CoV-2 individuals.

Figure 1: Enrolment of RDTs
RDT: SARS-CoV-2 antigen rapid diagnostic test
RT-qPCR: Quantitative reverse transcription-polymerase chain reaction

Figure 2: RDT performance influencing factors
RDT sensitivity (2A) and viral load (2B), stratified by vaccination status, symptoms, and VOC. Odds Ratio (2C) of potential RDT performance influencing factors considered in the Lasso regression model.
95% confidence intervals are indicated as error bars. The threshold for viral load of 106 SARS-CoV-2 RNA copies/ml, which was suggested as the threshold for infectivity, is added as a horizontally dashed line to 2B. The odds ratio of 1 is added as a dotted line to 2C.
p-values were only indicated for significant comparisons.
VOC: variant of concern.

Fig. 1

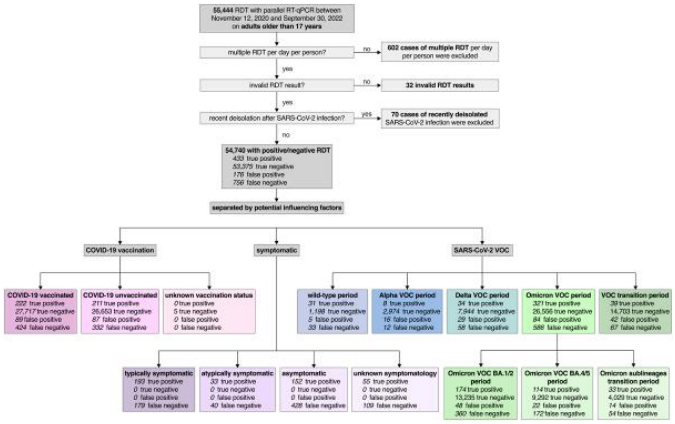
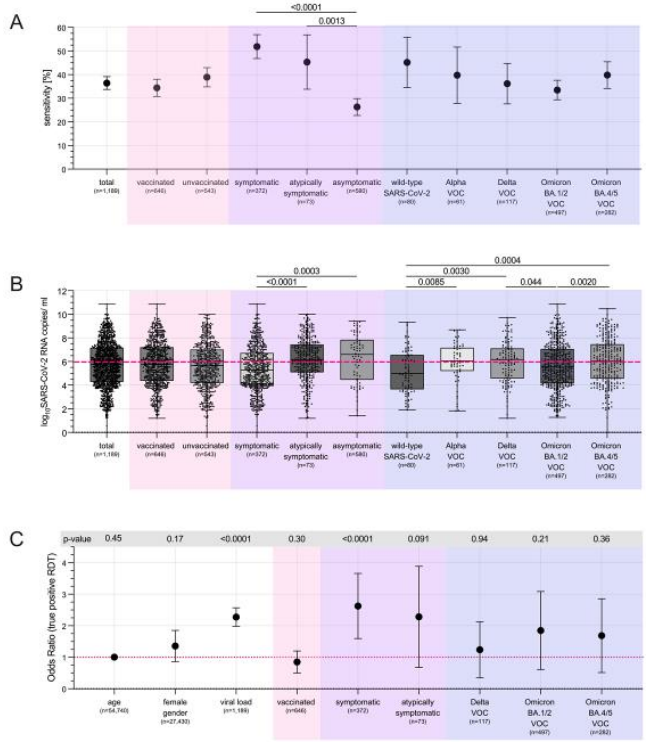


Fig. 2



PRHYV 127

Can washing bowls be reprocessed in bedpan dishwashers? - A hot discussion

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Introduction: The reprocessing of washing bowls in bedpan dishwashers (BDW) is controversially discussed. While some expert"s recommendations strictly exclude this, others explicitly allow reprocessing or recommends compliance with defined conditions for reprocessing. A main argument against reprocessing is the possible recontamination by pathogens from previous processes that have not been completely removed from the BDW unit. In this study, the rate of recontamination of washing bowls reprocessed in RDS was to be determined.

Material/method: A total of 25 BDW from different manufacturers placed in two hospitals were included. Before reprocessing, the level of contamination of each unit was determined by swabbing all spray nozzles, the door seal, as well as the rim of the pump sink and examining 50 mL of water from the pump sink. During the reprocessing process, the A0 value of the process was determined. After reprocessing of a sterile washing bowl, the inside of the bowl was wiped with a sterile moistened scratch sponge and grown on selective media after broth enrichment. The germs detected were categorized as intestinal flora, *Pseudomonas aeruginosa*, other water germs and skin/environmental flora. In each unit, 10 independent treatment processes were examined.

Results: With the exception of single processes in five units, an A0 value of 600 was exceeded in all reprocessing processes. Swabs of the spray nozzles and door seals showed no evidence of intestinal flora and rarely other bacteria. In the swab of the pump sink, intestinal flora and *P. aeruginosa* was detected in 19 and 40 of 250 examinations, respectively. In the pump sink water, intestinal flora and *P. aeruginosa* was detected in 41 and 78 of 250 examinations, respectively. The only detection of intestinal flora on a washing bowl after the reprocessing was the isolation of enterococci which were never identified in the respective BDW. None of the 250 processed washing bowls displayed growth of *P. aeruginosa*. In a single case a low pathogenic species also identified in the pump sink water could be identified on a washing bowl reprocessed in the one of the BDW units repeatedly failing an A0 value of 600.

Discussion: Of 250 washing bowls reprocessed in a BDW, two showed possible recontamination (< 1%). The only detection of the category intestinal flora (enterococci) occurred after reprocessing in an "inconspicuous" unit, indicating a possible secondary contamination during probe sampling. The only detection of the category water germs occurred after treatment in a device with constant detection in the water of the pump sink and unreliable achievement of the desired A0 value. Despite frequent detection of P. aeruginosa in the water, P. aeruginosa was not detected on any washing bowl. We see no indication of relevant recontamination of washing bowls by treatment in a BDW if an A0 value of 600 is reliably achieved.

PRHYV 129

Reduction of legionella contamination in toilet flushing cisterns

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Introduction: Immunosuppressed patients have an increased risk for legionella infections caused by inhalation of pathogen-containing aerosols from hospital plumbing systems. We previously reported a case of fatal hospital-acquired *Legionella pneumophila* infection caused by contaminated water from a toilet-flushing cistern. Here we provide experimental data on the growth of legionella in flushing cisterns and an easy protocol how to prevent it.

Methods: Growth of legionella in the buildings" hot and cold water system was monitored by quantitative bacterial culture on selective agar according to DIN EN ISO 11731: 2017.

Results: In order to monitor the growth of legionella in flushing cisterns we developed an experimental set-up with 4 toilets in which the influence of regular flushing versus no-use of toilets and the effectivity of one-time disinfection of flushing cisterns with peracetic acid (1%) could be investigated under controlled conditions. The cold water was supplemented with chlorine dioxin (0.1-0.2 ppm). In this experimental setting, the legionella concentration of the water in flushing cisterns was significantly ($p<0.05$) reduced by daily toilet flushing over a period of 21 days. After three weeks of daily flushing, the mean legionella concentration in the cisterns was 70% less compared to baseline. As expected, this was only a temporary effect. After three weeks of no-use of flushing cisterns, the mean legionella concentration increased by 260% and after a total of seven weeks no-use, the legionella concentration increased by 520% compared to the samples taken at the end of the daily flushing period. A more pronounced and long-lasting reduction of legionella growth without daily flushing was observed if in addition to chlorine dioxide disinfection of the cold-water systems the flushing cisterns were disinfected once for one hour with 1% peracetic acid. In this setting without daily flushing even four weeks after initial disinfection, the legionella concentration in all cisterns was still below the official German concentration limit for drinking water of 100 CFU/100 ml. To exclude the possibility that the lack of growth of legionella was because during no-use after the intensive disinfection protocol no recontamination of cisterns occurred, daily flushing was performed for 21 days. At the end of this three-week flushing period, no legionella were detectable in all four flushing cisterns.

Conclusions: As short-term measures, the combination of chlorine dioxin water supplementation together with one-time disinfection of highly contaminated cisterns with peracetic acid and daily toilet flushing strongly reduced legionella contamination in flushing cisterns and therefore could prevent inpatients from possibly fatal legionella infection.

PRHYV 130

Do external factors influence Legionella species contamination of drinking water in a large hospital?

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Question: *Legionella* are pathogenic gram-negative bacteria including *L. pneumophila* as a potential cause of legionellosis. The primary pulmonal form of this disease, Legionnaires' disease, can be fatal. As consequence, regular tests for *Legionella* in drinking water are mandatory by the German Drinking Water Ordinance, causing immense efforts and costs to society. Thus, it is of interest to identify external factors regularly influencing the occurrence and amount of *Legionella* spp. in closely and long-term monitored drinking water systems. Once such factors are identified, monitoring strategies can be optimized in collaboration with operators and authorities. Thus, the aim of this study was to measure how the concentration of *Legionella* spp. in a hospital's drinking water system depends on season and time of day with the latter as surrogate of water flow.

Methods: Over one year (August 2019 – July 2020), water sampling was executed monthly at 21 sites of the hospital"s drinking water system (seasonal influence). Additionally, at two points, samples were taken in the course of a week (September 2020, three times a day, 6 a.m. / 12 p.m / 5 p.m., influence of consumption). All samples were analyzed for *Legionella* spp. according to DIN EN ISO 11731:2018-03. Differences in the *Legionella* concentrations and their course were evaluated by statistical methods.

Results: Seasonal influence: Mean *Legionella* spp. concentrations did not differ significantly between the four seasons (spring, summer, fall, winter; $p=0.063$; Friedman and Dunn-Bonferroni-Post-Hoc tests). Monthly comparisons revealed a significant difference between February and June ($p=0.013$; Friedman test) with higher values in June. However, all other monthly comparisons ($N=65$) did not confirm this significance, and the February-June difference is interpreted as outlier effect of the June values.

Influence of consumption: At both sampling points, the *Legionella* spp. concentrations decreased during the daily course, illustrating a "rinsing effect" by increasing water consumption from morning until afternoon. However, differences between the sampling times were not significant ($p=0.247$ and $p=0.692$, respectively).

Conclusions: The legionella concentrations in our hospital"s drinking water system varied greatly over the individual samplings and sampling points. However, no trend was discernible over the course of the year, which is in line with publications characterizing legionella analyses as snapshots influenced by factors others than season. Our investigations throughout working days revealed, as expected, decrease of legionella contaminations with increasing water consumption. However, both results lacked statistical significance. Thus, the results do not offer ways to optimize legionella monitoring – neither in our water-carrying pipe system nor in any other.

PRHYV 131

Effect of UV-C disinfection in a dialysis unit – an intervention study

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Introduction: UV-C light has a known bactericidal effect and has been used for disinfection in hospitals in the USA for the past 10-15 years, but in Europe, UV-C light disinfection is not yet an integrated method in clinical departments. Microorganisms in patient areas can cause infections in patients, even if the patient area has been cleaned. Dialysis patients have weakened immune systems and frequent hospital visits, putting them at risk of serious infections and bacteremia. This study focuses on the preventive aspect of infection control and will therefore investigate:

Scientific question: Will cleaning and disinfection according to current standards in the dialysis units at Rigshospitalet, Denmark, followed by UV-C disinfection 5 nights a week:

1. Reduce the occurrence of bacteria on contact points and surfaces in the dialysis units?
2. Reduce the occurrence of bacteremia in dialysis patients?

Method and materials: A pragmatic crossover intervention study was conducted, where an automatic UV-C Disinfection Robot model C from Blue Ocean Robotics disinfects surfaces and contact points after regular cleaning. For 3 months, the UV-C robot operated in 2 dialysis units, while 2 corresponding dialysis units served as control departments, and this was reversed for the next 3 months. Tryptone Soya Agar (TSA) contact plates (OXOID - Thermo Fisher Scientific) were swabbed on 14 surfaces and contact points before and after cleaning and after UV-C light irradiation, totaling 574 samples. Data on bacteremia were obtained from the hospital's microbiology database. Excel and the statistical program Python were used for statistical analysis, and p-values were calculated using the Wilcoxon Rank Sum test and Fisher's Exact test.

Results: Cleaning and operation of the UV-C robot were performed by the department's regular cleaning staff. The microbiological cleanliness threshold is <2.5 CFU/cm2. Despite the cleaning staff being aware of the swabbing times, there were instances of exceeding the cleanliness threshold. However, all swabbing points had <2.5 CFU/cm2 after UV-C light irradiation. The cleaning staff learned to operate the robot during a 2-hour training session. However, there were technical, mechanical, and human factors that resulted in the robot's operation reaching 100% only in the 6th month. This could have been resolved by establishing in-house technical support. Data on bacteremia were based on 78 patients in the first 3 months and 75 patients in the last 3 months, representing a small patient population and a short intervention period. Therefore, the results for bacteremia are not valid.

Conclusion: UV-C light from a UVD-Robot model C from Blue Ocean Robotics, as a supplement to cleaning according to current standards, significantly reduces the occurrence of bacteria on contact points and surfaces. However, the intervention does not show a reduction in the occurrence of bacteremia in dialysis patients.

DKMDVP 132

Introduction of a premanufactured LAMP assay for the detection of *Toxoplasma gondii* in clinical samples

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Introduction: *Toxoplasma gondii* is the causative agent of Toxoplasmosis, a common opportunistic infection that affects mostly immunocompromised patients or women during pregnancy. Detection of the pathogen is mostly based on realtime PCR (qPCR) targeting a 529bp repeat sequence. Since 2013 commercial assays that use the technique of loop-mediated isothermal amplification of DNA (LAMP) are available. These assays offer a simple, sensitive and affordable solution for the detection of various clinically relevant pathogens in patients´ samples. The aim of the study was to establish a premanufactured LAMP assay for the detection of *Toxoplasma gondii* that saves time and that is easy to perfom and that meets the sensitivity of a commercial qPCR.

Methods: Overall, 53 samples from 42 patients were investigated (2 bronchoalveolar lavages, 23 EDTA blood samples, 21 cerebrospinal fluids and 5 vitreous aspirates). For qPCR 300µl of sample were processed using the Maxwell16 instrument (Promega). 10µl of DNA were used for qPCR (Real-TM *T. gondii*, Sacace). For the LAMP assay 125µl of sample, except for EDTA, were mixed with 125µl of a magnetic bead solution (Mag solution, Amplex) and incubated for 3min at 99.9°C. 125µl of clear supernatant were then added to an equal amount of RS solution (Amplex). 25µl were pipetted into premanufactured microtubes containing the lyophilized reaction mix (Amplex). For EDTA 25µl of blood were incubated in 500µl of RALF buffer (Amplex) as described above. Again, 25 µl of clear supernatant were used for the LAMP assay. For LAMP two different areas of the 529bp repeat sequence were chosen as target. LAMP was performed using a Genie II instrument (Amplex). Runtime was approximately 30min. To investigate the limit of detection (LoD) of the LAMP assay tenfold serial dilutions of a solution containing tachyzoites (Marc Hübner, Bonn) were processed with Mag solution as described above. Time-to-positivity of the LAMP assay was plotted against cycle threshold values (ct values) of the qPCR.

Results: QPCR was positive in 15/ 53 cases (28.3%) whereas LAMP was positive in 11/ 53 cases (20.8%). The agreement was 0.92. LoD of the LAMP assay was 10 tachyzoites per ml which matched with a ct value between 28-30 in qPCR.

Conclusions: The LAMP assay can be a useful technique for the detection of *Toxoplasma gondii*. Sensitivity in clinical samples could probably be improved by increasing the sample volume and by modifications of sample preparation which will be further investigated. However, the clinical significance of the detection of the pathogen with ct values greater than 32 is still unclear and is probably dependent on the site of infection.

DKMDVP 133

***Gardnerella vaginalis* in urine specimen of kidney transplant recipients- relevant or contaminant?**

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Background: Due to the advances in microbial diagnostics and pathogen detection by the use of total laboratory automation, the extent of urine specimen culturing *G. vaginalis* have been shown to be significantly increased. This has especially been the case in patients following kidney transplantation, who are in general at risk for urinary tract infections (UTI). *G. vaginalis* has traditionally been associated with bacterial vaginosis and its relevance in the context of UTI remains undefined and controversial. Therefore, the study aimed to analyze clinical presentation and laboratory findings as well as patient characteristics in kidney transplant recipients to determine the relevance of *G. vaginalis* in urine samples in these patients.

Materials and Methods: Kidney transplant recipients with one or more urine samples growing *G. vaginalis* in a two-year period from July 2016 until June 2018 were included in the study. Patient characteristics, laboratory findings, clinical presentation and immunosuppressive agents were analyzed and compared to a randomized, gender- matched control group of kidney transplant recipients, to clarify the relevance of *G. vaginalis* in urine cultures and identify possible associated factors. Chi-Square-, Mann-Whitney-U-Test or t- Test were used to asses statistical significance.

Results: During the study period, *G. vaginalis* was cultured in 70 urine specimens of 38 kidney transplant recipients, of which seven presented with repeated cultures with *G. vaginalis*. 86% of patients were female in both groups due to gender-matched randomization.

The mean age was significantly lower in the group with *G. vaginalis* (43 vs 50 years, p=0.003). 50% of samples with *G. vaginalis* had colony forming units (cfu) of $\geq 10^5$ per ml, but only 21% of samples in the control group. While the mean values of leucocytes in blood and C-reactive protein were not significantly different in the two groups (8.7 vs 7.9 leu/nl, p=0.09; 9.8 vs 8.8 mg/l, p=0.76), the number of leucocytes in urine were significantly increased in the samples with *G. vaginalis* (p=0.01). Typical Clinical presentation with dysuria, urinary urgency or frequency or suprapubic pain was not documented in the majority of cases.

Discussion: The presence of $\geq 10^5$ cfu/ml of a relevant pathogen and leucocyte count in urine are usually considered as typical laboratory characteristics of UTI, which was the case in the majority of samples in the presented study. The typical clinical presentation was not documented in most these cases, which questions the clinical relevance of *G. vaginalis*. However, several cases have been described with invasive infection caused by *G. vaginalis* that underline the possible relevance, especially in immunocompromised patients. In addition, *G. vaginalis* has been described as a risk factor for recurrent UTI. More studies are needed to determine their relevance in urine cultures regarding these aspects.

DKMDVP 134

Direct identification of bacteria from positive blood culture bottles by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: a systematic review and meta-analysis

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Introduction: We perform a systematic review and meta-analysis to evaluate identification protocols of bacteria directly from positive blood-cultures (BCs) from patients with bloodstream infections (BSI) using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) with following review questions:

What is the correct identification ratio (CIR) of MALDI-TOF based rapid identification of bacteria from positive BCs compared to the standard of care (SOC) from patients with BSI?

Are there significant differences in the CIR of MALDI-TOF based rapid identification of bacteria from positive BCs compared to SOC for (i) different mass spectrometer and BC systems and (ii) for different bacterial groups and species?

Methods: We included studies comparing MALDI-TOF based identification of bacteria directly from positive BCs with the identification involving subculture of positive BC bottles. The CIR was calculated for each bacterial species X according to CIR(X) = (n(correctly identified number of X)+0.0001)/(N(number of X according to SOC)+0.01). Influence analysis was performed to detect studies which influence the overall estimate of the pooled data most and to assess whether the identified studies distort the pooled effect.

Meta-analysis was performed for bacterial groups and species. Subgroup analysis is still pending and will be performed for different BC systems, mass spectrometer systems, separation methods, protein extraction methods.

Results: A total of 145 datasets from 93 studies were included. The most frequent blood culture system was the BD BACTEC™ system (64.8%). The mass spectrometry devices by the manufacturer Bruker were applied most often (80.7%). The most common separation method was centrifugation (82.1%). For the protein extraction method inhouse-protocols were used more often than commercial protocols (66.2%). The total CIR including every dataset was 0.72 (95% CI: 0.69-0.75). The CIR of gram-positive bacteria and gram-negative bacteria was 0.62 (95% CI: 0.58-0.66) and 0.85 (95% CI: 0.83-0.88), respectively. CIR of *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pyogenes* and *S. pneumonia* was 0.78 (95% CI: 0.73-0.83), 0.59 (95% CI: 0.54-0.64), 0.71 (95% CI: 0.65-0.78) and 0.36 (95% CI: 0.29-0.43). CIR of *Enterobacterales* was 0.90 (95% CI: 0.87-0.92) (*Escherichia coli* 0.94 (95% CI: 0.92-0.96), *Proteus* spp 0.87 (95% CI: 0.82-0.92), *Klebsiella* spp 0.92 (95% CI: 0.89-0.94), *Citrobacter* spp 0.85 (95% CI: 0.79-0.9)) and 0.86 (95% CI: 0.81-0.9) for *Pseudomonas* spp.

Conclusions: The findings of this meta-analysis show mass-spectrometry based bacterial identification directly from positive blood bottles is eligible to be used as a routine method for BSI but differences between bacterial species must be considered. Whether there are significant differences in the identification results for different (combinations of) BC systems, MALDI-TOF systems and extraction remains to be analyzed.

DKMDVP 135

Accurate and sensitive detection of microbial DNA and RNA targets using nanoplate dPCR

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Introduction: Microorganisms are highly diverse and have occupied countless ecological niches throughout evolution. One such niche is the human body, upon which colonization can have both beneficial and harmful effects. This is often associated with the presence of health-threatening virulence and resistance to antimicrobial agents. Therefore, the specific detection and quantification of microbes, virulence genes and antimicrobial resistance genes is of particular importance for human healthcare. Given this challenge, digital PCR in combination with specific detection methods is a powerful tool for accurate and sensitive quantification of human pathogenic microbes and their resistance and virulence genes. QIAGEN"s dPCR Microbial DNA Detection Assays are designed to test for the presence of microbes, resistance or virulence factor genes using digital PCR. The assays target over 685 bacterial, fungal, parasitic, viral, antibiotic resistance or virulence factor genes and can be run on the QIAcuity® in 2 hours with minimal hands-on time.

Materials & Methods: To test the accuracy of absolute quantification of microbial targets, gDNA from the bacterium *Shigella sonnei* was quantified. To demonstrate that multiplexing does not compromise accurate quantification, we tested a series of four assays in singleplex and multiplex using the same input templates. Moreover, a 5-plex detection of the viral RNA target SARS-CoV-2 together with four bacterial DNA targets normally found in wastewater samples was performed to include reverse transcription of RNA into DNA in the analysis.

Results: Accurate quantification is one of the core properties of digital PCR. The analysis of gDNA from *S. sonnei* showed strong concordance between measured and expected quantification, highlighting the high accuracy of dPCR over the entire dynamic range of 4 log levels. Multiplexing allows conservation of sample material, while detecting multiple targets in one reaction. Quantification results are the same regardless of single- or multiplexing, even over a dynamic range of 4 log levels. The performed 5-plex detection of the viral RNA target SARS-CoV-2

together with four bacterial DNA targets normally found in wastewater samples showed that the template dilution was accurately captured using dPCR.

Discussion: Digital PCR on the QIAcuity combined with the dPCR Microbial DNA Detection assay portfolio offers four key features:

- Coverage: A portfolio of 685 microbial assays detecting bacteria, fungi, viruses, parasites, plus AMR and virulence genes
- Accuracy: Accurate linear quantification across a 4-log dynamic range in singleplex and multiplex reactions
- Speed: A fast workflow with a turnaround time of less than 2 hours
- Multiplexing: Mix-and-match option for up to five targets and, in combination with QIAcuity OneStep Advanced Probe Kit, the possibility for simultaneous detection of DNA and RNA targets

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Guideline alignment for inoculated blood culture volumes in pediatrics – a pilot study

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Introduction: In pediatrics, pathogen detection by blood culture is crucial in many conditions. A positive correlation between inoculated blood volume (BV) and diagnostic yield (DY) has been demonstrated in adults (Lamy et al. 2016). A German national guideline focusing on patients with neutropenic fever recommends inoculation of 1-3ml, 5ml and 10ml for infants up to 10kgBW, up to 20kgBW, and more than 20kgBW (AWMF S2k guideline: 048/14). There is no guideline for patients in general pediatrics but in terms of inoculated BVs procedure is often based on the aforementioned guideline. The actually inoculated BVs in hospitalized children in Germany, is unknown.

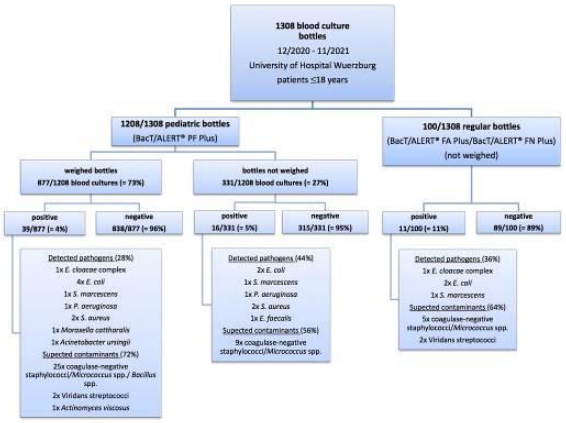
Objective: Evaluation of alignment along the aforementioned guideline regarding inoculated BVs, factors associated with inoculated low BVs and DY.

Methods: In this single center, prospective, observational study, type, weight and DY of blood culture bottles (BCB) of patients (<18 years) were documented between 12/2020 and 11/2021. Pediatric BCB were weighed by random sampling after inoculation and average bottle weight prior to inoculation was substracted. Approximate body weight was estimated by age specific 3rd percentiles (0-1 years: up to 10kgBW, 2-7 years: 10-20kgBW and ≥8 years: more than 20kgBW).

Results: A total of 1308 BCB were obtained during the study period. Of these, 100 were regular BCB (BacT/ALERT® FA Plus/BacT/ALERT® FN Plus) and 1208 were pediatric BCB (BacT/ALERT® PF Plus). Inoculated BV was documented in 877 out of 1208 bottles (72,6%).Cultures, compared to the guideline, were adequately inoculated in 64/505 (13%) children at the age of 0-1 years and 13/214 (6%) at the age of 2-7 years. Of 258 children 8 years or older 44 (17%) regular BCB were used.Of 877 weighed pediatric BCB, submitting department, number of bottles and average BV were: emergency n=224, 1,0 ml (95%-CI [0.8, 1.2]), oncology, n= 207, 3,8 ml (95%-CI [3.62, 3.98]), general pediatrics n= 126, 1,1 ml (95%-CI [0.76, 1.38]) and pediatric intensive care, n=87, 0,63 ml (95%-CI [0.32, 0.94]), others (neonatolgy, obstretics, pediatric surgery, n= 213).Bacterial growth was detected in 39/877 (4%) weighed, in 16/331 (5%) not weighed pediatric BCB and in 11/100 (= 11%) of the regular bottles. Rate of contaminants was high (72%, 56% and 64%). In the groups of children at the age of 0-1 years, 2-7 years and ≥ 8 years DY was 25/505 (5%), 11/214 (5%) and 3/158 (2%). DY of bottles inoculated with recommended volume and less volume than recommended was 4/77 (5%) and 35/800 (4%). The rate of suspected contaminants was 1/4 (25%) and 27/35 (23%).

Conclusion: Inoculated BVs, compared to guideline recommendation, were low. Average inoculated BV was highest in pediatric oncology, presumably due to the high rate of patients with central line catheters. The missing correlation between inoculated BV and DY may be attributed to the small sample size of this pilot project and should be investigated in a pediatric multicenter study

Fig. 1



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Evaluation of a MALDI-TOF MS-based rapid assay for detection of Colistin resistance in agar and blood culture derived *Escherichia coli* samples

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Introduction: For treatment of multiple drug resistant (MDR) Gram-negative bacterial infections, colistin is used a last-resort antibiotic and directly acts on the bacterial cell-membrane. Modification of Lipid A by cationic 4-amino-L-arabinose (L-Ara4N) or phosphoethanolamine (pEtN) groups leads to colistin resistance by preventing access to the cell-membrane due to electrostatic repulsion.

Broth microdilution is the gold standard method for antimicrobial susceptibility testing but is very time-consuming. Along with the growing incidence of MDR bacterial infections, there is an urgent need for rapid and accurate colistin resistance testing methods.

The aim of this study is to evaluate the performance of a novel MALDI-TOF MS-based colistin resistance detection assay for *Escherichia coli* strains derived from agar plates as well as positive blood cultures.

Materials/methods: 103 clinical *E. coli* isolates were tested by rapid colistin resistance assay with MALDI-TOF MS (Bruker, Germany) comparing two different starting materials: i) colonies

grown on agar plates, ii) bacteria derived from spiked positively flagged blood cultures purified by MBT Sepsityper Kit (Bruker, Germany).

Subsequently, lipid A molecules of the bacterial cell membrane were extracted using the MBT Lipid Xtract Kit (RUO; Bruker). The spectra acquisition and evaluation were automatically performed by the LipidART module (Bruker) of MBT Compass HT (RUO) on a MALDI Biotyper sirius™ (Bruker) in negative ion mode.

According to the ISO 20776-1:2019 guideline, broth microdilution was performed as reference method (MICRONAUT MIC-Strip Colistin, Bruker).

Results: Evaluation of agar grown isolates using the MBT LipidART module resulted in a sensitivity and specificity for the detection of colistin resistance of 97.7% (43/44) and 98.2% (56/57), respectively. Validity was 98.1% (101/103).

Blood culture grown isolates showed different variants of native Lipid A molecule compared to agar grown isolates and automated evaluation of spectra by the MBT LipidART module revealed sensitivity and specificity of 93.3% (42/45) and 94.7% (54/57), respectively. Validity of the test was 99.0% (102/103).

Discussion: Applying the Lipid Xtract kit in combination with MALDI-TOF MS showed excellent performance for detection of colistin resistance in *E. coli*. Especially for positive blood cultures, this MALDI-TOF MS-based method with 15 minutes hands-on-time and less than 30 minutes time-to-result provides a same-day-result of the colistin resistance status. The method has the potential to accelerate diagnostics in future, after appropriate development and regulatory approval.

DKMDVP 138

qPCR based detection of virulence associated *Legionella pneumophila* by *lag-1* gene amplification

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Question: Legionella are ubiquitous environmental bacteria. By inhaling legionella-containing aerosols, the pathogens are able to infect human cells and trigger atypical pneumonia called Legionnaires´ diseases (LD). L. pneumophila serogroup 1 mAb $3/1^+$ is described as particularly virulent and reported in the majority of clinical samples. MAb $3/1^+$ *Legionella* harbour the *lag-1* gene as a part of the Lipopolysaccharide (LPS) biosynthesis locus and are related with a significantly increased virulence. The finding that mAb $3/1^+$ strains are underrepresented in environmental isolates but responsible for the majority of community-acquired outbreaks underlines the increased virulence and thus the importance to detect and prevent potential infection sources in time. However, this contradicts to the time consuming and less sensitively standard culture based methods for Legionella diagnostic and prevention. For this reason we developed and validated a culture independent qPCR according to the correlation between the presence of the *lag-1* gene and mAb 3/1 reactivity.

Material/Methods: Two primer-probe sets for *lag-1* gene amplification were designed. Both assays were validated according to international standards (ISO/TS 12869:2019). The primer specificity was evaluated in silico and in vitro by testing environmental and respiratory microorganisms (n=114). Efficiency, limit of detection and quantification were defined using a plasmid carrying the *lag-1* gene (standard). The clinical evaluation was carried out by comparing currently used gold-standard ´mAb typing´ (cultivation and serotyping) and our investigations by *lag-1* qPCR in 100 patient and 50 water samples.

Results: We successfully designed and validated two primer-probe sets. According to ISO/TS 12869:2019 the Limit of detection (10 GU/reaction) and quantification (50 GU/reaction) were defined and no unspecific amplification of the 114 validation strains could be detected. The evaluation of clinical and environmental samples showed 100 % correlation with both methods and confirmed that the primers are able to amplify all genetic variations of the *lag-1* gene.

Conclusion: The *lag-1* qPCR is a new culture independent tool to detect mAb $3/1^+$ legionella strains and has the potential to improve the legionella risk management by including virulence associated markers. Unlike the current technical alert value of 100 cfu of legionellae in 100 ml by cultivation the described qPCR enables an additive risk assessment of water-bearing installations.

DKMDVP 139

Rapid bacteria identification from blood cultures using a centrifugation based sample preparation method and MALDI ToF MS in daily lab routine

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Introduction: Fast and accurate identification of microorganisms is crucial for analysis of positive blood cultures and plays a key role in the antimicrobial therapy of blood stream infections. Subculture-based analysis normally requires around 16 – 24 h from positive flagged blood culture to correct species identification via MALDI ToF MS. Established preparation methods for rapid species identification by MALDI ToF MS directly from blood cultures are either expensive, laborious and time consuming and are often not suited for all types of blood cultures. Therefore, we investigated an inexpensive, fast and undemanding method for direct identification which can easily be integrated into daily lab routine.

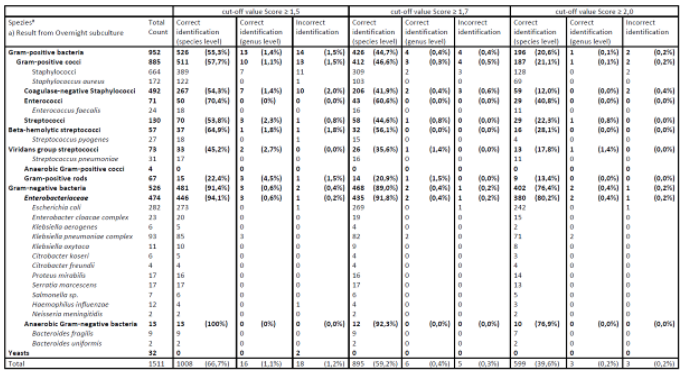
Material and methods: Positive blood cultures were analysed by transferring a sample into serum centrifugation tube with a gel barrier. After centrifugation (10 min, 3500 rpm), the supernatant was discarded. The pellet on top of the barrier gel was suspended in 1 mL ddH2O and decant into an Eppendorf tube, followed by another centrifugation step (2 min, 13.000 rpm). The resulting pellet was then used for protein extraction by resuspending in 70 % (v/v) ethanol followed by centrifugation, decantation and subsequent resuspending in 40 µL acetic acid and 40 µL acetonitrile. After a final centrifugation step the supernatant was then analysed via MALDI ToF MS twice for each sample. For species identification of bacteria, the manufacturer proposed cut-off value of ≥2.0 was used. A ROC analysis showed modified cut-off values of a score ≥1,5 for gram-positive cocci and for all other bacteria a score ≥1,7.

Results: We analysed over 1500 positive blood cultures so far using our rapid identification approach. Species identification was achieved for 66 % of all analysed blood cultures with 1 % of misidentifications using score ≥ 1,5 as cut-off. The identification of gram-positive cocci was successful for around 60 % (21 % with a cut-off score ≥ 2.0), 70 % of *Staphylococcus aureus* and enterococci, and 65 % for beta-hemolytic streptococci were correctly identified by applying modified cut-off values. For gram-negative bacteria species identification was obtained in 90 %, Enterobacterales in 94 % and bacteria with a chromosomal encoded AmpC beta-lactamase in 84 %. The method is not suited for identification of gram-positive rods and yeasts. By using this method in our daily lab routine, we could provide species identification up to 18 h earlier compared to our traditional subculture-based approach.

Discussion: Fast identification of bacterial agents in positive blood cultures is vitally important for targeted therapy. This In-house

method enables species identification from positive blood cultures via MALDI ToF MS in a simple, fast and cost-efficient way, providing the opportunity of rapid treatment adaptation.

Fig. 1



Species* a) Result from Overnight subculture	Total Count	cut-off value Score ≥ 1,5					cut-off value Score ≥ 1,7					cut-off value Score ≥ 2,0				
		Correct identification (species level)		Correct identification (genus level)		Incorrect identification	Correct identification (species level)		Correct identification (genus level)		Incorrect identification	Correct identification (species level)		Correct identification (genus level)		Incorrect identification
Gram-positive bacteria	**952**	**526**	**(55,3%)**	**13**	**(1,4%)**	**14**	**(1,5%)**	**426**	**(44,7%)**	**4**	**(0,4%)**	**4**	**(0,4%)**	**186**	**(20,6%)**	**1**	**(0,1%)**	**2**	**(0,2%)**
Gram-positive cocci	**885**	**511**	**(57,7%)**	**10**	**(1,1%)**	**13**	**(1,5%)**	**412**	**(46,6%)**	**3**	**(0,8%)**	**4**	**(0,5%)**	**187**	**(21,1%)**	**1**	**(0,1%)**	**2**	**(0,2%)**
Staphylococci	664	389		7		11		309		2		3		128		0		2	
Staphylococcus aureus	172	122		0		1		103		0		0		60		0		0	
Coagulase-negative Staphylococci	**492**	**267**	**(54,3%)**	**7**	**(1,4%)**	**10**	**(2,0%)**	**206**	**(41,9%)**	**2**	**(0,4%)**	**3**	**(0,6%)**	**59**	**(12,0%)**	**0**	**(0,0%)**	**2**	**(0,4%)**
Enterococci	**71**	**50**	**(70,4%)**	**0**	**(0%)**	**0**	**(0,0%)**	**43**	**(60,6%)**	**0**	**(0,0%)**	**0**	**(0,0%)**	**29**	**(40,8%)**	**0**	**(0,0%)**	**0**	**(0,0%)**
Enterococcus faecalis	24	18		0		0		16		0		0		11		0		0	
Streptococci	**190**	**70**	**(53,8%)**	**3**	**(2,3%)**	**1**	**(0,8%)**	**58**	**(44,6%)**	**1**	**(0,8%)**	**0**	**(0,0%)**	**29**	**(22,3%)**	**1**	**(0,8%)**	**0**	**(0,0%)**
Beta-hemolytic streptococci	**57**	**37**	**(64,9%)**	**1**	**(1,8%)**	**1**	**(1,8%)**	**32**	**(56,1%)**	**0**	**(0,0%)**	**0**	**(0,0%)**	**16**	**(28,1%)**	**0**	**(0,0%)**	**0**	**(0,0%)**
Streptococcus pyogenes	27	18		0		1		25		0		0		4		0		0	
Viridans group streptococci	**79**	**33**	**(45,2%)**	**2**	**(2,7%)**	**0**	**(0,0%)**	**26**	**(35,6%)**	**1**	**(1,4%)**	**0**	**(0,0%)**	**13**	**(17,8%)**	**1**	**(1,4%)**	**0**	**(0,0%)**
Streptococcus pneumoniae	31	17		0		0		16		0		0		11		0		0	
Anaerobic Gram-positive cocci	**4**	**0**		**0**		**0**		**0**		**0**		**0**		**0**		**0**		**0**	
Gram-positive rods	**67**	**15**	**(22,4%)**	**3**	**(4,5%)**	**1**	**(1,5%)**	**14**	**(20,9%)**	**1**	**(1,5%)**	**0**	**(0,0%)**	**9**	**(13,4%)**	**0**	**(0,0%)**	**0**	**(0,0%)**
Gram-negative bacteria	**526**	**481**	**(91,4%)**	**3**	**(0,6%)**	**2**	**(0,4%)**	**468**	**(89,0%)**	**2**	**(0,4%)**	**1**	**(0,2%)**	**402**	**(76,4%)**	**2**	**(0,4%)**	**1**	**(0,2%)**
Enterobacteriaceae	**474**	**446**	**(94,1%)**	**3**	**(0,6%)**	**1**	**(0,2%)**	**435**	**(91,8%)**	**2**	**(0,4%)**	**1**	**(0,2%)**	**380**	**(80,2%)**	**2**	**(0,4%)**	**1**	**(0,2%)**
Escherichia coli	282	273		0		1		269		0		1		242		0		1	
Enterobacter cloacae complex	23	20		0		0		19		0		0		15		0		0	
Klebsiella aerogenes	6	5		0		0		4		0		0		2		0		0	
Klebsiella pneumoniae complex	93	85		3		0		82		2		0		71		2		0	
Klebsiella oxytoca	11	10		0		0		9		0		0		8		0		0	
Citrobacter koseri	6	5		0		0		4		0		0		3		0		0	
Citrobacter freundi	4	4		0		0		4		0		0		4		0		0	
Proteus mirabilis	17	16		0		0		16		0		0		14		0		0	
Serratia marcescens	17	17		0		0		17		0		0		13		0		0	
Salmonella sp.	7	6		0		0		6		0		0		5		0		0	
Haemophilus influenzae	12	4		0		1		4		0		0		3		0		0	
Neisseria meningitidis	2	2		0		0		2		0		0		2		0		0	
Anaerobic Gram-negative bacteria	**15**	**15**	**(100%)**	**0**	**(0%)**	**0**	**(0,0%)**	**12**	**(92,3%)**	**0**	**(0,0%)**	**0**	**(0,0%)**	**10**	**(76,9%)**	**0**	**(0,0%)**	**0**	**(0,0%)**
Bacteroides fragilis	9	9		0		0		9		0		0		7		0		0	
Bacteroides uniformis	2	2		0		0		2		0		0		2		0		0	
Yeasts	**32**	**0**		**0**		**2**		**0**		**0**		**0**		**0**		**0**		**0**	
Total	1511	1008	(66,7%)	16	(1,1%)	18	(1,2%)	895	(59,2%)	6	(0,4%)	5	(0,3%)	599	(39,6%)	3	(0,2%)	3	(0,2%)

DKMDVP 140

Cell counts, genome copy numbers or estimated genome equivalents – Introducing a new nanoreactor-based digital PCR for detection and absolute quantification of bacterial pathogens

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Introduction: The basis for determining the limit of detection (LOD) of different molecular diagnostic methods is the absolute quantification of bacteria and followed by a theoretical calculation of their genome equivalents (GE). This is usually done either by cultivation and subsequent cell counting or by molecular methods such as quantitative or digital PCR. Direct quantification by cultivation or cell counting is affected by a possible presence of cell conglomerates or of dead cells. Molecular methods usually calculate genome equivalents based on DNA concentration and genome size or determine the copy number of genetic markers. For this study, the correlations between cell numbers during different growth phases and genome copies in *E. coli* were investigated using a new bead-based digital PCR.

Methods: Growth kinetics was analysed using the *E. coli* strain Nissle 1917. Cell numbers were determined by OD600, plating and most probable number (MPN) of samples from different (lag, log and stationary) phases. To quantify genome copies, DNA was isolated by bead beating without further purification and directly bound to nanoreactor beads out of crude extract. The chromosomal species marker *gad* (glutamate decarboxylase gene) was used in a digital PCR using nanoreactor beads.

Results: Cell number determination from plating and estimation by MPN gave equivalent results. During the growth experiment, samples were taken from the culture at the lag phase, the exponential phase and the stationary phase for the isolation of genomic DNA. Based on the assumption that each cell contains at least one and two complete genomes, a theoretical number of GEs was calculated by multiplying the number of cells by 1.5. Preliminary analysis of the lag phase sample showed a 10-fold higher number of genomic copies per microlitre than the absolute number of cells in the sample and more than 6-fold higher than the calculated GEs.

Discussion: The study shows the relationship between cell number and genome copies and the influence of growth phases. The described method allows an absolute quantification of genome copies in a sample due to the high number of nanoreactor beads. The binding of DNA without any purification step out of a lysed sample reduces the bias of sample preparation in comparison to column-based systems. The results confirm the occurrence of more than one genome copy per cell. For DNA-based diagnostic tests, this method allow a more accurate LOD calculation in terms of copy number and ultimately pathogen number.

DKMDVP 141

Successful PCR-based dermatophyte identification at species level in PAS-positive formalin-fixed skin biopsies and nail samples – a pilot study

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Introduction: In suspicion of superficial mycosis of the skin a diagnosis is usually made directly by microscopy (KOH preparations) and by culture of the scales of the lesions (1). Occasionally, however, an unexpected fungus is only discovered after histological examination of a skin biopsy taken under another tentative diagnosis, using periodic acid-Schiff staining (PAS). In addition, nail material is often sent immediately for histological examination with PAS staining with the question "Onychomycosis?".

Species identification is not possible histomorphologically. Since accurate pathogen identification is important for treatment and PCR analysis does not require vital fungal elements, we wanted to investigate in a pilot study whether dermatophytes in formalin-fixed, paraffin-embedded skin and nail preparations can be identified "retrospectively" in species level by PCR.

Material/Methods: We analysed archived formalin-fixed skin biopsies with PAS-positive fungal elements as well as formalin-fixed nail material (PAS-positive and PAS-negative). DNA-extraction was performed in a standardized manner and was followed by PCR diagnostics with a commercial assay.

Results: In 18 of the 27 skin biopsies, a dermatophyte was detected by our PCR analysis; *Candida (C.) albicans* was found in two other samples and *Fusarium (F.) solani* in one sample. In 15 of the 18 dermatophyte-positive cases, the species was determined: 14 x *T.rubrum*, 1x *T.mentagrophytes.*

In 35 of the 36 PAS-positive nail samples examined, dermatophytes were detected by PCR. In all cases, the species was identified: 32 x *T. rubrum*, 2 x *T. interdigitale* and 1 x *T. interdigitale* + *T. rubrum*. For comparison, 9 PAS-negative nail samples were examined: only in one sample *T. rubrum* was detected with a copy number of 41000, the other 8 samples were negative.

Discussion: The results show that the identification of dermatophytes from PAS-positive skin biopsies and nail material is successful in many cases. Successful dermatophyte identification from formalin-fixed skin samples with laboratory PCR probes was already described by Eckert et al. in 2016 (2).

Outstanding results were achieved with nail material, where PCR identified dermatophytes at species-level in 35 of 36 PAS-positive samples.

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und interdisziplinärer Konsensus. Molecular diagnosis of skin infections using paraffi n-embedded tissue – review and interdisciplinary consensus. *J Dtsch Dermatol Ges* 2018;16:139-147

(2) Eckert JC , Ertas B , Falk TM et al. Species identification of dermatophytes in paraffin-embedded biopsies with a new polymerase chain reaction assay targeting the internal transcribed spacer 2 region and comparison with histopathological features. *Br J Dermatol*. 2016;174:869 – 77

DKMDVP 142

Accurate and rapid antibiotic susceptibility assessment using a machine learning-assisted nanomotion technology platform

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Background: Antimicrobial resistance (AMR) renders formerly curable infections life-threatening. Many multi-resistant bacteria can no longer be conventionally treated with antibiotics, contributing to the rising number of sepsis-related deaths. Besides finding novel and alternative therapeutics, accelerating antimicrobial diagnostics can lead to faster treatment decisions and more selective usage of antibiotics. In sepsis patients where less critical antibiotics can be administered, quick de-escalation from last-resort antibiotics helps to control the spread of AMR. Most phenotypic antibiotic susceptibility testing (AST) detect bacterial growth, making them reliable but often too slow.

Methods: We used the Resistell Phenotech to develop a fast growth-independent yet accurate AST based on the measurements of bacterial nanomotions. Nanomotions were recorded for four hours for the antibiotics ceftriaxone, cefotaxime, ciprofloxacin, and ceftazidime-avibactam. Each antibiotic was measured at one concentration on a comprehensive set of E. coli and K. pneumoniae clinical isolates. Recordings were subsequently analyzed and classified using assisted machine learning.

Results: Bacterial nanomotions alter depending on their phenotype upon exposure to an antibiotic. For resistant bacteria, we observed an increase in the signal intensity, whereas the signal for susceptible bacteria stagnated or decreased. The antibiotic responses varied among isolates, necessitating machine learning to extract signal parameters and develop accurate classification models. A handful of signal parameters from the nanomotion signal sufficed to classify all strains with an accuracy of 90-100 %. We achieved perfect separation of susceptible and resistant isolates with only one signal parameter in the case of ceftazidime-avibactam.

Conclusions: An accurate AST based on nanomotion technology is measured at a single antibiotic concentration and is completed after four hours, making it significantly faster than gold-standard tests in hospitals. It changes paradigms of assessing antibiotic susceptibility and could supplement current methods in the clinic, offering treatment options faster and containing the spread of AMR for critical drugs.

DKMDVP 143

Project Presentation: Use of thermal imaging for differential diagnosis of cervical lymphadenopathies

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Introduction: The purpose of this study is to evaluate the use of thermal imaging to differentiate cervical lymph node pathologies using an artificial intelligence algorithm. Infectious and malignant differential diagnoses are discriminated, with a particular focus on the diagnosis of extrapulmonary tuberculosis.

Material and Methods: The study is planned as a multicenter study in Germany, Uganda, and Bangladesh. There will be one study arm with subjects with cervical lymphadenopathies and one arm with subjects with clinically inconspicuous lymph nodes. Three standardized images of the cervical region will be obtained from each study subject. In addition, relevant anamnestic data such as personal data, medical history and, if possible, the results of other diagnostic tests will be collected to confirm the actual diagnosis. An artificial intelligence will then be trained on the dataset to evaluate and differentiate pathologies of the cervical lymph nodes. In lymph nodes suspicious for extrapulmonary tuberculosis, fine needle aspiration cytology will be performed, this will be examined with routine diagnostics (GeneXpert, microscopy, histology), and furthermore, the left-over aspirates will be analysed with a newly developed isothermal amplification method. Finally, the entire project will be evaluated from a health economics perspective.

Results: Since the project has just started, only sample images are currently available (Figures 1-2). However, preliminary results are expected by late summer 2023 and will be presented at the conference.

Discussion: Thermography has been used in other contexts for obtaining relevant image information easily, quickly and without radiation exposure. It is characterized by wide availability and the application does not require any relevant personal training in advance. It is already being used successfully in other areas of medicine to diagnose various diseases and their consequences, e.g. peripheral artery disease in diabetes patients.[1] Combined with artificial intelligence to analyse and evaluate the images, thermal imaging may prove to be a useful diagnostic tool.

In case of a successful evaluation of the use of thermal imaging to discriminate pathologies of the cervical lymph nodes, an early decision on the need for lymph node extirpation could be made, and follow-up examinations could be performed without cross-sectional imaging and with very low radiation exposure. In addition, thermal imaging combined with isothermal amplification could be used as a mobile tuberculosis laboratory in regions with poorer medical infrastructure to improve the diagnosis of extrapulmonary tuberculosis.

References

Gatt, A. et al. (2018) "Establishing differences in thermographic patterns between the various complications in diabetic foot disease", International Journal of Endocrinology, 2018.

Figure Legends:

Figure 1: Thermal image of patient with lymphadenopathy

Figure 2: Thermal image of healthy subject

Fig. 1

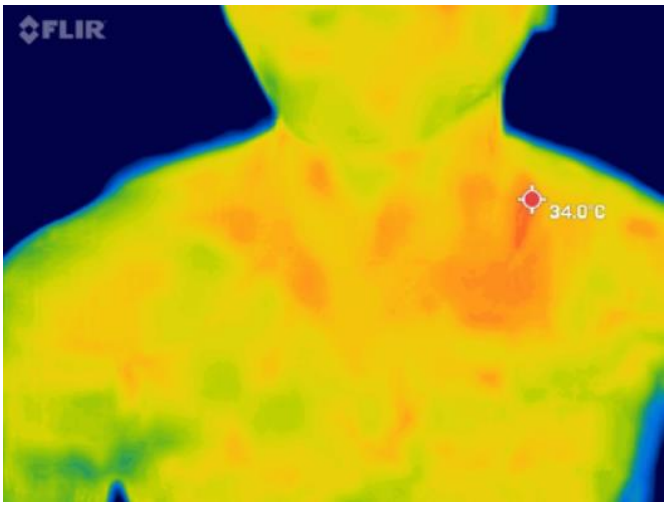
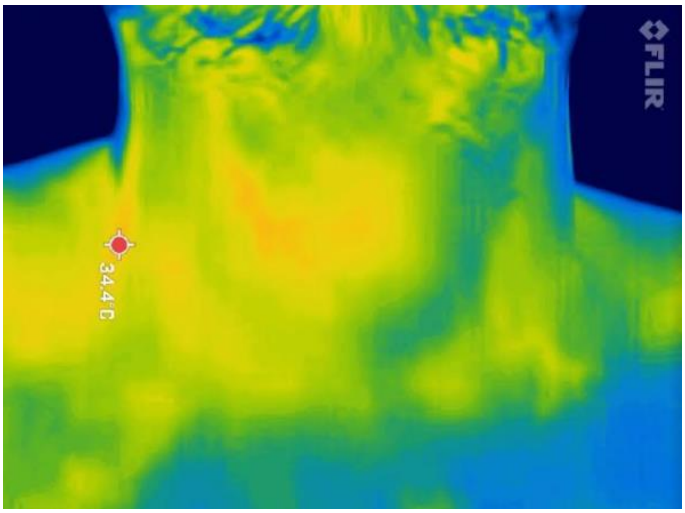


Fig. 2



DKMDVP 145

Performance of the Qvella FAST™ system together with MALDI-TOF, VITEK® 2 and the β-LACTA™ test for same-day identification and antimicrobial susceptibility testing of Gram-negative bacteria directly from positive blood cultures

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Introduction: Preparation of bacteria directly from positive blood cultures (BC) is used to shorten the time to identification (ID) and antimicrobial susceptibility testing (AST) of sepsis pathogens. The FAST™ system (Qvella Corporation Europe) automatically purifies a Liquid Colony™ (LC) from positive BCs for further applications. We aimed to test this system in combination with rapid ID, AST and phenotypic ESBL detection. The goal was to obtain all results within working hours (7:30 a.m. to 6:00 p.m.) on the day of BC positivity.

Methods: During five months, all BCs with Gram-negative rods that were reported positive by 12 o'clock were processed with the FAST™ system. The obtained LC was directly analyzed with MALDI-TOF (Bruker), VITEK® 2 (bioMérieux) and the β-LACTA™ test (Bio-Rad). The results were compared with MALDI-TOF and VITEK® 2 from short-term culture, and EUCAST rapid AST directly from positive BCs. AST results without categorical agreement (CA) were checked by broth microdilution (MERLIN Diagnostika).

Results: 119 positive BCs containing Gram-negative bacteria were processed. Ten BCs were excluded because of a mixed culture (n=6) or a FAST™ system failure (n=4; 3.4%) leaving 109 BCs for analysis. In total, 21 bacterial species were encountered, mostly *Escherichia coli* (56%). ID with MALDI-TOF from LC was correct at the species level in all cases (mean score 2.2; standard deviation 0.2). Most relevant antibiotics (piperacillin/tazobactam [TZP], ceftazidime [CAZ], meropenem [MEM], ciprofloxacin [CIP]) showed an essential agreement (EA) and CA above 96% and 97%, respectively. Only ampicillin/sulbactam (SAM) from LC failed to fulfill the FDA (<1.5%) and CLSI (<3%) requirements with a very major error (VME) rate of 3.3% (n=3). After six hours of incubation in the VITEK® 2, AST results were available for SAM (94.6%), CIP (91.8%) and CAZ (75.3%). In contrast, AST results of TZP and MEM were never available at that time-point. In 10.1% (n=11) of cases, complete AST from LC was available within working hours. The β-LACTA™ test from LCs correctly identified 14 (82.4%) of 17 isolates with resistance to 3rd generation cephalosporins within 15 min. EUCAST rapid AST from positive BCs allowed interpretation of MEM AST after six hours in all cases with very good CA (98.8%). In contrast, interpretation of TZP was rarely possible (26.3%) because most results were in the area of technical uncertainty.

Discussion: The FAST™ system provides a LC to speed up the laboratory workflow of positive BCs with minimal hands-on-time. The ID results were excellent and AST results were highly reliable. However, the goal of obtaining AST results on the same day was missed for the antibiotics most commonly used in Gram-negative sepsis, namely TZP and MEM. This may change if the working time is extended by 2 hours. A good method to determine resistance to 3rd generation cephalosporins from LC is the β-LACTA™ test.

EKP 146

Culture positive COVID-19-associated pulmonary aspergillosis (CAPA) in Germany: A retrospective evaluation of three tertiary care hospitals

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Introduction: Pulmonary aspergillosis is a feared complication in critically ill patients suffering from COVID-19 with an estimated CAPA prevalence of 0.7-7.7% and 2.5-39% in the ICU. Virus induced epithelial damage, interferon dysfunction and an impaired cellular defence may play major roles in its aetiology. Risk factors like COVID-19 therapy with corticosteroids or IL6 antagonists may further aggravate the clinical situation. Diagnosing CAPA remains difficult. Thus, we investigated the management of COVID-19 positive (ICU-) patients suffering from *Aspergillus* superinfection.

Material and Methods: We performed a retrospective analysis of clinical and microbiological findings focusing on three German

tertiary care hospitals during the COVID-19 pandemic in 2020 and 2021. Inclusion criteria were patients suffering from *Aspergillus* culture positive CAPA. The following criteria were subjects of query: Species, specimens, age, gender, underlying diseases, diagnostic methods, outcome and treatment. Data was processed with Excel and SPSS. Stat. significance was assessed with Fisher"s exact (FE) or chi-square test (Chi2).

Results: Within two years, 95 cases with a positive history of CAPA were observed in three hospitals. The prevalence of CAPA in COVID-positive patients was 0.84% (28/3349) for centre 1, 6.34% (50/790) for centre 2 and 2.07% (17/823) for centre 3. In the ICU, CAPA was more prevalent (centre 1: 3.39% (28/825); centre 2: 14.41% (50/347); centre 3: 4.63% (17/367)). *A. fumigatus* was the most abundant species (93%, 88/95). In 74 patients a galactomannan test (GM) was performed from pulmonal fluid, of which 79% (59/74) showed positivity. 83% of GM-positive patients (49/59, 83%) had high-level indices (>3). Nicotine abuse was reported in 17% (16/95). All patients received respiratory support with 52% (49/95) invasive mechanical ventilation and 48% (46/95) ECMO. Systemic glucocorticoid therapy was reported in 67 of 95 cases (71%), 15% (14/95) received monoclonal antibody therapy, 12% (11/95) received convalescent plasma, 40% (38/95) received antiviral therapy and 43% (41/95) were dialyzed. Antifungal therapy was administered as follows: 56% (53/95) received isavuconazole, 39% (37/95) echinocandins, 33% (31/95) voriconazole and 15% (14/95) amphotericin B. One patient each was treated with itraconazole or fluconazole.

CAPA had a fatal outcome in 60% (57/95) of cases. A significant association (p=0.015; Chi2) between GM indices >3 in BAL or TA fluid and mortality was observed. This was also found to be true for nicotine abuse and mortality (p=0.035; FE). Therapy with convalescent plasma, on the other hand, seemed to be associated with beneficial outcomes (p=0.024, FE).

Discussion: Our data reveal regional differences in prevalence, diagnosis, and treatment of CAPA. A high GM index in BAL or TA fluid, as well as nicotine abuse, was significantly associated with increased mortality.

EKP 147

Unlocking the immune enigma: Insights into Ebola virus persistence and recurrence

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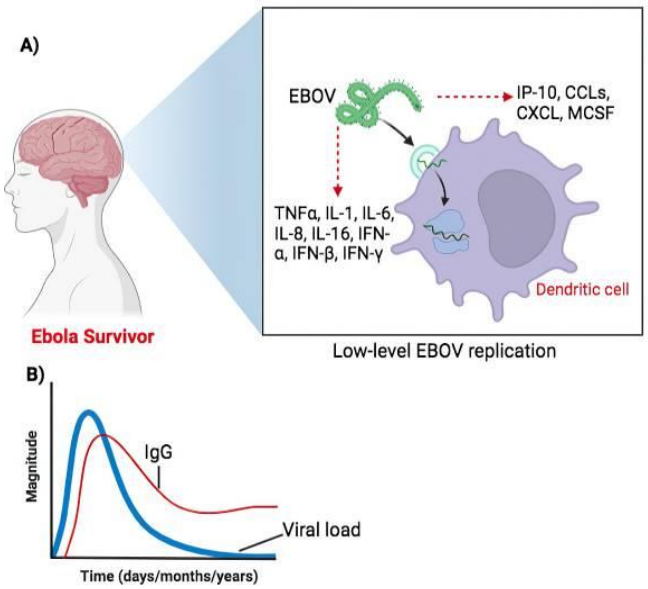
The immune response and immunological factors are pivotal in the pathogenesis and long-term outcomes of Ebola virus disease (EVD). However, the immunological mechanisms underlying post-EVD sequelae, including viral persistence and reactivation, remain poorly understood. This knowledge gap is crucial, especially considering the strategies employed by RNA viruses like Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

In this study, we explore the intricate interplay between the host innate and adaptive immune responses in EVD survivors, focusing on their implications for viral clearance and disease recurrence. Building upon our previous findings on post-EVD sequelae characteristics, we propose that the persistence and reactivation of the Ebola virus in survivors can be attributed to a complex interplay of virus and host mechanisms. Central to this process are extensive lymphocyte loss, deregulation of dendritic cell and T cell interactions, and potentially weaker inflammation profiles.

We hypothesize that a misbalance between immune cell surveillance and viral replication in infected tissues serves as a key factor in the occurrence of random disease recurrence. The expansion of viral replication may extend to other organs and tissues, ultimately establishing chronic EVD.

Understanding these intricate immunological factors is crucial for the development of targeted interventions aimed at enhancing viral clearance and preventing disease recurrence, which have been identified as triggers for recent Ebola outbreaks. Insights from our current findings contributes to a more comprehensive understanding of the immunopathogenesis of EVD and facilitate the development of effective strategies to control and mitigate the impact of the disease.

Fig. 1



EKP 148

Characterization of *Candida albicans* adaptation to inflammatory environments

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Introduction: *Candida albicans* is a commensal yeast of the normal human mycobiome. Yet, under certain circumstances it can become an opportunistic pathogen that can cause life-threatening infections in immunocompromised hosts. Particularly, the innate immune system is an important first line of defense against *C. albicans*. During the course of an infection, *C. albicans* is exposed to various cells of the innate immune response, first tissue resident macrophages, later recruited immune cells like neutrophils and monocytes, who all contribute to inflammation and aim at efficiently clearing the infection. However, commensal co-existence with the human host has set the stage for adaptation of both organisms to each other. Therefore, we hypothesized that *C. albicans* has evolved mechanisms to sense activation of inflammatory responses and engages a tailored immune-evasion program to secure its persistence.

Material/Methods: To set up an *in vitro* inflammation model for studying adaptations of *C. albicans* to this process, we isolated and/or differentiated an array of human immune cells and activated those with diverse infection-relevant stimuli, like inactivated morphotypes of *C. albicans* itself. The resulting supernatants (SN)s of those stimulations were used to simulate an inflammatory environment to grow *C. albicans* in. Thus, we screened the the SNs from neutrophils, Peripheral blood-mononuclear cells (PBMCs), and diversely differentiated moncyte-derived macrophages (MDMs) for their capacity to generate inflammatory envirenments that modulate growth and stress tolerance in *C. albicans*.

Results: The SNs from stimulated PBMCs with inactivated *Candida* morphotypes only moderately affected *C. albicans.* Interestingly, supernatants of yeast-stimulated neutrophils provided an environment, that boosted growth and resilience of *C. albicans* compared to the unstimulated control. Similarly, we observed that SNs of hyphae-stimulated M-CSF-differentiated macrophages supported growth and stress resistance of *C. albicans*, while this could not be accomplished with either using GM-CSF-differentiated macrophages instead or by stimulating M-CSF-differentiated macrophages with other stimuli like LPS or Pam3CSK4.

Discussion: Our results indicate that specific activation of innate immune cells leads to generation of inflammatory environments that unexpectedly can have protective properties for the opportunistic pathogen *C. albicans*. To understand the molecular underlying mechanism within the pathogen, we are currently working on generating a transcriptome dataset of the inflammation-adapted fungus. This will hopefully provide us with pathways responsible for adaptation to host inflammation, which might reveal novel targets for antifungal therapy.

EKP 149

Host albumin drives *Candida*-induced hyperinflammation underlying the pathogenesis of vulvovaginal candidiasis

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Vulvovaginal candidiasis is characterized by symptoms that are driven by inflammatory responses. The inflammatory response during vulvovaginal candidiasis is initiated by fungal pathogenicity mechanisms resulting in tissue damage. However, the persistence of inflammation and symptoms results from inefficient neutrophil-mediated fungal clearance leading collateral damage and the induction of immunopathology. It has become increasingly apparent that host-derived factors play crucial roles in the pathogenesis of vulvovaginal candidiasis and the associated dysfunctional inflammatory response. We investigated the impact of albumin, one of the most abundant proteins present in vaginal fluid, on fungal pathogenicity mechanisms as well as the induction of inflammatory responses.

In vaginal fluids from VVC patients we observed correlations between proinflammatory cytokines and albumin levels. To dissect this connection, we investigated the impact of albumin on *Candida albicans*-induced inflammatory responses relevant to VVC pathogenesis. We observed that albumin potentiates *C. albicans*-induced NLRP3 inflammasome activation, a crucial process driving hyperinflammatory responses during VVC. We observed that this increased inflammasome activation could be pinpointed to adaptations in the fungus to the presence of this host protein, specifically binding of albumin to the fungal surface adhesin Agglutinin-like protein 3 and increased secreted aspartic protease 1 expression. Using a murine VVC model we showed that the effects of albumin and associated inflammatory responses drive severity of the infection.

Collectively these data suggest host albumin as a major driver of the inflammatory responses that crucially underlie the pathogenesis of vulvovaginal candidiasis.

EKP 150

***In vitro* testing of new antifungals: Ibrexafungerp susceptibility in echinocandin-resistant clinical *Candida* species.**

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Introduction: Invasive *Candida* infections are a threat to vulnerable patients suffering from critical illness or immunosuppression. One of the first-line choices in treating these severe conditions are echinocandins. Resistances to echinocandins following therapeutic failure are mainly mediated by hotspot (HS) mutations of the *Fks*-gene, encoding the β-D-glucan synthase. In recent years, a rise of such phenotypes can be noticed. That is why, new treatment strategies comprising new substances -like the first-in-class antifungal ibrexafungerp (IBX)- are urgently needed. We challenged a library of 192 pheno-/ and genotypic echinocandin resistant *Candida* isolates assessing IBX susceptibility by broth microdilution according to EUCAST (BM).

Materials & Methods: The German National Centre for Invasive Fungal Infections (NRZMyk) provided strains and patient data. Species ID was confirmed by ITS sequencing. Anidulafungin (AND) and IBX BM was conducted in accordance with EUCAST.

Results: 192 invasive *Candida* isolates were tested from 184 patients. The sex ratio was computed to 56% : 44% (m : f). The majority of patients (83%) were between 41 and 80 years of age. Strains largely derived from abdominal specimen (39%) and blood-/CVC-tip culture (35%). Most prevalent species were *C. glabrata* (112/192) and *C. albicans* (63/192). 76% (145/192) of all relevant point mutations were detected both in positions F659 and S663 (43x / 48x in *C. glabrata*) and in F641 and S645 (15x / 39x in *C. albicans*). Mutations at the beginning of HS were associated with higher IBX MIC-values (F659 & F641 (MIC 50/90 in mg/l): >4/>4 & 2/4) in comparison to AND (F659 & F641 (MIC 50/90 in mg/l): 1/4 & 0.25/1). However, in downstream mutations MIC-values were almost equal for both substances (MIC50/90 values in mg/l for S663: 2/4 (AND & IBX); for S645: 0.5/1 (AND) and 0.25/1 (IBX)). Applying wt UL values for IBX, 39 of 145 echinocandin-resistant isolates could be reclassified as IBX wildtype.

Conclusion: IBX shows *in vitro* activity against echinocandin resistant *Candida* and thus is a valuable addition to the antifungal armory. However, our data suggest that this effect is more pronounced in strains harboring mutations, which affect the center of HS. *In vivo* data may help to further characterize resistant phenotyps.

EKP 151

Structure-function relationships underpin disulfide loop cleavage-dependent activation of *Legionella pneumophila* lysophosholipase A PlaA

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Introduction: *Legionella pneumophila*, the causative agent of a life-threatening pneumonia, intracellularly replicates in a specialized compartment in lung macrophages, the *Legionella*-containing vacuole (LCV). Secreted proteins of the pathogen govern important steps in the intracellular life cycle including bacterial egress. Among these is the type II secreted PlaA which, together with PlaC and PlaD, belongs to the GDSL phospholipase family found in *L. pneumophila*. Additionally, PlaA was previously described as a factor promoting bacterial exit in the absence of the type IVB-secreted effector SdhA. We here focus on the characterization of the phospholipase PlaA, investigate the mode of activation mechanism, and 3D structure of PlaA.

Materials and methods: 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: PlaA shows lysophospholipase A (LPLA) activity which increases after secretion and subsequent processing by the zinc metalloproteinase ProA at residue E266/L267 located within a disulfide loop. The 3D structure of PlaA shows a typical α/β hydrolase fold and reveals that the uncleaved disulfide loop forms a lid structure covering the catalytic triad S30/D278/H282. This leads to blockage of both substrate access and membrane interaction before activation; however, the catalytic and membrane interaction site gets accessible when the disulfide loop is processed. After structural modelling, a similar activation process is suggested for the GDSL hydrolase PlaC, but not for PlaD. Furthermore, the size of the PlaA substrate binding site indicated preference towards phospholipids comprising ~16 carbon fatty acid residues which was indeed verified by lipid hydrolysis, suggesting a molecular ruler mechanism.

Conclusion: Our analysis revealed the structural basis for the regulated activation and substrate preference of PlaA and suggests that protein activation of a potentially lytic enzyme may be important to maintain bacterial integrity.

EKP 152

The opportunistic pathogen *Candida albicans* takes advantage of host inflammation

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Introduction: *Candida albicans* is a common commensal fungus in humans, but during immunosuppressive therapy or dysbiosis of the microbiome it can cause life-threatening infections. The commensal-to-pathogen switch during invasion of the host tissue exposes the fungus suddenly to new host niches and promptly changing environments, driven by inflammatory responses. To persist, the fungus has to rapidly sense and integrate signals from its changing environment to appropriately adapt.

Material and Methods: To study how *C. albicans* can accomplish adaptation in the hostile surroundings of an inflammatory niche, we established an *in vitro* inflammation model. We stimulate human immune cells with diverse stimuli and use the supernatants as surrogate for an inflammatory environment, wherein we grow *C. albicans* and analyze changes in its pathogenic potential and

stress resistance. We further applied live cell imaging to dynamically record the immune cell stimulation process in real time and used proteomics to characterize the composition of supernatants in depth.

Results: Specifically, supernatants of macrophages, which were stimulated with fungal hyphae, induced stress resistance in viable *C. albicans*. Furthermore, this environment can train a commensal *C. albicans* strain to increase its escape from macrophages. Surprisingly, we observed that during supernatant generation, inactivated fungal hyphae strongly elicit inflammatory cell death of macrophages. Performing proteomics on the macrophage supernatants however revealed a distinct profile from macrophages lysed by freeze-thawing. These lysates were interestingly not sufficient to grant the same stress benefits for *C. albicans*, hinting at specific properties of the hyphal-triggered inflammatory environment. We are currently aiming at identifying single proteins released from macrophages after hyphal stimulation, that promote fungal resilience.

Discussion: Our findings demonstrate that an inflammatory environment counterintuitively can become an asset for an opportunistic pathogen to withstand physiologically relevant stresses and the encounter with immune cells. Identification of single host proteins that regulate this adaptation might help to establish new diagnostic biomarkers or targets for host-directed therapies, which could support the difficult treatment of invasive fungal infections in the future.

EKP 153

Systemic screening of *Candida albicans* protein kinases in infection and colonisation

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Introduction: The opportunistic pathogenic yeast *Candida albicans* exists as a commensal of the intestinal mycobiota. Dysbiosis of the intestinal microbiota can initiate *C. albicans* overgrowth – a major predisposing factor for disseminated candidiasis. Commensal bacteria such as *Lactobacillus rhamnosus* antagonise *C. albicans* pathogenicity. We are interested in the signalling pathways regulating *C. albicans* commensalism and pathogenicity. Virtually all signal transduction pathways that enable the adaptation of *C. albicans* are regulated by protein kinases. From its genome, *C. albicans* was predicted to have 108 protein kinases of which nearly 50% remain uncharacterised. Only a small subset of protein kinases has been investigated for their role during interactions with host cells. The aim of this study is to gain a broad insight into the role of *C. albicans* protein kinases during infection and commensalism.

Materials and Methods: To investigate both, commensal and pathogenic states of *C. albicans*, *in vitro* infection models monitoring the interaction of *C. albicans* and intestinal epithelial cells (IECs) with or without *L. rhamnosus* were used. A deletion mutant library of all 108 protein kinases was screened during infection of IECs in the absence or presence of pathogenicity antagonising *L. rhamnosus*. The ability of each mutant to damage the host tissue was quantified by measuring the activity of cytoplasmatic lactate dehydrogenase in the supernatant. In addition, existing transcriptome datasets of *C. albicans*-IEC interaction with or without *L. rhamnosus* were re-analysed for protein kinases expression.

Results and Discussion: Around 40% of all protein kinases genes showed differential expression during infection, whereas around 25% of all protein kinases genes showed changed expression when infecting *L. rhmanosus* colonised IECs. The cytotoxicity screen revealed around 30% of all kinase deletion mutants showing differential damage compared to the wild-type (12 increased damage and 28 decreased damage), indicating a role of the deleted kinases in pathogenicity and anti-virulence. In addition, 20% of kinase deficient strains showed increased susceptibility to antagonistic effects of *L. rhamnosus* against *C. albicans*-induced damage. The deleted kinases in these mutants may thus be important for *C. albicans* to support commensalism. As filamentation is the major driver of *C. albicans*-induced damage, mutants with altered damage on IEC with or without bacteria will be investigated. Further virulence attributes such as adhesion, invasion and translocation will be assessed. As fungal metabolic alterations are vital for *L. rhamnosus*-induced commensal behaviour, mutants with reduced damage potential when infecting *L. rhamnosus* colonised IECs may play important roles in metabolic adaptation.

Collectively, several protein kinases play crucial roles during infection whereas others play important roles during interaction with commensal bacteria.

MSP 154

Molecular surveillance of multidrug-resistant gram-negative bacteria in Ukrainian patients

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Background: Since the beginning of the Russo-Ukrainian War in February 2022, Ukrainians have been seeking for shelter in other European countries. Aim: This study investigates the prevalence and the molecular epidemiology of multidrug-resistant gram-negative bacteria (MDRGN) and methicillin-resistant *Staphylococcus aureus* (MRSA) in Ukrainian patients at admittance to University Hospital Frankfurt, Germany (UHF). Methods: Screening and observational analysis of n=103 patients within March and June 2022. Genomes of MDRGN-isolates were analyzed for antimicrobial resistance genes and phylogenetic relatedness. Results: Overall, n=34 MDRGN-isolates were found in n=17/103 patients (16.5%; 95% confidence interval = 9.9 – 25.1) of which n=21 MDRGN-isolates with carbapenem-resistant (CR) were found in ten patients. Of these, five patients were positive for >1 MDRGN+CR-strain. In total, eight MDRGN+CR isolates were found in four of six patients with war-related injuries. In n=6/10 patients with MDRGN+CR the respective strain was found in invasive materials (*e.g.* from surgical site). Genomic characterization of these MDRGN+CR revealed that these isolates harbored at least one carbapenemase gene, with six isolates harboring two carbapenemase genes. In total, these genes were *bla*NDM-1 (n=10), *bla*OXA-48 (n=4), *bla*OXA-72 (n=4); *bla*NDM-5 (n=3), *bla*OXA-23 (n=2), *bla*IMP-34 (n=1), *bla*KPC-3 (n=1) and *bla*OXA-244 (n=1). Additionally, *bla*GES-1 was identified in one *Pseudomonas aeruginosa* isolate. Core genome and plasmid analysis of isolates revealed no broad epidemiological connection between most of these isolates. No MRSA was found. Conclusion: Prevalence of carbapenem-resistant gram-negative bacteria in Ukrainian patients with war-related injuries and/or hospital pre-treatment is high. This has enormous impact on infection prevention and control strategies.

MSP 155

Hypervirulent *Klebsiella pneumoniae* isolates in a German tertiary hospital over 17 months – prevalence, resistance and genomic diversity

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Objective: Hypervirulent *Klebsiella pneumoniae* (hvKp) are spreading worldwide. They can cause invasive infections, e.g. pyogenic liver abscesses (PLA), in healthy patients of all ages, also in outpatient settings. The occurrence of hvKp seem to correlate with hypermucoviscous phenotype. The aim of this study was (i) to investigate the occurrence of putative hypervirulent and hypermucoviscous *K. pneumoniae* (hmKp) at a tertiary hospital, (ii) to analyze the corresponding patient data for associations and risk factors, (iii) to use whole genome-sequencing for in-depth analyses of isolates obtained from PLA cases.

Methods: For the study period of 17 months, all *K. pneumoniae* isolates from various clinical specimens were tested for hmKp phenotype by applying the string-testing. All isolates were tested for antibiotic susceptibilities. A multiplex PCR for common genes (*magA*, *iutA*, *rmpA* and *rmpA2*) associated with hmKp and hvKp was applied to all hmKp isolates. A *K. pneumoniae* isolate with a positive string-test and detection of the aforementioned genes was defined as "putative hvKp". Further, all isolates from patients with liver abscesses were added to the collection, regardless of hmKp phenotype. These were subjected to WGS (Illumina) to determine cgMLST and phylogeny. Subsequently, the Kleborate online tool was applied to screen for known hvKp-genes not included in the first screening PCR. Anonymized patient data including age, sex and sampling were overlaid with the micro-and molecular biological results.

Results: A total of 10.9% (331/3044) isolates with hmKp phenotype were detected. Among these, 67% were isolated from screening (urine and various swabs), while 33% were collected from invasive material (blood, punctures and BAL). The patients" age range was from 0 to 95 years, with a mean of 69 years. At 72%, the majority of isolates were susceptible to all antibiotics tested, 7% showed multi-drug resistance, including (1.2%) resistant phenotype to carbapenems. 13.3% (44/331) isolates was tested positive by PCR for genes associated with hvKp. cgMLST revealed most sequenced isolates belonged to international hvKp clonal lineages ST23, showing close phylogenetic relationships. Isolates of four cases of liver abscesses also belonged to ST23, ST25 and ST268, but without phylogenetic relatedness to other isolates.

Conclusions: Hypervirulent and hypermucoviscous *K. pneumoniae* were successfully identified within the study period with a prevalence of 1.4%. Some isolates showed multidrug resistances. Amongst the identified putative hvKp, no transmissions were identified, but isolates were assigned to the international hvKp lineage ST23, bearing the potential to further spread. Presented cases of liver abscesses seem to represent individual occurrences, so far.

MSP 156

Effect of mutations in the *fexA* resistance gene on the functionality and structure of the phenicol exporter FexA

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Introduction: The prototype *fexA* gene confers combined resistance to chloramphenicol and florfenicol. Since it was first described, however, *fexA* variants that only confer resistance to chloramphenicol have also been identified. This was also the case in a methicillin-resistant *Staphylococcus aureus* isolate obtained from poultry meat illegally imported into Germany. The *fexA* sequence of this isolate contained four mutations resulting in amino acid substitutions, compared to the *fexA* prototype. The functional effects of each mutation were investigated in this study.

Methods: Eleven *fexA* variants were generated by on-chip gene synthesis and targeted mutagenesis. This included the *fexA* prototype as well as *fexA* variants that had the previously described mutations either alone or in combinations. The synthesized constructs were integrated into a shuttle vector and transformed into three recipient strains of different species (*E. coli*, *S. aureus*, *Salmonella* Typhimurium). Subsequently, minimum inhibitory concentrations (MIC) of florfenicol and chloramphenicol were tested by broth macrodilution assays according to CLSI protocols. In addition, virtual protein models were created to visualize the structural effects of the mutations on the FexA protein.

Results: The results showed that the presence of a C110T and/or G98C mutation resulted in lower florfenicol MIC values. The effect was seen in all recipients. In *E. coli* and *S. aureus*, transformants with a C110T mutation showed the same florfenicol MIC values as the empty recipients. For G98C, an increase of a single dilution step was observed. As the *Salmonella* recipient strain used in this study was an *acrB* mutant with a deficient multidrug efflux system, it had a lower florfenicol MIC (1 µg/ml) than the other two recipients (4 µg/ml). In this strain, an increase of MIC to 2 µg/ml was still observed even in the presence of the C110T mutation. The generated protein models indicated a substitution by bulkier amino acids due to these mutations in the substrate binding site of the FexA variant. The remaining two mutations, A391G and C961A, had no effect on florfenicol sensitivity.

Discussion: Our study demonstrated that the C110T and G98C mutation impair the resistance-mediating properties of *fexA*, likely due to bulkier amino acids physically preventing florfenicol from being exported. Identical and similar variants of *fexA* as detected in our *S. aureus* isolate have been described recently. The same variant as in our isolate has been linked to a resurgence of chloramphenicol resistant MRSA in hospitals in Kuwait[1]. A reversion of the mutations and a restoration of florfenicol resistance is conceivable in the presence of florfenicol.

Reference: [1]Udo, E. E., et al. (2021). Resurgence of Chloramphenicol Resistance in Methicillin-Resistant *Staphylococcus aureus* Due to the Acquisition of a Variant Florfenicol Exporter (*fexAv*)-Mediated Chloramphenicol Resistance in Kuwait Hospitals. Antibiotics (Basel) **10**(10).

MSP 157

Colistin resistance and molecular epidemiology of multidrug resistant *K. pneumoniae* from ICUs in Vietnam

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Background: Emerging resistance to broad-spectrum antibiotics such as 3rd generation cephalosporins and carbapenems is a major issue in gram-negative bacteria worldwide. This often leaves few therapeutic options, one of them colistin. As the prevalence of colistin resistance is rising in regions where colistin is heavily used in animal husbandry and medical settings, reliable detection of colistin resistance is essential for initiating proper antibiotic therapy. The broth microdilution as the gold standard of susceptibility testing for colistin is time-consuming, expensive and requires trained laboratory staff. Therefore, having accurate and fast phenotypic testing and/ or genetic markers to predict phenotypic resistance would lead to better treatment of patients in high-prevalence settings. We aim to find genetic markers that can predict phenotypic resistance to colistin in *K. pneumoniae.*

Material/Methods: 112 specimens were collected from a hospital in Vietnam. Species identification was confirmed to be *K. pneumoniae* by MALDI TOF MS. Susceptibility testing was performed by broth microdilution with the Thermo Fisher Sensititre panel and with semiautomated BioMérieux vitek2 and AST-XN24. All Isolates were sequenced by Illumina MiSeq and Oxford Nanopore technology for hybrid assembly.

Results: About one third (43/112) of the *K. pneumoniae* isolates were phenotypically resistant towards colistin in the gold standard broth microdilution. The semiautomated susceptibility test achieved nearly the same results with only one false resistant isolate. Sequencing data showed that 77% of the isolates belonged to three sequence types, the predominant MLST being ST16 (70/112) followed by ST11 (9/112) and ST15 (8/112). We could identify the colisitin resistance mechanisms in 26 of 43 phenotypically resistant isolates, 23 with *mgrB* mutation and 3 with *mcr1*. No known resistance mechanisms were detected in any of the phenotypically susceptible strains. An in-depth data analysis for other potential mechanisms in the resistant strains is ongoing.

Discussion: Our results show that the colistin resistance determinants are heterogeneous in this study population, indicating no suitable genetic marker to predict phenotypic resistance in all colistin-resistant isolates. Therefore, reliable phenotypic susceptibility testing is still essential to identify phenotypic colistin resistance in *K. pneumoniae* in *mgrB* and *mcr* negative isolates. Our data suggested that the Vitek AST-XN24 could be a time and cost-effective alternative to broth microdilution. Our finding should be validated using other gram-negative bacteria. Further investigations are needed to study the genotypic underlying colistin resistance mechanisms.

MSP 158

Antibiotic resistance in *Enterococcus faecium* in Bavaria compared to Germany and Europe 2020-2022

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Introduction: Antibiotic-resistant pathogens are a serious medical issue of global importance. Continuous surveillance of the occurrence of antibiotic-resistant bacteria shall provide reliable data on the spread of antibiotic resistance and help to identify trends at an early stage. For this reason, the Bavarian Government decided to establish a Bavarian Antibiotic Resistance Database (BARDa) in 2017.

Material/method: Since 2020 to 2022 24 to 27 laboratories evenly distributed over Bavaria have been participating voluntarily in BARDa. They evaluate the results of antibiotic resistance testing according to the EUCAST standard and submit them anonymised electronically as SIR assessments to BARDa. For each patient, only the first isolate is included in the evaluation for a period of 90 days. Screening samples are excluded. Confidence intervals are calculated using Wilson score method. The resistance data of 11 bacterial pathogens in the in- and outpatient sectors of human medical care are evaluated and compared to other German (ARS by RKI, ARMIN by lower Saxony) and European (EARS-NET by ECDC) surveillance systems. For the latter, data for 2022 are not yet available.

Results: From 2020 to 2022 1,309,402 isolates were included in the BARDa reports with *Escherichia coli (*38.5%), *Staphylococcus aureus* (15.5%), and *Enterococcus faecalis* (14.9%) as the most prevalent species. *Enterococcus faecium* isolates have a share of 2.9%.

The resistance rates of *Enterococcus faecium* against ampicillin and imipenem are at a high level (approximately 90%). For gentamicin 500 (high level), a significant increase of resistant isolates to 26.5% is displayed in 2022 in BARDa compared to previous years. For ARMIN and ARS (blood cultures only), resistance is at a higher level (42.2% and 57.4% in 2021, respectively). Vancomycin and teicoplanin show a significant decrease in BARDa in 2022 compared to previous years to 24.0% and 12.4%, respectively. However, even in 2021, both levels are greater than in ARS and ARMIN. In general, German resistance values to vancomycin are above the European average (17.2% in 2021), with an upward trend. In detail, the highest resistance values for vancomycin in Bavaria in 2022 are found in intensive care units (26.7%) followed by nursing wards (25.7%) and outpatient hospitals (22.0%) with a declining trend from 2020 to 2022.

Discussion: Only *Enterococcus faecium* resistance rates are described here, as these are higher for vancomycin in Bavaria and Germany than the European average. For *Enterococcus faecium* a North-South and West-East gradient is evident in Europe for vancomycin resistance. In Bavaria, this resistance situation appears to be more strained than in Northern Germany. Enterococcal isolates in Bavaria are differentiated to species level, so that isolates from all materials can be included in the evaluation and thus differences in specific areas of human medical care can be shown.

Fig. 1

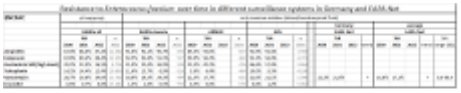
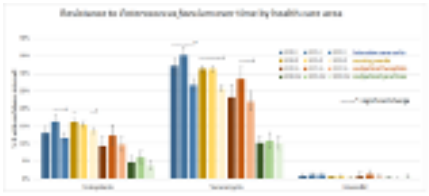


Fig. 2



MSP 159

In-patient diversity and environmental spread of intestinal vancomycin-resistant enterococci in haematological and oncological patients

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Background: Understanding vancomycin-resistant *Enterococcus faecium* (VREfm) dynamics in haematological and oncological patients is crucial for choosing optimal diagnostic strategies and designing preventive interventions.

Methods: We performed a prospective cohort study at four German haematology/oncology departments throughout 2019-2022. Study patients were screened for VREfm on admission and weekly thereafter by rectal swabs. Repetitive environmental sampling was performed in patients with VREfm colonization detected on admission. From a subset of VREfm positive patients, 10 colonies per sample were selected for further characterisation using whole genome sequencing (WGS, Illumina)) and core-genome multi-locus sequence typing (cgMLST, Ridom SeqSphere+) to assess clonal relatedness. Microbiome analysis was performed by 16S rRNA gene profiling on rectal swabs from patients with VREfm.

Results: From 162 patients, 18 were VREfm positive at baseline. Among 144 initially VREfm negative patients, in-hospital VREfm acquisition was observed in 44 patients (30.6%). WGS of multiple isolates per sample were performed for a subset of 33 patients with VREfm acquisition and 5 patients with positivity at baseline. Of note, two distinct VREfm clusters (>200 alleles different) simultaneously present in the same sample were detected in 7 of these 38 patients (18.4%).

Out of 75 environmental sample sets collected from 18 patients, 26 were VREfm positive with the bed control being the most frequently contaminated spot. Microbiome analysis of corresponding patient rectal swabs revealed a higher mean abundance of enterococci present in patients shortly before or after detection of environmental contamination (mean 33.7%) as compared to patient samples without corresponding environmental detection of VREfm (mean 13.7%) even though this was not statistically significant, (p=0.243).

Conclusions: In this multicentre cohort study, VREfm detection in the environment was associated with increased enterococcal abundance in the intestinal microbiota. Furthermore, WGS revealed a high frequency of in-patient diversity of VREfm, which has substantial consequences for future infection control and eradication strategies.

MSP 160

Evaluation of FT-IRS compared to cgMLST for assessing strain-relatedness of vancomycin-resistant *Enterococcus faecium*

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Introduction: Vancomycin-resistant *Enterococcus faecium* (VREfm) are increasingly isolated in the hospital setting, particularly in vulnerable patient groups, where they cause a variety of infections. Core-genome multi-locus sequence typing (cgMLST) has proven invaluable in typing VREfm and to determine bacterial transmissions, however, the time to result leads to several days delay. Fourier Transform-Infrared spectroscopy (FT-IRS) is a phenotypical method that has been demonstrated to show a high degree of typing resolution in different hospital associated pathogens within two hours. The aim of this study was to compare cgMLST clustering of VREfm against FT-IRS to determine its usefulness as a rapid screen to rule in/out epidemiological relatedness of bacterial isolates prior to cgMLST analysis.

Material/method: Eighteen VREfm from colonized patients and their environment were selected for this study. Isolates were previously sequenced (Illumina) and analysed by cgMLST (Ridom SeqSphere+). Three clusters of three related isolates, and 9 singletons were included (Figure 1). FT-IR analysis was performed using the IR Biotyper® system (Bruker Daltonics, Germany). Spectra were acquired from dried spots of bacterial suspensions in ethanol solution, in four technical replicates of three independent cultures on different days. Exploratory data analysis was performed by Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and Hierarchical cluster analysis (HCA), using the IR Biotyper software V4.0.

Results: The results are summarized in Figures 1 and 2. cgMLST clustering was mirrored in FT-IRS based clustering, with a few minor differences. The cgMLST cluster A included patient isolate Koe-A185 and environmental isolate Koe-A325 which were identical, while Koe-A182 from the same patient was 6 alleles different. However, by FT-IRS the patient isolates clustered closer. cgMLST clusters B and C were also mirrored in FT-IRS. A 4th cluster (cluster D) was seen in FT-IRS which was not reflected in cgMLST data where there were 17 allelic differences.

Discussion: These data suggest that FT-IRS has the potential to rule-in/rule-out clonality, yields a quick answer to the infection control team regarding the potential transmission of VREfm, and can be utilized as a pre-screen before sequencing. Future studies including more isolates are necessary to confirm these findings and to determine the optimum threshold for calling a potential transmission using FT-IRS.

Fig. 1

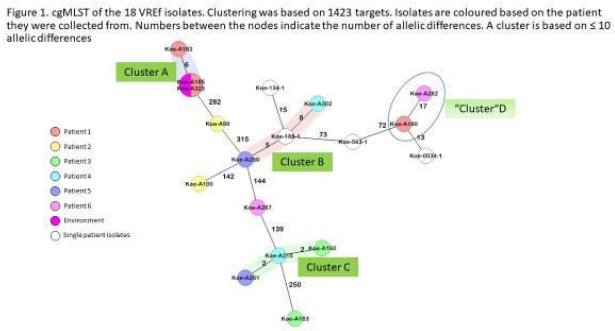
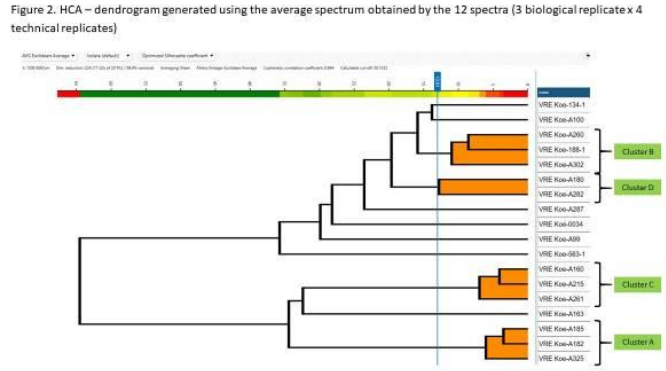


Fig. 2



MSP 161

The antifungal drug luliconazole inhibits the growth and biofilm production of azole-resistant *Aspergillus fumigatus* in *ex vivo* and *in vivo* infection models

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*Reports on i*nvasive fungal infections with azole-resistant *A. fumigatus* isolates (ARAF) increased recently. These infections have become a significant threat in clinical settings. Another factor known contributing to increased resistances is the biofilm formation capability of *A. fumigatus*. Developing novel strategies for the treatment of infections with ARAF has become crucial. Here, the activity of the imidazole luliconazole (LLCZ) against ARAF has been tested in novel *in vivo* and *ex vivo* models.

A *Galleria mellonella* infection model was used for *in vivo* treatment assays. To further demonstrate the anti-biofilm effect of LLCZ in conditions that simulate the human organism, models such as an *ex vivo* model with murine precision cut lung slices (PCLS) and a biofilm co-culture model with human epithelial cells (A549) have been used. The metabolic activity of the formed biofilm was quantified via an XTT assay in these models. The anti-biofilm of LLCZ effect was visualized via confocal laser scanning microscopy.

A single-dose LLCZ treatment of the *G. mellonella* larvae increased the survival of the treated larvae by 20 % over a 7-day infection period. The biofilm formation was decreased by roughly 50 % in the *ex vivo* PCLS model. LLCZ showed a strong inhibition of immature (2 h) biofilm (80 %) in the co-culture model, while mature biofilm (24 h) was not significantly inhibited (12 %).

This study is the first demonstrating the effect of LLCZ against ARAF infection and biofilm formation *in vivo* and demonstrates the efficiency of the drug in human-like conditions.

MSP 162

Whole genome sequence analysis of clinical *Escherichia coli* isolates with resistance to third generation cephalosporins from patients in hospitals and clinical practices in community medicine in Germany

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Introduction: *Escherichia coli* is the leading cause of community acquired urinary tract infections and multidrug-resistant strains pose a threat to hospitalised patients. Production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases is the main cause of these resistances that emerged in different clonal lineages worldwide. This study aimed to analyse the nationwide phylogeny of *E. coli* with resistance to third generation cephalosporins from hospitalised patients and outpatients.

Methods: *E. coli* isolates were collected prospectively at 45 diagnostic laboratories each in two time periods (October 2016 - March 2017 and October 2019 - March 2020) in the scope of the resistance surveillance study performed by the Paul Ehrlich Society for Infection Therapy. For whole genome sequencing (Illumina) all isolates with third generation cephalosporin resistance (study period 2016/17, n=160; study period 2019/20, n=145) were included. These isolates were from hospitalised patients (2016/17, n=123 and 2019/20, n=96) and outpatients (2016/17, n=37 and 2019/20, n=49). Genome sequences were assembled using unicycler and further analysed using core genome multilocus sequence typing (cgMLST). Resistance genes were identified using ResFinder.

Results: A total of 1038 and 1031 isolates were collected in 2016/17 and 2019/20, respectively. ESBL genes were detected in 152 (2016/17) and 134 isolates (2019/20); these belonged mainly to the CTX-M family. ESBL gene *bla*$_{\text{CTX-M-15}}$ was present in more than 50% of all third generation cephalosporin-resistant isolates from hospitalised patients and oupatients, and the proportion of ESBL gene *bla*$_{\text{CTX-M-27}}$ increased in the second study period (Table 1). MLST enabled assignment of various sequence types (STs) to the 305 resistant isolates and a close genetic relationship was detected only for a few isolates (cgMLST-based). The most frequent ST was ST131 with a higher proportion in the first study period and in isolates from outpatients (Table 1). Further, worldwide spread "high risk clones" of *E. coli* like ST10, ST38, ST73, ST405 and ST744 were present with more than one isolate in both study periods among both hospitalised patients and outpatients.

Discussion: *E. coli* isolates with resistance to third generation cephalosporins belong to various clonal lineages, whith a clear dominance of ST131 in both hospitalised patients and outpatients. The increased occurrence of ESBL gene *bla*$_{\text{CTX-M-27}}$ in the study period 2019/20 is mainly attributed to a shift of *bla*$_{\text{CTX-M-15}}$ to *bla*$_{\text{CTX-M-27}}$ in ST131 isolates. Apart from ST131 several STs that are known as "high risk clones" were present in this study and other causes of third generation cephalosporin resistance than ESBL production, e.g. CMY, DHA and SHV-1 overproduction, were found in only a few isolates.

Fig. 1

Table 1: Detected resistance determinants and sequence types in *E. coli* isolates from patients in hospitals and clinical practices in community medicine

Study period	2016/17 community		2019/20 community		2016/17 hospital		2019/20 hospital	
3GCR isolate number	37/460 (8.0%)		49/460 (10.7%)		123/578 (21.2%)		96/571 (16.8%)	
ESBL types	CTX-M-15	n=23	CTX-M-15	n=20	CTX-M-15	n=63	CTX-M-15	n=48
	CTX-M-27	n=6	CTX-M-27	n=14	CTX-M-27	n=4	CTX-M-27	n=16
	CTX-M-1	n=3	CTX-M-1	n=6	CTX-M-1	n=19	CTX-M-1	n=10
	CTX-M-14	n=2	CTX-M-14	n=1	CTX-M-14	n=27	CTX-M-14	n=10
	CTX-M-3	n=0	CTX-M-3	n=2	CTX-M-3	n=3	CTX-M-3	n=1
	CTX-M-8	n=0	CTX-M-8	n=1	CTX-M-8	n=0	CTX-M-8	n=0
	CTX-M-55	n=0	CTX-M-55	n=1	CTX-M-55	n=1	CTX-M-55	n=0
	CTX-M-65	n=0	CTX-M-65	n=0	CTX-M-65	n=0	CTX-M-65	n=2
	SHV-12	n=1	SHV-12	n=0	SHV-12	n=0	SHV-12	n=1
	TEM-52	n=0	TEM-52	n=0	TEM-52	n=0	TEM-52	n=1
Other types*	CMY-2	n=1	CMY-2	n=0	CMY-2	n=0	CMY-2	n=1
	AmpC**	n=1	AmpC**	n=0	AmpC**	n=2	AmpC**	n=3
	DHA-1	n=0	DHA-1	n=3	DHA-1	n=1	DHA-1	n=1
	OXA-244	n=0	OXA-244	n=2	OXA-244	n=0	OXA-244	n=2
	CMY-4	n=0	CMY-4	n=1	CMY-4	n=0	CMY-4	n=1
	CMY-42	n=0	CMY-42	n=0	CMY-42	n=1	CMY-42	n=1
	SHV-1**	n=0	SHV-1**	n=0	SHV-1**	n=3	SHV-1**	n=1
Sequence types (ST)	ST131	n=21	ST131	n=24	ST131	n=58	ST131	n=33
	ST38	n=1	ST38	n=3	ST38	n=6	ST38	n=6
	ST744	n=1	ST744	n=1	ST744	n=2	ST744	n=1
	ST10	n=1	ST10	n=1	ST10	n=4	ST10	n=1
	ST167	n=1	ST167	n=0	ST167	n=2	ST167	n=2
	ST617	n=1	ST617	n=0	ST617	n=1	ST617	n=1
	ST1193	n=1	ST1193	n=2	ST1193	n=3	ST1193	n=2
	ST405	n=1	ST405	n=4	ST405	n=1	ST405	n=1
	ST410	n=0	ST410	n=0	ST410	n=4	ST410	n=0
	ST73	n=0	ST73	n=1	ST73	n=6	ST73	n=4
	ST69	n=2	ST69	n=6	ST69	n=2	ST69	n=5
	ST88	n=1	ST88	n=0	ST88	n=4	ST88	n=1
	ST648	n=0	ST648	n=1	ST648	n=5	ST648	n=2
	Other STs	n=6	Other STs	n=6	Other STs	n=25	Other STs	n=37

*presence/co-presence of other bla genes or mechanisms resulting in 3rd gen. cephalosporin resistance (3GCR); ** *E. coli*-ampC overexpression due to promoter mutations or bla_{SHV-1} overexpression resulting in 3rd gen. cephalosporin resistance (3GCR)

MSP 163

Protein microarrays as tools for lateral flow development for multiplex beta-lactam and colistin resistance detection

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Introduction: The untargeted use of antibiotics, the lack of hygiene, insufficient availability of safe food and clean drinking water, as well as inadequate epidemiological surveillance, all play a major role in the development of resistance in bacteria. Also, the economic orientation of medicine, as well as war, displacement, poverty, hunger, and epidemic outbreaks, further exacerbate the problem by facilitating the spread of resistant pathogens. The WHO priority pathogens list for research and development of new antibiotics and monitoring includes these critical organisms, *A. baumannii*, *P. aeruginosa*, and carbapenem-resistant *Enterobacteriaceae*. Therefore, a key objective of our work is the fast, economic and direct identification of the currently most important beta-lactamases (KPC, OXA-23, OXA-48, OXA-58, VIM, NDM, IMP, CTX-M-9, and CTX-M-1), as well as the resistance enzyme for colistin MCR-1, through a cost-effective multiplex lateral flow assay. The results of such a test would be highly relevant for the epidemiology, hygiene, and treatment of infections caused by multidrug-resistant gram-negative pathogens.

Methods: The most important beta-lactam enzymes and MCR-1 have been sequenced, cloned, overexpressed, and purified. Functional antigens, tested by a nitrocefin-test, formed the basis for generating antibodies against ESBLs, carbapenemases and MCR-1. An essential goal is to utilize these antibodies after appropriate screening via antibody microarray-based tests into a multiplex lateral flow assay. A total of 59 different antibody clones were spotted onto the protein microarray for the 10 targets at various concentrations. This allowed for parallel screening of all antibody combinations under the same reaction conditions using recombinant antigens and native bacteria cultures. The latter were directly processed on microarray without any specific sample preparation.

Results: As a result, any cross-reactivity between the antibodies for different targets could be simultaneously captured. This enables antibody screening with minimal sample volume and reduced time compared to conventional ELISA testing. By creating a decision matrix with multiple antibodies per target, pairings between capture antibodies on the microarray and the biotinylated detection antibodies could be identified. The best antibody pairs in terms of specificity and sensitivity, identified through the screening process, were subsequently utilized for the production of a multiparameter lateral flow test. Recent results showed an excellent specificity and sensitivity for OXA-48, OXA-23, OXA-58 and MCR-1.

Discussion: The preliminary results once again confirm the efficiency of protein microarrays in simultaneously testing multiple antibodies for their specificity and sensitivity. The course of the recent experiments is promising and indicates that antibodies for the detection of ESBL and carbapenemases will also be found for further targets.

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Characterization of the novel beta-lactamase OXA-944 from *Acinetobacter guillouiae*

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Background: Carbapenem resistance in *Acinetobacter* species caused by the expression of OXA-type beta-lactamases is increasing worldwide and therefore a particular threat to health care systems. This study aims to characterize OXA-944, a novel variant of the intrinsic OXA-274 family, conferring carbapenem resistance from *Acinetobacter guillouiae*.

Material and Methods: Whole genome sequencing (WGS) was employed for species identification, detection of beta-lactamases and phylogenetic analysis. Antibiotic susceptibility of clinical isolates and transformants was evaluated by microbroth dilution. The gene *bla*OXA-944 or *bla*OXA-274 was cloned into an overexpression plasmid and the protein were purified using affinity chromatography. OXA-944 was crystallized and the structure was solved by X-ray. Molecular modeling was used to analyse the substrate binding. The impact of OXA-944 on carbapenem therapy was evaluated in the *Galleria mellonella* infection model.

Results: The clinical isolate *A. guillouiae* ACGU_2348 was recovered from a wound swab and showed decreased carbapenem susceptibility. Comparison of the genomes of ACGU_2348, the reference strain DSM590 and several additional clinical isolates revealed a core genome of 2,967 genes, 4,774 unique genes and 2,090 accessory genes. Analysis of the resistome of ACGU_2348 could not detect an acquired beta-lactamase but the presence of OXA-944, a variant of the intrinsic OXA-274 family. The sequences similarity of OXA-944 and OXA-274 was 97.5% on amino acid level. Production of OXA-944 increased carbapenem resistance significantly in *A. guillouiae* as well as *Acinetobacter baumannii* transformants but remain unaltered in *E. coli*, indicating a species specific impact. The structure of the purified OXA-944 protein could be solved by X-ray crystallography with a resolution of less than 2 Å. Infection of *G. mellonella* with a sublethal dose of *bla*OXA-944 expressing bacteria could not be cured by high-dose imipenem therapy indicating that OXA-944 mediates carbapenem resistance *in vivo*.

Discussion: Mutations in intrinsic OXA-type beta-lactamases can lead to increased carbapenemase activity of the enzymes and are most likely underestimated particular in *A. non-baumannii* species. Therefore, intrinsic OXA variants could serve as hidden reservoir of antibiotic resistance.

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Molecular and phenotypic characterization of *Klebsiella pneumoniae* carbapenemase (KPC) variants and their effect on the activity of imipenem/relebactam *in vitro* and *in vivo*

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Introduction: The emergence of ceftazidime/avibactam (CTV) resistance in *Klebsiella pneumoniae* carbapenemase (KPC) producing Enterobacterales has been linked to mutational changes in bla_{KPC}. The new carbapenem-beta-lactamase-inhibitor combination imipenem/relebactam (IMR) is a potentially effective agent for the treatment of infections caused by carbapenem-resistant *K. pneumoniae*, but the efficacy of this combination in imipenem- and/or CTV-resistant *K. pneumoniae* has not been investigated. Here, we provide data on the *in vitro* and *in vivo* activity of imipenem/relebactam against imipenem- and CTV-resistant *K. pneumoniae* isolates and additionally characterize *in vitro* generated mutational changes that are associated with imipenem/relebactam resistance in *K. pneumoniae*.

Methods: Clinical *K. pneumoniae* isolates carrying KPC (T2444, KPC-3, imipenem-resistant and CTV-susceptible; T2437, KPC-3^{D179Y}, imipenem-susceptible and CTV-resistant) were used for *in vitro* generation of mutants under IMR selection pressure. Mutant selection frequencies were determined. Mutations associated with IMR-resistance were identified using whole genome sequencing. Antimicrobial susceptibility testing, time-kill kinetics and the *Galleria mellonella* infection model were applied to evaluate the activity of IMR.

Results: All imipenem- and CTV-resistant KPC-producing *K. pneumoniae* mutants were susceptible to IMR. Time-kill response of an imipenem- and CTV-resistant *K. pneumoniae* isolate to IMR revealed bactericidal activity. *G. mellonella* infected with an imipenem- and CTV-resistant *K. pneumoniae* demonstrated significant higher survival rates under IMR treatment, compared to treatment with imipenem or relebactam alone. The selection frequency for IMR-resistant mutants was higher for clinical *K. pneumoniae* isolate T2444 expressing wild-type KPC-3 than for clinical *K. pneumoniae* isolate T2437 expressing KPC-3^{D179Y}. Sequence analysis of IMR-resistant mutants identified novel mutational changes in ompK36 associated with IMR-resistance in *K. pneumoniae*.

Discussion: Our experiments suggest that IMR is a promising treatment option for infections caused by imipenem- and CTV-resistant *K. pneumoniae*. However, prolonged IMR therapy may tend to select for IMR-resistant mutants and combination therapy may be warranted.

MSP 166

KPC-carbapenemases spread in surface waters

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Multidrug-resistant (MDR) Enterobaceriaceae and carbapenemase-producing bacteria show an increasing occurence in healthcare-settings and waste waters worldwide. However, these pathogens have rarely been isolated from urban surface waters in Germany. This surveillance follow-up report describes the evidence of KPC-carbapenemase carrying MDR Enterobacateriaceae in surface waters in western Germany.

In 2017 a MDR KPC-producing Klebsiella pneumoniae-outbreak at a hospital, originating from a nearly drowned patient, triggered the examination of the surface waters in a limited area of the federal state of Hesse.

The bacteria were cultivated on selective agars and characterized utilizing microdilution for antibiotic testing, PCR for verification of carbapenemase genes, and whole genome sequencing. Phenotypical extended-spectrum beta-lactamase (ESBL) producing bacteria were isolated in from all 19 sampling sites in Hesse. However, only if when the sampling site was located downstream of a waste water treatment plant, MDR carbapenemase producing isolates were isolated. In total, eleven strains harbored three different carbapenemase genes (bla KPC-2 , bla KPC-3 , or bla OXA-51). The transposon sequence Tn4401With the exception of the presence of an insertion element, a 30 kb part of the plasmid harbouring bla KPC-2 in the environmental isolate obtained in 2017 was identical in both an environmental isolate from rivers in 2017 and to the plasmid that of isolats which had been responsible for a huge waste water-associated clinical outbreak in Hesse in 2014. The bla KPC-3 gene was found in identical genetic area environment as the Frankfurt KPC-3 outbreak isolates in 2017. Furthermore, in 2023 the analyses in multiple sources from all 19 sampling sites (surface waters, hospital wastewaters) will be repeated for epidemiological clues, indicating that plasmid-mediated transmission events underlie KPC-2 spread. Whole-genome sequencing and long-read-resequencing on a subset of isolatesare used to characterize the plasmid content. Comparative and sequence-type based analysis of the genetic context of bla KPC-2-carrying elements will be performed.

Its persistence over long periods at different geographical locations and presence in environmental (surface water) samples, suggest that plasmid-encoded factors confer resilience in different ecological settings. Hidden reservoirs of KP-CPE in hospital wastewaters and the environment on a sequencing basis, are urgently needed to implement effective transmission-control protocols.

MSP 167

An in-depth comparison of bacterial strain typing methods: OXA-244-producing *Escherichia coli* in Germany, 2013–2021

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Background: Previously, we reported on the rapid spread of OXA-244-producing *E. coli* in Germany, predominantly driven by genetically clustered isolates of sequence type (ST)38, that was also observed in at least nine other European countries prompting the ECDC to publish a rapid risk assessment in 2020 and a subsequent update in 2021. However, the source and route of transmission remained unclear, which at least in part may due to the low genetic diversity of the ST38 cluster in combination with standard typing methods to identify subclustering of isolates. Here, we provide an in-depth comparison of typing methods by including all OXA-244-producing *E. coli* isolates received at the German National Reference Centre between 2013 and 2021.

Methods: A total of 523 non-duplicate OXA-244-producing *E. coli* isolates were subjected to Illumina whole-genome sequencing (WGS). Besides *in silico* multilocus sequence typing (MLST), analyses consisted of core genome (cg)MLST, single-nucleotide polymorphism (SNP)-based analyses, split k-mer analyses as well as a newly developed ad-hoc whole genome (wg)MLST scheme to identify subclusters among genetically-related ST38 isolates.

Results: Among the 523 sequenced OXA-244-producing *E. coli* isolates, a total of 27 different ST were detected, of which ST38 (n = 368; 70.3%) was the most prevalent, including 224 isolates

forming a distinct cluster, as revealed by cgMLST analysis. However, neither cgMLST nor SNP-based or split k-mer analyses were able to unambiguously dissolve clusters among genetically homogeneous ST38 isolates. In contrast, only results of an ad-hoc wgMLST revealed subclustering among ST38 isolates.

Discussion: Here, we provide an update on OXA-244-producing *E. coli* confirming the predominance of ST38, including the large cluster of genetically-related isolates. Comparison of typing methods showed substantial differences in their discriminatory power. Unlike others, only results of an ad-hoc wgMLST scheme revealed subclustering among isolates which may help to identify epidemiological links between OXA-244-producing *E. coli* ST38 isolates in Germany. In summary, we here demonstrate the importance of using different molecular strain typing approaches, particularly in complex outbreak scenarios.

MSP 168

Antibiotic concentrations in raw hospital wastewater surpass minimal selective and minimum inhibitory concentrations of resistant *Acinetobacter baylyi* strains

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Question: Even in concentrations below the minimal inhibitory concentrations of susceptible bacteria, antibiotics may select resistant bacteria, if they exceed the "minimal selective concentrations" (MSC). The MSC is the antibiotic concentration at which a resistant strain will start to outcompete a susceptible strain in competition experiments (Gullberg et al., 2011). At the MSC, the decreased growth rate of the resistant strain is balanced by the decrease in growth rate of the susceptible strain caused by subinhibitory concentrations of the antibiotic. Patients excrete a high percentage of administered antibiotics, making hospital wastewater one of the main sources of antibiotic pollution. We determined the MSCs of fluorescently labelled *Acinetobacter baylyi* strains with different resistance profiles and compared the values with antibiotic concentrations determined in hospital wastewater.

Methods: For the competition experiments, a number of test tubes containing a variety of different antibiotic concentrations were inoculated with a resistant and a susceptible *A. baylyi* strain with different fluorescence labels in a ratio of 1:1. After overnight incubation, cell counts were evaluated by fluorescence activated cell sorting. The ratios (resistant/susceptible cells) for different antibiotic concentrations of all measurements were and fitted to first- and second-order polynomials, employing a linear leasts-quare algorithm.

Results: Low MSCs were measured for the quinolone ciprofloxacin, for the carbapenem meropenem and for piperacillin. In comparison, the measured meropenem concentrations in the raw wastewater of a large maximum care hospital showed strong daily fluctuations but were always above the MSC and MIC values of the *A. baylyi* strains. In addition, the ciprofloxacin concentrations were in the range of the MSCs for about half the time and even the highest piperacillin MSC was reached twice within 24 h.

Discussion: In previous studies, we had isolated a great variety of multi-resistant bacteria from the drains and the wastewater of the same hospital displaying simultaneous resistance against all three antibiotics tested above. So far, it has been assumed that good hygienic measures should be able to avoid nosocomial infections of patients with such bacteria. However, the key aspect of our results is that fluctuating high concentrations of antibiotics are present in hospital wastewaters and that these concentrations surpassed or reached the MSCs of the model organism *A. baylyi*. In conclusion, the colonization of hospital drains and wastewater pipes with multi-resistant bacteria is probably driven by high antibiotic concentrations in the wastewater. Therefore, all attempts to remove the bacteria permanently from the drains will be difficult, as long as the antibiotic concentrations in this biotope are not taken into account.

Gullberg et al., Selection of resistant bacteria at very low antibiotic concentrations, PloS Pathog. 7, e1002158 (2011)

MSP 169

Frequency of *Acinetobacter* species isolated from clinical samples over a 34-month period

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Background: The majority of published reports on clinical *Acinetobacter* spp. involve *Acinetobacter baumannii*, particularly multi-drug resistant isolates. We sought to determine the frequency of all *Acinetobacter* spp. that were cultured from clinical samples in our routine microbiology diagnostic laboratory between November 2019 and September 2022.

Methods: All clinical specimens submitted to the diagnostic laboratory were processed using standard laboratory methods. Species were identified using MALDI-TOF, and *Acinetobacter baumannii* group isolates were further identified using *gyrB* multiplex PCR. Whole genome sequencing (WGS) was performed on a MiSeq. Assembled genomes (Velvet) were submitted to JSpeciesWS to confirm species identification. One unique isolate per patient was retained for this study.

Results: From a total of 512 consecutively obtained *Acinetobacter* isolates collected from a wide variety of specimen types (Table 1), 493 were determined as unique and were isolated from; blood, n=3; abdomen, n=17; rectal swabs, n=46; urinary tract, n=119; respiratory, n=148; and wound and skin swabs, n=160. Thirty-eight patients had >1 *Acinetobacter* species, or >1 unique strain within a species. The most frequently isolated species were members of the *A. baumannii* group, in particular *A. baumannii* and *A. pittii*, with *A. pittii* the most frequently isolated *Acinetobacter*, accounting for 43.2% of the total (Table 1). Other species such as *A. bereziniae*, *A. johnsonii*, *A. junii*, *A. lwoffii*, and *A. ursingii* were also found in >2% of isolates. Using the assembled genomes, we found that most of the *A. dijkshoorniae* (synonym of *A. lactucae*) were *A. pittii*. Similarly, five *A. seifertii* isolates were also found to be *A. pittii*. *gyrB* multiplex PCR correctly identified *A. baumannii*, *A. calcoaceticus* and *A. nosocomialis*. However, it did not differentiate between *A. pittii* and *A. dijkshoorniae*. Genomic data revealed several *Acinetobacter* species that were always misidentified by MALDI-TOF, although other species were correctly identified. Six isolates belonged to 6 unnamed *Acinetobacter* species.

Conclusions: The most common *Acinetobacter* sp. isolated in our hospital was *A. pittii*, followed by *A. baumannii*. MALDI-TOF was unable to correctly identify some *Acinetobacter* spp., especially *A. pittii*. We suggest using a molecular method to confirm *Acinetobacter* species identification.

Fig. 1

Table 1. Number of *Acinetobacter* species identified from 512 clinical specimens

	Number of isolates (%) identified by	
Species	**MALDI-TOF**	**WGS data**
A. baumannii	130 (25.4%)	132 (25.8%)
A. baylyi	2 (0.4%)	1 (0.2%)
A. beijerinckii	1 (0.2%)	2 (0.4%)
A. bereziniae	11 (2.1%)	11 (2.1%)
A. calcoaceticus	21 (4.1%)	8 (1.6%)
A. courvalinii	0	2 (0.4%)
A. dijkshoorniae	93 (18.2%)	8 (1.6%)
A. dispersus	2 (0.4%)	2 (0.4%)
A. geminorum	0	5 (1%)
A. guillouiae	2 (0.4%)	3 (0.6%)
A. gyllenbergii	2 (0.4%)	5 (1%)
A. haemolyticus	2 (0.4%)	2 (0.4%)
A. indicus	1 (0.2%)	1 (0.2%)
A. johnsonii	15 (2.9%)	13 (2.5%)
A. junii	18 (3.5%)	15 (2.9%)
A. lwoffii	15 (2.9%)	13 (2.5%)
A. nosocomialis	12 (2.3%)	6 (1.2%)
A. parvus	2 (0.4%)	2 (0.4%)
A. pittii	119 (23.2%)	221 (43.2%)
A. proteolyticus	10 (2%)	0
A. radioresistens	7 (1.4%)	8 (1.6%)
A. seifertii	6 (1.2%)	0
A. ursingii	28 (5.5%)	28 (5.5%)
A. variabilis	0	4 (0.8%)
Acinetobacter spp.	0	20*

* includes *A. guerrae* (n=1), *A. soli* (n=1), *A. vivianii* (n=1), *A. calcoaceticus*-like n=4), *A. courvalanii*-like (n=1), *A. dijkshoorniae*-like (n=1), *A. pittii*-like (n=2), Genospecies 16 (n= 3), and separate unidentified Genospecies/taxon (n=6)

Fig. 2

Table 2. No. of isolates from source material of the most common *Acinetobacter* species

Species	Abdomen	Blood	Rectal swabs	Respiratory	Urinary tract	Wound and skin swabs
A. baumannii	4	2	18	39	29	40
A. berezinae				5	2	4
A. johnsonii			1	7	3	2
A. junii			1	9	4	1
A. lwoffii			1	2	2	8
A. pittii	10	1	15	64	67	63
A. ursingii	1		2	11	6	8

MSP 170

Impact of *Mycobacterium tuberculosis* complex strain diversity on tuberculosis transmissions in a cosmopolitan low-incidence setting

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Understanding the factors driving tuberculosis (TB) transmission in a particular setting is essential to guide effective public health measures. Interestingly, only strains of some *Mycobacterium tuberculosis* complex (Mtbc) lineages occur globally (generalists), while others are only found in particular regions (specialists). This points towards differences in the Mtbc strain transmissibility, potentially influencing TB epidemiology in low-incidence settings by import of more transmissible strain types.

To understand interactions between TB transmission, host and pathogen type in a low-incidence setting, we analysed notified TB cases in Hamburg, Germany, from 1997 – 2021 in relation to strain type and transmission data inferred from whole genome sequencing data of the 3062 Mtbc strains.

Strains of Mtbc L1 to L6 and *M. bovis* were found in the strain collection, with a dominance of L4 strains (75%). Within L4, L4.1.2.1 ("Haarlem") and L4.8 ("mainly T") strains were most prevalent (32% and 20% of L4 strains, respectively) and also most frequently transmitted (30% and 18% of clustered L4 strains, respectively). Associations of ancestry and strain types, reflected in the transmission events were found at lineage and L4 sublineage level consistently over the entire 25-year period. For example, transmissions of L3 strains were mostly found between patients born in Eastern Africa, while L4.1.2.1 and L4.8 strains transmitted in patients with a wider range of origins.

Our findings support the hypothesis that strains of generalist lineages can efficiently transmit in wider range of host genetics background. L4.8 strains appear to be an important widespread generalist lineage.

MSP 171

Long time persistence and evolution of carbapenemase-producing *Enterobacterales* in the wastewater of a tertiary care hospital in Germany

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Introduction: Worldwide observations revealed increased frequencies of multi-resistant *Enterobacterales* and resistance genes in hospital wastewater compared to any other type of wastewater. Despite the description of clonal lineages possibly adapted to hospital wastewater, little is known about long term persistence as well as evolution of these lineages. In this study, wastewater isolates of different *Enterobacterales* species from a tertiary care hospital were investigated with 2.5 years distance.

Material and methods: Wastewater samples from a tertiary care hospital were taken and plated on selective agar to select for 3rd generation cephalosporin-resistant *Enterobacterales*. Whole Genome Sequencing (WGS) and resistance gene identification were performed for *E. coli*, *C. freundii*, *S. marcescens*, *K. pneumoniae*, *K. oxytoca*, and *E. cloacae* isolates (n=59), isolated in 2022 and compared with strains isolated from the same wastewater pipeline in 2019 (n=240).

Results: Individual clonal lineages with highly related isolates could be identified in all species identified more than once in 2022 that appear to persist in the wastewater drainage. A common motif of all persistent clonal lineages was the carriage of mobile genetic elements encoding carbapenemase in persistent clones in this environment observed over the 2.5-year period and multiple clones showed a rise in the amount of carbapenemase genes per isolate. In 2022 isolates *bla*VIM-1 replaced *bla*OXA-48 as the most common carbapenemase gene compared to 2019.

Discussion: Different carbapenemase genes could be detected within clonal lineages in both years, giving evidence for horizontal gene transfer. Interestingly, despite a similar abundance of carbapenemase genes (>80 % of all isolates) at both time points genes encoding extended spectrum β-lactamases decreased over time. This data indicates that hospital wastewater continuously releases genes encoding carbapenemases to the urban wastewater system. The evolution of the resident clones as well as the reasons for the selection advantage in this specific ecological niche needs to be further investigated in the future.

MSP 172

Nasal staphylococci community of healthy people in La Rioja (Spain): High frequency of toxigenic *S. aureus* and MSSA-CC398 subclade

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Introduction: The molecular epidemiology of nasal *Staphylococcus aureus* (*S. aureus*) could provide insight into the virulence potential, resistome, and host adaptation system in healthy humans. This study determined the nasal staphylococci diversity and characterized the *S. aureus* strains from healthy adults in La Rioja (northern Spain).

Materials & Methods: Nasal samples from 57 healthy people (with no antibiotic use or hospital visits or contact with animals in the last 6 months) were analysed for staphylococci recovery and isolates were identified by MALDI-TOF-MS. Non-repetitive *S. aureus* isolates (one isolate from each carrier or > one if they showed different AMR phenotypes) were selected and further characterized. The antimicrobial resistance (AMR) genes, *spa*-types, virulence determinants and immune evasion cluster (IEC) types of *S. aureus* strains were tested by PCR/sequencing. Core genome single nucleotide polymorphisms (SNPs) analysis was used to assess the relatedness of MSSA-CC398 strains.

Results: A total of 213 staphylococci (3-8 isolates per carrier) of seven species were identified. The frequency of species carriage in the participants was *S. epidermidis* (87.7%), *S. aureus* (36.8%), *S. hominis* (7%), *S. haemolyticus* (5.3%) *and S. warneri* (5.3%), *S. lugdunensis* (1.8%) and *S. pasteuri* (1.8%). About 98.2% of the participants carried ≥ one species. Of the 27 non-repetitive *S. aureus* strains, no methicillin resistance was detected but 14.8% presented a multidrug resistance phenotype. The following AMR rates were detected (percentage of isolates/ genes or mutations detected): penicillin (81.5/*blaZ*), erythromycin-clindamycin-inducible (25.9/*ermT*, *ermC*), ciprofloxacin (14.8%/ GrlA [p.S80F], GyrA [p.S84L]), erythromycin (7.4/ *msrA*), tobramycin (7.4/*ant4''*), tetracycline (3.7/*tetK*), sulfamethoxazole-trimethoprim (3.7/ *dfrG*), clindamycin (3.7/*lnuA*), mupirocin (3.7/*mupA*). Seventeen different *spa* types were identified and assigned to eight clonal complexes (CCs), of which CC398 (*spa* types t571, t1451 and t1998) was the predominant (33.3%), followed by CC121 (18.5%). About 19% of the *S. aureus* carriers harboured varied strains (with different AMR genes and/ or genetic lineages). In addition, among 77.8% of the CC398 strains, the *ermT* gene was carried by *rep*13 flanked by IS257 which were upstream of *cadR* and *cadD* genes. However, one of the MSSA-CC398 strains carried the *ermC* gene in *rep*10. Only the MSSA-CC398 (*ermT*-positive) strains were clonally related (SNPs <50) and carried the φ*Sa3* (IEC type-C). Other IEC types (A to F) were found among the non-CC398 strains with the predominant being type-B (27.8%). About 85.1% of the *S. aureus* strains carried one or more of *lukF/S-PV, tst, eta, etb, etd, sea, seb, sec, sed, see* and *sep* genes.

Discussion: The high rate of toxigenic, diverse *S. aureus* and clonally related MSSA-CC398 subclades highlight the ability of healthy people to carry (in their noses) and transmit pathogenic strains of *S. aureus*.

MSP 173

Phenotypic and genotypic characterization of azole-resistant *Aspergillus fumigatus* over 11 years

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Question: Invasive aspergillosis (IA) mostly occurs in immunocompromised patients, especially in patients with haematological malignancies or after allogeneic and solid organ transplantation. As first-line antifungal therapy, azole antifungal drugs are recommended which have been shown to be effective in the past. In recent years, the emergence of azole-resistant *Aspergillus fumigatus* strains (ARAF) has become a significant challenge in the treatment of IA. This study assessed the epidemiology of ARAF strains in the last eleven years within the University Hospital Essen, Germany.

Material and Methods: The epidemiology of ARAF was investigated during 2012–2022. All respiratory samples were plated on malt extract agar and incubated for 7 days at 30°C. Identification of isolates was performed using classical macro- and micromorphological characteristics. During the years of collection, all isolates underwent susceptibility testing for at least itraconazole or for both, itraconazole and voriconazole by gradient test. ARAF was defined as non-wild-type minimal inhibitory concentration (MIC). Analysis of mutations mediating resistance was performed using PCR. Patient records were analysed retrospectively regarding sex, age, underlying disease and 30-day in-hospital outcome.

Results: Over the 11 years, 196 ARAFs (6.1%) and 3002 wild type (WT) isolates of *Aspergillus fumigatus* were found. The number of ARAF cases remained consistent in the years from 2015 to 2019 until an increase in the years 2020 and 2021. 2021 was the year with the highest ARAF rate of 10.8%. Regarding seasonal distribution, non-ARAFs occurred mostly in summer and fall whereas most of the ARAFs were isolated in spring and summer. In total, ARAFs were mostly detected in male patients (n=108, 55%) but the gender distribution was variable over time. Median age was 44 years in patients with ARAF and 50 years in patients with non-ARAF. L98H/TR34 was the most prevalent mutation (33%) followed by T289/Y121 (5%). Results on 30-day in-hospital outcome and underlying disease will follow.

Conclusions: The findings of this study provide valuable insights into the epidemiological development of infections with ARAF within the last decade. It highlights the emergence of azole-resistant IA in North Rhine-Westphalia and underlines the importance for systematic antifungal susceptibility testing of *A. fumigatus*.

MSP 174

Resistance determinants against therapeutically relevant antibiotics in publicly available genome sequences from *Clostridioides difficile* isolates

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Introduction: *Clostridioides difficile* is the predominant pathogen that causes antibiotic-associated diarrhea and membranous colitis worldwide. The antibiotics fidaxomicin and vancomycin are recommended for *C. difficile* infection (CDI) therapy, whereas metronidazole had been the drug of choice for several decades until recently. While resistance to these antibiotics has been reported for *C. difficile* occasionally, little is known about the prevalence of *C. difficile* strains with reduced susceptibilities. As of April 2023, the *C. difficile* database on the EnteroBase platform held 23,028 publicly available genome sequences and associated metadata (https://enterobase.warwick.ac.uk/). Based on this dataset, we report on the occurrence of genetic determinants causing resistance to therapeutic antibiotics in *C. difficile*.

Material/Method: We screened 23,028 genome sequences from *C. difficile* isolates for genetic traits known to cause resistance against fidaxomicin, vancomycin, and metronidazole by using BLAST. Additionally, all genomes were screened using Resistance Gene Identifier (RGI) and Comprehensive Antibiotic Resistance Database (CARD). For selected strains, antibiotic susceptibilities were measured using E-test and broth-dilution, respectively.

Results: Reduced susceptibility to fidaxomicin caused by mutations in *rpoB* and *rpoC* was previously reported from a limited number of clinical *C. difficile* isolates. Even though fidaxomicin resistance emerged independently in different *C. difficile* genotypes, according to our results it does not seem to have spread widely. Similarly, our results show that the plasmid pX18-498, recently reported to alter vancomycin susceptibility has not yet disseminated internationally. In contrast, mutations in the genes *vanS* and *vanR* causing reduced susceptibility to vancomycin were detected in many *C. difficile* genomes from different geographical locations, and their frequency markedly increased since the 1990s, presumably caused by selection pressure due to vancomycin usage. The plasmid pCD-METRO, which confers metronidazole resistance, was detected in *C. difficile* genomes from Europe and the Americas. In addition, many *C. difficile* genomes carried incomplete fragments of plasmids pCD-METRO and pX18-498, but our susceptibility tests showed that these were not sufficient to cause phenotypic resistance to metronidazole or vancomycin, respectively.

Discussion: Our analysis of this large dataset provides genomic insights into the prevalence of resistance determinants for therapeutic antibiotics in *C. difficile*. While fully fledged antibiotic resistance in *C. difficile* is rare globally, reduced susceptibility to vancomycin seems to be emerging.

MSP 175

Surveillance, control and characterization of an NDM-1 *Acinetobacter baumannii* outbreak

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Background: NDM-producing carbapenem-resistant *Acinetobacter baumannii* (CRAb) are associated with nosocomial outbreaks, but, rarely in Germany. We have characterized a CRAb outbreak, investigated the source and routes of transmission, and successfully eliminated the pathogen from the hospital environment.

Materials/methods: Between May and September 2019, 11 CRAb isolates from ten patients were collected from four wards in the internal medicine department. Additionally, six CRAb isolates were obtained from extended environmental screening of high-touch surfaces and medical equipment. Antimicrobial susceptibility testing was performed using Vitek2/Etest (bioMérieux). Whole genome sequencing using the MiSeq (Illumina) and MinION (Nanopore) was performed. Genomes were analyzed using core-genome MLST (cgMLST) (Ridom) and *in silico* (Resfinder, Pasteur-MLST).

Results: 15 CRAb isolates encoding both $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-23}}$ on the chromosome, and two with only $bla_{\text{OXA-23}}$ were identified. One patient had two isolates; one co-harbouring $bla_{\text{OXA-23}}$ and $bla_{\text{NDM-1}}$, and the other encoded only $bla_{\text{OXA-23}}$. All isolates harboured the intrinsic $bla_{\text{OXA-66}}$ and were assigned international clone 2. All isolates were ST570. One isolate ($bla_{\text{OXA-23}}$ only) was ST2 and considered unrelated. By cgMLST the $bla_{\text{OXA-23}}$/$bla_{\text{NDM-1}}$ isolates differed in ≤ 3 alleles, indicating inter-ward transmission. The ST570 $bla_{\text{OXA-23}}$ isolate was identical to the $bla_{\text{OXA-23}}$/$bla_{\text{NDM-1}}$ isolates, and the difference in genotype was through loss of the Tn*125*-like encoding $bla_{\text{NDM-1}}$. The index patient, transferred from a hospital in Egypt was tested positive for the outbreak strain on hospital admission and although all hygiene measures were followed, four weeks later two more patients became infected with the CRAb on other wards without prior contact with the index patient. Environmental sampling revealed diverse transmission reservoirs, i.e., electrical socket, electrocardiogram leads, and the control panel of a respirator. A two-stage cleaning of the affected wards, performed independently by two different cleaners, plus microbiological control afterwards, and improved hand-hygiene compliance of the staff resulted in complete eradication and ended the outbreak.

Conclusions: This study describes the transmission of a $bla_{\text{NDM-1}}$-positive CRAb in the internal medicine department. The source of the outbreak was identified after environmental screening and was eradicated though strict infection control measures. These data highlight that the combination of high-resolution molecular surveillance (short- and long-read sequencing), extensive environmental screening, and thorough cleaning and disinfection, can effectively prevent further pathogen transmission in hospitals.

MSP 175a

Real-time genomics for rapid phenotypic resistance prediction in bloodstream infections – A case presentation of a KPC-55 Klebsiella pneumoniae infection

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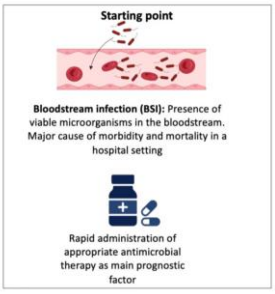
ES and CC contributed equally

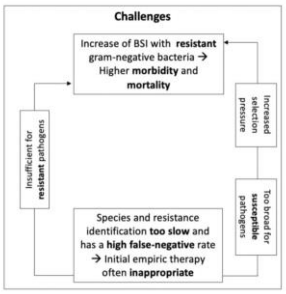
Bloodstream infections have become one of the main causes of hospital mortality with an estimated five million deaths per year globally [1]. Recent trends have shown an increase in the proportion of multi-drug resistant gram-negative bacteria as causative agents [2]. These strains represent the biggest challenge in treating bloodstream infections, as resistant infections are associated with higher mortality and longer hospital stays due to delayed and/or inappropriate therapy [3].

A rapid and accurate identification of the infection causing pathogen and its resistance patterns would facilitate early administration of appropriate antibiotic therapy leading to reduced resistance selection pressure and decreased mortality and morbidity (Fig. 1A). However, bloodstream infection diagnosis is currently mainly culture-based, which is typically time- and resource-consuming and has low clinical sensitivity and high culture bias [4]. In this study, we have therefore started implementing metagenomic approaches using nanopore sequencing to overcome the limitations of culture-based approaches (Fig. 1B). Briefly, nanopore sequencing is a portable long-read third-generation sequencing technology that makes accurate real-time resistance predictions based on near-complete genomes possible. We will here present our first results of sequencing two Klebsiella pneumoniae isolates from blood cultures of an ICU patient with a KPC-2 and a novel KPC-55 subtype, and compare the accuracy and the turnaround time of our real-time genomics approach to the current state-of-the-art culture-based methods (Fig. 1B). For this, we applied established genotypic species and alignment-based resistance prediction methods to the assembled long-read nanopore sequencing data. Our preliminary results comparing two genotypic resistance prediction methods show a promising overlap in taxonomic and functional predictions with culture-based approaches. Of note, we were able to accurately identify Klebsiella pneumoniae infection and correctly predict phenotypic carbapenem resistance within a shorter turnover time compared to the culture-based resistance detection.

Our long-term aim is to develop fully integrated genomic and computational solutions to bring about an accurate, data-driven, and cost-effective approach for fast in-depth assessments of bloodstream infections directly at the point of care. This project has the potential to maximise the efficiency of antimicrobial therapy reducing the use of broad-spectrum antimicrobials and increasing survival rate in the context of bloodstream infections.

A





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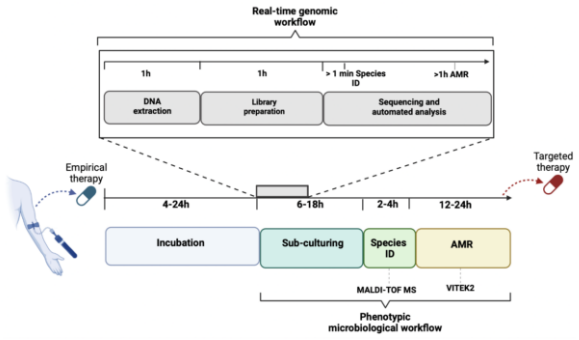

Fig. 1 A. Project background and motivation B. Workflow comparing a culture-based and a nanopore-based approach. ID: Identification, AMR: antimicrobial resistance, MALDI-TOF MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

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GIMPP 176

Manipulation of microvillar proteins during *Salmonella enterica* invasion results in brush border effacement and actin remodeling

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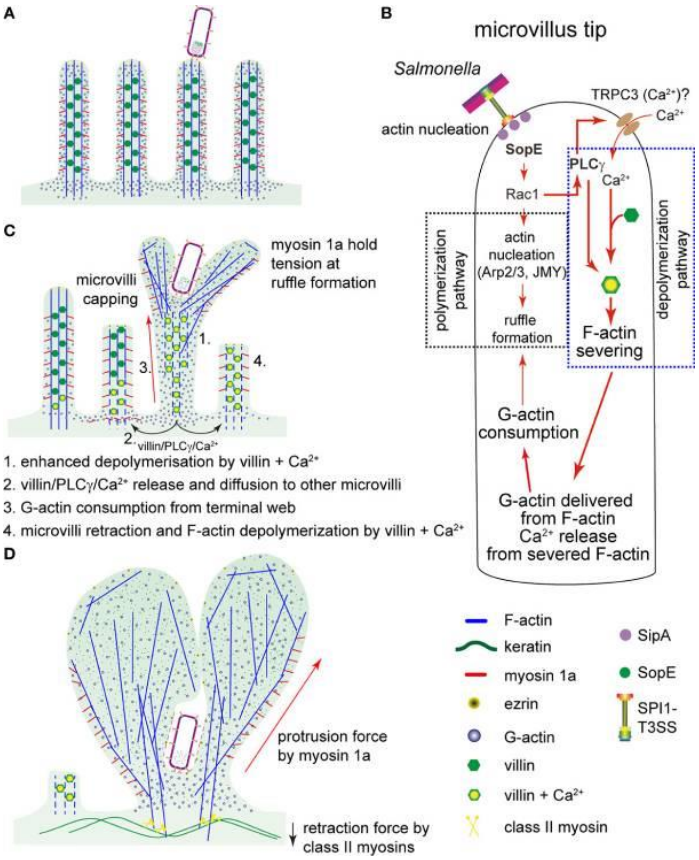
Enterocyte invasion by the gastrointestinal pathogen *Salmonella enterica* is accompanied by loss of brush border and massive remodeling of the actin cytoskeleton, leading to microvilli effacement and formation of membrane ruffles. These manipulations are mediated by effector proteins translocated by the *Salmonella* Pathogenicity Island 1-encoded type III secretion system (SPI1-T3SS). To unravel the mechanisms of microvilli effacement and contribution of SPI1-T3SS effector proteins, the dynamics of host-pathogen interactions was analyzed using live cell imaging (LCI) of polarized epithelial cells (PEC) expressing LifeAct-GFP. PEC were infected with *S. enterica* wild-type and mutant strains with defined defects in SPI1-T3SS effector proteins, and pharmacological inhibition of actin assembly were applied. We

identified that microvilli effacement involves two distinct mechanisms: i) F-actin depolymerization mediated by villin and ii), the consumption of cytoplasmic G-actin by formation of membrane ruffles. By analyzing the contribution of individual SPI1-T3SS effector proteins, we demonstrate that SopE dominantly triggers microvilli effacement and formation of membrane ruffles. Furthermore, SopE via Rac1 indirectly manipulates villin, which culminates in F-actin depolymerization. Collectively, these results indicate that SopE has dual functions during F-actin remodeling in PEC. While SopE-Rac1 triggers F-actin polymerization and ruffle formation, activation of PLCγ and villin by SopE depolymerizes F-actin in PEC. These results demonstrate the key role of SopE in destruction of the intestinal barrier during intestinal infection by *Salmonella*.

Front Cell Infect Microbiol. 13:1137062. doi: 10.3389/fcimb.2023.1137062. eCollection 2023

Fig. 1



GIMPP 177

Function of small basic protein (Sbp) in *Staphylococcus epidermidis* biofilm matrix assembly: molecular mechanisms and spatio-temporal patterning

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Introduction: *Staphylococcus epidermidis* is a common member of human skin microbiota epithelial microflora but also one of the most frequent nosocomial pathogens causing implant-associated infections. Biofilm formation is the major pathogenicity mechanism principal. It´s biofilm assembly relies on the production of an extracellular matrix that serves as glue to stabilize the multilayered bacterial architecture. The biofilm matrix consists of a plethora of various biomolecules, referred to as the matrixome. In effort to understand specific molecular interactions within the matrixome, a so far uncharacterized, 18 kDa protein was identified named small basic protein (Sbp).

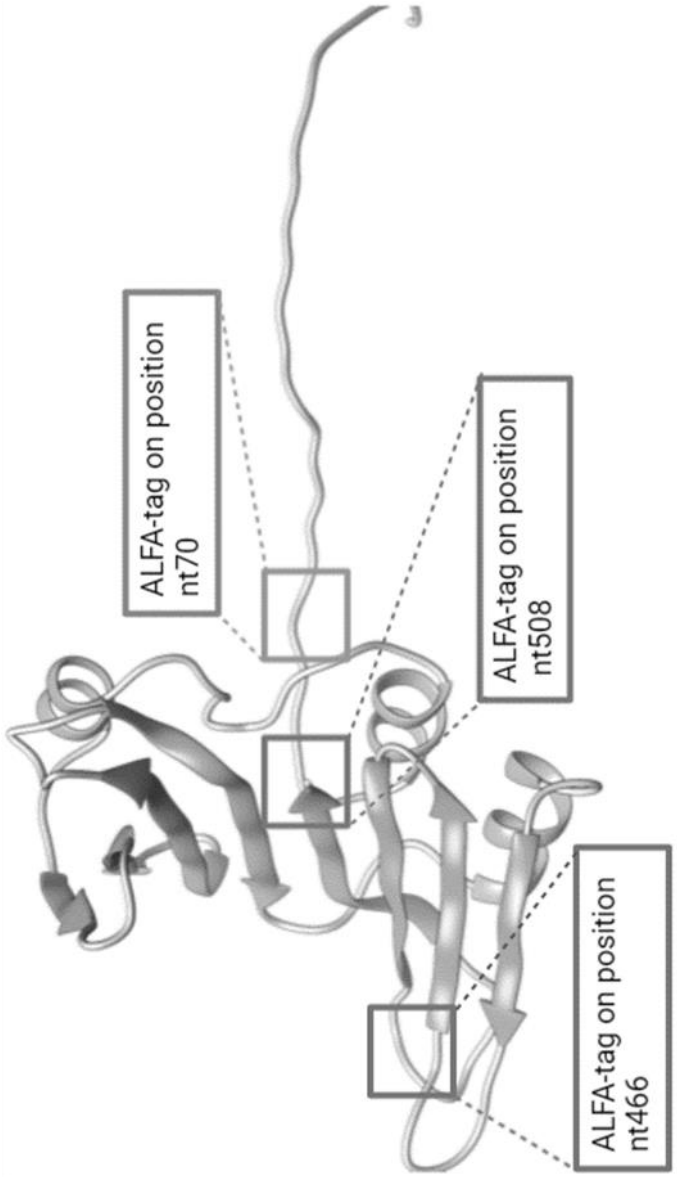
Material / Methods: To investigate the dynamic Sbp matrix assembly three different Sbp-isoforms were constructed in which Sbp is fused to an ALFA-tag located in different positions within the Sbp. The constructs were cloned in two different plasmid backbones, pRB473 (constitutive promotor) and pHB3 (xylose inducible promotor), and stability were tested (growth curves, biofilm formation Western Blot).

Results: Aiming at following Sbp matrix assembly by using live cell imaging, *sbp* was fused with an ALFA-tag encoding sequence. To identify suitable positions for ALFA tag insertion, Alphafold was employed for Sbp structure prediction. Identification of a head region, consisting of ß-sheets and α-helices, and an unstructured N-terminal tail (Figure 1) were in good agreement with CD-spectroscopy analysis. Based on these finding, ALFA tags were inserted at the junction of the unstructured tail (nt 70), at a predicted connecting loop between two ß-sheets (nt 466) and at the C-terminus (nt 505). Western blot analysis confirmed expression of sbp-ALFA in *S. epidermidis* 1457 and corresponding *sbp* knock-out 1457Δ*sbp*. Expression had no impact on bacterial growth, however, in trans expression of all isoforms rescued the impaired biofilm phenotype of mutant 1457Δ*sbp*. Currently, spatial distribution of wildtype sbp and sbp-ALFA fusion proteins in living biofilms is under investigation using CLSM and high resolution light microscopy.

Figure 1: Monomeric structure of Sbp using AlphaFold and establishment of three different Sbp-isoforms in which sbp is fused to an ALFA-tag which is located on different positions within the Sbp-protein-sequence.

Discussion: Here, we present successful set-up of sbp-ALFA fusions enabling the analysis of temporal-spatial dynamics of Sbp assembly in living *S. epidermidis* biofilms. Sbp-ALFA fusion proteins will be basis for future analysis of Sbp function and interaction networks supporting assembly of biofilm matrix architectures.

Fig. 1



GIMPP 178

Role of multi-drug-resistance Gram negative bacteria in development of acute-on-chronic liver failure (ACLF)

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Introduction: Infections with multidrug-resistant (MDR) Gram negative bacteria drive liver disease from a chronic and balanced status to an acute organ failure with high mortality rates. The dysbalance within the gut-liver axis leads results in translocation of bacteria and bacterial compounds culminating in the development of acute-on-chronic liver failure (ACLF).

Material and methods: Liver cells (HepG2) and intestinal cells (Caco-2) were infected either with *Klebsiella pneumoniae* ATCC 700603 and two clinical isolates (one MDR) or *Acinetobacter baumannii* ATCC 19606 and two clinical isolates (one MDR) for 3 and 6h hours. Supernatants were taken to analyse either the profile of secreted cytokines or bacterial metabolites with potential toxicity. These metabolites will be analysed further by HPLC / mass spectrometry. Conditioned medium from infected Caco-2 cells will be analysed for toxic effects on liver cells.

Results: Experiments revealed that the optimal multiplicity of infection (MOI) is 1 for *K. pneumoniae* strains and 100 for *A. baumannii* strains and the time for analysing infections is up to 6h hours. Infection of liver cells resulted in damage of the cell monolayer and this effect is more pronounced in infections with *K. pneumoniae* strains and multi-drug-resistance clinical isolates.

Discussion: We speculate that the translocation of (multidrug-resistant) bacteria or bacterial compounds or metabolites from the gut to the liver is decisive in the development of ACLF. Exact analysis of the role of inflammatory cytokines, bacterial compounds and metabolites might explain the pathomechanisms underlying ACLF and might represent an attractive target for intervention.

This work was supported by the LOEWE-Center "ACLF-I, project P5" (state of Hesse).

GIMPP 179

Characterization of *Coxiella burnetii*-induced modulation of the transcription factor EB (TFEB)

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Introduction: *Coxiella burnetii* is an obligate intracellular pathogen causing Q fever. The primary target of the bacterium are alveolar macrophages. Phagocytosis leads to the formation of a *Coxiella*-containing vacuole (CCV) which develops into a phagolysosomal-like compartment, permissive for replication. The development of this vacuole is an important process as it activates the bacterial metabolism and the translocation of type IV secretion system (T4SS) effector proteins into the host cell. One of these effector proteins is AnkG, which has anti-apoptotic activity. The deletion of *ankG* leads to reduced anti-apoptotic activity of *C. burnetii*, severe impaired CCV formation and a reduced replication ability. While the molecular mechanisms leading to AnkG-mediated anti-apoptotic activity are described, it is completely unknown how AnkG influences CCV formation and bacterial replication. Transcription factor EB (TFEB) is a master regulator of the autophagosomal and lysosomal development. The knockout of this transcription factor in *C. burnetii* infected HeLa cells leads to reduced bacterial load and drastically impaired CCV development, indicating that *C. burnetii* depends on TFEB activity. TFEB inhibited by mTORC1, which phosphorylates and thereby prevents TFEB migration into the nucleus. MTORC1 functions as a central nutrition and energy sensor and it becomes inactivated during lysosomal stress or nutrient deprivation. During *C. burnetii* infection mTORC1 is inactivated and cannot further repress the activity of TFEB. Aim of the work is to elucidate the role of AnkG, in CCV formation and its influence on the activation of TFEB.

Methods: We used the *C. burnetii* NMII wild-type and the deletion mutant Δ*ankG* to study how the infection affects the subcellular localization of TFEB by microscopy. Immunoblotting was used to analyze the phosphorylation pattern of TFEB and mTORC1 to investigate their activation status during infection. In addition we used confocal microscopy, to correlate the CCV size with TFEB activity.

Results: Our results show that an infection with *C. burnetii* triggers the translocation of TFEB into the host cell nucleus in infected HeLa cells in a time dependent manner. At 72 h post-infection TFEB is localized in the host cell nucleus of 100% of wild-type infected cells. This correlates with dephosphorylation of TFEB at S122 and S211. The deletion of *ankG* leads to a significantly increased phosphorylation of TFEB at S122 and S211, and as consequence to reduced translocation of TFEB into the

nucleus. Furthermore, we observed a correlation of nuclear TFEB and the size of the CCVs.

Discussion: As only nuclear localized TFEB are active, the data indicates that AnkG has a direct or indirect impact on the activation of the transcription factor. As TFEB is strongly involved in the regulation of the lysosome biogenesis, we assume that the establishment of the CCV in Δ*ankG* infections might be impaired due to reduced TFEB activation.

GIMPP 180

Revealing membrane insertion mechanisms of *Legionella*'s integral membrane effector proteins in host cells

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Question: A virulence strategy used by the intracellular pathogen *Legionella pneumophila* is to manipulate host cellular processes in order to survive within phagocytic host cells. Hence, more than 300 virulent effector proteins are secreted into the host cells by the specialized Dot/Icm type IV secretion system (T4SS). Many effector proteins harbor hydrophobic transmembrane-domains (TMDs) to fulfil their function in host cell membranes. However, the mechanisms T4-secreted TMD-effectors (TMEs) use to target and insert into the correct membranes of eukaryotic hosts remain to be elucidated.

Methods: To understand the relevance of host cell targeting factors and receptors involved in membrane insertion of bacterial TMEs, we will compare their localization after T4SS-assisted injection or in-host ribosomal translation either by live-cell fluorescence microscopy using the nanobody technology or self-labeling enzyme tags, or by subcellular fractionation of infected or transfected RAW264.7 macrophages. Moreover, to reveal membrane targeting and insertion pathways of bacterial TMEs, interactions with host proteins will be investigated by proximity biotinylation using the TurboID biotin ligase, followed by subsequent mass spectrometry-based protein correlation profiling.

Results: In order to co-localize T4SS-injected or plasmid-expressed TMEs with different host cell membranes by Western blotting, a protocol for subcellular fractionation of macrophages was established. This method combines differential centrifugation with a sucrose density gradient centrifugation, allowing the separation of most organelles based on their individual abundance distribution profiles in the gradient. In addition, the subcellular localization of in-host expressed TMEs was assessed by immunofluorescence microscopy, which revealed a similar membrane localization to that reported for T4SS-injected TMEs. To identify potential TME interaction partners involved in membrane targeting and insertion, a protocol for TurboID-mediated proximity dependent biotinylation was evaluated. The biotin ligase TurboID was fused to the TMEs, allowing the labelling of proximal or interacting proteins. Biotinylated proteins were then successfully captured on streptavidin beads and identified by mass spectrometry.

Conclusion: The methods established here will allow us to investigate the mechanisms by which bacterial TMEs target and insert into host cell membranes. In doing so, we aim to unravel the details of the intricate interplay between bacterial pathogens and eukaryotic host cells, which is crucial for the virulence of many pathogens. In addition, these findings may reveal new general principles for the targeting and delivery of integral membrane proteins directly to their site of action.

GIMPP 181

YgfB increases β-lactam resistance in *Pseudomonas aeruginosa* by counteracting AlpA-mediated *ampDh3* expression

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Introduction: The gene *ygfB* contributes to β-lactam resistance in multidrug resistant (MDR) *Pseudomonas aeruginosa (Pa)* strains by increasing expression of the cephalosporinase AmpC[1] that confers resistance to cephalosporins and other classes of β-lactams[2]. AmpC production is activated by 1,6-anhydro-N-acetylmuramyl-L-alanyl-D-glutamyl-meso-diaminopimelic acid peptides (anhMurNAc-peptides), that arise from the peptidoglycan recycling pathway and induce *ampC* by binding to the transcriptional regulator AmpR[3].

Methods: We combined transcriptomic, expression and promoter activity analyses with antibiotic susceptibility testing, LC-MS analysis of peptidoglycan precursors and protein-protein/protein-DNA interaction assays to unravel how YgfB contributes to AmpC overproduction and β-lactam resistance in *Pa*. Furthermore, we investigated the influence of YgfB on the combinatory effect of ciprofloxacin (CIP) and β-lactams in *Pa* by checkerboard assays. Transcriptomics were then used to broaden our insights into the role of *ygfB* in *E. coli* and to define a common response to CIP in *E. coli* and *Pa* with data analysis still ongoing.

Results: YgfB interacts with the transcription factor AlpA, inhibiting expression of the amidase AmpDh3. AmpDh3 degrades AmpR-activating anhMurNAc-peptides, therefore low AmpDh3 levels result in increased AmpC production. As AlpA production can be induced by DNA-damage, e.g. by treatment with CIP[4], we investigated the impact of *ygfB* deletion on the combination of CIP and β-lactams in the MDR strain ID40. Checkerboard assays showed, that *ygfB* prevents an effective combination of CIP and several β-lactams via the AlpA-AmpDh3 pathway. Preliminary findings suggest, that the role of *ygfB* in *E. coli* seems to be mostly related to flagella regulation and that the common CIP response mostly involves SOS-response genes.

Discussion: YgfB is a novel player in the complex regulation network of MDR in *Pa.* Differences in this regulatory network modulating *ampDh3* expression in various *Pa* strains might explain the strain-by-strain variations of the effectiveness of a ciprofloxacin/β-lactam combination. Given the plasticity of these regulation networks causing MDR, we think that this is an important step towards better understanding MDR in *Pa*. Results on the role of *ygfB* in *E. coli* and the common CIP response are still preliminary but promise some interesting insights.

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GIMPP 182

The role of proteases in the internalization process of *S. aureus* into non-professional phagocytes

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Introduction: Invasion of host cells is an important feature of *Staphylococcus aureus*. The main internalization pathway is the binding of the bacteria to host cells, *e.g.* endothelial cells, via a fibronectin (Fn) bridge between *S. aureus* Fn binding proteins and α5β1-integrin, followed by phagocytosis. We have previously shown that not the amount, but the supramolecular structure of fibronectin molecules deposited on the eukaryotic cell surface plays an essential role in the uptake of bacteria by host cells. Cells surrounded by a fibrillar Fn network (osteoblasts, fibroblasts) take up less *S. aureus* than cells with low Fn expression (epithelial cells). Destruction of the fibrillar Fn using trypsin significantly increased *S. aureus* uptake.[1] However, proteases targeting the Fn network are also expressed by both bacteria and host cells. The objective of this study was to analyze the role of bacterial and host cell proteases in the internalization process.

Material and methods: To study the internalization of *S. aureus* wild type strains as well as protease knock out mutants in epithelial and endothelial cells (+/- protease inhibitors) we used the lysostaphin protection assay. We investigated the impact of *S. aureus* supernatants +/- protease inhibitors on fibrillar Fn by fluorescence microscopy. Using RT-qPCR, we examined gene expression of selected host proteases after *S. aureus* internalization.

Results: Upon uptake of *S. aureus* into host cells, there was marked destruction of Fn fibrils on the surface of the endothelial cells. The extent depended on the *S. aureus* strain. Both Fn fibril degradation and bacterial internalization could be inhibited by a protease inhibitor cocktail. The serine protease inhibitor Pefabloc also significantly inhibited *S. aureus* uptake into host cells, whereas a cysteine protease inhibitor did not. The serine protease inhibitors aprotinin and alpha2-antiplasmin had no effect. After internalization of *S. aureus*, gene expression of the serine protease uPA increased, but the uPA inhibitor plasminogen activator inhibitor-1 had likewise no effect on bacterial internalization. An aureolysin mutant of *S. aureus* 8325-4 (aureolysin: a metalloprotease) was very poorly internalized compared to the wild-type strain; in contrast, an aureolysin knockout in *S. aureus* 6850 did not alter internalization. A mutant of *S. aureus* USA300 in which the major extracellular proteases were deleted was also taken up to the same extent as the wild type.

Discussion: To our knowledge, this is the first study to show the importance of proteases for the internalization of *S. aureus* into non-professional phagocytes. So far, however, the contribution of the extracellular proteases of *S. aureus* and/ or the (serine) proteases expressed by host cells remains elusive. [1]Niemann et al., 2021, mBio; DOI: 10.1128/mBio.01062-21

GIMPP 183

***Klebsiella pneumoniae* brain abscess caused by dissemination of a virulent subpopulation from a urinary tract infection**

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Simple point mutations that are acquired during infection can have significant consequences on the pathogenicity of bacterial and viral pathogens, as recently highlighted by the evolution of SARS-CoV-2 in an immunocompromised patient (Choi et al., N Engl J Med 2020). Here we present a case in which acquisition of simple mutations in the bacterial pathogen *Klebsiella pneumoniae* enabled dissemination from the urinary tract to the brain of a patient. Phenotype-driven genomics led to the identification of a subpopulation of hypercapsule producing mutants in urine which occured homogenously in a brain abscess. Hypercapsule producing isolates displayed phagocytosis resistance and were lethal in a mouse model of urinary tract infection. The combination of Illumina and Oxford Nanopore sequencing technologies established that the hyper and normal capsule producing populations were closely related and excluded the possibility of acquisition of virulence factors that could explain the virulence phenotype. Instead, single nucleotide polymorphisms in capsule biosynthesis genes were identified in the hypercapsule mutants, including point mutations in *wbaP*. Integration of a single *wbaP* point mutation in the parent isolate increased capsule production and virulence in the mouse model of urinary tract infection. This case illustrates that as whole genome sequencing of clinical isolates becomes increasingly accessible, we have the opportunity to understand the evolution of virulence within patients, with the potential to integrate such information into more sophisticated management of patients.

GIMPP 184

Comprehensive investigations into cytolysin A, a major virulence factor of typhoidal *Salmonella enterica* serovars

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Introduction: The Gram-negative bacterial pathogen *Salmonella enterica* is one of the most frequently isolated foodborne pathogens. Within the genus of *Salmonella enterica*, various serovars have been identified. These serovars differ significantly in their host specificities and pathogenicity. Broad host range serovars such as *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) cause short-term infections that mostly remain confined to the gastrointestinal tract in healthy humans. By contrast, closely related typhoidal serovars such as Typhi or Paratyphi A, the cause of (para)typhoid fever, are entirely human-adapted bacterial pathogens. They are more virulent and therefore can cause life-threatening systemic and life-long chronic infections. Comparative analyses of genome sequences between *S.* Typhimurium and *S.* Typhi revealed genomic islands that are present only in typhoidal serovars. One of these *Salmonella* pathogenicity islands (SPIs) is SPI-18, that includes a gene that encodes for cytolysin A (ClyA) and a potential invasine, referred to as TaiA. ClyA of *S.* Typhi has been identified as an active cytolysin with pore-forming properties on various host cells. ClyA heterogeneously expressed in *S.* Typhimurium caused atypical deep organ infections in mice and studies on humans infected with *S.* Typhi, detected significant amounts of ClyA in blood sera. These studies highlight the importance of ClyA and its contribution to the increased virulence of typhoidal *Salmonella* serovars. Despite these findings very little is known about ClyA expression and secretion in typhoidal *Salmonella*, which are adapted to an intracellular lifestyle. Therefore our research aims to investigate ClyA in the context of intracellular *S.* Paratyphi A.

Material/Methods/Results: Using immunofluorescence microscopy studies we detected and analysed the intracellular induction of ClyA. Isogenic mutants generated in various global regulators demonstrated their regulatory impact on ClyA production. Here the two-component regulator PhoP/Q as well as the transcriptional regulator SlyA could be determined for their impact on intracellular ClyA induction. Hemolysis assays, using horse whole blood samples, indicated that cytolysin A, once expressed, is secreted from S. Paratyphi A and is capable to induce lysis of erythrocytes. Furthermore, we examined whether outer membrane vesicles (OMV) play a role in the final release of ClyA from intracellular *S.* Paratyphi A. Here a contribution of an intracellularly induced muramidase (TtsA), shown to be essential for the secretion of typhoid toxin of intracellular *S.* Typhi, could been shown.

Discussion: Overall, this work established new paradigms of a barely investigated virulence factor of typhoidal *Salmonella* serovars. In the process, the knowledge gained by these studies may lead to the development of novel therapeutic strategies to combat infectious diseases caused by typhoidal *Salmonella.*

GIMPP 185

Different pathogenicity of *Fusarium* keratitis isolates in a 3D hemi-cornea-model

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Introduction: Amongst microbial keratitis pathogens especially the rare but fatal fungal species frequently lead to fatal patient outcome. Keratoplasty and even enucleations of the affected eye are frequently inevitable. Many species feature high antimycotic resistances. Thus, treatment options are strongly limited. *Fusarium solani* species are amongst the most prevalent fungal keratitis pathogens. Decisive differences of pathogenicity and genomic features are known for other pathogenic *Fusarium* species. In this work, we aim to identify and compare striking characteristics of *Fusarium solani* species complex (FSSC) members and common keratitis pathogens *F. falciforme, F. petroliphilum and F. keratoplasticum* in a 2D as well as a 3D corneal infection model.

Material & Methods: 2D monolayers of immortalized hTCEpi cells as well as a 3D hemi cornea model, consisting of a 500 µm thick stromal and a multilayer epithelium, were infected with conidia of *F. solani* keratitis isolates for 3 h to 48 h at 34 °C, 5 % CO2. Fungal invasion was visualized via transmission electron and fluorescence microscopy and histology. Cytotoxicity was measured via lactate dehydrogenase (LDH) assay.

Results: *Fusarium* conidia germination, adhesion and penetration of the epithelial layer in initiated within the first 3 h of infection. Fluorescence microscopy indicated a host cell reaction by actin accumulation engulfing invading hyphae. Transmission electron microscopy revealed the formation of trans cellular tunnels extending through up to 3 invaded hTCEpi cells. Fungal hyphae were encapsulated by multiple membrane like structures within the tunnels. *F. petroliphilum* and *F. falciforme* isolates showed a significantly lower cytotoxic effect than *F. keratoplasticum* (36.6 % [± 1.6] and 24.0 % [± 3.7] versus 49.8 % [± 1.8], p-value <0.001, n=3). Furthermore, the fungal burden within the stroma differed between the isolates.

Conclusion: Significant differences were found regarding adhesion, invasion and damage of *F. solani* keratitis isolates in 2D and 3D human corneal infection models. Fluorescence microscopy and transmission electron microscopy revealed a complex host response to the fungal invasion. Cytokine release measurements will lead to further quantification of the host response. Genomic and transcriptomic analysis will further elucidate underlying causes of these strain specific effects.

GIMPP 186

Surviving unknown waters: The journey of *Salmonella*`s integral membrane effector proteins SseF and SseG into eukaryotic membranes Sophie Schminke, Sarah Trenz, Samuel Wagner

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Introduction: *Salmonella* Typhimurium invades and replicates inside eukaryotic cells by injecting virulent effector proteins through two different type III secretion system (T3SS) into its host. While T3SS-1 is important for host cell invasion, the T3SS-2 is necessary for the replication of the bacteria inside the eukaryotic cell. Among the T3SS-2 injected effectors are the integral membrane proteins SseF and SseG. Both possess two trans-membrane domains and are essential for the survival of the bacterium inside the cell [1]. After their injection into the hosts aquatic cytosol, SseF and SseG are integrated into the membrane of the *Salmonella*-containing vacuole by an unknown mechanism.

During my PhD, I want to investigate the membrane insertion mechanism of SseF and SseG into eukaryotic membranes.

Methods: To unravel the highly dynamic process of SseF and SseG"s membrane insertion, I intend to compare their microenvironment after T3SS-2 injection and in-host translation. Therefore, I will use microscopy, biotin proximity labeling and proteomics.

To observe possible effector-cell organelle co-localizations, I will apply live cell microscopy using a turnover-accelerated chromobody (PepCb) conjugated to RFP [2]. Once the host cell expressed PepCb-RFP binds its antigen (Pep), membrane localization of SseF- and SseG-Pep constructs inside HeLa cells can be observed.

Further, *in vivo* biotin labelling of host cell proteins through the activity of biotin ligases will be used to detect also only transient protein interactions of SseF and SseG with proteins within a radius of 10 nm.

Results: First, I optimized the protocol for proximity biotin protein labeling using the biotin ligase TurboID (TID) [3], which was fused to the C-terminus of SseF and SseG.

So far, I showed that SseF and SseG were still injected via the T3SS-2 into host cell and then integrated into membranes after fusion with TID. After addition of biotin to infected HeLa cells, biotinylated proteins could be detected after their enrichment with Streptavidin-conjugated beads. Hence, protein biotin labelling by the effector-TID constructs was successful and will be further analyzed by mass spectrometry. Additionally, I am creating a stable cell line expressing PepCb-RFP to observe effector-organelle co-localization in live cells.

Discussion: The analysis of proteins in close proximity to SseF and SseG and their localization in the host membranes allow an insight into their microenvironment and indicate possible paths for SseF and SseG"s membrane insertion. This will help to further understand the role of SseF and SseG in the pathogenicity of *Salmonella*. Additionally, investigation of a mechanism allowing the pathogen to survive inside host cells could help the development of highly specific anti-infectives in the future.

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GIMPP 187

The PDZ2 domain of TSA47 from *Orientia tsutsugamushi*: Roles in intermolecular cross-linking and recruitment of α-tubulin

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Question: *Orientia tsutsugamushi* (OT) is a Gram-negative, obligate intracellular and human-pathogenic bacterium that replicates in the cytosol. As the causative agent of scrub typhus, a frequent febrile infection endemic in wide parts of Asia, OT has high medical relevance. In the absence of lipopolysaccharides and classical peptidoglycans, cross-linked aggregates of the outer membrane proteins confer OT with structural rigidity. One of these proteins, the type-specific antigen 47 (TSA47), shows high homology to human HtrA1, which is known to interact with microtubules. TSA47 consists of a trypsin-like serine protease domain and two PDZ domains. Recently, our group discovered that TSA47 disrupts the microtubular network and recruits α-tubulin. The present study aimed to investigate the kinetics of the interaction as well as the role of the PDZ domain in TSA47 cross-linking and its interaction with tubulin.

Methods: The codon-optimized TSA47 sequence was tagged with either a FLAG and/or a myc tag, cloned into a mammalian expression vector and transfected into human HuH7 cells. Expression of TSA47 in transfected cells was analyzed at 24 h post transfection (hpt) by Western blot, with and without the reducing agent DTT. To analyze co-localization of TSA47 with tubulin by epifluorescence and confocal laser scanning microscopy, cells were fixed at 12, 24, 36, or 48 hpt, permeabilized, and stained with antibodies against the tags and α-tubulin. Potential tubulin-binding sites of TSA47 were identified by in silico prediction. After identifying the PDZ2 domain as a candidate binding domain, it was removed using molecular cloning techniques, and the new construct was transfected and analyzed as previously described.

Results: Transfected TSA47 disrupted the microtubule network by forming punctate co-localization structures with α-tubulin. The recruitment of tubulin to TSA47-positive structures was observed as soon as TSA47 became expressed (12 hpt) and decreased with longer incubation periods. Intermolecular disulfide cross-linking of TSA47, potentially an oligomerization, was demonstrated by Western blot, as suggested by the presence of higher molecular structures that were abrogated upon addition of DTT. By in silico prediction, we identified two motifs as possible tubulin interaction sites. Deletion of the PDZ2 domain from TSA47 resulted in reduced ability to recruit tubulin. Also, DPDZ2 TSA47 showed a more heterogeneous pattern of higher molecular structures compared to the full-length protein, indicating that the PDZ2 domain orchestrates intermolecular cross-linking of TSA47.

Conclusion: Our results show that the PDZ2 domain of TSA47 contributes to coordinated cross-linking and facilitates the recruitment of tubulin by TSA47, thus hinting at a possible regulatory function of PDZ2 in TSA47 interactions. Although this study suggests a relevance of TSA47 as a pathogenicity factor, its precise mechanism of action remains to be elucidated.

GIMPP 188

Unraveling antibiotic resistance mechanisms and dynamics of antibiotic-resistant *Staphylococcus aureus* isolates during chronic airway infection in people with cystic fibrosis

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Introduction: *Staphylococcus aureus* is one of the most common pathogens isolated from the airways of people with cystic fibrosis (pwCF) and often persists for extended periods. A rising number of multidrug-resistant bacteria has been observed in pwCF. Antimicrobial resistance pattern of the bacteria involved in respiratory infections of pwCF is of increasing concern. We analyzed the diversity and dynamics of antimicrobial resistance in *S. aureus* isolates, which were collected over a long time period (e.g. 20 years).

Methods: 716 *S. aureus* isolates of 14 pwCF were tested for antimicrobial resistance by VITEK®2 or disk diffusion test. Depending on their resistance profile, the isolates were investigated by PCR for the presence of antimicrobial resistance genes or Sanger sequencing for the detection of mutations. Whole genome sequencing was performed for 370 isolates by Illumina or PacBio sequencing.

Results: We observed a high fluctuation of antimicrobial resistance in most of the 14 pwCF. The dynamics and pattern of antibiotic resistance was highly individual and differed in revealing constant resistance during the entire observation period, successive gain of resistance or loss of resistance. Some pwCF harbored isolates with different resistance genes and different mutations in single specimens. Interestingly, one patient was colonized with only one clone during the study period and a second clone with tetracycline resistance appeared exclusively during two observed visits with exacerbation of lung disease indicating special virulence. One pwCF gained a methicillin-resistant *S. aureus* after 11 years, which coexisted with a MSSA clone for 4 years and was later outcompeted by the MSSA clone. For most of the 14 pwCF, an increasing number of resistance genes/mutations during the time period was detectable. While not all instances of increased resistance correlated with antimicrobial therapy, in some events, gain of resistance appeared as side effect of antimicrobial therapy directed against coexisting *Pseudomonas aeruginosa*.

Discussion: Our investigation showed highly individual dynamics and turnover in the antimicrobial resistance pattern of *S. aureus* during chronic airway infection in 14 pwCF. While the main tendency was an increase in antimicrobial resistance, a loss of resistance (genes/target mutations) could be observed too. Emergence of resistance occurred mainly under antibiotic pressure, but we also observed evolution of increased resistance and persisting resistance without antibiotic therapy, indicating survival advantage of such resistant *S. aureus* isolates in this hostile niche of CF airways. Despite improved therapy options for pwCF, *S. aureus* remains a significant contributor to CF lung morbidity. Therefore, the individual fluctuation and dynamics of *S. aureus* isolates with changing resistance pattern should be considered in managing antimicrobial therapy for each individual pwCF.

GIMPP 189

Understanding the role of formyl-peptide receptors in skin colonization and inflammation

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Keratinocytes form a multilayer barrier that protects the skin from invaders or injuries. The barrier function of keratinocytes is in part mediated by the production of inflammatory modulators that promote immune responses and wound healing. Skin commensals and pathogens such as *Staphylococcus aureus* secrete high amounts of phenol-soluble modulin (PSM) peptides, agonists of Formyl-peptide receptor 2 (FPR2). FPR2 is crucial for the recruitment of neutrophils to the sites of infection, and it can influence inflammation. FPR1 and FPR2 are also expressed by keratinocytes but the consequences of FPR activation in skin cells have remained unknown. Since an inflammatory environment influences *S. aureus* colonization, e. g., in patients with atopic dermatitis (AD), we hypothesized that interference with FPRs may alter keratinocyte-induced inflammation, proliferation, and bacterial colonization of the skin.

To assess this hypothesis, we investigated the effects of FPR activation and inhibition in keratinocytes by measuring cytokines via ELISA and multiplex assay, performing microscopical wound healing and using an AD-simulating mouse model.

We observed that FPR activation induces the release of IL-8, IL-1α and promotes keratinocyte proliferation in a FPR-dependent manner. To elucidate the consequence of FPR modulation on skin colonization, we used an AD-simulating *S. aureus* skin colonization mouse model using wild-type (WT) or Fpr2-/- mice and demonstrate that inflammation enhances the eradication of *S. aureus* from the skin in a FPR2-dependent way. Consistently, inhibition of FPR2 in the mouse model or in human keratinocytes as well as human skin explants promoted *S. aureus* colonization.

Our data indicate that FPR2 ligands promote inflammation and keratinocyte proliferation in a FPR2-dependent manner, which is necessary for eliminating *S. aureus* during skin colonization.

GIMPP 191

Hypermutability in *Staphylococcus aureus* cultured from the airways of people with cystic fibrosis (pwCF) is associated with *Pseudomonas aeruginosa* co-colonization, increased antibiotic resistance, prevalence of small colony variants and nuclease activity compared to pwCF with non-mutating *S. aureus*

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Background: *Staphylococcus aureus* is one of the most common isolated respiratory pathogens from the airways of people with cystic fibrosis (pwCF). In order to persist in the hostile environment with abundant neutrophils, co-infecting pathogens and antibiotic therapy, *S. aureus* needs to adapt to these changing conditions. Hypermutability is known to play a major role in the adaptability of pathogens. This study investigates the prevalence of mutator isolates in a number of clinical isolates recovered during a recent prospective study [1], the underlying mechanism for higher mutation rates and the potential triggers for *S. aureus* to develop elevated mutation frequencies.

Methods: 67 *S. aureus* isolates from 14 pwCF and 3 reference strains were assessed for their mutation frequency using the rifampicin or streptomycin mutation assay depending on susceptibility. Phenotyping of all isolates was performed utilizing Columbia Blood and Congo Red agar. DNA isolation and PCR of the *mutS/mutL* operon were performed for all isolates and purified DNA was sent for sequencing. Nuclease activity, *P. aeruginosa* co-colonization and antibiotic resistance were retrieved from the recent study [1].

Results: 9 hypermutable isolates were identified in 4 pwCF. 4 out of 6 isolates, which possess the same nonsense-mutation in *mutL*, were hypermutators. The mutation frequency was significantly higher (α=0,021) if pwCF were co-infected by *P. aeruginosa*. An increased resistance rate was positively associated with the mutation frequency. Gentamicin, makrolide and lincosamide resistance was significantly higher (p=0,05) in hypermutator isolates. PwCF ever colonized with hypermutable *S. aureus* carried a higher prevalence of small colony variants (SCVs) (α= 0,024) and isolates with higher nuclease activity (α = 0,001). No correlation between lung function and mutation frequency was observed (rp = 0,059).

Conclusions: We identified a high prevalence of hypermutable *S. aureus* strains in the airways of pwCF. Co-infection with *P. aeruginosa* likely creates greater competitive pressure and therefore favors the emergence of adaptable hypermutators. *S. aureus* isolates from pwCF, who were ever positive for hypermutators, show characteristics beneficial for surviving such as increased antibiotic resistance, SCVs and nuclease activity.

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Funded by MedK Münster

GIMPP 192

Interaction of pathogenic and carrier isolates of *N. meningitidis* with Calu-3 cells in an Air-Liquid-Interface cell culture model

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Introduction: *Neisseria meningitidis (Nm)* is an obligate human bacterium and the leading cause of bacterial meningitis, mostly occurring in children and young adults. However, *Nm* can be found in 10 – 30% of the healthy population as part of the nasal microbiome [1]. *Nm* is grouped into different serogroups (Sg) based on their polysaccharide capsule. In addition, due to the high genetic variability, the population of Nm is structured into clonal complexes (cc). Among the Sg and cc there are pathogenic and carrier isolates. To this date, there is no clear evidence what distinguishes these two forms of *Nm* isolates. The aim of this study was to investigate the differences between carrier and pathogenic isolates in regard to their interaction, localization and transmigration through the epithelial barrier of the nasopharynx.

Methods: Human lung epithelial cells (Calu-3) were grown on cell culture inserts as an Air-Liquid Interface model, promoting the formation of an *in vivo* like epithelial barrier [2]. We used carrier and pathogenic isolates from the hypervirulent clonal complexes cc32 (belonging to Sg B) and cc22 (belonging to Sg W) to infect the cells for 24h [1]. qPCR of tight junction proteins, TEER measurements, permeability and transmigration assays were conducted to investigate the barrier integrity after infection, as well as the bacterial efficiency to overcome the barrier. To determine expression and secretion of important cytokines we used qPCR and ELISA.

Results: The infection with *Nm*, regardless of the strain, did not affect barrier integrity. In addition, we could show that pathogenic isolates were significantly more invasive and crosses the epithelial barrier more efficient as their corresponding carrier isolates. All strains tested induced an upregulation of cytokine expression, but to a different extent. While the pathogenic Sg B strain MC58 induces the overall strongest upregulation of cytokines, the Sg W carrier isolate led to stronger upregulation as the pathogenic DE13664 strain. The ELISA experiments, conducted for IL-8 and CCL20, confirmed the results from the qPCR.

Discussion: Our results demonstrate that *Nm* overcome the epithelial barrier most likely on a transcellular way, without affecting the barrier integrity. In addition, there are distinct differences in the transmigration rate between carrier and pathogenic isolates, suggesting a possible impairment of the carrier isolates to utilize cellular uptake and transport mechanisms through the cells.

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GIMPP 193

Expression and mode of action of pneumococcal serine proteases

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Introduction: Pneumococci colonize asymptomatically the upper nasopharyngeal cavity, but upon an external trigger, pneumococci disseminate and cause severe infections. Several studies, including our own, have already explored the impact of serine proteases HtrA, PrtA, SFP, and CbpG on the pathophysiology of pneumococci under *in vivo* conditions (Ali *et al*., 2021). However, the substrate specificities of pneumococcal serine proteases are still unknown. Computational structural analysis of the catalytic domain of each serine protease revealed conserved homology between PrtA and SFP as well as between HtrA and CbpG (Ali *et al*., 2021). In this study, we aimed to decipher the expression and regulation of these proteases during different growth phases. In addition, we heterologously expressed and purified the serine proteases, PrtA and SFP in *E. coli* and used them in *in vitro* activity studies with extracellular matrix (ECM) molecules as substrates.

Methods: After optimizing growth of pneumococcal strain serotype 4 (TIGR4) and 19F (EF3030) in minimal medium, we isolated total RNA from early, mid, and late exponential phase. The expression and regulation of serine proteases were analyzed using Northern blotting, qRT-PCR, and LC-ESI-MS/MS analysis. rPrtA and rSFP were expressed in *E. coli* and purified by affinity chromatography using N-terminal His-tag or Strep-tag fusion. Structural analysis will be performed for the SFP using X-ray crystallography. Furthermore, enzyme activity assays were performed with PrtA and SFP using different ECM proteins.

Results: Growth in minimal medium was optimized for the invasive serotype 4 TIGR4 strain and the colonizing serotype 19F strain EF3030. Northern blot analyses revealed that *htrA* is expressed as bicistronic and *prtA* as monocistronic mRNA, while *cbpG* is expressed as polycistronic mRNA. Transcripts were detected under various growth conditions. The *in vitro* activity assay with rPrtA showed specific cleavage of fibronectin, vitronectin, and thrombospondin-1. SFP was able to cleave vitronectin. So far, SFP crystals were obtained with diffraction at a resolution of 4 Å. These data will provide more structural insights into the mechanism of action on a molecular level.

Conclusion: The serine proteases HtrA, PrtA, and CbpG were expressed under all tested growth conditions and have a substrate specificity for different ECM proteins. Optimization of serine protease activity and the search for host protein substrates is continued and will shed light on the impact of serine proteases on pneumococcal host-microbe interactions.

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GIMPP 194

Unraveling the resilience of vancomycin resistant *E. faecium* through long-term desiccation and novel decontamination methods

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The World Health Organization (WHO) listed vancomycin-resistant enterococci (VRE) within high priority on the global list of antibiotic resistant bacteria, prioritizing the need for the development and research of new antibiotics. One representative is vancomycin-resistant *Enterococcus (E.) faecium*, an opportunistic pathogen that is part of the human intestinal flora but can be life threatening for immunocompromised patients. As a cause of endemic hospital outbreaks worldwide, VRE emerged as nosocomial pathogens which can, amongst others trigger bacteremia and endocarditis. Moreover, as vancomycin is the treatment of choice for these infections, therapy options are limited.

Here we tested the survival and metabolic activity after several months of desiccation of five representative *E. faecium* isolates. Additionally, novel and effective decontamination methods against these isolates were investigated. Therefore, the antimicrobial effect of blue light (408 nm) and copper coatings was determined. For all experiments five different *E. faecium* isolates were tested. Here one vancomycin susceptible type isolate, one vancomycin resistant reference isolate, two vancomycin resistant clinical isolates and one copper and vancomycin resistant isolate were implemented.

To estimate the risk of VRE and their role in an already existing pandemic of drug resistant infections it is essential to investigate VRE fitness and narrow down methods against the arising challenge of antibiotic resistant bacteria

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GIMPP 195

Isolate-dependent interaction of *N. meningitidis* with the nasopharyngeal mucus

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Introduction: *N. meningitidis* (*Nm*) is a human pathogen that colonizes the upper respiratory tract of approximately 10-30% of the healthy population. Nm is classified into 12 serogroups (Sg), six of which are primarily associated with disease, each with a regional difference in prevalence. In rare cases, *Nm* is able to cross the initial nasopharyngeal barrier, which is characterized by a pseudostratified columnar epithelium and specialized mucus secreting goblet cells. The mucus represents the first line of defense and is mainly composed of highly glycosylated mucins. In addition, high concentrations of antimicrobial peptides and human defensins can be found in the mucus. In order to efficiently cross the epithelial barrier, Nm must cross the mucus layer to come into direct contact with the cells. The aim of this study was to investigate the ability of different *Nm* Sg to interact with and utilize the nasopharyngeal mucus for efficient transmigration across the barrier.

Methods: To study the interaction between Nm and mucus, Calu-3 cells were grown in an air-liquid interface model, which leads to the formation of an *in vivo*-like barrier and promotes mucus secretion. The protective property of the mucus against *Nm* isolates from Sg B (MC58), C (8013) and W (DE13664) was analyzed by transmigration experiments with or without prior removal of the mucus. To test the ability of the different *Nm* strains to utilize the mucus for their proliferation, mucus was collected from non-infected cells and used for growth experiments over 24 h. A vacuum blot based western blot approach was then performed to visualize possible degradation products of the major mucin MUC5AC.

Results: The transmigration results showed that removal of mucus resulted in increased transmigration for MC58 and DE13664. For strain 8013, we observed an overall weak transmigration that was not affected by the presence or absence of mucus. For the growth experiments, we could see that the addition of mucus to the basal medium improved bacterial growth, with MC58 and DE13664 growing significantly better than the 8013 strain. Analysis of the growth samples by agarose gel western blotting using an anti-MUC5AC antibody showed that the three strains interact differently with the mucus. While MC58 and DE13664 produced a prominent band at a lower molecular weight, this was not detected for 8013.

Discussion: In this study, we were able to show that the mucus layer has protective properties against meningococcal infection, while the actual pathogenicity of the strain is only revealed upon direct contact with the cell. Interestingly, pathogenicity correlates with the ability of the bacteria to use the mucus for proliferation. This may be due to the fact that improved growth leads to an increased number of bacteria coming into direct contact with cells, where other mechanisms determine the efficiency of transmigration.

GIMPP 196

Impact of the *Klebsiella pneumoniae* capsular polysaccharide on fitness and virulence

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Background: *Klebsiella pneumoniae* is a Gram-negative, non-motile, rod-shaped bacterium colonizing mucosal surfaces of humans, but causes also 5% of all nosocomial infections. *K. pneumoniae* causes urinary tract and bloodstream infections as well as pneumonia and hypervirulent strains can cause liver abscesses and meningitis. The high antimicrobial resistance of *K. pneumoniae* strains complicates treatment and a vaccine for prevention is not available making development of new anti-virulence strategies important. One of the major virulence factors of *K. pneumoniae* is the capsular polysaccharide (CPS), which protects *K. pneumoniae* from uptake by host phagocytic cells and activation of the complement system. Hence, the CPS is crucial for immune escape and pathogenicity. A comparative analysis of the encapsulated wild-type with the isogenic unencapsulated mutant will shed new light how the CPS interferes with *K. pneumoniae* adhesion, survival in blood and in an experimental infection model.

Methods: The genome of *K. pneumoniae* ATCC BAA2146 was analysed *in silico* to identify genes in the CPS gene cluster that allow generation of a capsule-mutant. Gene specific primers were designed to amplify the highly conserved *wza* gene (polysaccharide export lipoprotein) and upstream and downstream regions by PCR. The PCR fragment was cloned into the pKOV vector and the *wza* gene sequence replaced by the *aad9* antibiotic resistance cassette. Adhesion of the *wza*-mutant to A549 cells, its uptake by THP1 monocytes as well as D*wza* survival in whole blood and in the wax moth *Galleria mellonella* infection model was compared to the isogenic wild-type.

Results: The unencapsualted geno- and phenotype of the *K. pneumoniae* CPS knockout strain (KP2146Δ*wza*) was indicated on

the molecular level and by scanning electron microscopy. Comparative growth analysis between wild-type and isogenic KP2146Δ*wza* showed no differences in the growth behaviour and generation time. Remarkably, KP2146Δ*wza* adherence to and invasion in A549 lung epithelial cells showed no significant differences when compared to the isogenic wild-type *K. pneumoniae*. However, phagocytosis assays and the experimental infection model demonstrated the impact of the CPS for survival.

Conclusion: This study reveals that the CPS of *K. pneumoniae* has no or only a minor impact on the physiology under *in vitro* conditions, while the CPS is indispensable for full virulence under infection related conditions and *in vivo*.

GIMPP 197

S*taphylococcus epidermidis* survival within primary human and mouse macrophages

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Introduction: *Staphylococcus epidermidis* is a leading pathogen in implant-associated infections. Biofilm formation protects *S. epidermidis* from innate immune effector mechanisms, and plays a key role for persistence in a hostile environment. Previous works from our lab have provided evidence that biofilm formation interferes with phagocytosis, bacterial killing and pro-inflammatory macrophage activation. So far, it is a common opinion that *S. epidermidis* is a strictly extracellular pathogen and lacks the ability to survive intracellularly.

Our aim was to determine the importance of bacterial aggregation and biofilm formation for *S. epidermidis* survival within human and mouse macrophages.

Methods: To study macrophage - biofilm interactions and the importance of polysaccharide mediated biofilm formation, wildtype *S. epidermidis* 1457 and the isogenic, biofilm-negative mutant 1457-10 was employed in infection experiments. The up-take and fate of phagocytosed bacteria was followed during the different phases of pathogen-macrophage contact (0 - 20 h) using quantitative culture, microscopic imaging and FACS approaches.

Results: The experiments confirmed that biofilm formation is critical for interference of *S. epidermidis* with phagocytic up-take from mouse and human macrophages, resulting in reduced uptake of *S. epidermidis* 1457 as compared to 1457-M10. This finding was consistently evident up to 6 hours after infection. Intriguingly, 20 hours post infection still living *S. epidermidis* 1457 cells were present in macrophages at relevant numbers (100 bacteria/macrophage). Of notice, living *S. epidermidis* were also recovered from macrophages infected with biofilm-negative *S. epidermidis* 1457-M10, indicating that polysaccharide dependent biofilm formation is not necessary for survival within macrophages.

Discussion: The findings provide novel evidence that *S. epidermidis* is capable to survive within professional phagocytes. Ongoing work aims at elucidating bacterial and host factors contributing to survival, as well as their importance for *S. epidermidis* pathogenicity during device-associated infections.

GIMPP 198

Type III secretion chaperones and transmembrane proteins: elucidating the mechanisms underlying correct targeting to secretion

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Introduction: Type III secretion (T3S) systems are needle-like molecular machines that allow the transport of proteins across gram-negative bacteria membranes directly into the host cell to ultimately promote bacterial survival. Among the delivered proteins are those containing transmembrane domains (TMD). T3S substrates with more hydrophobic TMD require binding of a cognate T3S chaperone (T3SC) which allows correct targeting to T3S and avoids incorrect targeting to bacterial membranes by outcompeting the components of the Sec-pathway. Thus, a precise orchestration of these processes is crucial for the efficient interaction of the chaperone/TMD-substrate pairs. Here, we aim to unveil the key features and processes that underlie T3S of TMD-substrates using as a model the *Salmonella*'s T3S chaperone/TMD-effector, SscB/SseF.

Methods: For the biophysical characterization of SscB and SscB/SseF, the purified proteins were analyzed by CD, Nano-DSF and SEC-MALS. To determine the key residues for complex formation, alanine scanning mutagenesis was used. Furthermore, protein interactions were analyzed by *in vivo* photocrosslinking and protein stability in *Salmonella* using a chloramphenicol-based assay. To assess T3S, a Nanoluc luciferase-based assay was developed for SPI-2 (Salmonella's pathogenicity island 2) inducing conditions.

Results: Previously, it was observed that *Salmonella*'s TMD-effector SseF required its cognate T3SC SscB to avoid erroneous insertion into bacterial membranes and to allow correct targeting to T3S. These proteins are encoded adjacently in SPI-2 (*sscB-sseF)*, and when gene order was changed a decrease in the T3S of SseF was observed, both in SPI-2 inducing conditions and during infection. Moreover, SscB was stabilized by SseF in *Salmonella*, and the presence of the chaperone binding domain (CBD) of SseF was sufficient for stabilizing purified SscB. The same was observed in SseF, which requires SccB to be stabilized. Furthermore, SscB interacts with SseF's CBD domain and first TMD, but not with the second TMD, in a stoichiometry of 1:1. Interestingly, SscB structural features resemble those of T3SC that bind translocators, also TMD-proteins. Furthermore, by performing alanine scanning mutagenesis, it was shown that SseF bore a "P/VXLXXP" consensus amino acid sequence in the CBD, which is conserved in the translocators of *Salmonella* and other bacterial species. Additionally, other *Salmonella*'s T3SC of translocators, although not able to promote SseF secretion, could stabilize SseF.

Discussion: Overall, these observations suggest that formation of SscB/SseF complex may occur rapidly since a specific gene organization is required and the proteins co-stabilized. This may be to allow for rapid protection of TMD and thus avoid mistargeting to the Sec-dependent pathway. Additionally, these results have shown that T3SC may have evolved to accommodate the structural characteristics of their respective interacting partners.

GIMPP 199

Determination of the prevalence of PIA-forming *Staphylococcus aureus* isolates in urines from patients with (catheter-associated) urinary tract infection and analysis of the underlying molecular mechanism

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Question: *Staphylococcus aureus* is a clinically important Gram-positive pathogen that can cause a wide variety of diseases, including medical-device-related infections (e.g. urine catheters). In people with cystic fibrosis (pwCF), *S. aureus* has been identified as a major respiratory pathogen and is well-known to adapt to the CF-environment by different strategies. Recently, we described a mucoid *S. aureus* phenotype (PT) for pwCF, which overproduced polysaccharide intercellular adhesin (PIA). The PIA-mediated biofilm forming capability was caused by a 5 bp-deletion (5 bp-del) in the intergenic region (IGR) of the intercellular adhesion (*ica*) locus [1] or by various mutations in *icaR* (unpublished observations), encoding for the repressor of the *ica*-operon. The aim of the study was to determine the prevalence of mucoid *S. aureus* in urines from patients with a urinary tract infection (UTI) or catheter-associated UTI (CA-UTI) and to investigate the underlying mechanism for mucoidy.

Methods: We initiated a prospective study between February 2020 and January 2021 to determine the prevalence of *S. aureus* with a mucoid PT, recovered from 87 urine cultures of 74 patients in a tertiary care hospital in Germany. Urine samples were sent to the central laboratory in Münster and streaked on Columbia blood and CNA agar, which suppresses growth of Gram-negative rods. From these plates, 10 *S. aureus* isolates per urine specimen were streaked on Congo Red (CR) agar, which allows to distinguish mucoid from non-mucoid *S. aureus* by the typical growth behavior [1]. All isolates were tested for biofilm formation in a 96-well plate assay, subjected to *spa*-sequence typing and to Sanger sequencing of the IGR and *icaR*. One selected *S. aureus* strain pair (mucoid/non-mucoid) of one patient was analyzed by whole genome sequencing (WGS).

Results: The prevalence of *S. aureus* in urine cultures was 1.6 % (n = 105), of which 87 were further evaluated for mucoidy. 10.4 % of these were identified being mucoid on CR agar. Mucoid isolates from one patient, whose urine was checked regularly throughout the year, were moderate biofilm producers when grown in brain heart infusion broth supplemented with 0.25 % glucose and 4 % NaCl in contrast to previously described mucoid *S. aureus* from pwCF, which produced high amounts of PIA in the same medium without NaCl. Sanger sequencing of the *ica*-operon revealed no mutations for all mucoid isolates. However, when employing WGS, mutations were identified in some genes, including *ebh* and *clfB*, which most likely caused mucoidy. Whether these mutations really have an impact on the mucoid PT, will be elucidated in further studies.

Conclusions: There was an unexpected low prevalence of *S. aureus* with a mucoid PT in (CA)-UTIs, which harbored neither the 5 bp-del in the IGR nor mutations in *icaR*, but mutations in genes encoding for proteins involved in bacterial attachment/adhesion.

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GIMPP 200

Fine tuning in T3SS export apparatus assembly

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Introduction: The type III secretion system (T3SS) is used by many Gram-negative bacteria to translocate a wide variety of effector proteins into host cells. It is a megadalton-sized protein-complex, composed of more than 200 subunits of ~20 different proteins (1). Due to its complexity, it requires a highly coordinated orchestration to ensure successful assembly of the machinery. T3SS assembly is nucleated by the assembly of the export apparatus in the inner membrane, which consists of five highly conserved subunits SctRSTUV. The assembly of export apparatus begins with the formation of $SctR_5T_1$ followed by addition of $SctS_4$ and subsequently $SctU_1$ (2–4). However, the export apparatus has a highly conserved gene order of *sctRSTU* which does not match the assembly order. In this study, we investigated the relevance of the gene order and fine-tuning mechanisms in assembly of the export apparatus in *Salmonella* Typhimurium.

Materials and method: We utilized several biochemical techniques along with genetics. Bacterial crude membrane was extracted and processed for analysis of the export apparatus and formation of needle complex by Blue Native (BN)- and SDS-PAGE. For functionality assessment, a luciferase based-assay (with SipA-NanoLuc fusion protein) was used to measure secretion (5).

Results: To assess the importance of the strict gene order conservation, each subunit of the export apparatus was deleted and *trans*-complemented. Strikingly, *sctT* displayed an overexpression phenotype in the absence of *sctS* whereas the presence of *sctS* upstream of *sctT* led to reduced expression. We found that the mRNA stem-loop structure in *sctS* regulates *sctT* translation. Upon disconnection of *sctST* genes or stem-loop destabilization, an SctT overexpression phenotype was observed. In such cases, a tendency towards reduced secretion efficiency was observed. On the other hand, other changes in gene order that did not result in SctT overexpression did not lead to a significant defect in secretion function.

Discussion: As pore-forming membrane proteins, it is crucial for bacterial survival and infection that the subunits of the export apparatus are synthesized in a highly regulated manner. While the gene order *per se* may not be strictly required for export apparatus assembly, *sctS* is strategically placed upstream of *sctT* to regulate SctT translation and to achieve 5:4:1:1 (SctR:S:T:U) stoichiometry while having equal gene dosages of 1.

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GIMPP 201

Aetiology of acute undifferentiated febrile illness (AUFI) at a tertiary care centre in eastern Uttar Pradesh, India

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Background: Acute undifferentiated febrile illness (AUFI) a common presenting complaint, can cause significant mortality and

morbidity if left undiagnosed. There is a regional variation in the aetiology of AUFI. Due to lack of access to diagnostic testing, fever may be treated empirically or by the patient themselves in areas with limited resources. Moreover, similarity in the symptoms makes an accurate clinical diagnosis difficult without laboratory confirmation. In order to target clinical work up and therapy, it is crucial to be aware of the local incidence of infections.Thus, this study was done to to identify the common aetiology of AUFI at a tertiary care centre in Eastern Uttar Pradesh, India.

Methods: This cross-sectional study was conducted in Sir Sunder Lal Hospital, Banaras Hindu University, Uttar Pradesh, India, between May 2021 and May 2022. All adult patients more than 18 year of age presenting with a fever less than 14 days without any localizing sign were included in this study. Clinical details were entered on a standard data collection sheet after obtaining a written informed consent. ELISA tests were performed on all samples by Leptospira IgM ELISA (NovaTec), Chikjj Detect TM IgM ELISA (InBios), Scrub Typhus Detect TM IgM ELISA (InBios), DENV Detect TM IgM Capture ELISA (InBios), as per the manufacturer protocol.

Results: During the study period, 121 patients admitted with AUFI were included in the study. Out of 121 participants, 70 (57.8%) were male and 51 (42.14%) were female. Mean age was found to be 29.05±9.11 years whereas mean duration of fever was found to be 5.81±3.97days. The most common aetiology found was Dengue comprising 41 (33.88%) of the total AUFI patients whereas 22/41 patients were found positive for dengue alone. Second most common aetiology were Leptospirosis and Scrub Typhus comprising 29 (23.96%) patients each whereas 8/29 patients were found positive for Leptospirosis alone and Scrub Typhus alone was found positive in 10/29 patients.The patients found positive for Chikungunya were 9 (8.49%) whereas only 6 were found positive for Chikungunya only. There were 13 (10.74%) patients which were not found positive for any of the aetiology stated above and needed further clinical investigation.

Conclusion: The most common cause of AUFI was viral. Availability of cost-effective serological test for dengue, scrub typhus and leptospirosis at primary health care setting would lead to early diagnosis and effective management of AUFI in this region. Although clinical diagnosis is not always sufficient to detect all febrile patients, active fever surveillance and serological testing is essential to anticipate epidemic preparedness in terms of resources and healthcare delivery.

GIMPP 202

Frequency of Panton-Valentine leucocidin (PVL) in Staphylococcus aureus (MRSA and MSSA) isolates from clinical samples

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Introduction: Some strains of *Staphylococcus aureus* (SA) produce Panton-Valentine leucocidin (PVL), a secreted toxin leading to neutrophil lysis [1]. PVL-producing SA strains are therefore associated with complicated skin infections as well as severe invasive infections [2]. However, the prevalence of PVL-producing MRSA or MSSA is assumed to be rather low (< 5%), respectively [1,3]. Despite its potential as a severe pathogen, the prevalence of PVL-positive SA in clinical samples is unknown [3]. This study aimed at detecting the frequency of PVL in SA isolates from clinical samples and at giving further information on patients affected.

Material and Method: Over a period of four months, we collected every SA isolate found during routine microbiological analysis (one isolate per patient). After identifying (MALDI-TOF©) and antimicrobial susceptibility testing (VITEK®2), we detected PVL in SA by using RT-PCR (r-biopharm©). In addition, we analysed basic information given on patients affected by PVL (see table 1 and 2). For retrospective statistical analysis we conducted Chi-Square test.

Results: We analysed 556 different SA isolates, 30 being PVL-positive (5.4%) and found in three different origins of samples. We found significantly more PVL-positive SA in samples from men and younger patients (both also in MSSA-subgroup) (see table 1). Most PVL-positive SA were found in skin samples from dermatology. However, PVL-positivity rates were highest in samples from originally sterile sites (7.9%) and in samples from paediatric surgery (24.3%). Also, PVL-positivity rate was higher in MRSA (22.6%) than in MSSA (3.6%) (see table 2).

Discussion: As an important result of this study, we showed that there is a considerable number of not only PVL-producing MRSA but also MSSA in clinical samples apart from skin samples. As a novelty, we detected highest PVL-positivity rates in samples from originally sterile sites and in samples from children undergoing paediatric surgery. Furthermore, MRSA-subgroup analysis showed an unexpectedly high PVL-positivity rate. Considering the present findings, we suggest that there is a gender- and age-related susceptibility to PVL-producing SA and we recommend physicians to establish a low-threshold testing attitude to PVL in order to start effective countermeasures.

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Fig. 1

Table 1: PVL-positivity rate and cohort's characteristics (MRSA and MSSA) (n=556)

Characteristics (n)	% PVL+ (n)	X^2(df), p-value[a]
Sex:		
Male (318)	7.9% (25)	$X^2(1)= 8.85$, p<0.01
Female (238)	2.1% (5)	
Age groups:		
<18 (121)	8.3% (10)	$X^2(4) =4.47$, p<0.05
18-29 (47)	8.5% (4)	
30-49 (99)	5.1% (5)	
50-69 (137)	4.4% (6)	
≥70 (152)	3.3% (5)	
Origin of clinical sample:		
Blood (51)	0% (0)	$X^2(5) =8.49$, p<0.01
Respiratory tract[b] (161)	3.7% (6)	
Skin, mucosa and soft tissue (277)	7.6% (21)	
Originally sterile sites of the body[c] (38)	7.9% (3)	
Urine (29)	0% (0)	
Clinical departments:		
Internal medicine (78)	2.6% (2)	$X^2(6) =32.49$, p<0.01
Surgery (67)	4.5% (3)	
Dermatology (150)	6.7% (10)	
Pediatrics (65)	0% (0)	
Pediatric surgery (37)	24.3% (9)	
Intensive care units (69)	2.9% (2)	
Other departments[d] (90)	4.4% (4)	
Overall PVL-positivity rate (556)	5.4% (30)	

[a] From Chi -Square test

[b] including bronchoalveolar lavage (BAL), tracheal secretion, sputum and nasopharyngeal swab

[c] including liquor, intra-vasal catheters, intraabdominal samples, muscle, joint and bone

[d] Gynecology, ear, nose and throat department (ENT) and neurology

Fig. 2

Table 2: Subgroup-analysis of PVL-positive MRSA and MSSA (n=556)

Characteristics (n=MRSA isolates)	%MRSA/PVL+ (n)	X^2(df), p-value[a]	Characteristics (n=MSSA isolates)	%MSSA/PVL+ (n)	X^2(df), p-value[a]
Sex:					
Male (34)	26.5% (9)	$X^2(1)= 0.79$, p=0.37	Male (284)	5.6% (16)	$X^2(1)=7.99$, p<0.01
Female (19)	15.8% (3)		Female (219)	0.9% (2)	
Age groups:					
<18 (15)	20.0% (3)		<18 (106)	6.6% (7)	
18-29 (2)	0% (0)	$X^2(4) =1.2$, p=0.88	18-29 (45)	8.9% (4)	$X^2(4) =10.3$, p<0.05
30-49 (11)	18.2% (2)		30-49 (88)	3.4% (3)	
50-69 (10)	30.0% (3)		50-69 (127)	2.4% (3)	
≥70 (15)	26.7% (4)		≥70 (137)	0.7% (1)	
Origin of clinical sample:					
Blood (4)	0% (0)		Blood (47)	0% (0)	
Respiratory tract[b] (33)	9.1% (3)		respiratory tract[b](128)	2.3% (3)	
Skin, mucosa and soft tissue (15)	53.3% (8)	$X^2(3)^c=16.1$, p<0.01	Skin, mucosa and soft tissue (262)	4.9% (13)	$X^2(4) =5.2$, p=0,39
Originally sterile sites of the body[d] (1)	100% (1)		Originally sterile sites of the body[d] (37)	5.4% (2)	
Urine (0)	0% (0)		Urine (29)	0% (0)	
Clinical departments:					
Internal medicine (15)	13.3% (2)		Internal medicine (63)	0% (0)	
Surgery (4)	50% (2)		Surgery (63)	1.6% (1)	
Dermatology (8)	37.5% (3)		Dermatology (142)	4.9% (7)	
Pediatrics (10)	0% (0)	$X^2(6) =17.0$, p<0.05	Pediatrics (55)	0% (0%)	$X^2(6)= 26.1$, p<0.01
Pediatric surgery (3)	100% (3)		Pediatric surgery (34)	17.7% (6)	
Intensive care units (6)	16.7% (1)		Intensive care units (63)	1.6% (1)	
Other departments[e] (7)	14.3% (1)		Other departments[e] (83)	3.6% (3)	
Overall (53)	22.6% (12)		**Overall** (503)	3.6% (18)	

[a] From Chi -Square test

[b] including bronchoalveolar lavage (BAL), tracheal secretion, sputum and nasopharyngeal swab

[c] Urine as origin of sample had to be excluded from statistical calculation because no MRSA found

[d] including liquor, intra-vasal catheters, intraabdominal samples, muscle, joint and bone

[e] Gynecology, ear, nose and throat department (ENT) and neurology

GIMPP 203

Application of high-throughput sequencing technologies for comprehensive analysis of bacterial genome methylation and epigenetics in the model bacterium *Helicobacter pylori*

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Background and Questions: Bacterial epigenetics is a recently expanding field of study, which has shown that numerous bacterial species express several methyltransferases (Mtases) which methylate genomic DNA at specific nucleotide motifs. It is well established that DNA methylation is contributing to genomic DNA integrity and replication, however many recent studies expanded Mtase functions also to genome-wide transcript regulation in bacteria. *Helicobacter pylori* is currently one of those bacterial species which possess the highest number and the most variably expressed set of DNA Mtases [1,2]. Although we have a basic understanding of *H. pylori* epigenetics, we need to better characterize the activity and basic biology of the set of methyltransferases under various environmental conditions. More quantitative measurements of global genome methylation are also wanted.

Methods and Results: We have used various methods, including long-read sequencing, enzymatically aided detection, biochemical methods, and mutant analyses, to quantitate specific methylation patterns of the *H. pylori* genome under various conditions. We have thereby collected information on strain-specificity, hemi-methylating and fully methylating Mtase enzymes, local methylation. Furthermore, we have identified basic conditions that modulate genome-wide nucleotide methylation in *H. pylori*.

Conclusions: We have obtained quantitative information on diverse genome methylation patterns in *H. pylori*. This not only helps our fundamental understanding but also makes bacterial epigenetic modulation more accessible for possible therapeutic approaches.

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GIMPP 204

The role of minor cofactor mycofactocin in establishment of mycobacterial dormancy

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Introduction: According to the WHO, one quarter of the world"s population is latently infected by *Mycobacterium tuberculosis*, a pathogenic microorganism - the causative agent of TB, which remains the leading infectious cause of death worldwide. Alcohols are commonly distributed disinfectants. However, a wide variety of bacteria, including Mycobacteria, counteract their activity by active degradation of alcohols. The exact biochemical pathways of ethanol catabolism in mycobacteria, however, are still elusive. It is particularly important to understand how alcohols are degraded or assimilated by metabolically inactive VBNC cells. Recently a new coenzyme, mycofactocin (MFT) was identified in mycobacteria to participate in ethanol oxidation1. The possible participation of mycofactocin congeners in supporting of mycobacterial dormancy was based on the proteomic studies, where the increased abundancy of a flavin/heme dehydrogenase *mftD (Rv0694)* – a product of *mft* gene cluster - in dormant *M. tuberculosis* cells was observed2. Therefore, we aimed to analyze certain mechanisms of mycobacterial adaptation to alcohols in transition of the bacteria to dormancy.

Materials and Methods: For the investigation of the mycobacteria"s physiological adaptation to alcohols" treatment a model microorganism *Mycolicibacterium smegmatis* was used, since this microorganism reflects biochemical treats of other mycobacteria and *M. tuberculosis* in particular3. Untargeted and targeted metabolic profiling of *M. smegmatis* cells, alongside with microbiological description (cell counting, microscopy, flow cytometry) were carried out.

Results: Transition to dormancy resulted in down-regulation of majority of metabolic processes, however adaptive processes, involved in peptidoglycan biosynthesis, accumulation of carbohydrates, porphyrin metabolism are up-regulated. Treatment with ethanol results in increased ROS production. Targeted analysis of mycofactocin congeners demonstrate gradual reduction of the oxidized species to the 3d day of transition to dormancy. Ethanol-treated cells demonstrate up-regulation of the subunits of succinate dehydrogenases and nitrate reductase. Correspondingly the growth of bacteria can be stimulated by addition of succinate or nitrate.

Discussion: Mycobacteria are known for redundancy of their genome, thus genome of *M. smegmatis* encodes 33 alcoholdehydrogenases, which are mostly uncharacterized (especially MFT-dependent ones). The available observations indicate on the coupling of alcohols oxidation to mycobacterial electron-transport chain and discloses the functionality of MFT-dependent alcoholdehydrogenases as ETC-dependent enzymes. Understanding of redox processes occurring in dormant state is a prospective for development of new redox-prodrugs active against latent TB.

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GIMPP 205

Altered AT2 cell functionality in TERC ko/ko mice leads to severe influenza A virus infection

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Introduction: Aging is associated with increased mortality in influenza A virus-associated pneumonia. The aging process in the lungs leads to increased vulnerability to respiratory infections due to the accumulation of cellular damage and increased senescence, leading to major changes in the lung environment and its mechanical function among other altered systemic factors.

A model to study the influence of aging is the use of TERC ko/ko mice. Here, the telomerase enzyme activity is dysregulated leading to cellular senescence, chromosomal instability and subsequently organism aging. TERC ko/o mice show accelerated telomere shortening, premature aging and age-related immune dysregulation and disease. Thus, defective telomere has been suggested as a driving force as well as a risk factor of aging-related pathology.

In lung tissue, alveolar type 2 (AT2) cells are progenitor cells and important for lung homeostasis as they produce pulmonary surfactant, a lipoprotein substance that is required for proper lung function. AT2 cells of old mice show signs of cellular senescence, increased inflammatory signaling and impaired surfactant metabolism. Therefore, these cells are of special interest in understanding the pathomechanism during influenza infection.

Methods: We infected TERC-ko/ko mice and WT mice (G3) intranasal with influenza A virus for 2 and 21 days. The viral load was quantified via plaque assay and secretion of cytokines was measured via flow cytometry. Additionally, mRNA-sequencing of infected AT2 cells from TERC-ko/ko mice was performed. To further determine the infection event, a murine *ex vivo* infection model of lung slices was established.

Results: We observed a more severe course of disease in TERC ko/ko mice compared to WT mice within the first 10 day of influenza A virus infection. This was predominantly characterized by the loss of body weight but also by general symptoms of disease. Both genotypes were able to recover until day 21 p.i.. During the acute phase of infection, the viral load in the lungs was higher in TERC ko/ko mice than in WT mice. Additionally, the pro-inflammatory cytokine response was higher in infected TERC ko/ko mice. The global transcriptome response of infected AT2 cells from TERC ko/ko mice revealed a dysregulation in the inflammatory host response and the cell cycle as demonstrated by the PI3/Akt pathway.

Conclusion: The use of TERC ko/ko mice is suitable to explore how the aging process impacts the immune response and disease manifestation during respiratory viral infection. The results showed that TERC ko/ko mice were especially vulnerable to influenza A virus infection.

In particular, AT2 cells of TERC ko/ko, provide evidence that telomerase dysfunction promotes a limited proliferation rate, increased inflammatory response, and surfactant dysfunction in the lung, supporting an impaired regenerative capacity and more severe disease progression with advancing age.

GIMPP 206

Quantitative proteome analysis of *Klebsiella pneumoniae* under infection-mimicking conditions

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Introduction: *Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram-negative nosocomial pathogen with multiple antibiotic resistances and high rates of mortality in humans. Because effective vaccines are currently not available, potential proteinaceous candidates for vaccine formulations or other treatment options have to be identified. In this work, proteome profiling of a capsule-deficient mutant *K. pneumoniae* ATCC BAA-2146Δ*wza* (*Kpn*2146Δ*wza*) was conducted to study the *K. pneumoniae* proteome under infection-mimicking conditions.

Methods: Unencapsulated *K. pneumoniae* 2146Δ*wza* was cultured in a supplemented RPMI-based minimal medium under physiological stress conditions. Heat and oxidative stress were induced by shifting the temperature from 37°C to 42°C or adding hydrogen peroxide (0.3 mM), respectively, after reaching an OD600nm of 0.5. After harvesting, bacteria were lysed with 2% SDS, incubated at 95°C and benzonase was added to degrade nucleic acids. Bacterial cell debris was removed and samples of the supernatants containing 5 µg protein were incubated with magnetic beads for the trypsin digestion. Peptides were separated via the UltiMate™ 3000 RSLCnano System and analyzed using a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, MA, USA). Samples were analyzed in data independent acquisition (DIA) mode and raw data were analyzed using Spectronaut® Software (Biognosis).

Results: A total of 2664 proteins were identified with at least two peptides during heat stress, representing about 52% of the entire proteome. Under oxidative stress conditions, 2834 proteins were identified with at least two peptides comprising around 55% of the entire proteome. Five proteins were exclusively found under heat stress conditions, including the small heat shock protein IbpB. The proteins with the highest altered abundances under heat stress conditions included heat shock proteins and chaperones representing the expected stress response to heat shock. Specifically, eight proteins have been found exclusively under oxidative stress, such as the ferric enterobacter transport system permease protein FepD. The specific analysis of lipoproteins located in the outer membrane led to the identification of at least 30 lipoproteins under all stress conditions analyzed.

Discussion: Our proteome analysis of unencapsulated *K. pneumoniae* 2146Δ*wza* revealed candidate proteins that might represent target molecules for drugs disarming the pathogen or, in case of the lipoproteins, potential vaccine candidates due to their conservation among *K. pneumoniae.*

GIMPP 207

The influence of established and novel antibiotics on the activity of the WalRK and LiaRS two-component systems of *Bacillus subtilis*

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Introduction: Due to the increasing number of antibiotic-resistant bacteria, the demand for new antibiotics and new antibiotic targets is growing. Two-component systems (TCS) consist of a histidine kinase that detects a signal and a response regulator that conveys adaptation of gene expression in response to environmental changes. WalRK represents the only essential TCS in the Gram-

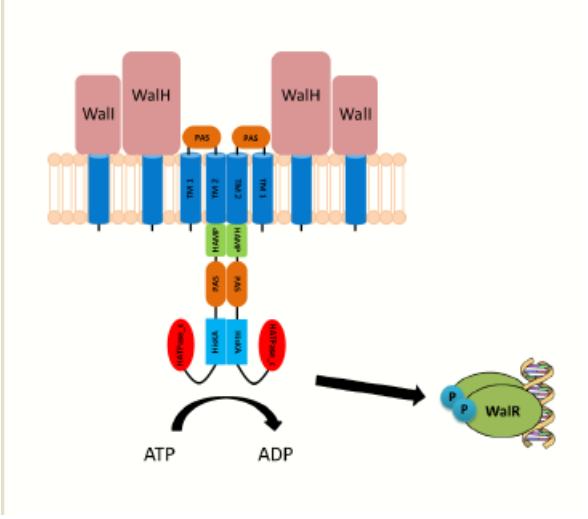
positive pathogen *Staphylococcus aureus* and, therefore, offers itself as a target for new and old antibiotics.. The system controls cell wall metabolism and the expression of autolysins, which are involved in cell wall modeling during cell growth and division. WalRK activates the expression of *ssaA*, encoding an essential autolysin in *S. aureus*. IseA is an inhibitor that blocks the activity of DL-endopeptidases in *Bacillus subtilis*. The expression of the *iseA* gene is negatively regulated by the WalRK system. The induction of *liaI* is an indicator for cell wall stress and represents binding to lipid II.

Material and Method: The upstream sequences of *liaI*, *iseA* and *ssaA* were fused to the luminescence gene cluster of *Photorhabdus luminescens* and integrated into the *B. subtilis* genome. With these reporter clones, luminescence assays were preformed using a TECAN plate reader in the presence of different antibiotics.

Results: Typical expression patterns for all three clones depended on the antibiotic class. The expression of *ssaA* was always downregulated. A strong upregulation of *liaI* and *iseA* was observed in the presence of lipid II binders. A lower activation of *liaI* and a strong upregulation of *iseA* was observed with β-lactams. In the presence of protein biosynthesis inhibitors the expression of all promoters was downregulated. Published WalRK inhibitors indicated a downregulation of *iseA* and *ssaA* at concentrations below the minimal inhibitory concentration and an upregulation of *liaI* at higher concentrations.

Discussion: Although the expected upregulation of *iseA* in the presence of published WalRK inhibitors was not observed, the expression patterns differed from the profiles observed in presence of established antibiotics. In conclusion, the role of the dephosphorylation activity of WalK in this connection remains to be elucidated.

Fig. 1



GIMPP 208

The pneumococcal NanA enhances pneumolysin induced platelet damage

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Background: *Streptococcus pneumoniae* (pneumococci, *S. pneumoniae*) is a major human pathogen and amongst others the causative agent of severe invasive diseases like community-acquired pneumonia. Pneumococci express pneumolysin (Ply), a cholesterol dependent cytolysin, which forms large pores in membranes of eukaryotic cells including platelets. Previously, we have shown that pore formation results in diminished platelet function, representing a new pathomechanism during pneumococcal pneumonia.. Furthermore, we demonstrated Ply neutralization with pharmaceutical human IgG (IVIG, Privigen). Besides Ply, *S. pneumoniae* expresses neuraminidase A (NanA), cleaving specifically sialic acid residues from eukaryotic glycoproteins. This increases binding of Ply to desialylated surface-associated platelet glycoproteins. Here, we aim to decipher how NanA activity affects platelet damage caused by Ply expressing pneumococci as well as their respective Ply deleted strain. In addition, inhibition of these effects by IVIG was analyzed.

Methods: Washed human platelets were incubated with different D39 and TIGR4 wild type and deletion strains (Δ*nanA*; Δ*ply*; Δ*cps*). Platelet desialylation, permeability and Ply binding was measured by flow cytometry after staining with Erythrina Cristagalli Lectin, anti-ply4 or anti-human ß-Tubulin antibody. All experiments were performed in the presence or absence of IVIG (5mg/ml).

Results: Compared to wild type D39 and TIGR4, desialylation of the platelet surface was reduced after incubation with *nanA* deletion strains. Additionally, Ply binding to platelets and platelet damage were nominally reduced when platelets were incubated with Δ*nanA* mutants (MOI0.1). Desialylation of platelets as well as platelet permeability were decreased in the presence of when IVIG.

Conclusion: Deletion of *nanA* reduces Ply binding, hence also weakening its destructive effect on platelets. Therapeutic doses of IVIG can prevent the effects of both, NanA and Ply, therefore representing a promising therapeutical intervention during severe pneumonia.

GIMPP 209

Studying the molecular basis of interaction for *Staphylococcus aureus* virulent adhesion factor SdrC, and subsequent binding effects on mammalian retinal physiology

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Introduction: The Gram-positive opportunistic bacterium *Staphylococcus aureus* is responsible for a plethora of community and nosocomial infections worldwide. Surface associated cell wall adhesion (CWA) proteins contribute to the success of *Staph. aureus* in bacterial virulence, attachment, biofilm formation and subsequent colonization of target host matrix substrates. These CWA adhesins comprise the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) featuring conserved regions, immunoglobulin IgG-like folded domains (N2 and N3 regions) and ligand binding domains. We are interested specifically in the SdrC protein belonging to the serine-aspartate repeat (sdr) subfamily of MSCRAMMs. It has been reported that SdrC interacts with the mammalian neuronal adhesin family of Neurexins localized at the presynaptic photoreceptor synapses.This raises the possibility that synapses could be important targets of adhesion upon bacterial infection, thus enabling such interactions to influence synaptic physiology.

Objectives: The details of molecular mechanisms for bacterial attachment to synapses remain elusive, which hinders novel pharmaceutical development and intervention strategies. Our study focuses on identifying novel receptors for SdrC at the synapse, and also exploring effects of SdrC-Neurexin interaction on synaptic physiology.

Materials and Methods: (I)Cloning and expression of bacterial virulence factor SdrC and respective mammalian receptor Neurexin in protein expression systems, followed by investigating putative interaction (protein pulldown assays, and immunofluorescence colocalization assays). (II) Characterization of the functional impact of the binding on synaptic physiology - synaptic vesicle cycling, Ca^{2+} influx, using genetically modified SypHy and SyGCaMP mouse line respectively. (III)Further identification of novel SdrC receptors in wild type mouse brain lysate via global protein pulldown, and Mass Spectrometry analyses.

Results: (I) We reconfirmed SdrC-Neurexin interaction, though we did not observe any enrichment of Neurexin in our pulldown assays or Mass Spectrometry analyses. (II) Our physiological studies are currently ongoing. (III) An interesting find from our global pulldown assays was identifying synaptic glycoprotein Neuroplastin65 as a novel binding partner to SdrC. Is it needless to say that essential controls were performed with housekeeping proteins like β-actin, fodrin, and GAPDH.

Conclusion: It is possible that Neurexin facilitates only bacterial attachment via SdrC, in turn accelerating the homophilic interaction between SdrC N2N3 domains thus helping in biofilm formation. We are investigating the nature of interaction (direct binding or via an interactome) between SdrC and Neuroplastin65 via further protein pulldown and co-localisation assays. Recent reports of Neuroplastin acting as an auxiliary unit to Plasma Membrane Ca^{2+} ATPases (PMCAs) functioning is also crucial to our study.

PRHYP 210

Schimmelpilzexposition von immunsupprimierten Patienten an einem großen deutschen Universitätsklinikum in den Jahren 2018-2022

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Hintergrund: Schimmelpilze kommen als Bestandteil der Außen- und Innenraumluft ubiquitär vor. Örtliche Gegebenheiten, Temperatur, Luftfeuchtigkeit und Jahreszeiten können die Konzentration von Schimmelpilze in der Luft beeinflussen. Der Einfluss dieser Faktoren auf die Belastung der Außen- und Innenluft in Krankenhäusern und das daraus resultierende Infektionsrisiko für niedrig bis mittelschwer immunsupprimierte Patienten ist unklar.

Material & Methoden: In der vorliegenden retrospektiven Analyse aus den Jahren 2018 bis 2022 werden die monatlich ermittelten Schimmelpilz-Belastungen der Außen- und Innenluft am Universitätsklinikum Frankfurt am Main mit der mittleren Lufttemperatur sowie der relativen Luftfeuchtigkeit gegenübergestellt. Infektionen von niedrig bis mittelschwer-immunsupprimierten Patienten einer hämatologisch-onkologischen Normalstation wurden klinisch und mikrobiologisch (*Aspergillus*-Galaktomannan-Antigen-Nachweis) ermittelt.

Ergebnisse: In den Sommermonaten (Mai-Oktober) waren im Vergleich zu den Wintermonaten (November -April) erhöhte Schimmelpilzbelastungen in der Außen- und Innenluft nachweisbar. Die Schimmelpilzbelastungen in den Patientenzimmern folgten in niedrigeren Konzentrationen den Nachweisraten der Außenluft. Im Beobachtungszeitraum traten bei 3.959 behandelten Patienten zwei nosokomiale *Aspergillus*-Infektionen auf.

Zusammenfassung: Das Risiko, an einer nosokomialen *Aspergillus*-Infektion zu erkranken, lag in unserem Beobachtungszeitraum und Patientenkollektiv bei ca. 0.05 % und erscheint vernachlässigbar. Aufgrund der hohen Schwankungsbreite der Schimmelpilznachweise in Außen- und Innenluft können exakte Grenzwerte für

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PRHYP 211

OprD is involved in virulence of *P. aeruginosa in vitro*

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OprD is a porin specific for arginine uptake of *Pseudomonas aeruginosa* directly involved in the transport of carbapenems into the bacterial cytosol. Strains of *P. aeruginosa* lacking OprD are more resistant to treatment with carbapenems, whereas deletion of *oprD* results in increased virulence colonization in mice. In view of the dual role OprD in colonization and virulence regulation in *P. aeruginosa*, we therefore investigated the contribution of OprD in the invasion of *P. aeruginosa* in cell lines. Isogenic mutant strains of *oprD* or *aguA* and PAO1 WT strain were acquired from the Manoil Laboratory, University of Washington, USA. Since OprD is directly involved in arginine metabolism, Δ*oprD* and WT strains were cultured in distinct nutritive broths and chemically defined media PCN. Growth of OprD-deficient strain in BHI, LB and in the defined medium PCN was similar to the WT-strain. Next, invasiveness of *P. aeruginosa* was assessed in the respiratory cell lines A549 and Detroit 561 (D561). Whereas the invasion of the Δ*oprD*-strain was 2-fold higher than the WT strain in type II alveolar cell lines A549, only slight differences were detected in D561 cells. Visualization of the infection of Δ*oprD* strain by confocal microscopy also revealed that cytotoxic cells infected by the Δ*oprD* strain were 3-fold more than in the WT-strain infected cells. Further invasion experiments over a time lapse of 2 h demonstrated that Δ*oprD* strain constantly invaded the host cells as the WT-strain but cytotoxicity was observed only after 1 h p.i. in cells infected by Δ*oprD* strain, as time-lapse live cell microscopy revealed. Since OprD is involved in arginine uptake, both strains were therefore cultured in PCN supplemented with arginine (PCN Arg) or PCN containing NH4Cl as nitrogen source. Invasion rate of the WT-strain cultured in PCN Arg was 50 % more invasive than in PCN or the Δ*oprD* strain cultivated either in PCN or PCN Arg. In contrast, addition of glutamate to PCN had no effect on invasion neither in the WT-strain nor in the Δ*oprD* strain. Since arginine is decarboxilated by AguA to produce agmatine, deletion of this enzyme considerably reduced the virulence of those strains cultured in PCN Arg. In conclusion, our results showed that OprD and the catabolism of arginine dominantly regulate the virulence of *P. aeruginosa*. Therefore, arginine transport by OprD may trigger the invasion machinery by a signalling circuit that would produce arginine catabolite intermediates that act as signal for upregulation of virulence genes.

PRHYP 212

Point-of-care SARS-CoV-2 antigen tests in private testing centres were rarely used for regular testing in the German asymptomatic screening strategy

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Introduction: In March 2021, Germany started providing cost-free severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen tests, and many day-to-day activities after the lockdown

required negative test results. But how often, when, and how regularly were such test opportunities used over time?

Methods: We analyzed a unique dataset including more than 50,000 pseudonymized records from eight test centers in a typical medium-sized city, one of which was constantly open for eight months. Data were analyzed according to age, origin, time, booking arrangement, frequency, and interchanges between centers. A negative binomial model was applied to assess which variables impact the number of tests.

Results: Centers showed specific types of visitors, of whom most were tested only once in the period investigated. If individuals had been tested repeatedly, they preferred the same location. The preference for spontaneous testing increased with the capacity for spare tests. Compared to the local demographic, tested people aged between 18 to 30 years were distinctly overrepresented. Statistical analysis showed that the implemented mitigation measures influenced the number of tests used.

Discussion: Cost-free testing provided by private testing facilities was implemented as an integral part of the German complementary screening strategy aimed to accomplish weekly testing of the population. According to our data, these facilities were scarcely used for regular testing but rather for meeting the requirements of certified tests. Systematic analysis of usage data in all parts of the strategy is crucial to align the desired effect of a large-scale screening with the actual outcome.

PRHYP 213

The "Municipal Reserve" as a future instrument in municipal pandemic and crises management

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Introduction: The SARS-CoV-2/COVID-19 pandemic has highlighted multifactorial deficits, not only in the public health departments, but in the entire health care system in the context of pandemic management. These deficits must now be identified and measures derived, that will help to ensure, that the public health system, and especially the local public health departments, become more resilient in the context of future pandemics and crises. Within the framework of the Federal Ministry for education and research (BMBF) joint project SARSCoVDx, the needs of those, who were directly involved in pandemic management on the ground, are to be identified and possible municipal structures to be implemented in the future are to be derived.

Material and methods: Sufficient pandemic and crisis management can only succeed, if the actors and decision-makers, working directly on the ground, are involved in the establishment of structures and measures. For this purpose, we will conduct a questionnaire-based needs assessment of the Thuringian health departments starting in May 2023. The questions shall relate to the personnel structure, the material-technical equipment, the Task Force work and the communication strategies, in the context of the pandemic. Furthermore, needs shall be surveyed in the context of questions about the individual challenges of the health departments, existing cooperations and new possibilities and instruments. In the second step of our research project, these results will be compared with the ideas and instruments we have developed, especially focused on Thuringia.

Results: As a result of the comparison of the survey of the Thuringian health departments, with the ideas and instruments developed by us, we expect to envolve a proposal for a practical, sustainable and lived pandemic and crisis management with a focus on a "Municipal Reserve" structure related to Thuringia.

Discussion: Successful pandemic and crisis management depends to a large extent on professional expertise and the technical conditions directly on site. The municipal public health departments have a key role to play here. However, the establishment of future instruments in pandemic and crisis management requires, not only personnel and technical prerequisites, but also optimized communication strategies. Transparency in communication and decision-making, as well as the participation of those who have to act and react directly on the ground, is seen as one of the main requirements for the future. This should be taken into account with our needs assessment of the Thuringian health departments and the subsequent derivation of structures to be established in the future.

PRHYP 214

Developing a rapid phenotypic assessment of phage susceptibility using a nanomotion technology platform

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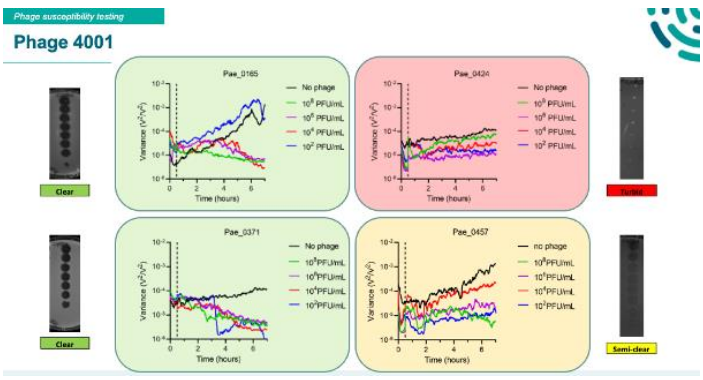
Question: Alternatives to antibiotics, such as bacteriophages, become essential. However, clinical implementation of phage therapy requires fast and reliable phage susceptibility testing (PST) of the infecting strain.

Methods: We propose nanomotion technology1-3 measuring the vibrations of bacterial cells based on their metabolic activity and viability changes for PST. To measure vibrations, we have developed the Phenotech device currently in clinical studies for antibiotic susceptibility testing (AST) in bloodstream infections (NANO-RAST and PHENOTECH-1).

Results: We present the first data obtained with our device measuring *Pseudomonas aeruginosa* isolates from lung infections. Our recordings show the dose-dependent lysis in real-time. For this, we analyzed the changes in the variance of the cantilever deflections over time. While decrease indicated phage-susceptibility, increase indicated phage-resistance. Results were compared to empirical drop test assays (DTA). Depending on the phage-bacterium pair, PST with the Phenotech was as fast as 4h, compared to 16-24h for the DTA.

Conclusions: We plan to extend our experiments to a larger set of *P. aeruginosa* isolates and phages to train classification models with supervised machine learning using common features from the nanomotion signal. These models will contribute to a rapid and reliable PST for optimal selection of phages in clinical protocols of phage therapy.

Fig. 1



PRHYP 215

Estimation of the number of unreported SARS-CoV-2 infections using different indicators

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Question: The incidence rate has been one of the most frequently used indicators to assess the epidemiological situation during the COVID-19 pandemic and to guide health policy measures. As accessibility, testing requirements and willingness to test changed during the pandemic, a increasing number of SARS-CoV-2 infections may remain undetected. The aim of this study is to estimate the actual SARS-CoV-2 incidence in the population by using different indicators as well as changes in the rate of unreported SARS-CoV-2 infections.

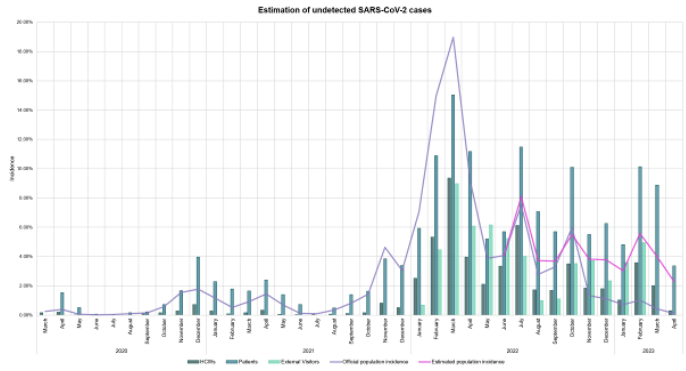
Methods: SARS-CoV-2 incidence rates of the Bavarian population between March 2020 and April 2023 were compared with three indicators: (I) Incidence in healthcare workers (HCW) of the largest tertiary hospital in the region with low-threshold access to PCR tests, (II) Incidence of inpatients of the universal admission screening of this hospital and (III) Incidence in a SARS-CoV-2 rapid antigen test (RDT) diagnostic center for visitors. The latter were corrected and extrapolated according to the limited sensitivity of RDTs.

Results: The incidence from the RDT diagnostic center only correlates moderately with the official incidence rates (r=0.63). There is a high correlation between the general population incidence and the inpatients incidence (r=0.91), as well as between the HCWs incidence and the general population incidence (r=0.94). Between April 2020 and June 2022, the general population incidence was on average 1.99% higher than the averaged incidence of the three indicators, after which the relationship reversed.

Conclusions: The reversal of the relationship between the data series of the Bavarian population and the three indicators in June 2022 points towards a large number of unreported SARS-CoV-2 infections. A possible reason for this could be that the general population is less likely to be tested than HCWs. In addition, the restrictions and measures were almost completely lifted at the same time. Further investigation on a larger scale may be useful to adequately predict actual incidences.

Figure 1: Regional official monthly SARS-CoV-2 incidence (purple) in comparison to the incidence derived from HCWs testing (dark green), patient screening (turkois), and visitor testing (light green). Population incidence extrapolated from HCWs testing and patient screening (pink)

Fig. 1



PRHYP 216

Impact of temperature and pipe material on *Legionella* sp.

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Question: The presence of pathogenic bacteria in potable drinking water pipelines, like *Legionella pneumophila*, can pose significant health risks to end users. Legionellae can be aerosolized by e.g. showers, cooling towers and dental chairs. Once inhaled they can cause severe pneumonia. Depending on the general health status of patients, infections can be fatal. To prevent growth of *Legionella* sp. it is recommended to maintain the temperature of the warm water (PWH) plumbing systems above 55°C.

We investigated how the PWH temperature in the circulation in combination with different pipe materials (PE, SST [stainless steel], CU) affect the amount of *Legionella* sp. in a test rig.

Material/Methods: We constructed a test rig consisting of a circulation ring line and distal pipes that represent the outlets for end users. The module was filled with potable water and flushed automatically. To mimic the highest risk for hypothetical users, low water use was simulated. The experiment consisted of 4 temperature periods (45,50,55,60°C), lasting 28 days each. Samples were taken every 7th day.

The presence of *Legionella* sp. was culturally determined according to DIN EN ISO 11731:2019-03 and additionally investigated via a live-dead qPCR, considering only intact legionellae for the interpretation.

Results: Water temperatures >45°C led to a reduction of cultivatable legionellae (CFU, colony forming units) within the circulation, PE- and CU-pipes, while the CFUs remained stable within the SST-pipe. Water temperatures of 55°C and 60°C led to a reduction of CFUs within the circulating pipe, until no more legionellae were provable. Within the distal pipes, the CFUs fluctuated around the technical action value (100 CFU/100 ml).

Via qPCR, more intact *Legionella* sp. than *L. pneumophila* were detected within all pipes. The GUs (genomic units) of *Legionella* sp. were for the distal pipes alike during all tested temperatures. Within the circulation, the GUs of *Legionella* sp. decreased between 45°C and 55°C. The GUs of intact *L. pneumophila* strongly decreased within the circulation with increasing temperatures, as within the CU- and PE-pipes. In contrast increased the numbers between 50°C and 60°C within the SST-pipe.

Discussion: Increasing the water temperature to ≥ 50°C led to a significant reduction of *Legionella* sp. within the circulation. The raised temperatures were less effective in the distal pipes, where the system did not always reach the desired temperature. Still, at

60°C the amount of *Legionella* sp. was reduced by 1 $\log_{10}$ unit, except for the SST-pipe. The legionellae within the biofilms seemed less affected by rising water temperatures, as the GUs of intact legionellae remained higher compared to the flowing water samples.

The trends within the distal pipes were alike for PE and CU, whereby the effect of increasing temperature was more obvious within the CU-pipes. Lowest temperature effects were observed for the SST-pipes.

PRHYP 216a

Automated pathogen detection– a new invention enforces risk management and drinking water safety

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Water-borne pathogens like *Legionella* or *Pseudomonas aeruginosa* cause serious illnesses, esp. of immunocompromised people. They are common in technical water systems. For risk management, culturing analysis as done for official monitoring are not suitable. Fast methods and shorter sampling intervals are needed. A R&D project aimed to develop an automated detection device that can be installed in buildings to monitor microorganisms, especially water-borne pathogens, in real-time.

A module based device was developed and constructed, that is able to detect target pathogens of interest in bulk water of household installations online and fully automated. The detection method used for this online monitoring system consists of three steps, which first were each developed and validated in laboratory scale: 1.) concentration of water microorganisms by filtration; 2.) separating and labelling of target species of interest by immuno-magnetic separation and fluorescence labelling, 3.) counting of target bacteria by imaging via miniaturized fluorescence microscopy. During the development process, the project focus is on legionellae to detect the most important water-borne pathogen in technical water systems, and on total bacterial counts to monitor general shifts/variations in water quality.

The filtration step is able to concentrate microorganisms by at least two $\log_{10}$ units by reducing water volumes from 10-100 litre to a final volume of 15 ml. For immuno-magnetic separation (IMS) and fluorescence labelling a mix of various polyclonal antibodies, which could be commercially acquired each, of important legionellae strains was used to ensure detection of various *Legionella* species. Inclusion and exclusion were tested successfully, using 54 different strains out of over 40 *Legionella* species and 131 non-legionella species. Lab scale manual IMS recovery rates were >80% up to 100%. Counting of separated and labelled target bacteria is done by automatic image recognition. A newly miniaturised fluorescence microscopy has been developed that is able to identify even one target cell within 200 µl each. In the second project phase, analysis modules will be brought together in one device for totally automated sample processing.

This newly developed device is a great chance to improve water safety. Because manual handling is not needed, and the device itself is totally capsuled, contact to contaminated water or pathogens during sampling or analysing cannot take place, and manpower with special expertise in hygiene, microbiology etc. is not needed. In combination with existing automated water disinfection systems and peripheral disinfectant sensors measuring near tap, which all can communicate to each other, enables the operator of a technical water system, to react immediately in case of inadequate hygienic-microbiological water quality. Those enabled temporary water disinfection only in case of microbial contamination covers highest health benefit for water users.

PRHYP 217

Surface sanitation in public environment using UVC irradiation and its effects on material stability

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Introduction: Pathogens survive on surfaces up to several years and hence pose a risk for infection transmission [DOI: 10.1186/1471-2334-6-130]. To minimize this risk in public, frequently touched surfaces are cleaned regularly, whereby UVC irradiation gained great attendance during Corona pandemic. However, long-term use of aggressive UVC can impair material stability, reduce its lifetime, and change surface characteristics, possibly posing the risk of increased adhesion of dirt and pathogens.

We investigated the microbicidal effect of UVC on materials used for surfaces in public environment and concomitant effects on material stability.

Material/Methods: Samples of commonly used materials for frequently touched surfaces in public (ABS, PVC, stainless steel) were inoculated with *Escherichia coli* and *Bacillus subtilis* spores as model organisms. For determination of UVC inactivation efficacy, specialized test regimes were developed by adapted surface inoculation and irradiation of dry surfaces using UVC LEDs (λ 272 nm, 80 mm distance between LEDs and sample surface). For assessment of the material stability against UVC irradiation, the colour, surface roughness, and the water contact angle were investigated.

Results: We showed fast reduction of the investigated microorganisms by UVC. Within 4 seconds of irradiation (approx. 0.4 kJ/m^2), a reduction up to 5.2 log was achieved for *E. coli*, for *B. subtilis* spores a reduction up to 3.8 log was verified. Furthermore, the amount of inactivation depended on the used sample material (ABS, PVC, or stainless steel).

Applying 120 kJ/m^2 UVC dose, the materials roughness was unimpaired, but optical discoloration was observed for ABS. PVC showed beginning of discoloration after applying a dose of 40 kJ/m^2. The wetting behaviour decreased slightly for all materials investigated.

Discussion: In accordance with numerous studies focusing on the inactivating effect of UVC, we demonstrated the successful inactivation of model microorganisms using UVC LEDs. We showed the dependence of inactivation efficacy from the materials inoculated. ABS, PVC, and stainless steel represent often used materials for high frequently touched surfaces in areas of public life. Besides the microbiological inactivation, we showed the effect of UVC on the materials characteristics and in which way long term use of UVC can impair material characteristics. The results show the importance of having a detailed look on the high frequent use of UVC for surface decontamination in areas of public life. Especially in context with the use of polymers, the UVC decontamination of surfaces should be used in reasoned cleaning intervals and, material stability under long term use should be kept tracked of.

The results are part of an internal funded project of the Fraunhofer Gesellschaft and a project funded by the Federal Ministry for Economic Affairs and Energy.

GIPWP 218

T. solium crude lysate modulates _H. pylori_-induced Akt signalling in gastric carcinoma cells

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Background: *Taenia solium*, a helminthic parasite infects humans and causes taeniasis, cysticercosis, and neurocysticercosis. The parasite is linked to Th2 immunity, which leads to reduced inflammation. *Helicobacter pylori*, a gram-negative bacterium, causes widespread global infection whose chronicity leads to gastric atrophy, dysplasia, metaplasia, and gastric adenocarcinoma. The co-infection of *H. pylori* and helminths is common in endemic areas although no studies have been conducted to understand the interplay of these two pathogens. Therefore, we aim to explore the effect of *T. solium* crude lysate (CL) during the infection of *H. pylori* on gastric adenocarcinoma (AGS) cells.

Materials and Methods: To investigate the relationship between *T. solium* and *H. pylori* coinfection we treated the AGS cell with CL of *T. solium* cyst and infected the AGS cells with *H. pylori*. We measured the inflammatory status by measuring ROS production and checked the cell survival through MTT, apoptosis, and cell proliferation assay. At the molecular level, we have checked the mRNA expression of mTOR, MAPK, JAK2, TYRO3, PDK1, TYK2, ERBB2, ERBB4, HIFs through RT-PCR and the activation of PI3K, AKT, mTOR, PTEN and HIF-1α through western blot.

Results: We have found that the CL reduced the *H. pylori*-induced inflammation by limiting intracellular ROS generation and limiting the *H. pylori*-induced cell proliferation and causing apoptotic death of AGS cells. Decreased *H. pylori*-induced proliferation was associated with the downregulation of mTOR, MAPK, JAK2, TYRO3, PDK1, TYK2,, and HIF1α mRNA expression and decreased activation of PI3K, AKT, mTOR, and HIF-1α while increased activation of PTEN.

Conclusion: Thus, our in vitro study suggests a negative correlation between *T. solium* and *H. pylori* in gastric inflammation and prior infection with taenia may decrease the progression of gastric carcinoma, however, this needs to be validated in clinical settings.

GIPWP 219

A three-year study of campylobacteriosis in outpatients from the Rhine-Ruhr region

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Introduction: The gram-negative rod-shaped *Campylobacter spp.* is considered the leading cause of bacterial gastroenteritis worldwide. Though it can be transmitted human-to-human, the most prevalent way of transmission is zoonotic or foodborne. Most frequently found in chicken, the increase in livestock production and carcass weight of chicken from 2000 to 2020 may explain this leading role of *Campylobacter spp.* Our study analyzed the demographic and geographic trends of campylobacteriosis in different parts of the Rhine-Ruhr region from 2021 to 2022. Thereby, it was possible to infer the transmission mode.

Methods: A total of 1181 *Campylobacter spp*. were isolated from stool samples between January 2021 and December 2022. Bacterial identification was performed by MALDI-TOF. Antimicrobial susceptibilities to azithromycin, ciprofloxacin, erythromycin, and tetracycline were assessed via disk diffusion method. The results were interpreted using EUCAST breakpoints. The frequency of different *Campylobacter* species was determined, followed by the differentiation of the patient cohort by sex and age (15-year intervals). A closer look was also taken at the seasonality of campylobacteriosis occurrences and the residence of the outpatients.

Results: The most prevalent *Campylobacter* species was *C. jejuni* (88%), followed by *C. coli* (10%). Most isolates originated from patients aged between 45 and 59 years (27.3%). Male patients showed a higher rate of campylobacteriosis (55.6%) than female patients (45.4%). Most isolates were collected in the summer months of July (14.3%) and August (14.9%). The patient residence did not influence campylobacteriosis frequency, the Ruhr Metropolis and the Rhineland showed uniform distribution of isolate-to-zip code ratios. Antimicrobial resistance (R) was highest for ciprofloxacin (70.5% R) and tetracycline (47.3% R) and lowest for erythromycin (1% R).

Discussion: The leading species causing campylobacteriosis in our study was *C. jejuni*, which agrees with other studies. Most isolates came from male patients aged 45–59 years. The prevalence of foodborne transmission could be one explanation for this, as more men eat meat daily. A Reason for the decreased occurrence in younger age groups could be that younger people have a higher awareness of climate change and its causes, and therefore, are more open to trying vegetarian or vegan meat alternatives, which carry less risk of transmitting *Campylobacter.* Apart from the climate, the high meat consumption and production also affects antimicrobial resistance. Intensive livestock breeding makes antibiotic therapy for animals inevitable, leading to the spread of highly resistant bacterial strains. Here, we showed that erythromycin is still the preferred option for treating campylobacteriosis.

GIPWP 220

Cytokine secretion and DNA damage in _H. pylori_ infection relies on sub-lethal signals in the mitochondrial apoptosis apparatus

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Introduction: *Helicobacter pylori* (*Hp*) colonizes the gastric mucus layer of around half of the world"s population. Although the infection is mostly asymptomatic, it is a major risk factor for gastritis, gastric and duodenal ulcers and gastric adenocarcinoma. *Hp* is recognized by epithelial cells and activates various signaling pathways through for instance pattern recognition receptors, resulting in inflammation. *Hp* can also induce DNA damage including double-strand breaks. The apoptosis system can be activated by infection, and pathogens have pro- or antiapoptotic effects. We have recently shown that various intracellular pathogens (bacteria and viruses) trigger sub-lethal signals in the mitochondrial apoptotic pathway.

Methods: We infected human gastric carcinoma cells (AGS) with *Hp* and tested for sub-lethal signals in the mitochondrial apoptosis pathway. We determined cytokine/chemokine release and DNA damage as a readout. We further investigated the release of mitochondrial cytochrome *c* and SMAC by Western Blot and confocal microscopy.

Results: Conditions could be established where infection with *Hp* did not induce cell death, but where sub-lethal signals in the mitochondrial apoptosis pathway could be identified. The pathway required the pattern recognition receptor NOD1 and specifically the BCL-2-family member BAK. We found that *Hp*-infection caused a pulse of release of the mitochondrial intermembrane-space protein SMAC apparently from the entire mitochondrial network into the cytosol. SMAC activated alternative NF-kB and was required for the normal secretion of chemokines upon infection. The activity of the mitochondrial apoptosis-pathway also caused DNA-ds-breaks, and in biopsies from *Hp*-patients we observed a correlation of evidence of sub-lethal signaling and the DNA-damage response.

Summary: The results identify sub-lethal signals in the mitochondrial apoptosis pathway during *Hp*-infection. With the release of mitochondrial proteins from the mitochondrial network, the results suggest a new model of mitochondrial signal. The evidence suggests that these signals contribute to inflammation and genome stress including mutations during chronic *Hp*-infection.

GIPWP 221

O-antigen diversification masks highly pathogenic STEC O104:H4

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Introduction: Shiga toxin-producing *E. coli* can give rise to a range of clinical outcomes from diarrhea to the life-threatening systemic condition, hemolytic uremic syndrome (HUS). Although STEC O157:H7 is the serotype most frequently associated with HUS, a major outbreak of HUS occurred in 2011, and was caused by a rare serotype, STEC O104:H4. Prior to 2011 and since the outbreak, STEC O104:H4 strains have only rarely been associated with human infections.

Materials and methods: Whole genome sequencing including bioinformatical and phylogenetical analysis was performed. Further, phylogenetic context, and a hypothesis concerning strain emergence were investigated. Virulence features were examined by cytotoxicity, adherence, and infection assays.

Results: From 2012 to 2020 intensified STEC surveillance was performed in Germany where subtyping of ~8,000 clinical isolates by molecular methods including whole genome sequencing was carried out. A rare STEC serotype O181:H4 associated with HUS was identified and, like the STEC O104:H4 outbreak strain, this strain belongs to sequence type 678. to sequence type 678.

Genomic and virulence comparisons revealed that the two strains are phylogenetically related and differ principally in the gene cluster encoding their respective lipopolysaccharide O-antigens but exhibit similar virulence phenotypes. In addition, five other serotypes belonging to ST678 from human clinical infection (OX13:H4, O127:H4, RKI9:H4, O131:H4, and O69:H4) were identified from diverse locations worldwide.

Discussion: Our data suggest the high virulence ensemble of the STEC O104:H4 outbreak strain remains a global threat because genomically similar strains cause disease worldwide, but that horizontal acquisition of O-antigen gene clusters has diversified the O-antigens of strains belonging to ST678. Thus, identification of these highly pathogenic strains is masked by diverse and rare O-antigens, thereby confounding the interpretation of their potential risk.

GIPWP 222

Impact of host-secreted antimicrobial molecules on tEPEC during infection

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Typical enteropathogenic Escherichia coli (tEPEC) is a highly prevalent cause of infantile diarrhea in developing countries. Hallmarks of tEPEC infection is the formation of bacterial microcolonies (localized adherence (LA)) and the formation of so-called attaching and effacing (A/E) lesions on the small intestinal epithelium. A recent comparative analysis of host transcriptomic datasets from mice infected with different clinical tEPEC strains has identified a pan-epithelial response to tEPEC infection. This gene set encodes for molecules with known antimicrobial activities such as RegIIIγ, RegIIIβ, SAA3 and C3, all downstream of IL-22 signaling. This IL-22-dependent response appears to be a broad-spectrum response, as a similar host response has been seen upon infection with two other enteropathogens (*Giardia lamblia* and *Salmonella enterica* sv. Typhimurium) despite their different phyla and infectious strategies. In this study, we aim to investigate the functional consequences of those antimicrobial molecules on tEPEC fitness and virulence.
First, RegIIIγ, RegIIIβ, SAA3 and C3 binding to tEPEC will be studied using immunofluorescence microscopy and FACS analysis. In addition, we plan to study the impact of various concentrations of recombinant RegIIIγ, RegIIIβ, SAA3 or C3 on tEPEC survival, growth and aggregation. Subsequent potential hindrance to attach to the epithelium - and therefore to form A/E lesions - due to bound antimicrobial molecules will be investigated using an immortalized small intestinal epithelial cell line *in vitro*. Ultimately, the synergistic effect of the cocktail of IL-22-dependent molecules on tEPEC pathogenicity will be studied using a 3D small intestinal organoid model. Here, tEPEC will be microinjected into the lumen of IL-22-stimulated enteroids and the ability of tEPEC to survive, grow, and form microcolonies and A/E lesions will be examined using microscopy, RT-PCR, and bacterial transcriptomics. Preliminary data shows no significant bactericidal effect of RegIIIγ on tEPEC *in vitro* and coating of tEPEC by C3 *ex vivo*. The other objectives of this work are currently being investigated. Overall, our data will help to understand the consequences of infection-induced host-derived antimicrobial molecules on tEPEC virulence and could help with the development of new therapeutics.

GIPWP 223

Methylome evolution through lineage-dependent selection in the gastric pathogen *Helicobacter pylori*

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Introduction: The bacterial pathogen *Helicobacter pylori*, the leading cause of gastric cancer, is genetically highly diverse and harbours a large and variable portfolio of restriction-modification systems. Our understanding of the evolution and functional roles of DNA methylation is limited.

Material/method: Here, we performed a comprehensive analysis of the methylome diversity in *H. pylori*, using a dataset of 541 genomes that included all known phylogeographic populations.

Results: The frequency of 96 methyltransferases and the abundance of their cognate recognition sequences were strongly influenced by phylogeographic structure and were inter-correlated, positively or negatively, for 20% of type II methyltransferases. Low density motifs were more likely to be affected by negative selection, as reflected by higher genomic instability and compositional bias. Importantly, direct correlation showed that methylation patterns can be actively enriched by positive selection and suggests that specific sites have important functions in methylation-dependent phenotypes. Finally, we identified lineage-specific selective pressures modulating the contraction and expansion of a m5c motif, revealing that the genetic load of methylation could be dependent on local ecological factors.

Discussion: Taken together, natural selection shapes both the abundance and distribution of methyltransferases and their specific recognition sequences, likely permitting a fine-tuning of genome-encoded functions not achievable by genetic variation alone.

GIPWP 224

Detection of *Clostridoides difficile* infection in samples derived from outpatients. Is there a role for routine testing?

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Introduction (background, relevance and question of the work): CDI is one of the most frequent causes of diarrhea in hospitals. However, its importance as a cause of diarrhea is not fully understood and remains the topic of debates. The guidelines of the german society of gastroenterology recommends the testing for CDI in Outpatients with diarrhea while the MIQ only supports it in cases with a high pretest probability. The objective of our study is to investigate the role of routine testing for CDI in adult outpatient with acute diarrhea.

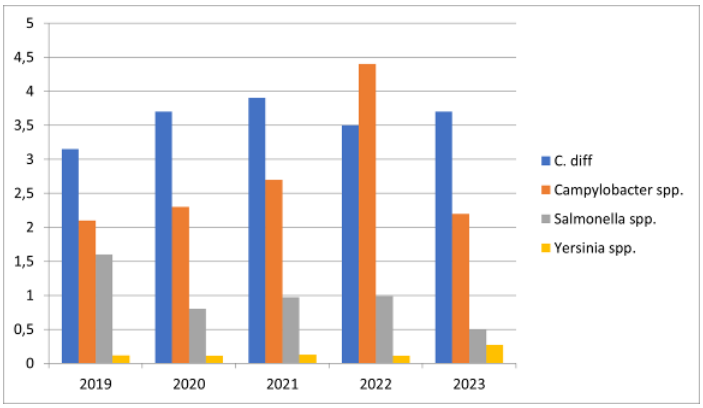
Material/method (patient collective, method, statistical procedures): All stool samples of adult patients sent from the outpatient setting to two private laboratories in the west and northeast of Germany between March 2019 and May 2023 for bacterial pathogens were tested for CDI according to an algorithm recommended by ESCMID. GDH EIA and a subsequent TOX EIA were performed in stool samples that were non-solid. This was followed by toxigenic culture or PCR in case of discordant results.

Results (results with data and statistics): During the study period a total of 85.600 Samples were tested for CDI. Overall 3.048 samples (3,6%) had a configurations suggestive of CDI. Isolated posititivity for GDH was detected in 1.226 cases (1,6%) of negative samples. A total of 1.010 samples (1,2%) were positive in the direct Tox EIA, while 2083 samples(2,4%) were positive in toxigenic culture and tox-PCR respectively. Im comparison with other bacterial pathogens detected in stool samples positivity rates were highest for CDI and did not change over time.

Discussion (Relevance of the work presented and conclusion): Constellations of test results suggestive of CDI are frequent in stool samples sent from outpatient providers. Detection of potential toxin production frequently occured only in a confirmatory step. These findings warrant further investigations wether routine testing for CDI should be recommended for stool samples sent from outpatient setting.

Figure 1. Positivity Rates for bacterial pathogens in outpatient stool samples.

Fig. 1



GIPWP 225

Hygiene measures during the COVID-19 pandemic temporarily reduce intestinal microbiota diversity and affect abundance of bacteria with probiotic potential: A case report

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Background: During the COVID-19 pandemic, adequate hand hygiene was an essential component of containing the spread of SARS-CoV-2 and preventing infection. However, it is not clear what impact increased hygiene measures have on the bacterial composition of the intestinal and respiratory tract. Understanding changes in the microbiome due to systemic changes in hygiene practices is important as the microbiota is a key regulator of host immunity. We present the results of a case study conducted to examine the isolated effect of hygiene measures on the nasal and gut microbiome.

Methods: A healthy participant followed typical hygiene practices recommended during the pandemic, such as frequent use of hand sanitizer and disinfectant soap, for four weeks without further behavioral changes including a constant diet. Nasal and fecal samples were collected every three to five days before, during, and after the experimental period to characterize the bacterial composition of each body site. 16S rRNA amplicon sequencing of the V3-V4 region was performed on the Illumina MiSeq platform. Microbiome changes were evaluated in terms of alpha and beta diversity and at the taxonomic level using Qiime2. Several alpha and beta diversity metrics, as well as a taxonomic analysis, were calculated to investigate the effect of hygiene measures on the reported microbiomes.

Results: For the gut, we observed a continuous reduction in microbial diversity metrics: observed features, Faith-PD and Shannon over the four weeks of intervention. A distinct qualitative change in gut microbiome composition was also observed during intervention. Microbial diversity and composition recovered during the washout period and were more similar to baseline again. (Jaccard, unweighted UniFrac).Interestingly, stronger effects were observed on the gut microbiome compared to the nasal microbiome. Changes in the nasal microbiome were less pronounced, as a change in diversity was only observed for Faith-PD metric, with a similar pattern as described above for the gut microbiome. No clear change in microbial composition was observed. A taxonomic assessment identified changes in key genera, for the intestinal and nasal microbiome, such as *Streptococcus*, *Bifidobacterium*, *Prevotella*, *Staphylococcus*, *Bacteroides*.

Discussion: In this case study, we were able to show that the hygiene measures used to reduce viral transmission during the Corona pandemic had a pronounced effect especially on the intestinal microbiome. Contrary to expectations, a significant change in the gut microbiome was observed using the different diversity metrics, while the nasal microbiome appeared to be more resilient to the intervention and the changes, when detectable, were minor. These results need to be taken into account in further microbiome research.

RKP 227

Emergence of zoonotic *Corynebacterium ulcerans* cases in Germany, 2010-2023: toxigenicity, antimicrobial resistance and molecular epidemiology of human and animal strains.

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Objectives: Diphtheria is still rarely observed in industrialized countries, but since the last 15 years we observe an increase of zoonotic *Corynebacterium (C.) ulcerans* infection in humans. *C. ulcerans* may (similar to *C. diphtheriae*) harbour lysogenic beta-corynephages bearing the *tox* gene encoding diphtheria toxin (DT). Toxigenic *C. ulcerans* may cause classical respiratory diphtheria or diphtheria-like syndromes as well as cutaneous diphtheria. Domestic animals such as cats and dogs and other wild and domestic animals serve as reservoirs for possible zoonotic *C. ulcerans* infection. We analysed toxigenicity, antimicrobial resistance and molecular epidemiology of more than 150 human and animal strains collected in the German Consiliary Laboratory for Diphtheria.

Materials & Methods: Strain identification was performed by biochemical differentiation and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Susceptibility testing was performed according to EUCAST guidelines. Toxigenicity was verified by real-time PCR and the optimized modified Elek-test. Next generation sequencing (NGS) analysis was carried out with MiSeq whole genome sequencing and data analysis by cgMLST.

Results: In case of wound infections *C. ulcerans* is frequently isolated in mixed infections. Asymptomatic carriage may occur in animals. Clindamycin resistance is prevalent in human and animal strains. NGS (cgMLST) analysis revealed more than 24 sequence types in animal and human strains. Analysis of NGS, epidemiological and clinical data indicated *C. ulcerans* strain transmission from different animals and their owners in wound infections and pharyngeal diphtheria-like illness.

Discussion: Since 15 years *tox+ C. ulcerans* has outnumbered *tox+ C. diphtheriae* human cases in Germany. *C. ulcerans* has a huge reservoir in wild, farm and pet animals. These animals can be asymptomatic carriers or suffer from local or systemic infections. Molecular strain typing methods have improved for the epidemiologic research of *C. ulcerans* infections in order to enable the confirmation of strain transmissions between animals and humans. Effective management of a *C. ulcerans* case requires coordination between human and animal health agencies.

RKP 228

Laboratory surveillance report of invasive *Haemophilus influenzae* infections in Germany 2019 to 2022

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Introduction: The National Reference Laboratory for Meningococci and H. influenzae (NRZMHi) carries out laboratory surveillance of invasive *H. influenzae* in Germany. The period 2019-2022 was marked by the COVID-19 pandemic, which led to significant changes in the number of invasive bacterial infections transmitted by airborne droplets..

Materials and Methods: The aim was to present epidemiological data of invasive *H. influenzae* infections from 2019 to 2022.

Isolates from blood and cerebrospinal fluid (CSF) are considered invasive and must be reported in Germany, but submission to the NRZMHi is voluntary. The NRZMHi performed species confirmation, serotyping and susceptibility testing for ampicillin and cefotaxime on all submitted isolates.

Results: From 2019 to 2022, invasive Hi were confirmed in 2202 cases; 2091 isolates were from blood, 101 from CSF only and 10 from both blood and CSF. Laboratory surveillance coverage was estimated by comparing NRZMHi submissions with notified cases at the RKI and ranged from 76% (2019) to 80% (2022). Most isolates were nontypeable *H. influenzae* (NTHi, 1718 isolates, 78.0%), followed by Hif as the most common capsular serotype (282 cases; 12.8%). Hib and Hie were at a comparable level with 72 cases (3.3%) for Hie and 68 cases for Hib (3.0%). Compared to previous years, Hia (59 cases, 2.7%) was found more frequently between 2019 and 2022. Hic and Hid were each found once in 2019 (0.05%). Among the analyzed cases, patients aged > 40 years were most affected (1870 cases; 84.9% of all cases). A significant percentage of cases (179 cases; 8-1%) was found in children aged <5 years. Ampicillin susceptibility testing revealed that 934 (42.2%) isolates were ampicillin-resistant (MIC > 1 µg/mL), 277 (12.6%) showed β-lactamase production. Cefotaxime resistance was found in 27 isolates tested (1.2%).

Discussion: The epidemiology of invasive Hi infections in Germany was strongly influenced by the COVID-19 pandemic in the reporting period. While case numbers in 2019 and early 2020 were at similar levels, restricted mobility and public health intervention during the pandemic led to a significant decrease in invasive Hi infections. Despite the low number of cases, the proportion of Hia cases remained high compared to previous years. The reduction in invasive cases did not impact neonatal infections due to NTHi, suggesting that airborne droplet transmission was prevented during the COVID19 pandemic. With increasing mobility, the number of cases rose again significantly by the end of 2022.

RKP 229

Comparison of monovalent Enzymimmunoassays (EIA) with the gold standard 19s-FTA-Abs-IgM

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Introduction: In 2019 more than 7,800 novel infections with treponema pallidum ssp. pallidum occurred in Germany showing an upward trend since 2010. To prevent clinical sequalae and further transmission, highly infectious patients in the early phase of syphilis have to be identified.

The current standard for the diagnosis of syphilis is a stepwise diagnostic procedure using a screening test followed by confirmatory tests. Treponema specific IgM-antibodies and non-treponema lipoid-antibodies are analyzed to estimate the activity of the infection. Quantification of these activity markers is necessary to confirm the success of therapy in follow-up testing.

Material and Methods: Syphilis-positive, -borderline, and -negative samples were analyzed to evaluate the sensitivity and specificity of three commercially available Enzymimmunoassays (EIA) for the detection of treponema-specific IgM-antibodies. In some cases, IgM immunoblots were added to confirm specificity. All results were compared to the gold standard, 19s-FTA-Abs-IgM-test (fluorescence treponema pallidum absorption). The EIAs were performed according to the manufacturer"s protocol. Spearman's Rho was calculated between EIA and FTA-Abs results for correlation.

Results: The results, presented in table 1 show a significantly decreased sensitivity in the three EIA compared to the 19-s-IgM-FTA-Abs-test.

Tab. 1: Sensitivity, specificity and correlation of IgM-antibodies measured with EIA of three different manufacturers in comparison to the 19s-FTA-Abs-IgM-test.

The correlation of the EIA-results compared to the different titer level (Fig. 1 A-C) indicates a failure of the EIA in detecting lower levels of IgM-antibodies.

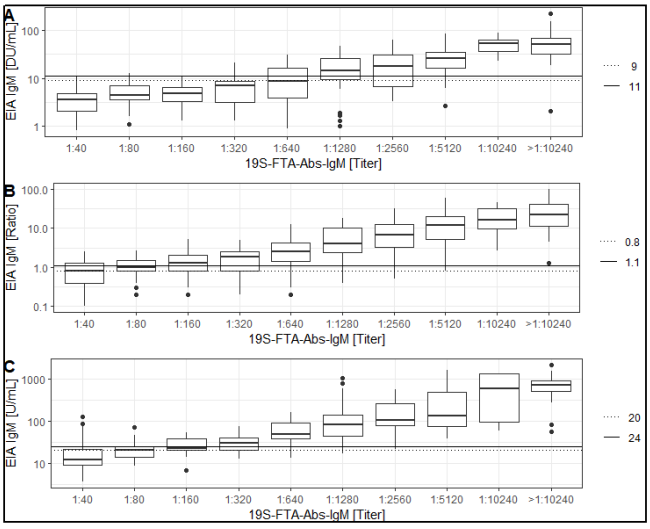
Fig. 1: The box plots show the correlation of the results measured by EIA from Demeditec (A), Euroimmun (B) and Mikrogen (C) compared to the different titer levels of positive samples in the 19s-IgM-FTA-Abs-test. The broken line demonstrates the beginning of the threshold range and the continuous line the boundary value for positive results corresponding to the manufacturer`s specifications.

Discussion: Especially in the early phase of active infection with low levels of IgM-antibodies the EIA is unable to identify highly infectious patients. One reason could be found in the antigen composition of the different test systems. The FTA-Abs-test works with the whole bacterial antigen in contrast to the recombinant antigens used for EIA. Therefore, it would be necessary to recommend more follow-ups for patients with negative results and typical symptoms if these tests are used. It is currently being investigated whether a reduction in thresholds will lead to an increase in sensitivity.

Fig. 1

	EIA IgM [DU/mL] (Demeditec)	EIA IgM [Ratio] (Euroimmun)	EIA IgM [U/mL] (Mikrogen)
Sensitivity [%]	44.24	82.94	76.28
Specificity [%]	97.37	94.74	97.22
Correlation to IgM-FTA-Abs	0.73 (p < 0.001)	0.79 (p < 0.001)	0.84 (p < 0.001)

Fig. 2



RKP 230

Increase of *Corynebacterium diphtheriae* cases among migrants arriving in Germany, 2022

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Background: Early August 2022, Germany"s national public health institute, the Robert Koch Institute (RKI) and the national consiliary laboratory for Diphtheria (NCLD) noticed an upsurge in diphtheria cases: 26 cases were notified in that year, 8 of which among migrants – compared to an annual average of 22 cases in the previous three years, including 3 migrants. Here, we reconstruct qualitatively the outbreak detection, and evaluate how routine surveillance contributed to it. The outbreak description with quantitative epidemiological data is already published elsewhere [1;2].

Methods: RKI initiated an investigation to confirm an outbreak including analysing cases reported in 2022 and comparing with previous years; assessing databases and scientific literature to check alternative explanations; compiling epidemiological data from routine surveillance and microbiological data from Germany"s NCLD. Simultaneously, RKI exchanged information with federal and local health authorities, ECDC and WHO/EURO.

Results: The increase of diphtheria cases among arriving migrants was disproportionally higher than among the local population. Seven out of 8 migrant cases were notified since late July. Early September, RKI publicly announced the detected signal and informed the expert audience via Germany"s Epidemiological Bulletin [3]. Subsequently, whole genome sequencing by the NCLoD and migration route analysis suggested: Affected migrants had acquired toxigenic *Corynebacterium diphtheriae* along the Balkan route. Meanwhile, further European countries reported via ECDC similar cases and sequencing results.

Conclusions: Within six weeks of the event"s beginning it became clear: The rising number of diphtheria cases in Germany was not just an upsurge – but a veritable outbreak, even part of an international one. Germany"s relatively quick detection was facilitated by obligatory, timely conducted single case controls of each diphtheria case reported to RKI, and voluntary data exchange between RKI, NCLD and ECDC. This outbreak is presumably ongoing. Case under-ascertainment is likely. Epicentres are not identified yet. Therefore, active case finding and outbreak detection in supposedly unaffected countries are of utmost public health relevance. We recommend increased awareness among clinicians, microbiologists, and institutions working with migrants; and intensified sample collection from wounds and laboratory diagnostics.

1] Badenschier F et al. Outbreak of imported diphtheria with Corynebacterium diphtheriae among migrants arriving in Germany, 2022. Euro Surveill. 2022;27(46):pii=2200849.; [2] Badenschier et al. Sudden increase of diphtheria with Corynebacterium diphtheriae among migrants arriving in Germany, 2022: an outbreak or just a statistical outlier? ECCMID 2023; [3] Robert Koch Institute. Häufung von Fällen mit Hautdiphtherie in Deutschland und Europa. [Upsurge of cases of cutaneous diphtheria in Germany and Europe]. Epidemiol Bull. 2022; (36):27-8.

RKP 231

Identification of hemolysis-related pathogenicity factors of *Bartonella bacilliformis*

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Introduction: *Bartonella bacilliformis* is the causative agent of Carrion's disease, a vector-borne biphasic illness restricted to the South American Andes. In the acute phase, bacteria infect erythrocytes causing a severe hemolytic anemia with case-fatality rates as high as 90% in untreated patients. Erythrocyte invasion is the most important step in the pathogenesis of Carrion's disease and results in the destruction of erythrocytes (hemolysis) which is contributing to the high mortality rate in humans. Exact knowledge of these processes is crucial for therapeutic drug development.

Methods: To identify genes involved in hemolysis, a Tn5 transposon library was generated and screened for hemolytic activity. Hemolysis knock-out mutants were isolated, the affected genes identified and systematically analyzed by loss of function / gain of function experiments. For this, markerless deletion technology was established for *B. bacilliformis* and the generated deletion and complementation mutants were tested for their hemolytic activity in a novel *in vitro* based hemolysis assay using human erythrocytes. Mechanisms of *B. bacilliformis* hemolysis were further elucidated by testing bacterial culture supernatants and cell lysates. A two-chamber infection model was established to analyze whether direct cell contact is needed to cause hemolysis. Furthermore, a library of active compounds was systematically screened for anti-hemolytic activity.

Results: Two genes were identified that are involved in the process of hemolysis. These mutants demonstrated that the loss of one of the two hemolysis-related genes leads to the complete inhibition of hemolysis whereas the hemolytic activity was restored by plasmid-based complementation. Furthermore, it was demonstrated that hemolysis is a contact-depended process and no extracellular or secreted compounds are involved. Screening of an inhibitor library revealed first anti-hemolytic compounds.

Conclusions: Two hemolysis-related genes / proteins have been identified and were functionally characterized. These findings indicate that both factors are essential for the hemolytic activity of *B. bacilliformis*. The identification of an inhibitor would represent an anti-virulence strategy that prevents a key process in the pathogenicity of *B. bacilliformis.*

RKP 232

***Borrelia tillae* – an *Ornithodoros zumpti* associated *Borrelia* species described decades ago in South Africa**

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Introduction: *Zumpt and Organ published a paper in 1961 that describes the first isolation (into mice) of a new relapsing fever* Borrelia *species that showed some differences in a complement-fixation test and in biological traits to* Borrelia duttonii. *The isolate showed good infectivity in laboratory mice and multimammate*

rats, but failed to infect adult guinea-pigs and vervet monkeys and had low infectivity for rabbits. Interestingly, like B. duttonii, *the isolate proved to be neurotropic in the four-striped rat (Zumpt and Organ, 1961, S Afr J Lab Clin Med 7, 31-35).*

Ornithodoros zumpti Heisch and Guggisberg 1953 is a soft tick species that has been found in nests of *Otomys* rats in the Eastern Cape province of South Africa. As *Borrelia tillae* was successfully isolated from *O. zumpti*, it can be assumed that this is the vector for this *Borrelia* sp.. In particular in view of unsuccessful attempts to infect *O. moubata*, the main vector of *B. duttonii*.

Materials and Methods: In our culture collection at the National Reference Center for Borrelia, Germany, we revived bacteria from a tube labelled *B. tillae* that came from the laboratory of H.E. Krampitz in 1984. We sequenced PCR products of 16S rRNA, *flaB* and multilocus sequence typing (MLST) loci, conducted online database searches and generated phylogenetic trees in MEGAX. An *O. zumpti* colony was screened by PCR targeting the *Borrelia* 16S rRNA locus and sequencing the PCR product.

Results: No sequence records exist for *B. tillae* in online databases, and no close match of 16S rRNA or *flaB* was found in BLAST searches. In 16S rRNA and *flaB* phylogenies sequences of the isolate revived by us cluster on their own branches. MLST sequences of *B. tillae* are not similar to any of the *Borrelia* spp. present in that database which holds several species of relapsing fever and related species. In fact, they show considerable differences indicating that this species differs from all so far sequenced Borreliae including *Candidatus* B. kalaharica. Borrelial 16S rRNA sequences from an *O. zumpti* colony were identical to the sequence obtained from the cultured isolate.

Discussion and conclusion: 16S rRNA, *flaB* and MLST sequences provide evidence that the recovered isolate differs from all *Borrelia* spp. for which sequence data exist. Initial screening of an *O. zumpti* colony recovered identical 16S rRNA sequences to the cultured isolate strongly supporting the notion that we recovered indeed *B. tillae*, a species described several decades ago. To consolidate our findings with previous descriptions, we would need to get an isolate from *O. zumpti* and do infection experiments with the respective animals that were used in the original work. Whole genome sequencing is currently in progress.

RKP 233

An ensemble genome reconstruction pipeline enables to fully resolve the complex *Borrelia* genome

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Introduction: Bacteria of the *Borrelia burgdorferi* sensu lato complex are tick-borne agents of Lyme borreliosis. Different genospecies vary in their vector/host associations and pathogenicity. The genetic basis for these adaptations is unresolved and requires complete genomes for comparative analyses. The *de novo* assembly of the complete *Borrelia* genome especially of the plasmids is challenging due to high number of plasmids and sequence similarity. As plasmids are dynamic even within isolates of the same species, there are no references available. We developed a high fidelity approach to reconstruct the *Borrelia* genome improving assemblies. The workflow (ensemble pipeline) includes different sequencing technologies and assembly strategies, several manual curation steps and reconciling different assemblies (Figure 1). By analyzing 27 *Borrelia* isolates, we determined if this approach enables the reconstruction of a fully resolved genome of all isolates, including the complete set of plasmids.

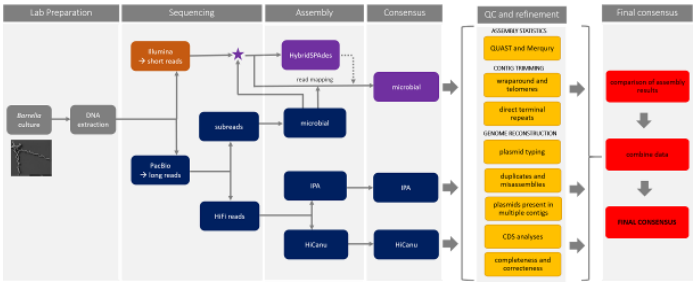
Material and methods: We generated long-read sequence data (PacBio continuous long reads and PacBio HiFi data) and short-read data (Illumina) for 27 isolates belonging to the genospecies *B. bavariensis* (n=16), *B. garinii* (n=9) and *B. valaisiana* (n=2). Three assemblers were used for genome reconstruction: 1) PacBio Microbial Assembler, 2) PacBio Improved Phased Assembler (IPA), 3) HiCanu (modified Canu Assembler). Several quality control and refinement steps were conducted, and assemblies were compared regarding genome completeness and correctness. Final consensi were generated by data combining and analyzed for fully resolved genome elements.

Results: Despite the latest technologies, the use of only one sequencing and assembly method is not sufficient to define a finished *Borrelia* genome. Plasmids that were incomplete or not recovered from one assembler were sometimes completely reconstructed using another assembler. Initial analyses of the final combined consensi of 8 samples to date showed that the ensemble pipeline (Figure 1) successfully resulted in fully resolved genomes of all samples (*B. valaisiana* n=2, *B. bavariensis* n=5, *B. garinii* n=1).

Discussion: Our study demonstrates that the ensemble reconstruction pipeline enables the complete reconstruction of chromosome and plasmids, even for complex genomes such as *Borrelia*. The presented pipeline may be of interest for the assembly of further complex microbial genomes.

Figure 1: Schematic overview of ensemble pipeline for *Borrelia* genome reconstruction. Grey: Lab preparation steps. Dark blue: Data based on PacBio sequencing. Orange: Data based on Illumina sequencing. Purple: A combination of PacBio and Illumina data. Yellow: QC and refinement steps. Red: Steps to generate the final consensus.

Fig. 1



RKP 234

Comparative antimicrobial activity of ceftazidime-avibactam and cefiderocol against *Burkholderia cepacia* complex species isolated from cystic fibrosis patients

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Chronic infection with *Burkholderia cepacia* complex (BCC) is associated with increased morbidity and mortality, and represents a significant challenge to clinicians treating people with cystic fibrosis (CF). So far, no objective therapeutic guidelines exists neither for eradication therapy nor for the antibiotic treatment of exacerbations or chronic BCC lung infection. The BCC currently comprises at least 22 closely related species that are typically inherently resistant to the majority of available antibiotics. Moreover, antimicrobial susceptibility testing of BCC is notoriously problematic and so far no clinical breakpoints for BCC organisms have been established by EUCAST. The aim of this study was to assess the susceptibility of BCC to ceftazidime/avibactam and cefiderocol (the testing of other agents is ongoing), representing new therapeutic options for the treatment of multidrug-resistant gram-negative organisms. BCC isolates (n = 99) from individual CF patients (about 30% *B. multivorans*, 30% *B. cenocepacia* and 30% other BCC species) were tested by reference broth microdilution (BMD) as recommended by EUCAST. Both, ceftazidime-avibactam and cefiderocol were found to be highly active with susceptibility rates of 92% (using ≤ 8g/mL as a tentative breakpoint as in case of. *Pseudomonas aeruginosa*) and 90.5% (using ≤ 2 µg/mL as breakpoint), respectively. Minimal inhibitory concentration (MIC) ranges, MIC_{50}- and MIC_{90}-values were as follows: 0.25 µg/mL - 32 µg/mL; 2µg/mL, and 8µg/mL (for ceftazidime/ avibactam) and 0.03 µg/mL - ≥ 64 µg/mL; 0,125 µg/mL, and 2µg/mL (for cefiderocol). Comparison of cefiderocol and ceftazidime/avibactam MIC test stripes (Liofilchem, Italy) with BMD showed a categorical agreementof 96.8% and 97%, respectively, with only 3,2% and 1% very major errors. Thus, ceftazidime/avibactam and cefiderocol may be relevant therapeutic alternative options to treat BCC infections in CF.

RKP 235

Genomic surveillance of human listeriosis in Germany, 2018-2021

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Introduction: Listeriosis is a foodborne disease caused by the ubiquitous bacterium *Listeria monocytogenes* and characterized by high fatality rates of up to 30%. Consequently, the monitoring of outbreaks and the identification of contaminated food sources is vital for maintaining public health. Within the German listeriosis surveillance system, infection related isolates are collected and their genomes sequenced at the Consultant Laboratory for *Listeria* located at Unit 11 of the Robert Koch Institute in Wernigerode (1). We here present an in-depth analysis of the population structure of clinical *L. monocytogenes* isolates from 2018-2021 with respect to important outbreak clusters, incidence of certain phylogroups and their genomic and clinical characteristics.

Material/Methods: Clinical isolates were subjected to whole genome sequencing (WGS). Assembly and further WGS-based subtyping as well as a scan for the presence of virulence and resistance genes was performed. Closed genome sequences of the most relevant subtypes were reconstructed by hybrid Illumina and MinION sequencing. Clinical information on disease manifestations was used for the calculation of risk for developing different disease manifestations among the various sequence types.

Results: Between 2018 and 2021, 1802 genome sequences of clinical *L. monocytogenes* isolates were acquired and analyzed revealing a variety of 109 different multi locus sequence typing (MLST) sequence types (STs) and 188 core genome MLST (cgMLST) clusters, of which 21 comprised more than ten isolates. Five large outbreak clusters could be identified consisting of 32-132 isolates (2-4), from which for two the infection source is still unknown. Furthermore, our work provides an overview of genomic traits, outbreak-relatedness and diseases manifestation among clinical isolates from a genomic pathogen surveillance program over four consecutive years.

Discussion: Molecular surveillance of listeriosis in Germany helps to identify epidemiologically linked cases and their geographical distribution, which facilitates the identification of contaminated food sources and thereby enables timely intervention and initiation of public health measures. By looking at the outbreak clusters on a molecular level, further insights into the properties of certain phylogroups such as e. g. the virulence potential can be gained, although the contribution of other factors in addition to the genetic prerequisites must be considered. Our work also provides the basis for the further analysis of different *L. monocytogenes* phylogroups to identify the genetic basis of hypo- and hypervirulence in experimental infections.

1 – Halbedel *et al.* ***J Clin Microbiol***. 2018 May 25;56(6):e00119-18.

2 – Halbedel et al. ***Emerg Infect Dis.*** 2020 Jul;26(7):1456-1464.

3 – Lachmann et al. ***Clin Microbiol Infect.*** 2021 Jul;27(7):1035.e1-1035.e5.

4 – Halbedel et al. ***Microbiol Spectr.*** 2023 Apr 10:e0352022.

RKP 236

European multi-center study to establish MIC and zone diameter epidemiological cut-off (ECOFF) values for *Bacillus anthracis*

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Background: *Bacillus anthracis*, the etiologic agent of anthrax, is a zoonotic microorganism that mostly affects herbivorous mammals, but can be transmitted to humans by contact with infected animal or their products. It is endemic almost worldwide, and it is considered one of the most important biothreat agents. If not promptly treated, *B. anthracis* infections can lead to death within few hours, underpinning the importance of a timely and

effective antimicrobial treatment. By now, for this microorganism, no antimicrobial susceptibility testing (AST) standards are available. Therefore, in this study, we aimed at setting up, in collaboration with EUCAST (European Committee on antimicrobial susceptibility testing), epidemiological cut-off (ECOFF) values to distinguish between wild-type or not microorganisms.

Materials/methods: Under the framework of an EU-funded Joint action 335 *B. anthracis* isolates (17 to 146 per center) from human, environmental and animal origin were tested at six study sites against ten therapy relevant antimicrobials (amoxicillin, benzylpenicillin, ciprofloxacin, clindamycin, doxycycline, levofloxacin, linezolid, rifampicin, tetracycline and vancomycin) by means of disc diffusion (DD) method and broth microdilution (BMD) with user-defined commercial BMD plates. Each center validated the methods testing three quality control (QC) strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) over 10 days and comparing the results with the EUCAST QC tables. AST was performed according to EUCAST, but with reduced incubation time compared to ISO 20776-1. *B. anthracis* MIC and DD distributions were submitted to EUCAST and aggregated results were curated in accordance with EUCAST SOP10.2.

Results: For each drug investigated, ECOFF values were defined based on 330 to 335 observations. DD and BMD data distributions, revealed a wild-type phenotype for the majority of the isolates. Three strains with benzylpenicillin MIC values of 32 mg/L were found, indicating resistance to this drug. MIC values above the defined ECOFF values were observed in a few strains, indicating the presence of low level resistance to benzylpenicillin, clindamycin, doxycycline, levofloxacin, linezolid and tetracycline. The genetic background to the resistance mechanisms and the phenotypic shifts observed remain to be investigated.

Conclusions: In this multi-centre study, we validated the use of BMD and DD methodologies for AST of *B. anthracis* and determined MIC and zone diameter ECOFFs for ten antimicrobial agents. The ECOFFs can now be used to distinguish between wild-type (WT) and non-WT *B. anthracis*. Together with clinical data our results will pave the way for EUCAST to determine clinical MIC breakpoints for this microbial target.

RKP 237

European multi-centre study to establish MIC and zone diameter epidemiological cut-off (ECOFF) values for *Brucella melitensis*

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Background: *Brucella* (*B.*) *melitensis*, the causative agent of brucellosis, is a zoonotic agent causing about 500,000 human cases annually and is endemic in the Mediterranean basin, the Middle East, parts of Central and South America, Africa and Asia. The disease is associated with a high risk of chronification and relapses and requires a long-term antimicrobial combination therapy. *B. melitensis* is a laboratory Risk Group 3 organism, the most frequent cause of bacterial laboratory infections and a potential biothreat agent. Antimicrobial susceptibility testing (AST) standards for *B. melitensis* are not available and it is not yet listed in the EUCAST breakpoint tables. In this study, we aimed to establish minimal inhibitory concentration (MIC) and zone diameter distributions of wild-type strains from different origins to set epidemiological cut-off (ECOFF) values for *B. melitensis* using EUCAST methodology.

Materials/methods: Under the framework of an EU-funded Joint action 499 *B. melitensis* isolates (20 to 247 per centre) from human and animal origin were tested at six study sites against nine antimicrobials (ceftriaxone, ciprofloxacin, doxycycline, gentamicin, levofloxacin, rifampicin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole) by disc diffusion (DD) method and broth microdilution (BMD) with user-defined commercial BMD plates. Each centre validated the methods with three QC strains (*E. coli* ATCC 25922, *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213) and compared results with EUCAST QC tables. BMD was performed according to ISO 20776-1, but with prolonged incubation as described for *B. melitensis* before (Tscherne *et al.* 2022). DD was performed with EUCAST Mueller-Hinton agar for fastidious organisms. *B. melitensis* MIC distributions were submitted to EUCAST and aggregated results were curated in accordance with EUCAST SOP10.2.

Results: For each drug investigated, ECOFF values were defined based on 249 to 499 observations. DD and BMD data distributions, revealed a wild-type phenotype encompassing the majority of the isolates. Single strains showed slightly increased MICs, above the ECOFFs for ceftriaxone (n=3), doxycycline (n=2), rifampicin (n=2), streptomycin (n=2) and trimethoprim-sulfamethoxazole (n=3). If they have acquired resistance mechanisms will be further analysed genotypically.

Conclusions: In this multi-centre study, we have validated the use of BMD and DD methodology for AST of *B. melitensis* and determined MIC and zone diameter ECOFFs for nine antimicrobials. The ECOFFs can now be used to distinguish between wild-type (WT) and non-WT organisms. Together with clinical data they will serve as background data to set clinical MIC breakpoints and disc diffusion correlates for *B. melitensis*.

Reference:

Tscherne A, Mantel E, Boskani T, *et al.* Adaptation of *Brucella melitensis* Antimicrobial Susceptibility Testing to the ISO 20776 Standard and Validation of the Method. Microorganisms. doi:10.3390/microorganisms10071470

RKP 238

Impact of the COVID-19 pandemic on invasive meningococcal disease in Germany: a laboratory surveillance report 2019-2022

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Introduction: The implementation of non-pharmaceutical interventions against COVID-19 also influenced invasive infectious diseases caused by bacteria transmitted by respiratory droplets, including meningococci. Thus, not only in Germany, but worldwide, the number of cases declined significantly **(**Brueggemann et al., Lancet Digit Health 2021)**.** The measures had a sustained impact on invasive meningococcal disease (IMD) in

Germany, where case numbers remained at a very low level in 2022. Here we report the epidemiology of the German IMD cases submitted to the reference laboratory 2019-2022

Methods: Data on IMD isolates and clinical samples of suspected IMD cases sent to the reference laboratory were analysed according to age, serogroup, antimicrobial susceptibility, and whole genome sequencing (WGS).

Results: In comparison to 2019, the number of IMD cases decreased by more than 50% in 2020. In the following year a further decrease by around 50% was noted. Only after May 2022, the number of IMD cases increased slowly.

During the pandemic, infants were most affected. Thus, the proportion of IMD cases in this age group increased significantly from 10% in 2019 to 21% in 2021 and 2022. The proportion of IMD cases in toddlers increased from 13% in 2019 to 19% in 2021 but dropped again to 12% in 2022. Among adolescents, a minor decrease of cases was noted in 2020 and 2021, whereas the proportion of IMD cases in 2022 (15%) was higher than in 2019 (10%).

Serogroup B was dominant in each year at a percentage between 56% (2020) and 69% (2021). The percentage of serogroup C cases dropped from 11% (2019) to 4% (2022). After a doubling of serogroup W cases from 8% (2019) to 16% (2021) the percentage reduced to 6% in 2022. In 2019 and 2021, the percentage of serogroup Y cases was around 20%, but dropped to 3% in 2021. Finally, the percentage reached 24% in 2022.

Based on WGS, eight genetic clusters were detected in 2019, two in February 2020 and none in 2021.

No major changes in antimicrobial susceptibility were detected.

Conclusions: Germany experienced an unimaginable decline of IMD cases, not only in 2020 and 2021, but also in 2022 when most COVID-19 containment measures already had ended. Case numbers only increased in 2023.

RKP 239

Insights into the integrated genomic surveillance (IGS) of STEC in Germany 2020-2022

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Question: Shigatoxigenic *E. coli* (STEC), including enterohemorrhagic *E. coli* (EHEC), belong to the top ten pathogens for food- and waterborne caused diseases. Since 1995, the German National Reference Center (NRC) at Robert Koch-Institute has been performing molecular surveillance for clinical STEC/EHEC and in 2015 whole genome sequencing (WGS) was introduced.

Methods: All STEC/EHEC samples (n=1,243) analyzed at NRC (respective to 85% of reported cases in Germany from SurvNet@RKI) were genome sequenced between 2020 and 2022. Analysis of sequences for surveillance and cluster detection with the common cgMLST scheme of Enterobase for *E.coli* was performed by using RIDOM seqsphere software (at least version 8.5.1). Sequence data were uploaded in database miGenomesurv (https://www.medizin.uni-muenster.de/migenomesurv/home.html).

Results: We found a high diversity of serotypes including the most important and those commonly associated with human diseases, as well as rare serotypes like O2:H6, O156:H25 and O182:H25.

Dominant serotypes in this period were O26:H11 (16%), O146:H21/H28 (12%), O91:H14 (9%) and O157:H7 (8%).

Nine new serotypes were found, as well as hybrid pathovars, like EHEC/ETEC, and a great number of different virulence associated genes (VGAs). About 30% of the isolates contained *stx*1-gene phages (71% *stx*1a, 27% *stx*1c, 2% *stx*1d) and 70% were positive for *stx*2-gene phages (44% *stx*2a, 36% *stx*2b, 9% *stx*2c, 1% *stx*2d, 6% *stx*2e, 3% *stx*2f, 1% *stx*2g).

Approximately 25% of all sequences were integrated into clusters (threshold allelic distance > 10) including two to ten isolates. The majority of cases for this period of time were sporadic. Identification of the source of infection was successful in two cases.

Conclusion: WGS was successfully implemented to assess cluster detection, virulence gene analysis, serovar prediction and detection of STEC hybrid variants as well as the emergence of novel pathogen types. Several infections with *stx*2f gene positive strains, which additionally showed a plasmid-encoded novel attachment protein BfpA-2 (serovars O128:H2, O125ac:H6 and O145:H34), were detected during the indicated period. Of further interest were human strains with *stx*2g gene content of serovars O38:H39 and O187:H28, which were previously detected primarily in flour. For the first time, isolates with *stx*2d gene of serovars O80:H2, O115:H21, O168:H8, O17:H45 and O91:H10, as well as O8:H9 isolates in combination with stx2a and stx2d genes were detected, indicating possible novel pathogens with increased virulence potential. Novel recently published O antigens OgN1, OgN14, OgN10, OgN14, OX18, RKI1, OX18, OX25, RKI3, RKI6 and RKI7 were found, which can only be detected by sequence analysis. Identification of the source of infection was successful in two cases (ready-to-eat salad (RASFF) and catering service).

RKP 240

MRSA on the decline..... tasks of an National Reference Center (NRC) for staphylococci 2.0

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Background: Fortunately, in recent years we have seen a continuous decrease in MRSA rates in hospitals, but also in outpatient settings. This is the case in Germany, but also for large parts of Europe and beyond. At the same time, we are encountering an increasing number of isolates of *S. aureus* and other staphylococcal species with multi-resistance or resistance to antibiotics of the last resort at the NRC.

Materials: Since the beginning of the 2000s, the NRC for staphylococci has been analysing between 2500 and 4500 staphylococcal isolates annually from submissions of local laboratories and from a wide variety of studies. All incoming isolates are phenotypically characterized and subjected to resistance testing using broth microdilution according to EUCAST. In addition, all *S. aureus* isolates are typed using *spa*-typing. Since 2015, WGS has been increasingly used for molecular characterization of isolates. For *S. aureus* and *S. capitis*, core genome MLST (cgMLST) schemes are available for molecular typing. For other species, we use in-house ad hoc cgMLST schemes to analyse strain relatedness. Since 2020, all MRSA from blood cultures are subjected to WGS.

Results: Alongside the development of MRSA rates, MRSA submissions have decreased over time. These submissions in part still relate to outbreak analyses, but also to classic surveillance tasks that can be processed with NGS at significantly higher resolution. On the other hand, submissions of MSSA with "unusual" resistance phenotypes or resistance to last resort

antibiotics are increasing. We are also seeing an increase in submissions of coagulase-negative staphylococci, which often show multidrug resistance phenotypes. These are often *S. epidermidis*, but also increasingly other species (*S. haemolyticus*, *S. hominis*, *S. capitis*, *S. pettenkoferi*....).

Discussion: Infections with MRSA and *S. aureus* still pose a significant risk to patients and challenge infection prevention and control. Thus, constant surveillance remains essential. However, in recent years, we have noticed a significant change in the requests addressed to the NRC for staphylococci. It will be interesting to see to what extent the shifting focus on MSSA and multi-resistant coagulase-negative staphylococci is also reflected in a change in clinical perception of these staphylococcal variants. In any case, the NRC will be prepared to perform appropriate surveillance functions now and in future.

RKP 241

Transformation and challenges: Commissioning a new biosafety level 3 laboratory for the National Reference Center for Mycobacteria

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In 1992, the Department of Diagnostic Mycobacteriology at the Research Center Borstel was appointed as the National Reference Center for Mycobacteria (NRC) by the Robert Koch Institute for the first time. By focusing on the identification, susceptibility testing and molecular surveillance of mycobacterial bacteria, we aim to improve the care of TB patients, uncover infection chains and coordinate measures in the fight against tuberculosis. In addition, WHO designated the NRC as a Supranational Reference Center responsible for nine countries with low and middle income setting. More than 10000 samples are processed annually. However, work with aerosol-transmissible pathogens must be carried out in specialised laboratories of biosafety level 3 (BSL3) which requires a complex technology system. In 2017, after 25 years of NRC, the new construction of the NRC was approved by the federal and state governments. After a 5-year planning and construction period, the new BSL3 laboratory could be occupied at the end of 2022.

The commissioning of the new BSL3 building included fundamental innovations across various aspects of the diagnostic workflow on (i) the technical level (technical components), (ii) the operational level (maintenance), (iii) the approval process as well as (iv) the diagnostic work processes and (v) staff-related changes. Technical commissioning including the ventilation systems, compressed air and gas supply systems as well as the airlock system started nine months prior to the official move. The process was closely monitored by the engineers, and an external advisory board evaluated interfaces, integral functional testing and the testing of operating and malfunction situations. Inhouse facility management was trained in parallel. Relocation of the laboratory equipment was successfully accomplished within two days, resulting in only a brief interruption of diagnostic activities. However, relocation of the inhouse reference sample collection had to be postponed. The diagnostic workflow was reorganized to accommodate the new BSL3 laboratory concept, which provides a staff-independent sample flow. Adaptation of these processes are still ongoing, as the implementation of new features in daily routine diagnostics poses ongoing challenges. All technical and functional changes underwent discussion and evaluation by the responsible approval authorities, as well as specialists in occupational safety, biosafety, fire protection, and biosubstance ordinance. Overall, the commissioning of the new BSL3 laboratory and the associated changes to the diagnostic workflow have required meticulous planning, coordination, and collaboration with relevant stakeholders. The implementation of these changes into daily routine diagnostics continues to be a challenging and ongoing process.

ZOP 242

Go with the Flow: *Streptococcus canis* adheares to endothelail cells under physiological flow conditions

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Introduction: *Streptococcus canis* (*S. canis*) is known as opportunistic pathogen colonizing dogs and cats, but also causes zoonotic diseases such as endocardites and septicaemiae in humans.

In the course of wound infection, *S. canis* enters the bloodstream and adheres to the heart endocardium as well as to the vascular endothelium thereby inducing inflammation and cell damage. Several virulence factors of *S. canis* have already been identified including the *S. canis* M-protein (SCM). In previous studies, the M-protein of *S. canis* has been characterized as bacterial surface receptor interacting with host-derived fibrinolytic components such as plasminogen, displaying strong binding affinity for the Fc-domain of immune globulins, and mediating bacterial aggregation via hemophilic protein-protein-interaction.

Material/Method: Following inflammatory bacterial infection, vascular cell regeneration is mediated by cell proliferation and cell migration. We aim to determine the impact of *S. canis* and the M-protein SCM in particular on endothelial wound healing and bacterial attachment during cell culture infection. The chamber separation cell migration assay (CSMA), enables live cell imaging of the endothelial wound healing process after infection with streptococci. Additionally, differential immunofluorescence staining followed by confocal laser scanning microscopy visualizes bacterial cell attachment.

Results: We report here that incubation of human umbilical vein-derived endothelial cells (HUVEC) with clinical *S. canis* isolates induces substantial cell damage and significantly inhibites endothelial gap closure, whereas endothelial wound healing is significantly less affected by incubation with the isogenic SCM-deficient mutant.

Moreover, based on these data, we aimed to analyse the effect of *S. canis* infection on endothelial wound healing under physiological flow conditions present in the blood circulation. Therefore, we combined the CSMA with a formerly established microfluidic pump system. This enables the application of defined shear stress values, thereby mimicking the vascular blood flow. Equally to infection under static conditions, circulating *S. canis* significantly inhibited endothelial gap closure at a defined shear stress.

Discussion: The developed CSMA technique in combination with the microfluidic pump system proved to be ideally suited for the analysis of *S. canis* infection in the vascular system simulating systematic disease progression *in vitro*.

ZOP 243

Human kidney cells demonstrate differences in the permissiveness for pathogenic and non-pathogenic orthohantaviruses

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Introduction: Members of the genus Orthohantavirus (family *Hantaviridae*) are characterized by organ specificity and a broad range of pathogenicity. Hantaan virus (HTNV) and Puumala virus (PUUV) cause a severe and mild form of haemorrhagic fever with renal syndrome (HFRS), respectively. Whereas Tula virus (TULV) is a non-pathogenic member, for which only rare cases with very mild symptoms are described. However, the pathogenic risk for humans for the majority of hantaviral species is not known. They were identified in host reservoirs, which were chronically and asymptomatically infected. Orthohantaviruses share high genetic similarity but virus-specific pathogenicity factors that influence the virulence and the renal manifestation of orthohantavirus infection are not known and a prediction of virulence potential is not possible so far.

Methods: To identify determinants of organ tropism and pathogenicity, we studied the in vitro replication competence of different orthohantaviruses in human renal cells as relevant cell culture model. We examined the surface receptor expression by flow cytometry and analysed replication kinetics in human tubular epithelial cells, podocytes and glomerular mesangial cells with pathogenic orthohantaviruses PUUV and HTNV and with the non-pathogenic species TULV.

Results: Tubular and glomerular (podocytes, mesangial cells) cells express integrin avb3 and integrin b1, which are described as entry receptors for orthohantaviruses. The pathogenic viruses HTNV and PUUV demonstrated an initial infection of all renal cells at day two post infection that was increasing over time and reaching infection rates of more than 50 percent. In contrast, TULV did not infect tubular epithelial cells nor podocytes and the infection of mesangial cells was very low (< 5%) and abortive.

Discussion: Human kidney cells exhibit a cell type- and virus-specific permissiveness for orthohantaviruses despite the surface expression of hantaviral receptors integrin avb3 and integrin b1 in all examined renal cells. The use of relevant human renal cell culture models will allow the identification of pathogenicity determinants and will help to predict the virulence of novel hantavirus species identified in animal reservoirs.

ZOP 244

Identifying virulence factors in canine infective endocarditis caused by *Streptococcus canis*

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Introduction: Infective endocarditis (IE) is defined as bacterial infection of the heart valve endothelium or endocardium. Bacteraemia and endothelial disruption, caused by underlying cardiac defects like subaortic stenosis, are required for infective endocarditis development. *Streptococcus canis* (SC) was found to be involved in almost a quarter of canine IE cases. SC colonizes the skin and mucosae of asymptomatic cats and dogs, and is associated with both superficial and severe infections. Cases of SC causing severe infection or IE in humans have been described after bite or scratch injuries from dogs or cats. The overall prognosis of dogs with IE is poor. This is often caused by biofilm formation on the affected heart valve. Bacteria in biofilms are 10-1000 times more resilient against antibiotic treatment.

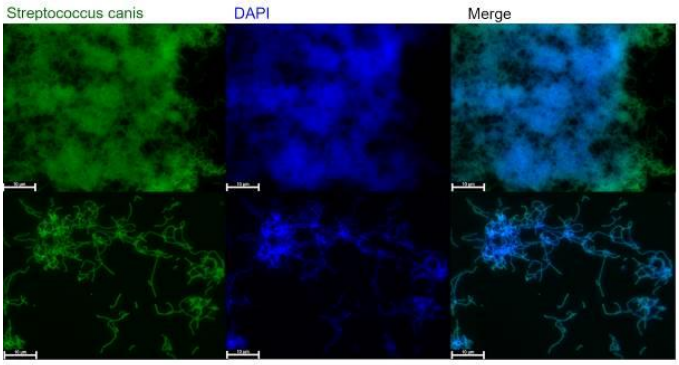
Methods: In this study, we use a random transposon mutant library to discover virulence factors in the adhesion and invasion of SC to endothelial cells. This input library was made by transposon directed insertion-site sequencing, starting from a clinical endocarditis SC strain. After *in vitro* infection of HUVEC cells, the mutants that did not adhere or invade were sequenced and this output pool was compared to the input library. By comparing output and input, potential virulence factors can be identified. As biofilm formation is another important factor in IE and an indicator of the severity of disease, virulence factors in biofilm formation will also be discovered using this method. As a selection assay, a biofilm will be formed on a fibrin matrix to create a pool with the biofilm-forming mutants and a control pool with the mutants in the supernatant.

Results: The biofilm-formation assay was tested with several SC strains and their M-protein knock-out strains, as the M-protein is a known virulence factor for biofilm formation in streptococci. We could show that indeed the ability to form biofilms disappeared when removing the M-protein of SC. Analysis of this assay was done through fluorescence microscopy, as is shown in Fig. 1, with a wild-type SC strain in the upper panel and the same strain without M-protein below. Adhesion of streptococci to the fibrin matrix still occurs, but there is no biofilm without the M-protein.

Discussion: The first and only well-described virulence factor of SC is its M-protein. We confirm that it also plays a role in biofilm formation. After sequence analysis and identification of the other virulence factors of SC in adhesion, invasion, and biofilm-formation, we will confirm these factors by mutagenesis. This will not only help us understand the development of IE better but will also give us predictive factors for disease severity and treatment success.

Figure 1: *S. canis* G361 (above) and G361ΔSCM (below) grown for 48h on a fibrin matrix, stained with α-SC (green) and DAPI DNA staining (blue). Upper panel shows a multi-layer biofilm structure, the lower panel shows a microcolony and some bacterial chains.

Fig. 1



ZOP 245

Effects of Stx2 phage on *Escherichia coli* O104:H4 FliC expression and metabolism

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Introduction: The Shiga toxin 2 (Stx2) is the main virulence factor of the 2011 enterohemorrhagic *Escherichia coli* (*E. coli*) O104:H4 outbreak strain, which is encoded on a prophage. Stx2-encoding prophages can infect and lysogenize other bacterial strains. Our former investigation showed that lysogenization of *Escherichia coli* K-12 MG1655 with prophage ϕO104 elevated its FliC expression and altered its metabolism. Still, it remains unclear, whether ϕO104 causes these alterations in its natural background. Therefore, ϕO104 was transducted into the Stx2 phage-cured *E. coli* O104:H4 isolate C227-11ϕcu and its effects was assessed by phenotypic assays.

Materials/methods: ϕO104 Δ*stx2* from *E.coli* isolate LB226692 was transducted in C227-11ϕcu and MG1655, the latter served as a control. A semi-quantitative Western blot analysis was done to assess the effects of the presence of the phage on FliC expression in both strains. The blots were imaged using the ChemiDoc MP System and the Volume Intensities of the signals were analyzed via ImageLab. To assess the ϕO104 Δ*stx2*-dependent changes in carbon source metabolism in MG1655 and C227-11ϕcu, BIOLOG phenotype microarray system and its plate PM1 MicroPlate™ Carbon Sources were used. BIOLOG assay results were analyzed using opm package in Rstudio with an one-way ANOVA followed by a Tukey test.

Results: First, we analyzed the effect of ϕO104 carriage on FliC expression in total protein samples of overnight culture grown under standard laboratory conditions (LB medium, 37 °C, 180 rpm).

We observed 2.1- and 1.7-fold stronger expression of FliC in MG1655::ϕO104 Δ*stx2* and C227-11ϕcu::ϕO104 Δ*stx2*, respectively, in comparison to the naive strains. Next, BIOLOG assay with PM1 showed that MG1655 and MG1655::ϕO104 Δ*stx2* assimilated 58 of the 95 tested substrates differently. For all of the significant differences found, the presence of ϕO104 Δ*stx2* in MG1655 decreased growth. However, the current results differed for 9 carbon sources when compared to our former findings. In contrast, BIOLOG assay with C227-11ϕcu and C227-11::ϕO104 Δ*stx2* did not show any significant differences.

Discussion: The Western blot results of MG1655 and MG1655::ϕO104 Δ*stx2* were very similar to our former results of MG1655 and MG1655::ϕO104 and thus confirmed the positive effect of ϕO104 carriage on FliC expression. Moreover, the carriage of ϕO104 has a similar positive effect in *E. coli* O104:H4 C227-11ϕcu background. Interestingly, the BIOLOG assay results of MG1655 and MG1655::ϕO104 Δ*stx2* were very comparable to our former results. However, differences were not found in the results of C227-11ϕcu and C227-11ϕcu::ϕO104 Δ*stx2*. Ongoing experiments will clarify if and which differences are responsible for the missing metabolic effect of ϕO104 carriage in *E. coli* O104:H4 genomic background.

ZOP 246

Scrutinizing the association of the unique EHEC subtilase cytotoxin with OMVs and its significance for host cells

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) is the etiologic agent of the life-threatening hemolytic-uremic syndrome. The cardinal virulence factor of EHEC is the Shiga toxin (Stx). In addition, it can encode various accessory toxins, which are often freely secreted but can also be associated with outer membrane vesicles (OMVs). Whether the subtilase (SubAB) cytotoxin, which is usually encoded by non-classical EHEC serovars, is also associated with OMVs, remains unclear. SubAB is an AB5 cytotoxin like Stx categorized into different subtypes with the main target being the host ER chaperone BiP. Therefore, our goal was to examine whether SubAB is associated with OMVs and how OMV-associated SubAB effects host cells compared to freely secreted SubAB.

Methods: We first screened our strain collection for strains being exclusively positive for single SubAB subytpes. Using an established multi-step ultracentrifugation protocol including a density gradient we then prepared the potentially SubAB-positive OMVs and applied them in Western blotting using the bacterial outer membrane protein OmpA as an OMV marker to determine the association of SubAB with OMVs. Additionally, we characterized the OMVs in size and concentration by nanoparticle tracking analysis. Next, we tested with cytotoxicity assays using non-human vs. human cell lines growing in serum-free vs. serum-containing media whether SubAB-positive OMV effects are dependent on the SubAB receptor *N*-glycolyl neuraminic acid (Neu5Gc). In addition, cytopathic consequences of SubAB-positive OMVs were detected by Western blotting using, first, the cleavage of the host protein BiP and, second, the activation of the three-pronged ER stress pathway via phosphorylation as markers. Finally, potential inflammatory effects were tested by using multiplex ELISAs.

Results: Analyzing our strain collection we recognized that SubAB2 subtypes often occur in tandems of two different alleles and is combined with specific combinations of Stx subtypes. Western blotting showed that all SubAB subtypes are associated with OMVs. Moreover, similar to Stx the impact of SubAB-positive OMVs on host cells is in fact dependent on the Neu5Gc receptor, which cannot be synthesized by human cells and is only supplied by diet (or serum in case of cell culture). Likewise, OMV-associated SubAB is capable of cleaving BiP and inducing the phosphorylation of the ER stress signaling pathways similar to free SubAB as well as the secretion of different cytokines including IL-8.

Conclusions: Taken together, these results clearly reveal that SubAB is an additional OMV-associated EHEC exotoxin. Additionally, OMV-associated SubAB showing analogous effects as free SubAB resembles the similar effects of free and OMV-associated Stx and is unlike EHEC-hemolysin, which was demonstrated to show differential effects. Finally, our study illustrates the significance of profiling virulence factors also from non-classical EHEC serovars.

ZOP 247

The effect of RpoS on core gene expression and metabolism in *Escherichia coli* O104:H4

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Introduction: *Escherichia coli* (*E. coli*) O104:H4 caused in 2011 in Germany the enterohemorrhagic *E. coli* outbreak with the highest incidence rate of hemolytic uremic syndrome worldwide. A main aim of our research is to investigate the factors and mechanisms contributing to *E. coli* O104:H4 hypervirulence. We recently described an *E. coli* O104:H4 isolate that had acquired a single nucleotide polymorphism (SNP) in the start codon (ATG>ATA) of *rpoS* coding for the alternative RNA polymerase sigma factor S (RpoS), which resulted in its enhanced virulence gene expression. Here, we further analysed the effect of the *rpoS* ATG>ATA allele on core gene expression and metabolism in *E. coli* O104:H4.

Methods: RNA-seq was performed with total RNA from *E. coli* O104:H4 wild type and *rpoS* ATG>ATA cells grown in LB medium at 37oC to mid-log phase (OD_{600} = 0.4) and early stationary phase (OD_{600} = 3). Illumina reads were processed using READemption and differences in the gene expression were determined by DESeq2. *E. coli* O104:H4 protein coding genes homologous to *E. coli* K-12 MG1655 were identified using OrthoFinder and referred to as core genes. Differentially expressed core genes were analysed using the STRING database of protein-protein interactions. Phenotype microarray assays were performed with BIOLOG PM1 Carbon Sources plate.

Results: In total, the expression of 23% (1135 genes) and 37% (1839 genes) of all *E. coli* O104:H4 protein coding genes were found to be affected (log2fold > 1; adjusted p-value of < 0.01) by the *rpoS* ATA>ATG SNP at OD_{600} = 0.4 and OD_{600} = 3, respectively. Interestingly, among the upregulated genes in *E. coli* O104:H4 *rpoS* ATG>ATA at OD_{600} = 3 were numerous metabolic genes, e.g phenylacetate catabolism, tricarboxylic acid (TCA) cycle and ribonucleotide biosynthesis genes. Moreover, genes encoding ribosomal and translation-related proteins were found significantly upregulated in the mutant strain. On the contrary, transmembrane transport-related genes were found downregulated at both growth phases in *E. coli* O104:H4 *rpoS* ATA>ATG, with sugar transporter genes as a prominent example. Further analysis with the Biolog phenotype microarrays revealed a strong correlation between the gene expression and metabolic profile of the strains. For example, *E. coli* O104:H4 *rpoS* ATA>ATG assimilated more efficiently virtually all tested amino acids and the majority of carboxylic and dicarboxylic acids (TCA cycle substrates), whereas the wild type strain grew better with sugars like arabinose, galactose and fucose.

Conclusion: Our data indicate that RpoS has a profound effect on *E. coli* O104:H4 core gene expression and metabolism. Namely, RpoS serves as a negative regulator of the TCA cycle and amino acid catabolism, whereas positively regulates the assimilation of certain sugars. If the observed RpoS-dependent changes in gene expression and metabolism have an impact on *E. coli* O104:H4 colonization and pathogenicity *in vivo* remains to be further elucidated.

ZOP 248

Update of the CoxBase-plattform with new, improving features for genomic *Coxiella burnetii* analysis

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The zoonotic pathogen *Coxiella (C.). burnetii*, affects small ruminants and humans, causing *C. burnetii*-mediated disease (coxiellosis and Q fever). Due to the worldwide distribution of this pathogen, periodicc outbreaks are still prevalent across different geographic regions. Epidemiological tools and methods to study, monitor as well as ensure its control are therefore essential.

For this reason, we have developed a platform called CoxBase (https://coxbase.q-gaps.de) which has been designed to address several aspects of the genomic analysis of *C. burnetii*, such as epidemiological surveillance, metadata summarization via visualisation, *in silico* implementation of multiple genotyping systems, genotyping data management, and genome annotation.

The CoxBase platform provides multiple query interfaces to extract genotyping metadata information of different *C. burnetii* hosts. The platform can also be used to retrieve plasmid primers for genotyping analysis and presently contain more isolate data and metadata than any other existing *C. burnetii* resources. The platform also implements features that remove barriers to data exchange via implementing bookmarkable result pages and inadvertently ensure that analyses carried out on the platform are reproducible.

The platform has recently been updated with a new suite of features including the prediction of antibiotic resistance genes using a machine learning approach as well as recent genotyping data information from a plethora of hosts.

We will continue to update the platform with new data sources and hope the platform continue to serve as a useful resource to researchers

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ZOP 248a

Human microbiota-associated IL-$10^{-/-}$ mice: a valuable enterocolitis model to dissect the interactions of *Campylobacter jejuni* with host immunity and gut microbiota

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Introduction: Secondary abiotic (SAB) IL-$10^{-/-}$ mice generated by broad-spectrum antibiotic pretreatment constitute a valuable *Campylobacter jejuni*-induced enterocolitis model. Given that the host-specific gut microbiota plays a key role in susceptibility of the vertebrate host towards or resistance against enteropathogenic infection, we surveyed immunopathological sequelae of *C. jejuni* infection in human microbiota-associated (hma) and SAB IL-$10^{-/-}$ mice.

Methods: In order to generate hma mice, SAB IL-$10^{-/-}$ mice were subjected to peroral transplantation of fecal microbiota derived from healthy human donors on three consecutive days. One week following engraftment of the fecal transplants, hma and SAB IL-$10^{-/-}$ mice were infected with *C. jejuni* by oral gavage.

Results: Following oral challenge, *C. jejuni* readily colonized the gastrointestinal tract of hma and SAB mice, but with lower numbers in the former versus the latter. Whereas hma mice were clinically less severely compromised,both, macroscopic and microscopic inflammatory sequelae of *C. jejuni* infection including histopathological and apoptotic cell responses in the colon of IL-$10^{-/-}$ mice were comparably pronounced in the presence and absence of a human gut microbiota at day 6 post-infection. Furthermore, *C. jejuni* infection of hma and SAB mice resulted in similarly enhanced immune cell responses in the colon and in differential pro-inflammatory mediator secretion in the intestinal tract which also held true for extra-intestinal including systemic compartments. Notably, *C. jeuni* infection of hma mice was associated with distinct gut microbiota shifts.

Discussion/Conclusion: Hma IL-$10^{-/-}$ mice represent a reliable *C. jejuni*-induced enterocolitis model to dissect the interactions of the enteropathogen, vertebrate host immunity and human gut microbiota.

MSZOP 249

Antimicrobial resistance properties of *Aeromonas* spp. isolates from German patients

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Introduction: Bacteria of the Gram-negative genus *Aeromonas* are ubiquitously distributed in aquatic environments and can be isolated from insects, livestock, and retailed foods of animal or plant origin. Some strains can cause human intestinal or extraintestinal infections. Despite sometimes severe courses of infection in immunocompromised hosts, data on antimicrobial susceptibility of German clinical *Aeromonas* isolates are missing. Due to the demographic development of the German population, our study aimed to investigate the occurrence of antimicrobial resistances in *Aeromonas* spp. isolates from German patients.

Materials and Methods: We selected a representative pool of well documented clinical strains (n = 61) associated with intestinal and extraintestinal infections between 2016 and 2019. All strains were verified on genus level by MALDI-TOF MS analysis using direct transfer method. Antimicrobial susceptibility was determined by

disk diffusion (DD) and broth microdilution (BMD) methods according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Results: The majority of German clinical *Aeromonas* isolates were susceptible to most of the 27 antimicrobial agents tested. Non-susceptibility was found towards aminopenicillins. Besides, a noticeable proportion of strains showed intermediate to full resistance to cephamycins, carbapenems, polymyxins, quinolones, and/or tetracyclines. Non-susceptibility towards second-, third- or fourth-generation cephalosporins, monobactams, aminoglycosides, folate pathway inhibitors, macrolides, phenicols, and fluoroquinolones, respectively, was sporadically observed.

Discussion: Antimicrobial agents recommended for treatment of *Aeromonas* infections were found to be effective *in vitro*. However, the occurrence of sporadic resistances towards third- or fourth-generation cephalosporins as well as fluoroquinolones highlights the need for systematic monitoring of antimicrobial susceptibility in *Aeromonas* spp. in Germany taking into account all aspects of the One Health approach.

MSZOP 250

Whole genome analysis of bovine mastitis-related MRSA in Thuringia, Germany

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Background: In animal husbandry, acute and chronic staphylococcal mastitis cause massive financial losses for the producer due to the reduced milk yield of the infected cow, price reductions with reduced milk quality up to the milk sales ban and significantly increased premature loss of animals. The current study aimed to understand more about the genetic diversity, antimicrobial resistance profiles and virulence factors of *Staphylococcus* (*S.*) *aureus* isolated from clinical bovine mastitis in dairy farms in Thuringia, Germany. Additionally, the study aimed to draw conclusions for a current situation of bovine clinical and subclinical mastitis infections in dairy herds in the state and the potential public health risk. Furthermore, mapping the possible phylogenetic relations between MRSA strains from various farms as well as within one farm was analysed.

Early identification of carriers is a key factor in controlling outbreaks. In this context, a multiparameter staphylococcal rapid test is to be developed that can be used in both human and veterinary medicine with the ability to adapt the target gene structures quickly, so that the rapid evolution of resistance can be considered.

Methods: With the help of whole genome sequencing (WGS), forty *S. aureus* isolates from clinical bovine mastitis cases from 17 Thuringian dairy farms were phenotyped and genetically described.

Results: Thirty (75%) of the 40 *S. aureus* isolates tested positive for methicillin resistance. The isolates had high levels of resistance to benzylpenicillin, tetracycline, and oxacillin, correspondingly 77.5, 77.5, and 75%. Moxifloxacin, ciprofloxacin, gentamicin, and trimethoprim/sulfamethoxazole all had lower resistance rates, at 35, 35, 30, and 22.5%, respectively. Contrarily, erythromycin and clindamycin resistance were only infrequently encountered (5 and 2.5%, respectively). Linezolid, teicoplanin, vancomycin, tigecycline, fosfomycin, fusidic acid, and rifampicin were all effective against all isolates. Five distinct sequence types of isolates were identified: ST398 (n = 31), ST1074 (n = 4), ST504 (n = 3), ST582 (CC15) (n = 1), and ST479 (n = 1).

Resistance genes *bla*Z, *bla*I, and *bla*R associated with benzylpenicillin resistance were found in 31 (80%) strains, all were belonging to ST398. Methicillin resistance associated *mec*A, was identified in 30 (96.8%) isolates of ST398.

Conclusion: In this study, five different sequence types and seven clusters were identified in the federal state of Thuringia and ST398 had the highest prevalence in bovine mastitis cases. The circulation of some clusters within the same locale over a few a long time appears the determination of cluster-associated contamination in spite of the seriously therapeutic care. On the other hand, a few locales had distinctive clusters at the same year or amid diverse a long time.

MSZOP 251

Prevalence and molecular typing of *Salmonella enterica* subsp. *diarizonae* in sheep intended for slaughter in Germany

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Introduction: Salmonellae are one of the most important zoonotic pathogens and causative agents of foodborne infections in Europe. Contaminated food such as meat and meat products are the main source of infection for humans. The serovars 61:k:1,5,(7) and 61:-:1,5,(7) of the subspecies (subsp.) *Salmonella* (*S.*) *enterica* subsp. *diarizonae*, also known as sheep-associated *Salmonella enterica* subsp. *diarizonae* (SASd), are commonly found in sheep, causing mostly subclinical infections. SASd extremely rarely cause disease in humans, but a chronic proliferative rhinitis can be attributed to SASd from sheep. The subspecies *S. enterica* subsp. *diarizonae* often shows atypical growth characteristics compared to other salmonellae, therefore identification may be difficult. The aim of our study was to determine the prevalence of SASd in slaughter sheep in Germany and to test different selective enrichment and culture media for their suitability for the detection of these serovars.

Materials and Methods: For this purpose, the tonsils of sheep and lambs were removed shortly after slaughter and examined according to method L 00.00-20 "Horizontal method for the detection of *Salmonella* spp. in food" as well as by conventional polymerase chain reaction. Rappaport-Vassiliadis medium with soya (RVS) and Muller-Kauffmann tetrathionate novobiocin (MKTTn) broth were used as selective enrichment media and suspensions were spread on xylose lysine deoxycholate (XLD) and brilliant green phenol red lactose sucrose (BPLS) agar after 24 ± 3 h of incubation. Presumptive colonies were sent to the National Reference Laboratory for *Salmonella* (Federal Institute for Risk Assessment) in Berlin for final confirmation, serotyping and susceptibility testing. Confirmed isolates were further characterized by pulsed-field gel electrophoresis (PFGE) in order to investigate their clonal relationship.

Results: Percentages of 15% of lambs and 76% of sheep were found to be SASd positive. The isolates were susceptible to all tested antibiotics and characterization by PFGE revealed both clonal spread among isolates from the same region and differences in banding patterns. Selective enrichment in MKTTn broth was much more effective in detecting the serovars 61:k:1,5,(7) and 61:-:1,5,(7) than the RVS medium, while no significant differences were observed between the two selective culture media XLD and BPLS agar.

Discussion: Our results showed that SASd are present in German sheep populations and that in particular adult animals are a reservoir for these pathogens. The standard protocol commonly used for the detection of *Salmonella* spp. in food is suitable for isolation of the sheep-associated serovars as well, with selective enrichment in MKTTn broth as a preferred method. Isolated SASd

should be further characterised and screened for their virulence potential to better assess the public health risks posed by SASd.

MSZOP 252

A MALDI-TOF-based inhibitor approach for the detection of beta-lactamase activity in Gram-negative bacteria

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Introduction: With the increasing number of multi-drug resistant isolates, rapid and reliable tests for the detection of antibiotic resistance are of utmost importance. In particular, the development of new drug combinations like meropenem/vaborbactam (MEV) or imipenem/relebactam (IMR) necessitates targeted use to avoid the development of new resistances. Currently, the detection of MEV/IMR resistant strains is usually carried out by PCR, which is rapid but costly and yields no information on phenotypic resistance.

Here, we evaluated the use of the MBT STAR-Carba assay combined with the inhibitor relebactam to quickly identify carbapenemase-carrying isolates, which are sensitive and therefore suitable for the therapy of a carbapemem/inhibitor combination.

Materials/methods: 25 carbapenem resistant clinical Gram-negative bacterial isolates comprising different species (*Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Providencia*, *Acinetobacter*), and carbapenemases (KPC, VIM, NDM, IMP, and OXA) were pre-characterized by gradient testing (Liofilchem, italy) for their minimal inhibitory concentration against meropenem, as well as by PCR (Bruker, Glasgow). The selected strains were then tested with the MBT STAR-Carba Kit (Bruker, Germany) with and without addition of 40 mg/mL relebactam during the sample incubation. MALDI-TOF MS measurements were performed in MBT Compass HT on a MALDI Biotyper system. LogRQ values were used to evaluate inhibitory effect according to the hydrolyzation-status of the antibiotic.

Results: For a total of 18 isolates carrying VIM-, NDM-, IMP-, and OXA-carbapenemases no inhibition of enzymatic hydrolyzation activity (MBT STAR-Carba) was detected, which is in concordance with the MIC information obtained from gradient testing. In contrast, clear inhibition of enzymatic activity for both approaches was observed for the seven strains containing a KPC, which reflects the information on the inhibition capabilities of carbapenemases by relebactam reported in the literature.

Conclusions: Combining the already established MBT STAR-Carba assay with an inhibition approach facilitates the rapid and reliable detection of KPC bearing clinical isolates that are predestinated for the application of respective carbapenem inhibitor therapy without employing expensive and more time-consuming molecular diagnostics. Compared to PCR, this approach addresses the enzymatic activity rather than just the presence of a resistance gene with no conclusions on its expression status. However, clinical evaluation of this approach will be necessary before implementation into routine diagnostics.

MSZOP 253

***In vitro* antibacterial activity of natural products against zoonotic bacterial pathogens**

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Introduction: Antimicrobial resistance (AMR) is considered one of the greatest threats to both human and animal health. Efforts to address AMR include implementing antimicrobial stewardship programs and introducing alternative treatment options. Nevertheless, effective treatment of infectious diseases caused by bacteria will still require the identification and development of new antimicrobial agents.

Material/method: Eight different natural products were screened for antimicrobial activity against seven pathogenic bacterial species (*Brachyspira* sp., *Chlamydia* sp., *Clostridioides* sp., *Mannheimia* sp., *Mycobacterium* sp., *Mycoplasma* sp., *Pasteurella* sp.).

Results: While most products inhibited bacterial growth only at high concentrations or not at all, three natural products (celastramycin, closthioamide, maduranic acid) displayed activity at concentrations <2 µg/mL against *Pasteurella* sp. and two of them (celastramycin, closthioamide) also against *Mannheimia* sp. Those results were confirmed by testing a larger collection of strains encompassing 64 *Pasteurella* and 56 *Mannheimia* field isolates originating from pigs or cattle. *Pasteurella* isolates with a known AMR phenotype against commonly used therapeutic antimicrobial agents exhibited higher MIC50 and MIC90 values against celastramycin, closthioamide, and maduranic acid than isolates with unknown AMR profiles.

Conclusion: This study demonstrates that screening natural products can yield novel scaffolds with broad- or narrow-spectrum antimicrobial activity against important Gram-negative veterinary pathogens with zoonotic potential.

MSZOP 254

Comparison of broth microdilution and agar diffusion for antimicrobial susceptibility testing of *Dermabacter hominis* Tim R. Blum[1], Dennis Knaack[2], Sören Schubert[3], Uwe Groß[4], Robin Köck[5], Frieder Schaumburg[1]

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Introduction: *Dermabacter hominis* is a short gram positive rod, which is part of the normal human skin flora. In immunocompromised patients, or patients with significant comorbidities, it may cause infections (e.g. wound and skin infections). Currently, there are no validated breakpoints for broth microdilution and agar diffusion for *D. hominis* available. The objectives of this study were to asses the susceptibility rates using EUCAST clinical breakpoints for *Corynebacterium* spp. or PK/PD breakpoints and to deduce breakpoints for agar diffusion.

Methods: We tested 24 isolates from five laboratories in Germany. The isolates derived mostly from tissue (n=11) and superficial or deep swabs (n=3). All isolates were identified by MALDI-TOF (Bruker)

For broth microdilution, we used commercial 96-well plates (MERLIN Diagnostica GmbH, MICRONAUT-S GP varia complete MIC) and followed the guidelines given by EUCAST and ISO 20776-1. The minimal inhibitory concentration (MIC) was interpreted either with clinical breakpoints for *Corynebacterium* spp. or PK/PD-breakpoints if *Corynebacterium* spp. breakpoints were not available. For agar diffusion, we followed EUCAST recommendations for the testing of *Corynebacterium* spp. and used Mueller-Hinton-Fastidious-agar (BD) and antibiotic disks (Oxoid) with antibiotic concentrations as recommended by EUCAST. *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619 and *Enterococcus faecalis* ATCC 29212 were used as quality control (QC) strains and were continuously within the QC range.

Results: All isolates were susceptible to vancomycin, rifampicin and linezolid (100%, n=24/24). The majority of the isolates 75% (n=18/24) were susceptible against fosfomycin, whereas only some isolates showed a susceptibility against clindamycin 25% (n=6/24), gentamicin 25% (n=6/24) and levofloxacin 17% (n=4/24). Benzylpenicillin showed a poor activity against *D. hominis* (8%, (n=2/24)).Based on scatter plots of MIC values vs. inhibition zone diameters the following susceptibility breakpoints for agar diffusion were deduced: Clindamycin (2 µg, ≥10 mm), gentamicin (10 µg, ≥ 21 mm) and levofloxacin (5 µg, ≥19 mm). Due to limited variances in both MIC values and inhibition zone diameters, no disk diffusion breakpoints could be deduced for vancomycin, rifampicin and linezolid.

Discussion: Vancomycin remains the treatment of choice for infections caused by *D. hominis*. Should a resistance against this antibiotic occur, rifampicin and linezolid could be a second-line therapy. Based on a good correlation between MIC and inhibition zone diameter, we were able to deduce disk diffusion breakpoints for clindamycin, gentamicin and levofloxacin.

MSZOP 255

Time trends of skin and soft tissue infections caused by methicillin-resistant *Staphylococcus aureus* in Lambaréné, Gabon

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Background: According to the recent Global Burden of Disease Study 2019, *Staphylococcus aureus* was the leading bacterial pathogen associated with more than one million deaths worldwide. Methicillin-resistant *S. aureus* (MRSA) was the second leading cause of death worldwide due to bacterial antimicrobial resistance. While *S. aureus* is particularly common in skin and soft tissue infections (SSTIs) in Africa, data on MRSA rates are scarce and reports vary widely across the continent (5%-80%). In this study, we describe the proportion of MRSA causing SSTIs in Lambaréné, Gabon, over an 11-year period.

Methods: We retrospectively analyzed data of 731 bacterial specimens from patients with SSTIs collected from the microbiology laboratory and medical records of the Albert Schweitzer Hospital of Lambaréné, Gabon, between January 2009

and December 2019. We determined changes in the temporal prevalence of MRSA and identified risk factors for SSTI with MRSA compared to methicillin-susceptible *S. aureus* (MSSA).

Results: Out of 731 specimens, 499 (68,2 %) yielded *S. aureus* of which 7.0% (36/499) were identified as MRSA and 54.7% (273/499) originated from the community. The median age of patients with MRSA-associated SSTI was 22 (0-76), while the proportion of MRSA was higher in adults compared to children (15.4% vs. 4.2%).

We found that patient age above 18 (OR 4.14; CI [2.02 to 8.48]; p<0.001), admission to the pediatric ward (OR 9.59; CI [1.21 - 47.9]; p=0.032), and suppurative infections (OR 0.08; CI [0.00-0.64]; p=0.014) were significantly associated with MRSA as the causative agent.

After an initial decline from 7% in 2009, there was a significant increase in the proportion of MRSA from 3% to 20% between 2012 and 2019.The rate of resistance to erythromycin was significantly higher in MRSA than in MSSA (72% vs. 10.3%, p<0.001), and clindamycin resistance was detected exclusively in MRSA isolates (8.33%).

Conclusions: We observed an alarming trend in the proportion of MRSA causing SSTIs over the 11-year period analyzed in our study. The percentage of MRSA isolated from culture-positive specimen increased from 7% in 2009 to 20% in 2019, posing a neglected public health threat in our study area. Continuous surveillance of MRSA lineages in the hospital and community along with antibiotic stewardship programs could address the increasing trend of MRSA in Lambaréné.

MSZOP 256

Molecular characterization of *Salmonella enterica* across the poultry production chain in Costa Rica

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Introduction: Globally, *Salmonella enterica* are important foodborne pathogens, showing increasing trends of antibiotic resistance. Data on the frequencies, virulence, phenotypic and genotypic antibiotic resistance of these zoonotic bacteria along the food chain in Costa Rica is scarce. This study therefore aims at analyzing the frequency of *Salmonella enterica* across the poultry production chain and to characterize its antibiotic resistance and virulence.

Material/Methods: In a cross-sectional study from August to December 2019, samples were collected at two levels along the poultry food chain in Costa Rica, including retail food (chicken meat samples) and food-producing animals (broiler caecal samples). In total 65 chicken meat samples and 171 chicken caecal samples were collected and analyzed. *Salmonella* strains were identified by culture and biochemical methods and the antibiotic susceptibility was tested by disk diffusion. 71 antibiotic resistant *Salmonella* isolates were selected and subjected to whole-genome sequencing analysis (WGS), allowing the in-silico detection of antibiotic resistance and virulence markers, and multilocus sequence typing.

Results: High frequencies of *S. enterica* in chicken meat 58,5% (n/N=38/65) and in poultry farms 36,8% (n/N=63/171) were found. The 89,5% (n/N=34/38) and the 93,6% (n/N=59/63) of these *Salmonella* strains isolated from chicken meat and caecum samples exhibited multidrug resistant (MDR). 94% of the sequenced *Salmonella* isolates were identified as serovar Infantis and 3% belong to the serovars Anatum and Kentucky. AMR and plasmid analyses reveal the presence of a large pESI - like plasmid in 92% (n/N=65/71) of the *S.* Infantis isolates. In addition, our data displayed a high concordance between the genotypic and phenotypic susceptibility testing profiles with an agreement of approximately 98% when comparing ESBL AMR resistance markers

Discussion: The pESI - like plasmid found is known to carry several virulence and resistance markers and was found as a major source of antibiotic resistance in *Salmonella* Infantis strains which have spread in poultry in North and South-America. The high concordance between the genotypic and phenotypic susceptibility testing profiles found underlines the applicability of NGS analyses to monitor and track AMR patterns and prevalence. The high frequency of MDR *Salmonella* found highlight the need to improve and integrate the foodborne pathogens and antimicrobial resistance surveillance systems along the food production chain in Costa Rica.

IIP 257

Impact of chlamydial glutamate transporter on mitochondrial activity in *C. pneumoniae* infection

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Introduction: *Chlamydia pneumoniae* (*C. pneumoniae*) is an obligate intracellular gram-negative bacterium that causes respiratory tract infections in humans and animals. Mitochondrial activity and related metabolites such as glutamate play a crucial role in chlamydial development. However, the role of glutamate in *C. pneumoniae* infection remains unclear. In this study, we established an easy-to-handle genetic manipulation tool to elucidate the effect of glutamate on development of *C. pneumoniae*.

Material and Methods: HeLa cells and HEp-2 cells were used in this study. Plasmid shuttle vector encoding the gene of glutamate transporter was transformed into *C. pneumoniae*. Mitochondrial activity was measured by Mito Stress test kits following Seahorse Bioscience manufacturer"s instructions.

Results: We constructed a plasmid shuttle vector encoding genes for the glutamate transporter, green fluorescent protein (GFP) and ampicillin resistance (AmpR). After transformation, we confirmed a strong GFP signal in transformed *C. pneumoniae* inclusions. Glutamate transporter protein was also successfully induced in transformants. In the assay of mitochondrial respiration, cells infected with the shuttle vector-transformed strain exhibited significantly upregulated basal respiration and ATP-linked respiration compared to cells infected with the untransformed strain. Under similar infection rates, the transformant significantly increased production of progeny compared to the wild-type strain.

Discussion: Utilizing this shuttle vector, we demonstrated that the glutamate transporter plays a key role in enhancing mitochondrial activity and promoting the growth of *C. pneumoniae*.

We conclude that our shuttle vector system is a useful tool for genetic manipulation of *C. pneumoniae*.

IIP 258

Orientia tsutsugamushi induces inflammation via TLR13-mediated recognition of bacterial RNA in murine macrophages and dendritic cells

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Introduction: The intracellular bacterium *Orientia tsutsugamushi* (OT) is the causative agent of scrub typhus, a potentially life-threatening zoonotic infection in humans with rising impact on global health. Severe cases of scrub typhus go along with an exaggerated immune response characterized by highly elevated serum levels of TNF-a and IL-6 as well as a strong activation of macrophages (MF) and dendritic cells (DCs). Interestingly, OT lacks most classical bacterial PAMPs like flagellin and LPS. Hence, the bacterial structures eliciting innate inflammation, as well as the immunoreceptors involved in its recognition, have remained elusive. Previous data from our group suggests the involvement of the RNA-sensing toll-like receptor (TLR) 13 in the recognition of heat killed (hk) OT by murine BMDCs.Therefore, the aim of this project was to investigate the roles of bacterial RNA as a PAMP of OT and TLR13 as its respective host receptor in mice.

Methods: In order to study the immune response of MF challenged with hkOT and RNA isolated from live OT, we implemented immortalized murine MF (mMF) as a model system. Using CRISPR-interference, we generated TLR13-knockdown (TLR13kd) mMF. After challenging wild type (WT) mMF, TLR13kd mMF, as well as *Trif-/-*mMF and *Trif-/-Myd88-/-*mMF with hkOT and OT RNA, cytokine induction was analyzed by ELISA and qPCR. To study the response of primary murine phagocytes to OT, we differentiated BMDCs as well as plasmacytoid DCs (pDCs) from murine bone marrow using GM-CSF and Flt3-Ligand, respectively. Differentiation was confirmed by flow cytometry. The cytokine responses of pDCs and BMDCs to OT RNA were analyzed by ELISA and qPCR.

Results: We found that OT RNA and hkOT induced a potent TNF-a and IL-6 response in mMF. Using *Trif-/-*mMF and *Trif-/-Myd88-/-*mMF, we demonstrated that the recognition was MyD88-, but not TRIF-dependent. In addition, we showed that TNF-a and IL-6 induction by OT RNA and hkOT was drastically reduced in TLR13kd mMF compared to WT mMF, confirming a TLR13-dependent recognition of OT RNA. We successfully differentiated murine CD11c+B220+CD317+ pDCs. In contrast, BMDC comprised a heterogeneous population, consisting of F4/80+CD115+ MF and CD135+MHCIIhigh classical DCs. Both BMDCs and pDCs showed a strong cytokine induction upon OT RNA transfection, confirming the capacity of OT RNA to act as an immunogenic bacterial PAMP in two different primary phagocytes, BMDC and pDCs.

Discussion: We provide the first description of OT RNA as a highly immunogenic PAMP in mice, and the role of TLR13 in its recognition. TLR13 is known to recognize a 12mer motif in the 23S rRNA of *S.aureus*, which is conserved in OT. In addition, we demonstrated that cDCs, pDCs and MF are involved in the recognition OT RNA. Further studies are needed to elucidate the mutual contributions of MF and DC subpopulations in shaping the inflammatory response to OT RNA, in experimentally infected mice as well as in severe human cases of scrub typhus.

IIP 259

C5a receptor antagonists protect human neutrophils from *Staphylococcus aureus* Panton-Valentine leukocidin

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Background: The *Staphylococcus aureus* pore-forming toxin Panton-Valentine leukocidin (PVL) is associated with a range of diseases including (recurrent) skin and soft tissue infections (SSTIs) and necrotizing pneumonia. The pathogenesis of these infections may be determined by the cytolysis of granulocytes by PVL, eventually leading to tissue destruction. The bi-component toxin consists of the LukS and LukF subunits, while LukS binds to the C5a receptor (C5aR) and LukF to CD45 on neutrophils, monocytes, and macrophages. Initial binding of LukS and secondary recruitment of LukF leads to subsequent hetero-oligomerization, resulting in the formation of an octameric pore that inserts into the cell membrane. In this study, we tested the neutralizing effect of small molecule C5aR and CD45 antagonists on the toxicity of PVL.

Methods: Human polymorphonuclear leukocytes (PMNs) were freshly isolated and exposed to serially-diluted recombinant PVL after pre-incubation with log-dilutions of commercially available C5aR or CD45 antagonists. The cytotoxic effect of PVL was measured as percentage of propidium iodide (PI)-stained cells by FACS. The neutralizing capacity of the competitors was determined by non-linear regression analysis of changes in the dose-response curve and calculation of shifts in the half-maximal effective concentration (EC_{50}) of PVL.

Results: The tested C5aR antagonists exhibited differential competitive potencies. While treatment with 10 µM Avacopan, PMX205 or W-54011 shifted the dose-response curve and resulted in a 3.4-fold, 3.1-fold, or 4.3-fold increase in the EC_{50} of PVL ($p<0.001$), respectively, DF2593A or BM213 were unable to neutralize the cytotoxic effect of PVL. For some of the competitors, preliminary results suggest an inverse effect at low competitor concentration, where the cytotoxicity of PVL is enhanced.

Conclusion: The tested C5aR antagonists showed different potencies to neutralize the cytotoxic effect of PVL on human granulocytes. This suggests that different modes of interaction or potentially induced changes in the receptor conformation may influence the capacity to compete for the toxin-receptor interaction or result in an altered susceptibility of the granulocytes to pore-formation by PVL. We show that the use of small molecules antagonizing the interaction of PVL with its receptor offers a protective potential against PVL toxicity. However, further studies in suitable animal models are needed to assess possible therapeutic approaches.

IIP 260

SLUSH peptides in *Staphylococcus lugdunensis*: hemolytic potential and implications for Iron acquisition

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Background: *Staphylococcus lugdunensis(S. lugdunensis)* contains a three numbers of short peptides (SLUSH A, -B, -C) , called *S. lugdunensis* synergistic hemolysins (SLUSH) and OrfX which is present in SLUSH locus. SLUSHs are known to lyse human erythrocytes and steal iron from erythrocytes for their growth. SLUSH peptides are similar to PSMs in Staphylococcus

aureus (*S. aureus)*. SLUSHs are only hemolytic factors present in *S .lugdunensis*.

Methods: Erythrocytes from human blood was for growth curve in iron restricted medium and hemolysis assay for S.lugdunensis strains (HKU09-01 WT and HKU09-01 D*SLUSH*). SLUSH recombinant peptides are also used with erythrocytes in growth curve. RT-PCR was perfomed for expression.

Results: Deletion of SLUSH genes in HKU strain lost its hemolytic activity in Blood sheep plate and in hemolysis assay, revertion of gene complemented the phenotype. Growth curve of S.lugdunesis with erythrocytes allowed the strains to break the cells and use the iron for their growth, SLUSH mutant lost their ability to steal the iron. Addition of SLUSH recombinant peptides in the presence of erythrocytes complemented thr growth defect for mutants. RT-PCR analysis revealed that the SLUSH genes expression and Agr regulated.

Conclusions: SLUSH peptides are only hemolytic factors presented in S.lugudunensis and they behave similarly to PSM in S.aureus and regulated via Agr system

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IIP 261

Bivalent BNT162b2mRNA original/Omicron BA.4-5 booster vaccination: adverse reactions and inability to work compared to the monovalent COVID-19 booster

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Question: For the bivalent mRNA-1273.214 vaccine (Wuhan-Hu-1/BA.1) slightly higher rates of the predominant adverse reactions have been reported. However, due to approval without an additional clinical study to date no evidence is available on adverse reactions and inability to work following a BA.4-5 adapted, bivalent COVID-19 vaccination.

Methods: This non-randomized controlled study examined adverse reactions, PRN (pro re nata) medication intake and inability to work after a fourth vaccination (i.e. second booster) among HCWs (healthcare workers) of the prospective CoVacSer study. The second booster was performed with the monovalent BNT162b2mRNA vaccine or the bivalent BNT162b2mRNA original/Omicron BA.4-5 vaccine.

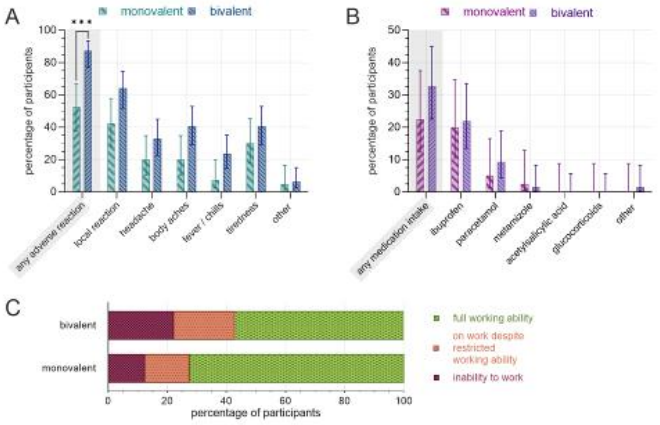
Results: 76 HCWs received a fourth dose of COVID-19 vaccination between the 13th of August 2021 and the 14th of October 2022. The rate of adverse reactions for the second booster dose was significantly higher among participants receiving the bivalent 84.6% (95% CI 70.3%-92.8%; 33/39) compared to the monovalent 51.4% (95% CI 35.9-66.6%; 19/37) vaccine (p=0.0028, *Figure 1*). Bivalent vaccinated participants further reported higher rates of adverse reactions in all subcategories. Also, there were more frequent intake of PRN medication and numerically higher rates of work ability restrictions in the bivalent vaccinated group.

Conclusions: Individuals receiving a second COVID-19 booster vaccination with the bivalent BNT162b2mRNA original/Omicron BA.4-5 vaccine reported adverse reactions more frequently compared to those receiving the monovalent vaccine. Also, there was a trend towards an increased rate of inability to work and intake of PRN medication following bivalent vaccination. In the light of preprints reporting inconclusive results in neutralizing antibody levels between the compared vaccines, our results, and further studies on safety and reactogenicity of bivalent COVID-19 booster vaccines are highly important to aid clinical decision making in the choice between bivalent and monovalent vaccinations.

The work has been published in Clinical Microbiology and Infection: https://doi.org/10.1016/j.cmi.2023.01.008

***Figure 1**: Post-vaccination adverse reactions, PRN medication and inability to work following the second COVID-19 booster administration, separated by vaccine. **A)** rate of adverse reactions by subcategory, **B)** rate of PRN medication, **C)** work ability restrictions. Monovalent: BNT162b2mRNA (n=37), bivalent: BNT162b2mRNA original/Omicron BA.4-5 (n=39). **: p<0.01, *: p<0.05.*

Fig. 1



IIP 262

Socioeconomic factors influencing vaccination coverage of children tested at school entry examinations in Schleswig-Holstein

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Question: High vaccination coverage protects the individual and the community from vaccine-preventable infectious diseases. The Robert-Koch-Institute (RKI) currently recommends twelve vaccines for children aged 5-6 years. The aim of this study was to quantify the vaccination coverage in Schleswig-Holstein"s pre-school children and to identify socioeconomic factors influencing them. The results may serve as a suggestion for possible countermeasures in case of low vaccination coverage.

Methods: At school entry examinations (SEE), data on vaccination status are routinely collected from all pre-school children. In a first part, children aged 5 and 6 years who were examined at SEE in Lübeck from 2012/13 to 2020/21 were included in this cross-sectional study. Lübeck – the second-largest city in the state of Schleswig-Holstein – is located northeast of Hamburg. Its population of 219,645 inhabitants lives in eleven postal code

districts. Currently data on the whole state is merged and undergoes analysis. Completeness of vaccination was assessed by comparing the number of received vaccination doses to the recommendations of the RKI. Multiple logistic regressions were used to examine exploratively the influences of different socio-economic predictors on the completeness of the vaccination status overall and per vaccine, vaccination refusal, and vaccination over-supply.

Results: Of 13,241 included children, a total of 11,883 (89.7 %) presented their vaccination card. The overall vaccination coverage in Lübeck was 78.2 %, but higher for individual vaccines. The highest vaccination coverage was achieved with the polio vaccine (94.1 %). The lowest vaccination coverage, regarding recommended vaccines in the study period, was seen with the pneumococcal vaccine (84.8 %). A total of 263 (2.3 %) of children were not vaccinated at all.There were differences in vaccination coverage by year, especially in the times of the COVID19-pandemic. Being born outside of Germany, having parents with the highest secondary education, having a currently non-working father, not attending all nine German medical check-ups, having a therapy recommendation, and living in certain postal code districts had a significant negative influence on full vaccination coverage. Gender, parents" country of birth, mother tongue, family structure, and BMI had no significant effect on the overall completeness of the vaccination status.

Conclusions: Vaccination coverage in Lübeck was satisfactorily high, with slightly lower coverage for newer vaccines. The specific eradication goals were reached except for vaccination against pertussis, hepatitis B, measles, varicella, and pneumococci.This research contributes to our understanding of predictors for childhood immunization at the local level. Strategies to increase vaccination coverage should be adapted to children who experience disadvantages regarding vaccination.Further results for Schleswig-Holstein will be available in the course of 2023.

Fig. 1

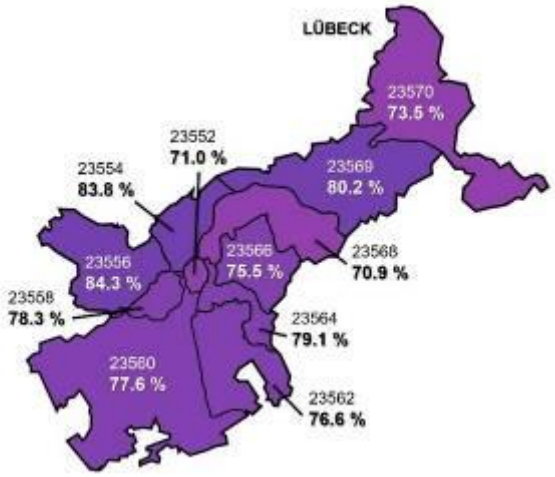
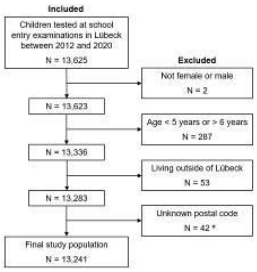


Fig. 2



IIP 263

The relationship between mental health, sleep quality, and the immunogenicity of COVID 19 vaccinations

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Question: Sleep modulates the immune response and sleep loss can reduce vaccine immunogenicity. Vice versa immune responses impact sleep. We aimed to investigate the influence of mental health and sleep quality on the immunogenicity of COVID-19 vaccinations and, conversely, of COVID-19 vaccinations on sleep quality.

Methods: The prospective CoVacSer study monitored mental health, sleep quality, and Anti SARS CoV 2 Spike IgG titres in a cohort of 1,082 healthcare workers from September 29th, 2021, to the December 19th, 2022. Questionnaires and blood samples were collected before, 14 days, and three months after the third COVID-19 vaccination as well as in 154 participants before and 14 days after the fourth COVID-19 vaccination.

Results: Healthcare workers with psychiatric disorders had slightly lower Anti-SARS-CoV-2-Spike IgG levels before the third COVID-19 vaccination. However, this effect was mediated by higher median age and body mass index in this subgroup. Antibody titres following the third and fourth COVID-19 vaccination ("booster vaccinations") were not significantly different between subgroups with and without psychiatric disorders. Sleep quality did not affect the humoral immunogenicity of the COVID-19 vaccinations (Figure). Moreover, the COVID-19 vaccinations did not impact self-reported sleep quality (Figure 2).

Conclusions: Our data suggests that in a working population neither mental health nor sleep quality relevantly impact the immunogenicity of COVID-19 vaccinations and that COVID-19 vaccinations do not cause a sustained deterioration of sleep, suggesting that they are not a precipitating factor for insomnia. The findings from this large-scale real-life cohort study will inform clinical practice regarding the recommendation of COVID-19 booster vaccination for individuals with mental health and sleep problems.

The work has been published in the Journal of Sleep Research: https://doi.org/10.1111/jsr.13929

Figure 1: Anti SARS CoV 2 Spike IgG levels following COVID–19 booster vaccinations stratified by sleep quality. 1A) before (pre vaccination) and after (14 days and 3 month follow up) the third COVID 19 vaccination. 1B) before (pre vaccination) and after (14 days) the fourth COVID 19 vaccination. Sleep quality is stratified in five categories (very dissatisfied to very satisfied).
Anti SARS CoV 2 Spike IgG logarithmically scaled. BAU/ml: binding antibody units per millilitre. Medians are indicated as horizontal bold line, quartiles as dotted lines.

Figure 2: Sleep quality before and after COVID 19 booster vaccinations (left: absolute numbers, right: relative share) 2A) before (pre vaccination) and after (14 days and 3 month follow up) the third COVID 19 vaccination. 2B) before (pre vaccination) and after (14 days) the fourth COVID-19 vaccination. Sleep quality is stratified in five categories (very dissatisfied to very satisfied).

Fig. 1

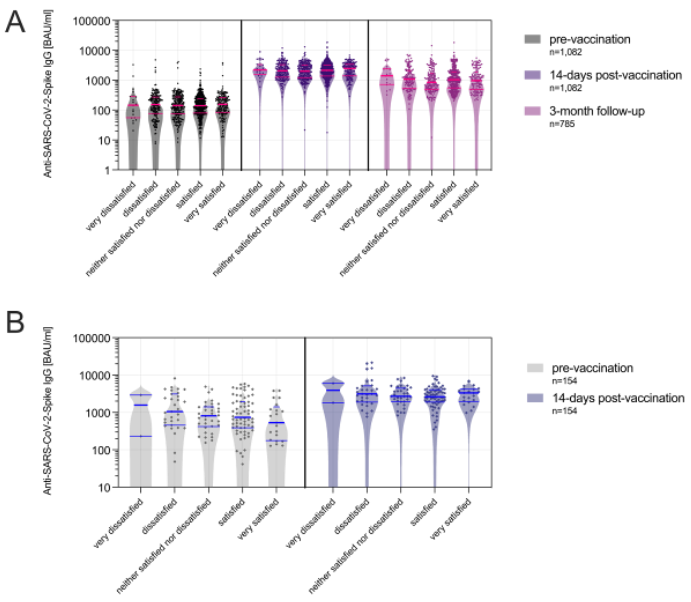
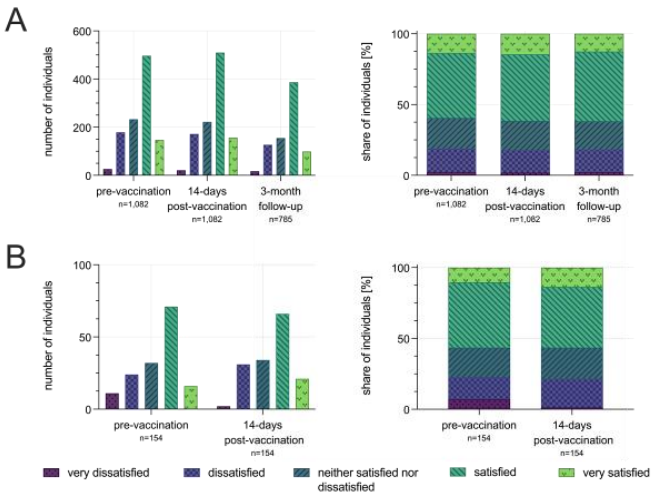


Fig. 2



IIP 264

Host determinants for the spread of PVL-positive *Staphylococcus aureus* in Sub-Saharan Africa

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Background: *Staphylococcus aureus* produces a variety of toxins, including leukocidins, to evade the host immune defence and establish an infection. The Panton-Valentine leukocidin (PVL) is a bi-component pore forming toxin that targets the membrane of neutrophil granulocytes, macrophages, and monocytes. PVL is associated with severe skin and soft tissue infections (e.g. pyomyositis) and the prevalence of PVL-positive *S. aureus* is higher in Africa than Europe (approx. 50% vs 5%). However, the determinants accounting for differences in this prevalence are unknown. In this study, we compared the cellular and humoral response to PVL in African and European volunteers.

Methods: In a multi-center cross-sectional study (2019–2023) in Africa (Gabon, Nigeria, Congo) and Germany (Münster, Jena), we obtained blood and serum samples, as well as nasopharyngeal swabs from African (n=447) and German (n=325) participants with no signs or symptoms of an *S. aureus* infection. We measured the susceptibility of isolated granulocytes to different PVL concentrations by FACS. Inflammasome activation after PVL stimulation of isolated monocytes was assessed by ELISA quantification of secreted IL-1ß. The anti-PVL-antibody levels in serum were quantified by ELISA and nasopharyngeal *S. aureus* colonization was determined by bacteriological culture and molecular identification.

Results: After exposure to PVL, granulocytes were more likely lysed in Africans compared to Germans, and this difference was most significant at a concentration of 0.5 nM PVL (median, 51.2% vs. 24.1%, p<0.0001). The median induction of IL-1ß secretion was significantly higher in Africans than in Germans (4097 pg/ml vs. 103 pg/ml, p<0.0001) in response to sub-cytotoxic concentrations of PVL (0.5 nM). Also, the levels of anti-PVL-antibodies were significantly higher in Africans compared to Germans (16.7 AU vs. 5.1 AU, p<0.0001), while *S. aureus* nasopharyngeal colonization rates were comparable in both groups (24% vs. 39%).

Conclusions: The stronger inflammasome activation by PVL in African monocytes compared to German could result in an enhanced recruitment of neutrophils to the site of infection via the release of IL-1ß. Combined with the higher susceptibility of granulocytes from Africans to PVL, this could result in increased tissue damage and might be associated with the higher incidence of *S. aureus* SSTI in Africa compared to Europe. The higher anti-PVL-antibody level in the African participants might be due to higher exposure to PVL in Africans.

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Myeloid cells in a mouse model of chlamydial infection

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Objectives: *Chlamydia trachomatis* (*C. trachomatis*) are pathogenic intracellular bacteria that infect the female genital tract and may cause permanent tissue damage, e.g. scarring and infertility. However, the exact mechanism of bacterial clearance and development of tissue damage in the genital tract is still unknown. Our previous work has focused on the role of the innate immune system in general and of neutrophils on bacterial clearance and tissue destruction. Little is known about other myeloid cells in the female genital tract both at steady state and during chlamydial infection. In this project, we will characterize the recruitment of myeloid subpopulations and their impact on chlamydial infection and tissue damage.

Methods: In a *Chlamydia muridarum* (*C. muridarum*) infection mouse model, we investigated the infiltration of myeloid cells in the genital tract by flow cytometry and histopathology. Bacterial load and tissue damage were also analyzed. The contribution of neutrophils to bacterial clearance and tissue damage was characterized in mice deficient in mature neutrophils (Mcl-1 mice). The effects of other myeloid subpopulations were analyzed in related knockout mice (e.g. CCR2-KO mice, which are deficient in the recruitment of inflammatory monocytes), and reporter mice.

Results: Myeloid cells infiltrated the genital tract at early time points of infection, with a maximum for neutrophils and monocytes/macrophages at 7 dpi, and for dendritic cells at 14 dpi. The chlamydial DNA was already detected early in the upper genital tract. Chlamydia was efficently cleared at 31- 35 dpi from all parts of the genital tract. Mcl-1 mice lacking neutrophils showed substantial qualitative and quantitative alterations of the inflammatory infiltrate and had higher chlamydial burden and reduced tissue damage. In wt mice, upon entry into the genital tract, inflammatory monocytes differentiated into macrophages, which sequentially gave rise to different subpopulations, and this progression was accompanied by the upregulation of MHCII and downregulation of Ly6C. In addition, we have identified three different dendritic cell (DC) subpopulations differing in CD11b and CD103 expression, which increased in absolute numbers at later time points compared to macrophages. In CCR2-KO mice, the accumulation of macrophages upon infection was strongly reduced. This indicates that the increase in macrophages in infected tissue is largely caused by recruitment and differentiation of inflammatory monocytes. However, no effect of CCR2-deficiency on recruitment of dendritic cells was observed.

Conclusions and Outlook: Neutrophils or probably also other myeloid cells play a substantial role in chlamydial clearance and long-term tissue destruction. The preliminary data revealed several interesting myeloid subpopulations, which will be characterized in

detail regarding their effects on chlamydial infection, recruitment of other immune cells and on development of tissue damage.

IIP 266

Bringing together what belongs together: The mouse-adapted *S. aureus* strain JSNZ enables persistent neonatal *S. aureus* colonization in mice

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Introduction: *Staphylococcus aureus* (*S. aureus*) has a long evolutionary history with a broad host range. Specific *S. aureus* lineages are associated with particular host species. Our recent studies have revealed that laboratory and wild mice are natural hosts of *S. aureus*. These murine strains have adapted to their host by acquiring novel virulence factors, eliminating genes encoding for superantigens and human-specific immune evasion factors, usually located on mobile genetic elements. The whole-genome sequencing of 230 *S. aureus* isolates from laboratory mice worldwide revealed that CC88 is the predominant lineage. The prototypical CC88 isolate, JSNZ, was isolated from a colony of C57BL/6 mice, where it persisted for 2.5 years. We recently demonstrated that JSNZ persistently colonizes adult mice upon intranasal inoculation, while colonization with human-adapted *S. aureus* strains is only transient.

Aim: We aimed to establish and characterize a neonatal persistent *S. aureus* colonization model using the mouse-adapted *S. aureus* CC88 strain JSNZ.

Method: Adult female and male C57BL/6NRj mice were colonised intranasally by applying 10^8 CFUs of the *S. aureus* JSNZ strain and mated seven days later. Neonatal colonization of their offspring was achieved by vertical transmission. The bacterial load was determined in homogenized organs and faecal samples. Cytokines and chemokines were quantified with a bead-based assay (LEGENDplex). Infected preputial glands were paraffin-embedded and stained with H/E or antibodies against *S. aureus* and Gr1 as well as DAPI.

Results: All breeding pairs were persistently colonized in the nose. Males exhibited stable bacterial loads in the stools over time, whereas colonization density in females was variable. Notably, the offspring exhibited persistent colonization with high bacterial densities in the nose and the gastrointestinal tract for a lifetime. Colonization was usually symptom-free, but some older males developed a spontaneous infection of their preputial glands (preputial gland adenitis, PGA), characterized by pus formation and enlargement of the gland. This infection was often unilateral and lasted for several months. PGA induced the atrophy of the glands, as evidenced by dilation and ectasia of the preputial gland ducts. In addition, it was associated with massive neutrophil infiltration and high *S. aureus* loads, triggering a strong release of IL-1α, IL-1β, IL-17, MIP-1α, and KC within the glands. *Spa* typing confirmed JSNZ as the causative agent. PGA triggered a strong Th17-dominated T-cell response.

Discussion: The mouse-adapted strain JSNZ is an excellent tool to mimic natural colonization in mice and understand the interplay between *S. aureus* and the host. The spontaneous development of PGA allows studies into factors that shift the delicate balance between colonization and invasion.

ZOLMP 267

Growth and toxin production of bacterial pathogens in plant-based milk alternatives

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Introduction: Currently, a large number of vegetarian or vegan foods are offered on the market. Within these, the most important group is vegan milk alternatives. In 2020, approximately 224 million litres of plant-based milk were sold in Germany with a turnover of approximately 351 million euros, which corresponds to a doubling compared to 2018. The consumption of vegan yoghurt and cheese alternatives is also increasing significantly. Among the most popular products are oat, almond and soy drinks. Soy, almonds and cashews are the favourites for vegan cheese. Current research is mainly focusing on the nutritional value, i.e. health benefits or risks of consuming these alternative products. In comparison, there has been very little work to date on the microbiological safety of plant-based milk, cheese and yoghurt alternatives. It is generally assumed that the microbiological risk is relatively low, as these products are mostly subjected to natural or controlled fermentation. The addition of probiotic starter cultures would also suppress the growth of potentially pathogenic germs. Moreover, various heating and preservation processes contribute to a further increase in stability. However, a general microbiological harmlessness cannot be attributed to these novel milk substitutes. This was shown by a case from last year, when there was a major recall of a fermented and matured cheese alternative with almond milk in July after the detection of *Listeria monocytogenes*. The initial aim of this project was to clarify how relevant pathogenic bacteria such as *L. monocytogenes*, *Salmonella enterica* and *Bacillus cereus* behave during the shelf life and storage period in plant-based drinking milk alternatives, depending on the underlying raw materials and nutrient availability.

Methods: 25 plant-based milk alternatives and a conventional cow's milk were purchased from local supermarkets. Total aerobic, mesophilic bacterial counts were determined. Growth of *L. monocytogenes*, *S. enterica* and *B. cereus* was tested firstly under "optimal" growth conditions (10 h, 37 °C, 120 rpm) and secondly under "storage" conditions following DIN EN ISO 20976-1:2019-09 (6 weeks, 22 °C) by plating serial dilutions on plate count agar. The toxin-forming capacity of *B. cereus* was also tested using enzyme immuno assays.

Results: With a few exceptions, the products purchased were germ-free. In general, the three selected pathogens could grow in all milk alternatives, but there were species-specific differences. Furthermore, depending on the food matrix and the isolate used, *B. cereus* was able to form relatively high amounts of enterotoxins.

Conclusions: This study contributes to a better understanding of the survival and growth potential of pathogenic bacteria in the increasingly popular vegan milk alternatives. Furthermore, additional insights into the toxin-forming capacity of *B. cereus* in these novel food matrices were gained.

ZOLMP 268

PVL-positive *Staphylococcus aureus* isolates in food, livestock and wildlife

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Introduction: Among different virulence factors enabling *Staphylococcus (S.) aureus* to cause skin- and soft-tissue infections, the Panton-Valentine leukocidin (PVL) is well-known for its effect in recurrent, large skin abscesses which can lead to severe

infections and stigmatization. Community-acquired cases are often associated with PVL-positive *S. aureus* and infections often occur in young, healthy individuals. Especially travelling to tropical and subtropical countries has been associated with a higher risk for PVL-positive *S. aureus* infections. While mostly PVL coincide with a Methicillin-sensitive *S. aureus* (MSSA) phenotype in infected people, the coincidence with a Methicillin-resistant *S. aureus* (MRSA) phenotype can result in difficult-to-treat and even more severe infections. As zoonotic agent, *S. aureus* can be transmitted from humans to animals and vice versa. Although food is not considered a relevant source for human colonization with *S. aureus*, the potentially grave consequences of infection with PVL-positive *S. aureus* require monitoring of food for this kind of bacteria.

Methods: Since 2014, the German National Reference Laboratory for Coagulase-positive Staphylococci incl. *S. aureus* (NRL-Staph) has screened all *S. aureus* isolates that were provided in frame of the national zoonoses monitoring program or sent in for other reasons, concerning the occurrence of the *lukS-PV* gene as marker gene for PVL. All *lukS-PV*-positive isolates were further *spa*- and SCC*mec*-typed as well as characterized regarding their antimicrobial resistance profile by broth microdilution. Additionally, whole genome sequencing allowed the detection of further (host-associated) virulence factors and to decipher phylogenetic relationships.

Results: In total, 31 *S. aureus* strains have been identified as positive for *lukS-PV* (30/4144 investigated MRSA strains and 1/681 investigated MSSA strains). More than half of the strains originated from studies that targeted imported, farmed fish (*Tilapia, Pangasius*; n=13) or imported prawns (n=8). The other *lukS-PV*-positive isolates originated from beef (n=7) with half of them being imported, and one each from chicken breast, insect (*lukS-PV*-positive MSSA) and an unknown matrix. Further characteristics of the isolates will be presented.

Discussion: PVL-positive *S. aureus* and especially MRSA are rare in human clinical infections in Germany in contrast to non-European countries and have been mostly described in community-acquired and travel-associated infections. However, the number of PVL-positive *S. aureus* infections is increasing. Our results indicate that PVL-positive MRSA/MSSA are usually not found in food and food-producing animals from Germany, even though MRSA rates can be quite high. In contrast, the rate of PVL-positive MRSA strains is relatively high in imported food and could cause a public health threat as clonal lineages differ considerably from common livestock-associated MRSA strains in Germany.

ZOLMP 269

Occurrence of lactic acid bacteria during spontaneous sauerkraut fermentations

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Introduction: Traditional sauerkraut fermentation is the result of the spontaneous growth and activity of the microbiota of white cabbage. In this study, the microbiota of lactic acid bacteria during spontaneous sauerkraut fermentations was examined in the brine of four fermentation barrels, namely barrel A, B, F, and G. All four fermentations were carried out under comparable conditions. Sampling out of barrels A, B, and G during the whole fermentation time and out of barrel F during the first seven days gave an overview of the diversity and succession of the microorganisms.

Material and Methods: Samples of the natural brine of the three barrels A, B, and G were taken from the beginning to the end of the fermentations and of barrel F during the first seven days. The pH values were measured. The samples were analyzed for lactic acid bacteria. Diluted samples were spread-plated on MRS agar with natamycin and GYP agar with $CaCO_3$ and incubated at 30 °C for three days anaerobically or aerobically, respectively. Optically distinguishable colonies were selected randomly. The isolates were confirmed as lactic acid bacteria by Gram staining and catalase test.

Isolates of lactic acid bacteria were identified by comparative sequence analyses of the 16S rRNA genes. After enrichment of the strains in MRS broth, extraction of the DNA, amplification of the genes by PCR and sequencing was carried out. The sequences were analyzed using the Basic Local Alignment Tool (BLAST) within the database of sequences of 16S rRNAs of the National Center for Biotechnology Information (NCBI).

Results: Starting from the second day of fermentation high colony counts of lactic acid bacteria were found, which correlated with the decreasing pH value of the brines. Out of heterofermentative lactic acid bacteria *Lentilactobacillus diolivorans, Levilactobacillus brevis*, *Levilactobacillus parabrevis*, *Leuconostoc citreum*, *Leuconostoc holzapfelii*, *Leuconostoc mesenteroides*, *Leuconostoc mesenteroides* ssp. *jonggajibkimchii*, and *Leuconostoc pseudomesenteroides* were identified. Out of homofermentative species of lactic acid bacteria *Lactiplantibacillus fabifermentans*, *Lactiplantibacillus paraplantarum*, *Lactiplantibacillus plantarum*, *Lactiplantibacillus xiangfangensis*, *Latilactobacillus curvatus*, *Latilactobacillus sakei*, *Lactococcus lactis* and *Pediococcus parvulus* were identified.

Mostly heterofermentative lactic acid bacteria were found in barrels F and G during the first stage of the fermentations. Homofermentative species of lactic acid bacteria mostly replaced heterofermentative lactic acid bacteria in the later stages of the fermentation in barrel G.

Discussion: Diversity and succession of lactic acid bacteria isolated out of spontaneous sauerkraut fermentations were different in the barrels. Succession of heterofermentative and homofermentative lactic acid bacteria during fermentation of sauerkraut could be shown in barrel G.

ZOLMP 270

Development of the methodology and evaluation of the sensitivity of a reverse hybridization-based detection of pathogenic bacteria on fresh produce

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Introduction: Food safety plays an essential role in the food sector and should be ensured to protect consumers from health issues. To facilitate safety assurance in fresh produce, a new methodology was developed, which is based on rapid DNA extraction and a PCR with a following reverse hybridization. This methodology should ensure a fast and user-friendly handling. The PCR amplifies species-specific bacterial DNA followed by hybridization, which is based on probe strips, visualizes the presence of specific bacteria through colour detection. To test the sensitivity of this test system, different bacterial concentrations were used, which derive from the guideline or warning values, respectively, of the DGHM including warning values for clinically relevant species. The aim of this project was to develop a uniform methodology based on an adapted combination of different microbiological, molecular and biochemical procedures and should be close to practice and everyday life and thus lead to representative results. The strip currently contains probes for six food-associated bacteria - *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enterica*,

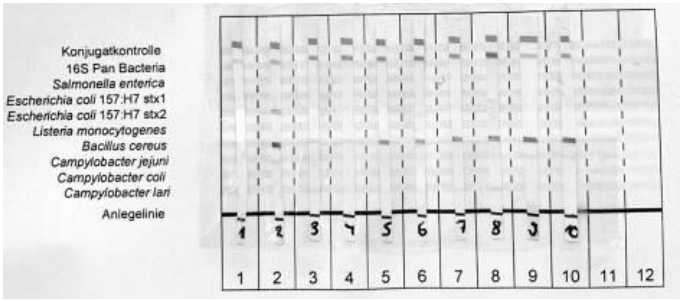
Campylobacter coli, C. jejuni and *C. lari* as well as the *Escherichia coli* stx1 and stx2 genes.

Method: Iceberg lettuce was used as experimental food samples, which were spiked with different concentrations of bacteria. The leaf-wash of lettuce samples was filtered through membranes, which then underwent direct DNA extraction. The specific bacterial genes were amplified by an adapted PCR and were then used in the reverse hybridization. After different washing and incubating steps, the signal was visualized by enzymatic reaction. To control the performance, gel electrophoresis was conducted.

Results: A uniform methodology for the facultative anaerobic (*B. cereus, L. monocytogenes* and *S. enterica*) and microaerophilic (*C. coli, lari* and *jejuni*) bacteria was developed. The hybridization strips showed a good sensitivity for low concentrations that are clinically relevant. The lowest concentrations, which are clearly detected on the strips, are for *B. cereus* 10^1 CFU/g, for *L. monocytogenes* and *S. enterica* 10^2 CFU/g. In the case of the three *Campylobacter* species, the highest dilution level, starting from a bacterial suspension with an optical density (OD_{600}) of 1, which could be detected was 10^{-6}. As an example, the result of the strips for *B. cereus* is shown in the figure. The strips 3 and 4 are representing the concentration 10^1 CFU/g and show the weakest but still clear colour signal.

Discussion: The established assay represents a significant contribution to the microbiological control of fresh plant products. To make the probe bands appear more distinct, bacterial concentrations could be enriched so that any so far distinctly weak bands could be strengthened. As a summary, the developed detection method has good prospects of being frequently used in quality control, especially in the food industry.

Fig. 1



ZOLMP 271

Survival of foodborne pathogens in kitchen sponges and cross-contamination of surfaces

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Introduction: Kitchen hygiene is considered as the last line of defense to prevent foodborne infections. Kitchen sponges can be massively contaminated with bacteria, leading to cross-contamination of food-contact surfaces during the process of cleaning. Even though foodborne pathogens are frequently found in kitchen sponges, there is limited knowledge on their survival and spatial distribution within the sponge tissue. We aimed to analyze the ability of foodborne pathogens to colonize kitchen sponges with an established background microbiota and to investigate the risk of surface cross-contamination through contact with contaminated sponges.

Material and Methods: We artificially contaminated sponges with 10 bacterial species commonly identified in used kitchen sponges including *Acinetobacter* spp., *Moraxella osloensis* and *Brevundimonas diminuta* to establish a suitable background microbiota (day d0). On d1, the sponges were inoculated with different doses of *Salmonella* Enteritidis, *Staphylococcus (S.) aureus* or *Escherichia (E.) coli* ranging from 5×10^2 to 5×10^4 cfu/sponge (4.5×10^1 - 4.5×10^3 cfu/sponge section [13.2 cm^3]). Bacterial uptake and colonization of the sponge tissue were determined on d0, d1, d3, d7, d9 and d14 applying three complementary approaches: quantitative bacteriology, metagenome analysis and confocal laser scanning microscopy (CLSM) of fluorescence in situ hybridization labeled bacteria. Sponges were routinely incubated in a humidity chamber at 22°C. From d3 to d7, sponges were incubated without humidity. The transfer of pathogens to surfaces by the use of colonized sponges was analyzed by qualitative and quantitative bacteriological methods.

Results: Even the lowest spiking concentrations tested for *E. coli* and *Salmonella* Enteritidis led to a stable colonization of sponge tissue, reaching a plateau on d7 with 10^8 cfu/sponge section. Sponge desiccation between d3 and d7 had no influence on the bacterial load. For *S. aureus*, higher spiking concentrations of 4.5×10^2 cfu/sponge section were necessary for a stable colonization. Cross-contamination of smooth surfaces after brief contact with a colonized sponge under moderate pressure was observed for each pathogen that reached a cell density of at least 5×10^3 cfu/sponge section. CLSM revealed a heterogeneous colonization of the sponge tissue with partly dense patches of multiple species on its surface. Metagenomic analyses identified reproducible shifts in the sponge microbial communities dependent on sampling time and experimental condition.

Discussion: Our data point towards the need of frequent replacements or appropriate sanitization of sponges to maintain kitchen hygiene. The model established in our study can help to identify suitable measures for a better sponge hygiene in households and to develop clear recommendations for consumer protection.

KMP 272

Tuberculosis associated with haemophagocytic lymphistiocytosis (HLH)

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Introduction: Haemophagcytic lymphohistiocytosis (HLH) is a clinical syndrome characterized by excessively activated macrophages. Intensive care treatment is often required and mortality is high. Secondary HLH may be caused by various infections.

Material: Data were collected form reviewing the patient`s chart as well as the hospital and laboratory information system.

Results: A 32-year old female presented with fever, back pain, pleural effusion, ascites, and paresis of the lower extremities. Sepsis and spondylodiscitis were suspected and initially she was treated with Flucloxacillin and Ceftriaxon followed by Meropenem and Vancomycin. Hämoglobin (6.7 mg/ dl) and thrombocytes

(37.000 /µl) were low, ferritin was markedly increased (> 30.000 µg / l). Il-6, CRP, PCT were elevated. ENA (extractable nuclear antigen) SS-A/Ro- and anti-DNA-antibodies were positive. Bronchoalveloar lavage (BAL) showed *Candida dubliniensis*, stain for acid fast bacilli (AFB) was negative. Bone marrow biopsy revealed haemophagocytosis and granuloma with epithelioid cells and central necrosis. A kidney-biopsy showed glomeruli that stained positive for IgA, IgG, IgM, C1q and C3c, and revealed tubuloreticular inclusions of glomerolar endothelium. HLH and systemic lupus erythematosus (SLE) were diagnosed, and she was started on immunosuppressive therapy (methylprednisolone, cyclophosphamide, Il1-receptor antagonist). However, deterioration of the patient continued and she required intubation and invasive ventilation. Her ferritin levels rose to >80.000 µg/l.

Tomography of her chest revealed inflammatory infiltrations of the right lung, as well as multiple enlarged mediastinal and axillary lymph nodes. Biopsy of lymph nodes stained positive for acid bacilli, nucleic acid amplification techniques (NAT) revealed *Mycobacterium tuberculosis complex*. The patient received anti-tuberculous therapy with Rifampin, Isoniazid, Ethambutol and Pyrazinamide. Subsequently, *Mycobacterium tuberculosis* was cultured from both the lymph node biopsy and the initial BAL. The patient improved on anti-tuberculous therapy and immunosuppressive therapy could be tapered down.

Discussion: This patient had TB presenting with HLH. She had no history of SLE and the SLE features might have resulted from overactivation of the immune system, as there was full recovery after initiation of tuberculostatic therapy. High mortality of tuberculosis associated with HLH has been reported exceeding 50% of cases. Here, early lymph node biopsy and BAL led to rapid diagnosis of TB, and instant treatment of the patient with anti-tuberculous therapy.

KMP 273

***Auritidibacter ignavus* might be an overlooked cause of ear infections**

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Introduction: *Auritidibacter ignavus* was first isolated in 2011 from a 28-year-old man with fulminant otitis externa. Since then, the species has received little attention. Therefore, limited information is available about this ear pathogen so far.

In our diagnostic institute, the species was first detected in 2021 via 16S-PCR in a culture-negative tissue sample from a patient with mastoiditis. Since the species was added to Bruker's MALDI database in April 2022, the species has been identified multiple times, so far only from ear canal and mastoid specimens. This suggests that this little-known species may cause ear infections more frequently than previously assumed.

Methods: The strains originate from clinical samples of patients with ear infections, submitted by a tertiary hospital for diagnostic purposes. The isolates were identified by MALDI-ToF MS (Bruker). The MIC of the strains was determined via broth microdilution. The results were interpreted according to the non-species related PK-PD breakpoints of EUCAST. For substances for which no corresponding breakpoints were available, the interpretation was based on typically sensitive bacteria instead.

Hybrid assemblies were created for all strains, using sequencing data from an Illumina and an Oxford Nanopore sequencer. The chromosomes were annotated with PATRIC to identify antibiotic resistance genes and virulence factors.

Results: Pinpoint colonies grew after 16 hours, colonies of 1 mm after 40 hours. The isolates were morphologically indistinguishable from corynebacteria or coagulase-negative staphylococci.

All strains were susceptible to all beta-lactam antibiotics (except oxacillin), trimethoprim-sulfamethoxazole, tigecycline, vancomycin, teicoplanin, linezolid and rifampicin and resistant to oxacillin, fluoroquinolones, fosfomycin and mupirocin. In contrast, gentamicin, erythromycin, clindamycin, fusidic acid and daptomycin were sensitive in some strains and resistant in others.

PATRIC predicted a highly conserved resistome in all strains. Further, the virulence factor *icl* was detected in all strains.

Discussion: *Auritidibacter* appears to be intrinsically resistant to oxacillin, fosfomycin, mupirocin and fluoroquinolones. This can be a problem, as ear infections are frequently treated topically with fluoroquinolones. Instead, cephalosporins could be a good option for empiric therapy, as these are usually well tolerated and no resistances have been detected to date.

The virulence factor *icl*, which encodes the glyoxylate cycle key enzyme isocitrate lyase, can help *A. ignavus* to persist in the body. Hereby, the species could possibly also lead to chronic ear infections.

Since the species seems to grow slowly and cannot be distinguished morphologically from bacteria in the local flora, it would be advisable to incubate ear samples for two days and to carry out differentiation even in bacteria with inconspicuous morphology grow.

KMP 274

Isolation of *Pseudoglutamicibacter cumminsii* from clinical outpatient samples

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Introduction: *Pseudoglutamicibacter cumminsii* (formerly *Arthrobacter cumminsii*) is a coryneform Gram-positive aerobic bacterium commonly found in soil. Its role in the pathogenesis of infectious diseases is poorly understood, and there are few reports of *P. cumminsii* or *Arthrobacter spp.* as the cause of infections. We evaluated the epidemiological characteristics of *P. cumminsii* isolates from outpatients and determined the antimicrobial susceptibility.

Methods: Specimens were cultured using conventional methods. Identification of bacteria was performed at genus and species levels using MALDI-TOF. Antimicrobial susceptibility testing (AST) was performed for 16 antimicrobials using the broth microdilution MIC method. Results were interpreted using EUCAST breakpoints. MIC 50- and MIC 90-values were calculated for all tested substances. The patient cohort was differentiated by sex and age (15-year intervals). Demographic and patients" data were extracted from the LIS MOLIS (v. 4.40) and statistics suite HyBASE® (v.6.2023.01.01).

Results: We isolated 116 *P. cumminsii* from clinical samples from January 2021 to February 2023. Female patients contributed 65.5%. Most specimens were found in the age ranges 60-74; 27.5% and 75-89; 23.2%. *P. cumminsii* was detected in 58.6% urine samples, 30.1% wound swabs, and the remaining 11.2% in samples from varying origins. In only two cases it was found in pure culture, in urine and knee synovial fluid sample. In most cases, *P. cumminsi* was detected in a mixed culture with *Escherichia coli* (33.6% of all samples), *Proteus mirabilis* (25%) or *Enterococcus*

faecalis (10.3%). Susceptibility (S) of *P. cumminsii* was tested for β-lactam antibiotics (97.4% S), linezolid (98.1% S) and quinolones (43.9% S). AST was conducted on 46.6% of isolates.

Discussion: *P. cumminsii* has not been described as inhabiting human skin or mucous membranes. Nevertheless, in our study *P. cumminsii* was mostly detected in urine samples from older women (≥ 60 years) alongside at least one typical uropathogenic bacterium, being able to consider *P. cumminsii* as contamination of the sample. However, in cases of a pure culture of *P. cumminsii*, its pathogenic or colonizing role should be considered, as described in different studies. AST showed *P. cumminsii* being susceptible to most of the tested substances. *P. cumminsii* is most likely eradicated during the antibiotic treatment of other bacteria. For this reason, this type of study is important to define the pathogenesis of this type of bacteria and to monitor resistance patterns in probable underdiagnosed emerging pathogens.

PRHYP 275

UV-C Lamps for Disinfection of Neonatal Incubator Surfaces: A Comparison of Effectiveness and Material Compatibility

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Background: Neonatal incubators are essential for providing a controlled environment for premature or low birth weight infants. However, they can also act as reservoirs for pathogenic microorganisms, posing a significant infection risk. The toxicity of disinfectants prohibits their use while incubators are in operation, presenting an ongoing challenge for achieving safe and effective disinfection in the neonatal intensive care unit. UV-C irradiation is a non-chemical disinfection method with the potential to eradicate microbial surface contamination in hospitals. UV-C light-emitting diodes (LEDs) have advanced, offering a flexible application due to their physically small size, making them attractive for medical use compared to low-pressure mercury vapor lamps (LP) as a UV-C light source. We evaluated the efficacy of UV-C LEDs for disinfecting incubator surfaces and compared the stability of incubator materials exposed to UV-C light from both LED and LP lamps.

Method: Polyvinyl chloride (PVC) and polymethyl methacrylate (PMMA), commonly used in neonatal incubators were exposed to UV-C light from LP and LED sources. The samples were treated with a UV-C dose of 20 MJ/m², equivalent to five years of irradiation at 15 kJ/m² with five weekly exposures. Subsequently, we conducted microscopic examinations, FTIR-ATR analysis, water contact angle measurements, and color assessments. Additionally, we evaluated the efficacy of UV-C LED disinfection against three different organisms: *E. coli*, *S. aureus*, and *E. faecalis* using test carriers made of PVC and PMMA. These carriers were inoculated with microbial contaminants, air-dried, and exposed to 265 nm UV-C light at a dose of 15 kJ/m2.

Results: The utilization of UV-C LED treatment has proven to be highly effective in disinfecting all three bacterial strains, resulting in an inactivation of > 5 log10. However, it is important to note that PVC surfaces showed visual changes, such as tarnishing, due to irradiation. Infrared spectrum analysis revealed an increased intensity within the spectrum, indicating the presence of plasticizers. Both PVC and PMMA exhibited increased hydrophilicity without significant color changes. On the other hand, PMMA surfaces experienced significant changes and deterioration when exposed to LP lamps, with visible tarnishing and stickiness. It is noteworthy that no such effects were observed when utilizing LEDs.

Conclusion: Our study demonstrated the efficacy of UV-C LED treatment in disinfecting neonatal incubator surfaces contaminated with pathogens. However, PVC surfaces exhibited material changes, suggesting potential degradation regardless of the UV-C source. The use of LP lamps may result in significant deterioration of PMMA surfaces, making the use of LEDs a more favourable option for neonatal incubator disinfection. As such, it is crucial to carefully evaluate the suitability of UV-C as a disinfection method before implementation.

PRHYP 276

Environmental persistence of vancomycin resistant enterococci in the hospital setting

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Question: Vancomycin resistant enterococci (VRE) pose a serious threat to the hospital setting, whereby patient surroundings and contact surfaces are potential sources of transmission. Studies focusing on the transmission of VRE demonstrated that VRE of certain multilocus sequence typing (MLST) sequence types (ST), namely ST80, ST117, ST192 and ST203, are predominantly found as microbial contaminants of surfaces in the hospital environment. Hence, we here examined whether VRE of different ST vary regarding their properties of persistence in the hospital environment.

Methods: VRE of the most prevalent ST in the hospital environment, namely ST80, ST117, ST192, ST203, ST721 and ST1489 derived from hospital inpatients and contact surfaces were analyzed. In order to investigate the persistence of VRE on surfaces, plastic trays were contaminated and incubated at 36°C for 24, 48 and 72 hours with each strain. The numbers of remaining bacterial load was quantified using CASO contact agar plates. In a second setup, the resistance of VRE of the above-mentioned MLST ST to the disinfectants glucoprotamine (IncidinTM plus) and peracetic acid (Schülke mikrozid® PAA wipes) was investigated. Surfaces were contaminated and disinfected with the respective agent in a damp wipe manner. After evaporation of the applied disinfectant, the remaining contamination was sampled quantitatively using CASO contact agar plates. Finally, we produced mixed solutions, each containing one colony of ST117 and one colony of one of the other MLST ST in liquid culture medium. After incubation overnight, surfaces were contaminated with the bacterial solution. Contact sampling was performed after 24 hours with CASO agar plates. After incubation, five CFU were isolated randomly from each CASO agar plate in two technical replicates and subjected to characterization of ST via whole genome sequencing.

Results: Survival rates of VRE did not differ significantly after 24, 48 and 72 hours of incubation neither depending on their ST nor their origin (patient vs. hospital environment). The effect of disinfectants on VRE load differed significantly ($p<0.001$). Glucoprotamine and peracetic acid caused a reduction of the bacterial load by an average of 98.17% and 100%, respectively. The decrease in bacterial load after disinfection did not differ significantly with respect to the ST. In all mixed-culture approaches, types other than ST117 were identified predominantly. In combination with ST203 and ST1489, ST117 was identified in only around 10% of sequenced colonies, while ST117 was not detected in combination with ST80, ST192 and ST721.

Conclusion: Our preliminary results do not suggest a ST specific survival or persistence advantage of VRE in the hospital environment. Further in depth-research is necessary to investigate microbial factors of VRE that contribute to environmental persistence and selective advantages in the hospital environment.

PRHYP 277

Mirror, Mirror on the wall, who is the fastest of them all? – The problem of *C. acnes* as target organism in microbiological quality control testing of cell-based preparations

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Introduction: Paul Ehrlich Institute approval for the manufacture of cornea tissue preparation (ctp) requires method validation of the microbiological examination according to the European Pharmacopoeia (Ph. Eur.) using an automated culture system to detect test strains in the presence of the cell-based product. Slow growing bacteria species as *Cutibacterium acnes* (*C. acnes*) are a challenge in this setting, especially due to potentially inhibiting effects of the preparation used. Besides the mentioned *C. acnes* ATCC 11827 strain, the Ph. Eur. also opens the use of other strains. Therefore, we compared the time to detection (TTD) in a semiautomated blood culture bottle system (BACTEC™ FX) of 24 *C. acnes* strains, preferring clinical isolates.

Material and Methods: Anaerobic blood culture bottles (bcb) were spiked with less than 250 CFU of 23 *C. acnes* strains and incubated until positive or for up to 14 days. To test reproducibility, this procedure was repeated 4 times for 5 strains with the fastest (fg-strains), respectively 4 strains with the slowest initial detectable growth (sg-strains). To compare the TTD also in the presence of the ctp the 5 fg-strains, 2 sg-strains and the control strain ATCC 11827 were spiked into ctp-containing and non-containing bcb, and then incubated until positive or for up to 14 days.

Results: The initial repeated measurement without addition of ctp to bcb (n=5) showed a spectrum of TTD from 164,3 h to no-detectable growth within the default 336 h. The 5 fg-strains had an average TTD of below 250 h ($mean_{min}$=190,56 h, $mean_{max}$=242,48 h). For further examinations 2 sg-strains were excluded due to repetitive non detectable growth (4 of 5), respectively a standard deviation (SD) higher than 50. The remaining 2 sg-strains showed repetitive a high TTD ($mean_{min}$=308 h, $mean_{max}$=320,04 h). The repeated measurement (n=2) of the TTD with and without addition of ctp to the bcb, the 8 *C. acnes* (7 isolates of the further examination plus *C. acnes* ATCC 11827) showed a higher TTD in bcb with ctp. 4 of the 5 fg-strains showed a lower TTD in bcb containing ctp than the sg-strains and the *C. acnes* ATCC 11827. The *C. acnes* ATCC 11827 was detectable in one out of two bcb without ctp and not detectable in those containing ctp during the default time slot of 336 h.

Discussion: The Ph. Eur. suggested ATCC-strain was non-detectable in ctp-containing bottles, which is consistent with the predescribed high TTD for *C. acnes* and leads to a problem for laboratories doing microbiological quality control. We could demonstrate that certain C. acnes strains have a lower TTD than the ATCC-strain. Given the possibility of using other *C. acnes* strains for quality control, an alternative, well-characterized *C. acnes* strain could improve quality control and thus the care of patients in need of cornea transplantation.

PRHYP 278

Exploration of architectural interventions for nosocomial infections prevention in emergency departments and intensive care units - An expert interviews based study

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Background: Nosocomial pathogen transmission remains a big challenge for hospitals, specifically at critical units like the emergency department (ED) or the intensive care unit (ICU). Architectural measures may be an effective preemptive approach in reducing the risk of such transmissions. This study aimed at exploring suitable interventions for infection prevention that might help in reducing pathogen transmission in each ward.

Methods: We applied a mixed-method approach using qualitative interviews, modified CATI (computer assisted zoom interviews) and ranking surveys. Physicians from critical wards and architects (G1) thereby identified high-risk situations and suggested architectural interventions. Experts on Infection Prevention and Control (IPC, G2) later evaluated the resulting information and estimated transmission risks according to G1 assumptions, focusing on SARS-CoV-2, *Pseudomonas aeruginosa* and enterococci. Finally, another group of IPC experts (G3) reflected specific transmission routes and potential impact of identified interventions.

Results: A total of n=34 (G1), n=13 (G2) and n=7 (G3) experts completed the interviews. In the ED setting, most SARS-CoV-2 transmissions were expected during "shock room treatment" with a minimum risk of 5%, while the interventions "increase of the air exchange rate" and "increase of the room's volume" were evaluated to be the theoretically most effective (both 20%). For *P. aeruginosa* and enterococci, most transmissions were expected during the "regular treatment" (0.6% and 1.3% median risk respectively), with the most effective intervention being "room separation" (42% and 51%, respectively).

In ICUs, the highest transmission risk was expected during "time spent in a shared patient room" for SARS-CoV-2 (minimum risk 5%), and *P. aeruginosa* (3%). In contrast, "invasive treatment" was mainly expected risk for enterococcal transmission (median risk 3%). The most effective measure for all three pathogens was "room separation" (85%, 83%, 80%, respectively).

For both wards, G3 estimated health care workers to attribute for more transmissions compared to patients. While interventions such as increasing the air exchange rate or increasing the overall room size were considered to be most effective for reducing SARS-CoV-2 transmission, the separation of multi-bed patient rooms into single-bed patient rooms, and the removal of sinks from patient rooms were judged to be most effective (*P. aeruginosa* or enterococci transmission prevention).

Discussion: Expert opinion of architectural interventions may help understanding procedures aimed towards reducing ED and ICU pathogen transmission. Separation of care rooms were judged to be most effective for all pathogens in the ICU and for bacterial pathogens in the ED. It remains elusive whether this is feasible without hampering adequate care procedures and increased cost.

Further work is therefore required to investigate such interventions in a real-world setting.

PRHYP 279

Implementing a personalized feedback report on outpatient antibiotic prescribing for physicians from high volume prescribing specialties in the statutory health insurance sector, Germany 2023

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Background: Approximately 85% of all antibiotics in Germany are prescribed to outpatients. Physicians in the outpatient sector are thus a group highly relevant to best practice prescribing and antibiotic stewardship measures in Germany. Individualized feedback on prescribing habits on a nationwide scale has not been available thus far. We explored means to provide individualized feedback to increase awareness.

Methods: We sought partners who could (1) provide data on antibiotics prescribed in Germany and (2) facilitate a feedback reporting mechanism compliant with data protection. Individualized reports should be concise and provide timely feedback to physicians from medical specialties with a high volume of antibiotic prescriptions.

Results: We partnered with the National Association of Statutory Health Insurance Funds (GKV-Spitzenverband) which receives reimbursement data of all prescriptions to patients with the statutory health insurance, i.e. approx. 87% of the population. The Joint Monthly Drug Utilisation System (GKV-Arzneimittel-Schnellinformation [GAmSi]) provides quarterly reports to approx. 100.000 physicians in Germany via the Association of Statutory Health Insurance Physicians (Kassenärztliche Vereinigungen). We selected the J01 group of the anatomical-therapeutic-chemical classification (ATC) as the most relevant in terms of total volume of antimicrobial prescriptions.

A personalized report consisting of 3 tables and 3 graphs will be included in all quarterly reports for physicians treating outpatients in specialties with high prescription volume (primary care, internal medicine, paediatrics, obstetrics/gynaecology, ear-nose-throat, ophthalmology). Physician peer group is used for comparative benchmarking.

The number of prescriptions issued is shown over time, by patient age group, and by ATC group. The WHO"s AWaRe ("Access Watch Reserve") classification[1] is used to show the proportion of "access" drug prescriptions among all antibiotics. The ratio of narrow- to broad-spectrum antibiotics focuses on best practice prescription habits. A table with the most frequently prescribed antibiotics, including their AWaRe category, reflects the scope of each physician"s prescribing repertoire. All data are shown in comparison with the respective peer group.

Conclusion: For the first time, an individualised antibiotic prescribing report for selected specialties will be offered nationwide starting in mid 2023. Integrating 3 pages on antibiotic use into an existing report is a novel and sustainable method to provide individualized feedback. While we cannot comment on (1) the number of prescriptions as denominator patient data is unknown and (2) the appropriateness of prescribing as patients" diagnoses and comorbidities are unavailable, we aim to raise awareness and encourage reflection and discussion on prescribing habits.

[1] https://www.who.int/publications/i/item/2021-aware-classification

PRHYP 280

Evaluation of MLST ST117 as an independent risk factor for VRE long-term colonization

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Introduction: Vancomycin-resistant enterococci (VRE) have become a major concern for public health, particularly in healthcare settings. Hospitalised patients with VRE colonisation are often subjected to extended hygiene measures, such as isolation and the use of protective equipment. In the absence of effective decolonisation measures for VRE, it is necessary to wait for the spontaneous clearance of the VRE carrier status to end extended infection prevention measures. In a previous study, our group developed the PREVENT-Score to assess the risk for a long-term persistence of VRE carrier status in VRE colonised patients based on clinical factors. However, we hypothesize that the long-term colonization may depend also on pathogen associated factors. Hence, we here investigated whether patients colonized with VRE of the Multilocus Sequence Typing (MLST) sequence type (ST) 117 are at higher risk for a VRE long-term persistence.

Materials/Methods: Over a two-year time-period (October 2016 – October 2018) all patients that were admitted to the University Hospital Münster with a history of VRE-colonization were included in our study. Clinical risk factors were recorded, and VRE screening was performed upon admission, defining patients as long-term carriers if still colonized > 10 weeks after first detection of VRE. As part of our routine surveillance for multidrug-resistant pathogens, all first-time VRE detections were subject to whole-genome sequencing (WGS). Based on the WGS data, the MLST ST117 was determined. Univariable and multivariable statistical analyses were performed, investigating whether ST117 carriage is an independent risk factor for long-term VRE persistence.

Results: In total, 341 patients with a history of VRE colonization were included in our study. Of these, 160 (47%) showed a VRE long-term colonization status. 220 patients could be identified as MLST ST117-carriers (65%) of whom 100 were defined as VRE long-term carriers (45%). Univariable analysis comparing VRE long-term and short-term carriers with and without carrying MLST ST117 showed no significant differences ($p = 0.464$). Furthermore, multivariable analysis including MLST ST117 and known independent risk factors for long-term VRE colonization (age of or over 60 years, respectively, hemato-oncological disease, cumulative antibiotic treatment for over 4 weeks, and VRE infection) was not significant, either ($p = 0462$).

Conclusions: Our study could not confirm MLST ST117 as an independent risk factor for long-term VRE carriage. Further studies are required to identify other pathogen-associated risk factors contributing to a persistence of VRE colonization.

PRHYP 281

Interactive hygiene training using license free open source software

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Background: Digital, interactive learning formats have become increasingly popular in medical education as opposed to classic

teaching methods such as frontal-class-teaching. However, not only medical students can benefit from modern learning methods. Hygiene trainings that are mandatory in many healthcare facilities can also benefit from digital learning formats. This allows medical students and healthcare professionals to learn about hygiene measures and infection control procedures through self-paced online formats. But especially in resource-limited settings the implementation and integration of such formats might bear a series of technological and financial challenges.

Methods: We developed an interactive hygiene training using only license free, open source software. The hygiene training was mainly developed using the HTML-5 package (H5P) based "course presentation" content type. H5P content can be integrated in a variety of different Learning- and Content-Management systems. Here, the content has been created in a Moodle-based Learning-Management-System. The course presentation was rolled out to employees of a tertiary care hospital in Germany such as nurses, physicians and technical assistants as well as medical students in the clinical part of their studies.

Results: The hygiene training was developed with thematic emphasis on basic hygiene measures. It includes pre-recorded videos on personal protective equipment (PPE) handling, port handling and hand hygiene amongst others. Interactive elements such as quizzes on hand hygiene indications, multi drug resistant organisms and virtual ward inspections were integrated into the interactive presentation format. Direct Links to documents in the in-house quality management software were added as well. Participation could be verified with Moodle reporting tools to be able provide feedback to clinicians.

Conclusions: H5P-Interactive Course Presentations in Moodle Learn-Management-Systems are a feasible, low-threshold and cost-effective way to not only allow medical students but also healthcare professionals to learn about infection prevention measures in mandatory hygiene trainings. As opposed to commercially or publicly available solutions, this type of content can be easily replicated by infection preventionists in different type and sized settings. In order to suit and teach facility-specific needs and regulations the content can be modified accordingly.

Fig. 1

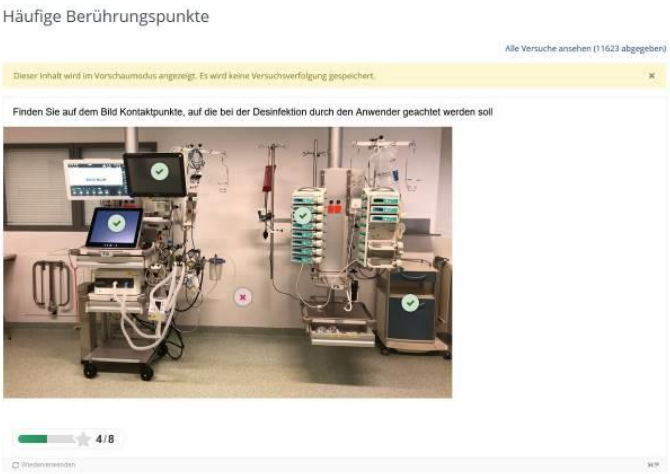
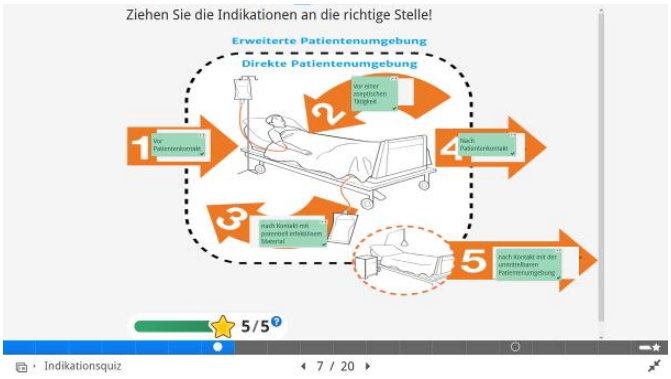


Fig. 2



PRHYP 282

Contamination risks of infant nutrition for premature and newborn infants in hospitals: Evaluation of workflows based on Interviews with interdisciplinary experts

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Question: *Cronobacter sakazakii* has been documented as a cause of life-threatening infections, predominantly in neonates, presumably caused by contaminated infant nutrition (IN). There are various risk factors and specific areas with increased risks for transmission of pathogens on neonatal intensive care units. These include the kitchens, where IN is prepared for premature and newborn infants, who are particularly susceptible to Hospital Acquired Infections (HAI), associated with an increased risk of mortality.
Our issue in the present work was to evaluate the different processes existing in handling of human milk and formula for newborn infants regarding risks of contamination and potentially subsequent HAI.

Methods: We conducted qualitative problem-centered interviews. A maximum variation sampling was chosen in order to be able to combine the various practical experience of the experts. After consultation with the data protection officers and a clearance from the ethics committee, two scientists interviewed a total of nine experts (2022/04/28-2022/06/22), who work on neonatal wards of hospitals in Lower Saxony (neonatology/pediatrics), are members of a public health service or work as medical specialists in infection prevention and control (IPC). The interview partners gave informed and written consent. The interviews were recorded, transcribed, pseudonymized and coded with the assistance of MAXQDA software.

Results: The interviewees, responsible for centralized (usually supplying several wards with IN) or decentralized IN kitchens (located directly on the ward) pointed out the differences between the two systems: not only the processes differ, but also the jurisdiction of the regulatory authorities. This requires a target group-specific definition for internal guidelines and for supervisory authorities' checklists for process optimization. They listed what they considered to be the greatest risks for contamination of IN in clinical settings (Table 1: Ranking (1=highest risk, 12=lowest risk)). Secondly, they suggested modifications and possibilities for optimization of processes to reduce the risks of contamination (Table 2: Potential process optimization). Disruptions in the organizational process were named as the greatest risk, followed by employees as the "human factor" and water. The experts see the greatest benefit for process optimization in compliance with proper

hygiene measures, adapted concepts and a better involvement of the parents.

Conclusions: Regarding the differences between different hospitals and centralized versus decentralized IN kitchens, respectively, consistent standards and process optimization should be developed to avoid contamination of IN for premature and newborn infants and to reduce the risks of HAI and outbreaks. Furthermore it is necessary to establish a Hazard Analysis and Critical Control Point (HACCP) concept to identify and control critical situations during the named processes.

Fig. 1

Rank	Source	Median	Average
1	Disruption in the organizational process	2	2,7
2	Employees	2,5	3,3
3	Water	3	4
4	Vessels	3,5	4,5
5	Surfaces	4	4,1
6	Parents	4,5	4,8
7	Devices	5	5,4
8	Additions, ad hoc	6	5
9	Paths	6	6,6
10	Additives, commercially dosed	7	6,2
11	Air	8	7,7
12	Other patients	9,5	8,5

Fig. 2

Ideas for reducing the risk of contamination	
"Good hygiene", proper hand hygiene, adherence to cleaning and disinfection schedules	4
Labeling systems, regular inspection of food warehouses, milk labeling	3
Handout for parents, pass-through to prevent parents from accessing decentralized kitchens, restrict access altogether, milk pumps: evaluate concepts and allow only equipment with check valve	3
Schedule time and rest for employees	2
Promote and control personnel hygiene	2
Personnel separation and/or segregation of duties of the station and the kitchen	2
Provide/review employee training, proper hygiene and food on a regular, mandatory basis	2
Use hot water, monitor rapid cooling, centrally monitored refrigerators, ensure cold chain, install air conditioners	2
Use sterile milk products, prefer single-use devices	2

PRHYP 283

Current state of digitalisation and automation of infection prevention and control in German clinics

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Background: Surveillance of healthcare associated infections is a cornerstone of infection prevention and control. Healthcare associated infections are relevantly contributing to patients" morbidity and mortality. In German hospitals, the majority of surveillance efforts has been heavily relying on manual processes like manual chart review.

We aimed to assess the current state of digitalisation and automation in German hospitals of different care levels.

Methods: Semi-structured interviews with healthcare professionals were conducted between December 2022 and January 2023 to capture a snapshot of the current surveillance organisation and digitalisation in infection prevention and control. Healthcare professionals from seven hospitals of different care levels were interviewed about the current general surveillance organisation, access to digital data sources, access to software specifically for infection control, most time-consuming steps in the surveillance process, if there are plans to implement automatic solutions, and perceived impediments for the implementation of such solutions.

Transcription and qualitative analysis of the interviews were done using MAXQDA 2022.

Results: General surveillance organisation, access to digital data sources and software specifically for infection control varied notably, not only between different care levels but also between different hospitals in the same care level. Outside of research projects, neither automatic software nor solutions utilising artificial intelligence have been implemented in clinical routine. Where specific software to aid infection control is used, experiences and perceived usefulness for clinical practice varied. Stated impediments for implementation compromised financial, technical (data quality, data integration) as well as regulatory (data protection) reasons.

Conclusion: While an increasing amount of healthcare professionals in infection prevention and control are having access to digital data sources and software to specifically aid the surveillance of healthcare associated infections, the surveillance process is still in large parts relying on manual labour. Even though the pandemic has been a catalyst for the digital transformation of clinical processes and many promising solutions are becoming available there is an implementation gap in clinical practice.

Especially healthcare facilities of lower care levels face the need to overcome obstacles through interdisciplinary cooperation to transform their surveillance process.

PRHYP 284

Standardized compliance assessment for infection prevention measures when using peripheral venous catheters reveals specific need for training

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Introduction: National guidelines for the prevention of PVC-associated infections describe evidence-based measures for the insertion and care of PVCs and are adopted in in-house standard operating procedures. The aim of this study was the development of a standardized method for determining compliance in addition to examining the self-reported knowledge and degree of implementation of infection prevention measures among healthcare providers (HCP) regarding PVC management.

Methods: We developed a checklist based on the recommendation from the German Commission on Hospital Hygiene and Infection Prevention at the Robert Koch Institute (KRINKO) on the "Prevention of infections arising from vascular catheters". We evaluated five parameters on a dichotomous scale: the insertion site, the dressing, the presence of a catheter extension set, the presence of a plug and the documentation of catheter insertion. The checklist was applied to 14 normal wards in 2019, and after feedback of the results was given to ward staff, was used again in 2020 on the same wards. For the retrospective data analysis, we developed a new instrument called PVC-quality index (PVC-QI). After completing the second evaluation, we carried out a survey with a custom-designed standardized questionnaire for the observed wards" HCP.

Results: The evaluation of 627 PVCs showed a significant increase in compliance related to the presence of an extension set (p=0.049) and the documentation (p<0.001) in the second investigation period. The mean quality index of 14 wards increased significantly from 4.7 in 2019 to 6.6 in 2020 (p<0.003). We identified one ward with a low PVC-QI in both periods (1.85 in 2019 and 2.18 in 2020).The PVC-QI allows both the comparison between different observed wards and a comparison of the results of different periods. The survey participants (N = 82) were slightly aware of the in-house standard operating procedure, with a mean of 4.98 on a 7-point Likert-scale (1 = not aware, 7 = completely aware). Only 62.2% of the participants knew that the documentation of the PVC insertion procedure is necessary, but the awareness of recommended measures was better for PVC placement than for PVC care.

Conclusion: The PVC-QI is a valuable tool for the assessment of compliance regarding PVC management in daily practice and can reveal knowledge gaps and lack of guideline adherence in distinct teams. Feedback for the ward staff regarding the results of compliance assessment improves PVC management. Taking the PVC quality index into account, wards could be specifically selected for targeted surveillance.

PRHYP 285

Tailoring implementation interventions of three orders in infection prevention and control (IPC): an extension of the logic model IPC-CASCADE to include tailored interventions for the senior management level

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Question: Implementation interventions in infection prevention and control (IPC) differ by their recipients. The cascadic logic model IPC-CASCADE [1] has distinguished two target groups: front line workers directly involved in patient care and, on the mid-level of management, IPC professionals as proxy agents, i.e., implementation support practitioners. While in both cases, implementation interventions aim to promote compliance with clinical interventions to prevent healthcare-associated infections (HAI), their tailoring may be vastly different, e.g., due to different behavioural outcomes. To overcome this gap and improve conceptual clarity, IPC-CASCADE distinguished between interventions BY IPC professionals FOR front line workers, and interventions BY the senior management FOR IPC professionals (first- and second-order implementation interventions, respectively). However, so far the model did not include interventions FOR senior managers.

Methods: Logic modelling.

Results: The extension of the model's basic logic - improving professional practice on a specific level by addressing its prospectively-assessed determinants by interventions tailored to the specific target group by selecting appropriate behaviour change techniques - to senior managers is unproblematic. However, certain specificities have to be taken into account. First, since on this level there are fewer players (stakeholders and protagonists) than e.g. on the front line worker level, assessments of the determinants of practice usually will have to more frequently use mixed methods approaches. Second, the scope of actions of senior managers is even more directly influenced by regulations defined by the health care system than those of other levels. Third, the question of providers of interventions on this level arises.

Conclusions: Based on the proposed extension of the IPC-CASCADE model, the concept of tailoring interventions is made accessible to all management levels within hospitals, including top-level senior managers. At the same time, the role of the health care system context for effective IPC is highlighted. Since the Cochrane review on tailored interventions showed that they had 1.6 times higher odds of leading to the desired outcomes than non-tailored interventions [2], tailored interventions on all levels including third-order interventions are essential.

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PRHYP 286

How are KRINKO recommendations perceived by infection prevention and control professionals? A pilot survey

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Introduction: In Germany, infection prevention & control (IPC) recommendations for health care facilities are made by the Commission for Hospital Hygiene and Infection Prevention (Kommission für Krankenhaushygiene und Infektionsprävention; KRINKO) according to § 23 of the German Protection Against Infection Act (Infektionsschutzgesetz (IfSG). These recommendations apply for a diverse range of settings and professions. However, it is unknown how these recommendations are perceived by their target audiences.

Methods: We conducted an anonymous online pilot survey to explore the perception of KRINKO recommendations by IPC professionals including hospital hygienists (*Krankenhaushygieniker:innen* (KHH), IPC nurses (*Hygienefachkräfte* (HFK)), IPC link doctors (*Hygienebeauftragte Ärzt:innen* (HÄ)) and IPC link nurses (*Hygienebeauftragte in der Pflege*, HBP) and was open from Sept. 19th, 2022 to Oct. 31th, 2022. A questionnaire containing 19 questions was prepared and supported by representatives of target professions and public health services. The questionnaire assessed information about respondents, perception of KRINKO recommendations and their development in general as well as the perception of the document "Infektionsprävention im Rahmen der Pflege und Behandlung von Patienten mit übertragbaren Krankheiten" using the VOXCO-tool. While being one of the target groups was necessary to take part in the survey, following questions could be skipped. Data was extracted and analyzed using RStudio.

Results: A total of 159 KHH, 600 HFK, 66 HÄ and 223 HPB (1048) started the questionnaire. Respondents reported a good knowledge of recommendations and strongly preferred a digital access to the documents. Knowledge about the development process of recommendations and the evidence categories was highest in KHH and HFK. KHH and HFK reported that they read the original recommendation documents frequently, while HÄ and HPB relied more often on the internal hygiene standard (Hygieneplan) of the facility. The recommendation "Infektionsprävention im Rahmen der Pflege und Behandlung von

Patienten mit übertragbaren Krankheiten" was generally perceived well in terms of structure, language and understandability.

Discussion: Our pilot survey is the first impulse to systematically assess perception of KRINKO recommendations. Overall, respondents reported a good perception of KRINKO recommendations in general, and of the specific focused document in particular. However, data suggest that different groups of IPC professionals would benefit from a more targeted communication strategy. This implies not only the content of recommendations but also knowledge about how a recommendation is prepared. We will use the results to improve communication and also plan to expand these surveys to gain further insights from other target groups.

PRHYP 287

Microbial contamination in healthcare facilities: Reflection from water Sanitation and hygiene assessment in India

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Introduction: Water, sanitation, and hygiene (WASH) services are integral components of one health as it is vital for the optimal health of humans and animals and for maintaining a healthy environment. Even after including the provision of WASH services to all in Sustainable Development Goals globally, many countries and healthcare facilities (HCFs) lack in achieving essential services. Thus, this study aims to assess the WASH status with respect to the anti-microbial resistance (AMR) in two Indian states, Assam & Gujarat.

Methods: The cross-sectional study was conducted in two different states of India i.e. Gujarat and Assam. A total of 10 HCFs from Gujarat and 60 from Assam were assessed in this study. In addition to the WASH assessment, microbiological swab samples were collected from various pre-decided environmental surfaces of the maternity ward, labour rooms and cleaning materials to document the AMR pattern. A total of 251 environmental samples were collected from Gujarat and 1182 from Assam.

Results: The findings of the microbiological surveillance shows, 31.1% and 69.7% of collected samples were contaminated with the microorganisms in Gujarat and Assam, respectively. In both, states, cleaning mops were found mostly contaminated, followed by the maternity beds. In Gujarat, *acinetobacter speccies, staphylococcus species, pseudomonas species, pseudomonas aeruginosa, klebsiella species* and *E.coli* were found while in Assam *staphylococcus species, bacillus subtilis, bacillus megaterium, pseudomonas aeruginosa, klebsiella species* and *E.coli* were found.

Discussion: WASH is one of the important components to maintaining infection prevention and control at HCFs still neglected most of the time, and very little attention is given to the microbiological surveillance of the environmental surfaces of the HCFs as part of the hygiene. Currently, only visual assessment is used for hygiene assessment, which is not a proxy for safety. This is recommended to keep a mandate of routine microbiological surveillance in the Indian HCFs to track the AMR pattern.

PRHYP 288

Professional challenges for infection prevention and control (IPC) teams accociated with managing resistance, emotions and task ambiguity: results of a national survey on associations with work strain

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Question: IPC teams are constantly confronted with intense emotions in their daily work, as they are significantly involved in many change processes with frontline staff, e.g., when promoting compliance with basic IPC measures. In addition, they are particularly confronted with contradictions due to their role as interface communicators. To identify IPC team members' perceptions of work strain and interpersonal challenges (managing emotions, resistance, and task ambiguity) in hospitals in Germany. In addition, the aim was to identify their needs in terms of further education.

Methods: In the IP-POWER study (Infection Prevention with head and heart: Psychological empowerment of infection prevention and control teams, German Clinical Trial Register ID DRKS00031879), a nationwide online survey was conducted among IPC team members in hospitals in autumn 2022. Contact data were compiled on the basis of a directory of hospitals published by the Federal Statistical Office of Germany. Customized items for the online survey were developed within the project.

Results: N=465 IPC team members from hospitals in all 16 federal states participated. Overall, work strain was relatively low (mean of 2.4 on a scale of 1 to 6), while role clarity and self-efficacy in managing the emotions/motivation of frontline staff, and resistance to IPC interventions were higher (means between 4 and 5). Multiple linear regressions showed that for both physicians and nurses, self-efficacy in dealing with resistance had a stronger protective effect on work strain than in dealing with emotions (physicians:β=-0.41, $p<0.001$, vs. -0.03, ns; nurses: β=-0.33, $p<0.001$, vs. -0.13, $p=0.02$), whereas task ambiguity was a risk factor (β=0.34 and 0.27, $p<0.001$). The greatest need for further education was reported for managing resistance and emotions, whereas it was least for IPC skills training.

Conclusions: IPC team members in German hospitals report substantial task ambiguity. Work strain is strongly affected by lack of self-efficacy to deal with frontline staff resistance, followed by task ambiguity. Based on these findings, implications for the IP-POWER intervention are derived.

PRHYP 289

Emergence of novel ST1299 *vanA* Lineages as possible cause for the striking rise of vancomycin-resistance in invasive strains of *Enterococcus faecium* at a German university hospital

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Background: The rate of vancomycin-resistant *Enterococcus (E.) faecium* (VREfm) strains amongst all *E. faecium* isolated from blood cultures has increased in the period 2015-2020 from 11.9% to 22.3% in German hospitals (https://ars.rki.de). As recently as 2018, the rate of vancomycin resistance in *E. faecium* isolates from blood cultures of in-patients treated at the University Hospital

Erlangen (UKER) amounted to 9.8%. Since 2019, however, the proportion of VREfm is constantly above 40% (2019: 46.3%; 2020: 41,9%; 2021: 40.7%). The aim of the present study was therefore to investigate possible causes for the striking rise of vancomycin-resistance in invasive *E. faecium* strains at UKER. In detail, we collected clinical information about UKER in-patients with bloodstream infection caused by *E. faecium* during one year. In addition, the genetic diversity of VREfm and vancomycin-susceptible *E. faecium* (VSEfm) isolates from blood cultures of the above-mentioned patients was analyzed by core genome multilocus sequence typing (cgMLST) with the purpose to detect or exclude the spread of new clonal lineages.

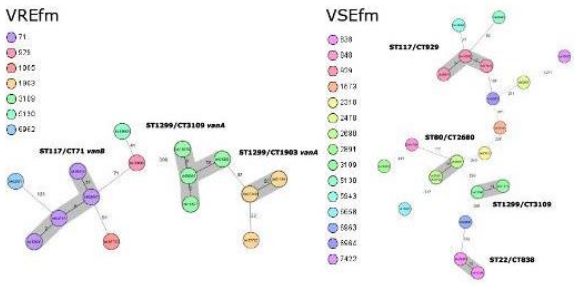
Materials & Methods: All isolates were investigated for genetic diversity by whole genome sequencing (WGS) using the Illumina MiniSeq platform (Illumina Inc., San Diego, USA). Complex types (CTs) were identified by cgMLST using SeqSphere+ software version 8.5.1 (Ridom GmbH, Muenster, Germany).

Results: Between January and December 2022, 37 consecutive non-duplicate isolates were included in this study. Resistance to vancomycin occurred in 15 isolates (40.5%). The median age of the patients was 65 years and 26 of them (70.3%) were males. In-hospital mortality rate was not significantly different in patients with bloodstream infection caused by vancomycin-resistant *E. faecium* (VREfm) compared to vancomycin-susceptible *E. faecium* (VSEfm) (33.3% vs 27.3%, P=0.69). A higher genetic diversity could be shown by cgMLST in VSEfm (15 clonal lineages) than in VREfm (7 clonal lineages). The majority of the VREfm (73.2%) belonged to three clonal lineages: ST117/CT71 *vanB* (n=4), which is the most commonly detected lineage in Germany, and two novel ST1299 *vanA* lineages classified as CT3109 (n=4) and CT1903 (n=3).

Conclusion: The high rate of vancomycin-resistance in invasive isolates of *E. faecium* at UKER could be associated with the emergence of novel ST1299 *vanA* lineages. Future studies designed to clarify the spread of ST1299 *vanA* at UKER should focus on the prevalence and genetic diversity of VREfm in the community.

Figure 1. Minimum-spanning-tree of all *Enterococcus faecium* isolates of this study based on cgMLST. Each circle represents isolates with identical allelic profile. The circles are colored according to the complex types listed in the column. The number on connecting lines represents the number of alleles that differ between the connected genotypes. Genotypes belonging to the same clonal group (alleles difference ≤ 20) are grey-shadowed

Fig. 1



PRHYP 290

From 3MRGN to 4MRGN: Monitoring changes in antimicrobial resistance plasmids

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Introduction: Propagation of antimicrobial resistance (AMR) is a public health risk and an economic burden. For prediction and prevention, a better understanding of molecular mechanisms how AMR develop and spread is needed. So far, little is known about the microevolution of AMR harbouring plasmids in the clinical setting. As part of the routine surveillance of multi drug resistant bacteria (MDRB), we identified a MDRB gaining resistance against carbapenems by long read whole genome sequencing (lrWGS). Here we illustrate the use of lrWGS for molecular surveillance of MDRB in the hospital setting.

Material/method: Bacterial isolates were collected during routine surveillance of MDRB. Genomic DNA was sequenced with PacBio® Sequel IIe system. *De novo* assembly of lrWGS data was performed using SMRT® Link software suite v.10. Contigs were analysed using MOB-Suite v.3.1 and pMLST-2.0 Server, cgMLST was determined using Ridom SeqSphere+ v.8.5.2 and plasmids were annotated with DFAST v.1.6.0. Additional analyses was performed with NCBI AMRFinder Plus v.3.11.2, SnapGeneViewer v.6.2.1 and alignments were done using progressiveMauve v.20150226.

Results: During surveillance of MDRB we discovered a 3MRGN *E. coli* ST410 isolate from an anal swab that acquired resistance against carbapenems within 12 days during the hospital stay. We identified four plasmids in the initial 3MRGN isolate and two plasmids in the subsequent 4MRGN isolate. Using cgMLST, the two isolates differed by a single allele confirming the clonality. Alignments of the plasmid sequences showed highly similar 82 kb plasmids in both isolates and partially highly homologous plasmids p3 of 3MRGN *E. coli* (63 kb) and p1 of 4MRGN *E. coli* (108 kb) with an insert of 45,553 bp including the two flanking IS26 transposase genes. Additional eight IS26 transposase genes were found in the insert sequence. One IS26 transposase gene was also detected on p1 and identified as the point of insertion. In-depth analysis of these plasmids p3 and p1 were characterized as mobilizable with a type MOBF relaxase and replication type IncFIA, IncFIB, IncFII, IncFII and pMLST Sequence Type *[F1:A1:B49]* and an additional IncQ1 for p1. The insert carried 15 AMR genes in total, coding for example for an extended-spectrum beta-lactamase (*bla*CTX-M-15), a carbapenemase (*bla*NDM-5) and quinolone resistance (*aac(6')-Ib-cr5*). Furthermore, we found the recovery of many point mutations in AMR genes next to the insert. The section includes recovered AMR genes for tetracycline (*tet*B, *tet*C, *tet*R), sulphonamide (*sul1*) and streptomycin (*aad*A2).

Discussion: This case report underlines the impact of plasmids acquiring AMR genes. The findings suggests the insertion of AMR genes by one or more mobile genetic elements because of the amount of IS26 transposase genes. lrWGS allows an accurate identification of the localization of AMRs on plasmids, making monitoring of different evolutional events possible as part of routine hospital surveillance.

PRHYP 291

Admission prevalence of colonization with methicillin-resistant *Staphylococcus aureus*, multidrug-resistant gram-negative bacteria and vancomycin-resistant enterococci among patients of a geriatric hospital in southwest Germany, 2015-2018

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Question: Multidrug-resistant (MDR) organisms (MDRO) are a major public health concern worldwide with an estimated 55.7/100,000 annual deaths associated with antimicrobial resistance in high income countries. Age has been identified age as a risk factor for colonization with MDRO in Germany, but the colonization prevalence among patients of geriatric hospitals has rarely been addressed in studies. From January 2015 through October 2018, all patients admitted to a geriatric hospital in southwest Germany were routinely screened upon each admission for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA), MDR gram-negative bacteria (GNB), and vancomycin-resistant enterococci (VRE). The objective of this study was to calculate the admission prevalence (AP) of these MDRO in the geriatric cohort in order to inform future infection prevention and control measures.

Methods: In a retrospective cohort study we calculated overall and annual AP and respective 95% confidence intervals (CI) as the proportion of patient-cases screened positive for MRSA, MDR-GNB and VRE, respectively. We evaluated changes in the annual AP using the Chi2 test for linear trend. In additional analyses we explored potential risk factors for colonization with MDRO using the Chi2 test. The study was approved by the ethics committees of the State Medical Associations of Baden-Wuerttemberg (S-220/2022) and Hesse (2022-2950-zvBO).

Results: A total of 10,429 (90.3% of all admitted) cases were screened for MDRO within 72 hours after admission and were included in the study. Their median age was 80 (IQR 77-88) years, 65% were female. Overall AP was 1.3% (95%CI 1.1-1.5%) for MRSA, 7.5% (7-8%) for MDR-GNB, and 8.1% (7.6-8.7%) for VRE. A total of 160 (1.5% of all and 9.1% of MDRO-positive) cases were simultaneously colonized with at least two MDRO, 127 of these (79.4%) including VRE. Male cases had a higher AP than females for VRE (10.2% vs. 6.9%, Chi2 test $p<0.00001$) and MDR-GNB (8.4% vs. 7%, $p<0.01$). From 2015 to 2018, annual AP for MRSA decreased from 1.9% to 1.1% (Chi2 test for linear trend $p<0.01$), remained stable for MDR-GNB (6.3-8.2%, $p=0.23$), and increased for VRE from 3.9% to 12% ($p<0.0000001$). The most prevalent gram-negative species were *Escherichia coli* (77% of all GNB) and *Klebsiella pneumoniae* (11%). Whereas in 2015 only 1% of MDR-GNB were carbapenem-resistant, the proportion was 7.6% in 2018.

Conclusions: AP of colonization with MRSA and MDR-GNB in our geriatric inpatient study population was comparable to that of patients in respective age-groups in general hospitals in Germany. In contrast, AP for VRE was higher in our study population, possibly due to regional differences in VRE colonization prevalence, resulting in an increased prevalence of simultaneous colonization with at least two MDRO. Our results suggest that geriatric hospitals can apply the same screening strategy for MDRO as general hospitals in Germany.

PRHYP 292

Genomic surveillance study of multidrug-resistant isolates from Ukrainian patients in a German health facility

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Question: Antimicrobial resistance is a pressing issue in Ukraine, with healthcare-associated infections caused by multidrug-resistant organisms being a major concern. A recent prospective multicenter study revealed a staggering rate of 48.4% antimicrobial resistance to carbapenems among Enterobacterales causing a healthcare-associated infection. We conducted a systematic survey to investigate the incidence rate and incidence density rate of carbapenemase-producing Gram-negative bacteria (CPGN) among refugees and war-wounded Ukrainians in connection with the German health system.

Methods: From the onset of the war until November 2022, seven Ukrainian patients were admitted to our hospital. Upon admission, screening samples and samples from the focus of suspected infection were taken from all seven patients. The incidence rate and the incidence density rate of CPGN were calculated as a result of the microbiological findings. We sequenced all CPGN using Illumina technology.

Results: The incidence rate of CPGN at our hospital was 0.06 for 2021 and 0.18 for 2022. All seven Ukrainian patients were infected or colonized with at least one CPGN, including *K. pneumoniae* (14/26), *P. aeruginosa* (6/26), *A. baumannii* (2/26), *Providencia stutartii* (1/26), *C. freundii* (1/26), and *E. coli* (2/26). Genomic surveillance revealed that i) most frequently detected carbapenemases among all sequenced isolates were *bla*NDM (17/26) and *bla*OXA-48 (6/26), ii) most commonly observed plasmid replicons among the *K. pneumoniae*isolates recovered from Ukrainian patients were Col(pHAD28) (12/14), IncHI1B(pNDM-MAR) (9/14), IncFIB(pNDM-Mar) (12/14), and iii) clonal relation between the pathogens of the Ukrainian isolates, but not for the isolates from our hospital surveillance system.

Conclusions: The rising prevalence of community-acquired colonization and infection with CPGN is having a direct effect on the infection prevention measures, such as higher number of isolations, reprocessing of patient rooms, additional microbiological testing, and overall organization within hospitals.

PRHYP 293

Mutations of the periplasmic chaperone SurA increase carbapenem resistance in *Klebsiella pneumoniae* with *bla*$_{\text{OXA-48}}$

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Background: In Germany, *Klebsiella pneumoniae* with *bla*$_{\text{OXA-48}}$ are the most clinically significant carbapenemase-producing Enterobacterales, as shown in the annual reports of the National Reference Centre for Multidrug-resistant Gram-negative Bacteria. Interestingly some of these isolates show high resistance to carbapenems whilst some show low minimal inhibitory concentrations (MIC) of carbapenems and are categorized susceptible according to EUCAST breakpoints. The aim of this study was to reveal possible genetic causations for varying MICs of carbapenems in *K. pneumoniae* expressing *bla*$_{\text{OXA-48}}$.

Methods: Twenty clinical *K. pneumoniae* isolates with *bla*$_{\text{OXA-48}}$ showing low MICs of carbapenems were put under gradually rising selective pressure of meropenem to select for mutants with elevated MICs. Whole-genome sequencing was performed with the low MIC primary isolates and the high MIC spontaneous mutants. A hybrid assembly of short-read Illumina and long-read Oxford

Nanopore sequencing data was carried out to generate whole genome sequences. Sequences of low MIC primary isolates and high MIC selected mutants were compared and screened for Single Nucleotide Polymorphisms (SNPs) or other genomic alterations like deletions or new insertion elements. Found mutations were edited in the corresponding primary clinical isolate using CRISPR/Cas9 and resistance levels, expression of resistance determinants and fitness of these mutants were determined.

Results: Mutations possibly leading to elevated MICs were predominantly found in genes of outer membrane proteins (OMP), as already described in the literature. But not all mutants revealed defects in OMP genes. Two isolates had either an insertion or deletion of one basepair in *surA*, coding for a periplasmic chaperone, resulting in a nonsense protein. Production of nonsense SurA led to an enhanced expression of *rpoE* alongside with a downregulation of porin gene *ompK36* after 6 h of incubation in the spontaneous and genetically constructed mutant. Diminished production of OMPs could also be seen in SDS-PAGE. These mutations led to elevated MICs of carbapenems and cephalosporins, but also to lower growth rates.

Discussion: A few mutations can have a huge impact on MICs of carbapenems in *K. pneumoniae* with *bla*$_{\text{OXA-48}}$. The resistance levels can be biased not only by mutations in the porin genes themselves, but also via an altered transport of porin proteins to the outer membrane. This is an important point to remember for future approaches to predict resistance levels from WGS data.

PRHYP 294

Transmission of *Enterobacter cloacae* complex in a neonatal intensive care unit uncovered by whole genome sequencing

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Introduction: Enterobacterales are relevant pathogens in the field of neonatal medicine, as newborns are especially vulnerable to colonization and infections. We present a cluster investigation of potential transmission events of *Enterobacter cloacae* complex (ECC) in a neonatal intensive care unit (NICU). Because sink drains are a common reservoir for Gram-negative bacteria like ECC, the existence of waste water systems in NICUs is currently a subject of discussion.

Methods: In January 2023, we noticed an increase of ECC isolates in the screening results of NICU patients. Hence a cluster investigation was initiated including screening of patients and their surroundings on the ward. Species identification was performed via MALDI-TOF MS. For antimicrobial susceptibility testing the Vitek2 automated system was used and the minimal inhibition concentrations were interpreted according to EUCAST clinical breakpoints. All isolates from screening and environmental testing were subjected to whole genome sequencing (WGS) and core genome multilocus sequence typing (cgMLST) to analyse their genetic relationship. Isolates that differed in ≤ 5 alleles were considered related. Presence of resistance genes was analysed with the NCBI AMRFinderPlus.

Results: In total, 10 patients showed colonizations with ECC in throat and anal swabs (n=19 isolates). CgMLST revealed two separate genetic clusters, namely one *E. hormaechei* cluster (7 patients, 13 isolates) and one *E. ludwigii* cluster (3 patients, 6 isolates). Three environmental isolates originating from two ward sink drains were related to the *E. hormaechei* cluster. All isolates showed phenotypic resistance to amoxicillin, ampicillin/sulbactam and cefpodoxim. Four isolates exhibited additional phenotypic resistances to piperacillin and third generation cephalosporins and were classified as "2 MRGN Neo Päd" as per German guidelines (n=3 *E. hormaechei*, n=1 *E. ludwigii*). One isolate (*E. hormaechei*) showed phenotypic resistance to ertapenem. The different resistance phenotypes were found in both genetic clusters without association to a particular cgMLST genotype. WGS analysis revealed in all isolates the presence of β-lactamase genes but no carbapenemases were detected. No additional resistance genes were identified in "2 MRGN Neo Päd" isolates compared to isolates displaying less resistant phenotype.

Conclusions: Our results illustrate that transmission events can best be differentiated by means of WGS, since two separate events involving ECC were identified in 19 isolates. The analysis of clinical routine screening for colonization showed transmissions of ECC isolates in the NICU setting. Environmental investigations pointed to sink drains as a possible reservoir of *E. hormaechei*. Differences in genotypic versus phenotypic resistance might be explained by antibiotic-induced expression of ß-lactamases. The sole use of phenotypic resistance test results in the analysis of transmission events can be misleading.

PRHYP 295

First characterisation of newly discovered metallo-beta-lactamase NWM-1

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Background: The worldwide increase of multidrug-resistant gram-negative bacteria has become an important clinical challenge. Resistance against carbapenems is of particular concern. It can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. A worrying trend is the dissemination of Ambler class B metallo-beta-lactamases (MBL). In 2020, a carbapenem-resistant clinical *P. aeruginosa* isolate was referred to the National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria. This isolate harboured the novel *bla*$_{\text{NWM-1}}$ MBL gene (North Rhine-Westphalia Metallo-beta-lactamase). Here we present first characteristics, like the resistance pattern and genetic background from the recently discovered MBL.

Material/methods: The carbapenem-resistant *P. aeruginosa* isolate NRL-63282 was whole-genome sequenced by Illumina-sequencing and the genome was screened for beta-lactamase-like proteins. The *bla*NWM-1 MBL gene was cloned into a pBK-CMV vector and the resulting expression vector was cloned into *E. coli* TOP10 cells. MICs were tested for *E. coli* TOP10 with and without the NWM-1 expression vector and for the NRL-63282. The new MBL was also characterized *in silico* via Prot Pi and SignalP.

Results: The *bla*$_{\text{NWM-1}}$ MBL gene is located chromosomal, flanked by two IS-91 elements. It belongs to the B3 subclass MBLs. The calculated size is 32,5 kDa and it possesses a lipoprotein signal peptide (Sec/SPII) which suggest that this MBL is membrane-bound. The *E. coli* TOP10 harbouring the NWM-1 expression vector showed no activity against Aztreonam or Doripenem. In comparison to *E. coli* TOP10 cells with the empty pBK-CMV vector, the cells expressing the NWM-1 showed four times increased MICs against Imipenem and 32- fold increased MICs against Meropenem. They show also 4-6- fold increased MICs for most cephalosporins except for 4th generation cephalosporins.

Conclusion: The characterization of NWM-1 shows the image of a subclass B3 MBL that seems to be very effective against meropenem and less effective against imipenem. The localisation between two IS elements harbours the possibility of horizontal gene transfer of this carbapenemase. Further studies are necessary to characterise the NWM-1 kinetically as well.

PRHYP 296

Design, development and verification of a new lateral flow Assay for the fast and direct detection of PBP2a from clonal cultures of *Staphylococcus aureus*

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Introduction: Since the initial therapy of staphylococcal infections often relies on beta-lactams, methicillin resistance, accompanied by resistance to almost all β-lactams in clinical use, has become increasingly common in *Staphylococcus aureus* worldwide. It is associated with high mortality and increased costs to healthcare systems, and infection control measures such as isolation of affected patients and barrier nursing are warranted. Because of the high clinical impact of methicillin/β-lactam resistance on therapeutic decisions and infection control measures, newly identified MRSA cases need to be confirmed by a second method. This includes molecular means (i.e., PCR for the detection of mecA/C) or a detection of the PBP2a protein.

Materials/Methods: A new Lateral-Flow (LF) assay was investigated that allows the detection of MRSA strains quickly and cost-effectively, without special laboratory equipment, directly from culture plates. Monoclonal antibodies were produced by immunizing mice with recombinant PBP2a antigen and screened against the antigen. Lateral flow assays were prepared using the best pair of antibodies identified. Staphylococcal cells were harvested directly from overnight cultures on Columbia blood agar and inoculated in a designated buffer. After incubation at room temperature, the bacterial suspension was applied directly to the LF assay. Following an extensive protocol optimization using fully characterised reference strains, the lateral flow assays were tested with 60 clinical isolates. All isolates were also genotyped using microarrays (Inter-Array GmbH, Bad Langensalza, Germany) which allowed the determination of their mecA/SCCmec carriage as well as the assignment to clonal complexes and epidemic strains.

Results: The test panel consisted of 28 MRSA, 4 coagulase negative staphylococci carrying the mecA gene, and 28 MSSA isolates. The MRSA and MSSA strains belonged to 27 different clonal complexes and sequence types. The results of genotyping and lateral flow assay were compared. All 32 mecA positive strains were detected by the lateral flow assay yielding strongly coloured bands. Of the 28 MSSA strains, 6 were gave false positive signals although the bands in the LF assay were very weak. The LF assay thus achieved a diagnostic sensitivity and specificity of 100% and 79%, respectively.

Discussion: The antibodies used were found to be suitable for the task, although the concentrations and details of the protocol still need to be slightly modified to reduce false positivity. The design as a LF assay in combination with a gentle lysis protocol that does not rely on boiling allows for the future addition of species-specific control markers as well as of other targets of interest, such as PVL.

PRHYP 297

IS*4* family elements can cause AmpC beta lactamase hyperproducing mutants in *Morganella morganii* by disrupting the *ampD* gene

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Introduction: Insertion sequences (ISs) are small DNA segments that can jump or copy themselves into various genomic sites via an encoded transposase. The IS*4* family is sporadically present in genomes of Enterobacterales.

Morganella morganii is an opportunistic bacterial pathogen associated with nosocomial infections, predominately urinary tract or post-operative wound infections. This species possesses an inducible AmpC beta lactamase. Resistance to 3rd generation cephalosporins may develop by mutation of regulating genes and subsequent overexpression of the chromosomal *ampC* gene. However, mutation rates are estimated to be low in *M. morganii* and the basis for derepression has not been thoroughly examined yet.

Methods: Clinical isolates of *M. morganii* strains were collected and divided into groups depending on phenotypical susceptibility profiles to 3rd generation cephalosporins (wildtype: WT, n= 12; *in vivo* mutant: MUTC, n= 5). All strains were tested for AmpC activity and MIC. *In vitro* mutants (MUTL, n= 10) of WT strains were obtained via selection from dense broth cultures on selective agar. *In vitro* mutants were isolated for 7/13 WT strains. Sequence data was generated via whole genome sequencing on Illumina and Oxford Nanopore Technology systems to enable hybrid assemblies.

Results: Without exception, differences between WT and MUTL were detected in *ampD*. For 8/10 MUTL strains changes in *ampD* were caused by insertion of an IS*4* family element into the gene while 2/10 had point mutations causing an amino acid exchange (Asp170Gly, Ile33Ser). All strains held several copies (4-30) of the IS*4* family elements in their chromosomes. If MUTL strains held additional copies compared to their corresponding WT, then one of the additional copies was always inserted into *ampD*.

Insertion of an IS*4* family element into *ampD* was also detected in 2/5 MUTC strains. Translated *ampD* sequences of the remaining 3/5 MUTC strains aligned to WT strains revealed changes in amino acids (Arg80Trp, Val88Leu) that were unique to MUTC strains.

Discussion: Mutations leading to hyperproduction of the AmpC beta lactamase in *M. morganii* were exclusively located in the *ampD* gene. Point mutations were close to the putative active site of the encoded enzyme (AmpD). Insertion of an IS*4* family element into *ampD* was found in several *in vitro* and some *in vivo* mutants suggesting that possession of IS*4* family elements could increase the probability of *ampD* disruption and subsequent AmpC hyperproduction. Mutation rates of such strains should be examined to validate this claim.

The *ampC* gene is normally repressed by the LysR type repressor AmpR. A defective AmpD is a plausible cause for overexpression of *ampC*, since the accumulation of its substrate induces *ampC* by affecting the repressor AmpR. Defective *ampD* genes are also frequently encountered in AmpC hyperproducing *in vivo* mutants of *Enterobacter* species. IS elements disrupting *ampD* have not been described so far.

PRHYP 298

Molecular characterisation of cefiderocol resistance determinants in *Enterobacterales isolates*

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Objective: Cefiderocol is a novel antibiotic with a cephalosporin and siderophore structural component. The structure of cefiderocol and its mechanism to cross the outer membrane of gram-negative bacteria provide protection against common resistance mechanisms of carbapenem-resistant Enterobacterales such as loss of porins. However, first reports of resistance to cefiderocol have already been published and a further increase of resistant strains can be expected with increasing use of this antibiotic. The focus of this study is the molecular identification and characterisation of resistance mechanisms leading to a reduction of susceptibility to cefiderocol in Enterobacterales.

Methods: Cefiderocol-susceptible carbapenemase-producing and non-carbapenemase-producing clinical Enterobacterales isolates were selected from the strain collection of the German National Reference Centre (NRC) for multidrug-resistant gram-negative bacteria. These isolates were exposed to successively increasing concentrations of cefiderocol to select for spontaneous mutants with increased cefiderocol resistance. Whole genomes of clinical isolates and spontaneous mutants were sequenced using Illumina and Oxford Nanopore technology and cefiderocol resistance was determined using phenotypic assays such as broth microdilution and agar diffusion tests.

Results: Cefiderocol-resistant spontaneous mutants of one *E. coli*, one *P. mirabilis*, one *K. oxytoca* and two *K. aerogenes* clinical isolates could be selected. Sequencing revealed that three of the highly resistant spontaneous mutants, here one *E. coli* and two *K. aerogenes*, had mutations in the colicin I receptor (*cirA*) due to a base substitution, deletion of a base or even deletion of the whole gene. The colicin I receptor is a TonB-dependent iron transporter. In addition, a base deletion was detected in the gene encoding for the TonB subunit of a highly resistant spontaneous mutant of *P. mirabilis*. The elongated TonB subunit transfers energy derived from the proton motive force to TonB-dependent outer membrane receptors that allow the entry of nutrients into the periplasm. Finally, a mutation in the *dacB* gene encoding PBP4 was detected in a highly resistant spontaneous mutant of *K. oxytoca*. The spontaneous mutants showed minimal inhibitory concentrations of cefiderocol that were on average 3-4 dilution steps higher than the corresponding cefiderocol-susceptible clinical isolate.

Conclusions: As previously described in the literature, mutations in the *cirA* and *dacB* genes lead to reduced susceptibility to cefiderocol in other species. In addition, mutations causing an altered TonB subunit may also contribute to increased MICs of cefiderocol. Sequencing of additional isolates is planned to identify further mechanisms of resistance to cefiderocol.

PRHYP 299

Establishing a point-of-care testing system with customized microfluidic chip for detection of bacterial outbreak clusters

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Introduction: Outbreaks of antibiotic-resistant bacteria raise morbidity and mortality rates and entail high costs for adequate patient care and extensive hygiene measures. Nowadays, whole genome sequencing (WGS) is more frequently used to identify bacterial outbreak strains isolated from patient and environmental specimen. However, this time-consuming and expensive technique is not suitable to screen hundreds of samples, which usually arise during an outbreak event. Instead, real-time PCR-based point-of-care testing (POCT) systems that enable detection of outbreak strains via cluster-specific single nucleotide polymorphisms (SNPs) directly from swab samples represent a fast, user-friendly, and cost-efficient alternative screening method.

Material/Methods: SNPs uniquely present in an *Acinetobacter baumannii* cluster, a *Staphylococcus aureus* cluster, and an *Escherichia coli* cluster were identified with the software Ridom SeqSphere+. Per cluster, three SNPs were selected for design of modified TaqMan assays using the software OligoArchitect. Next to forward and reverse primers, each reaction comprised a FAM-labeled SNP-specific probe targeting the cluster isolates and an unlabeled probe binding to the alternative nucleotide at SNP position in non-cluster isolates. In addition, for assay control a reaction targeting the 16S rRNA gene of different species was designed. Functionality and chip compatibility of real-time PCR was tested in 10 µl reactions in 96-well plates using a real-time cycler and in polymer chips comprising 16 5 µl reaction cavities, respectively.

Results: SeqSphere+ identified 194, 45, and 33 SNPs for the *A. baumannii* cluster, the *S. aureus* cluster, and the *E. coli* cluster, respectively. The modified TaqMan assays designed for three SNPs per cluster were functional and compatible with the chip material. In both the 96-well plate and the polymer chip, all assays specifically detected down to 100 copies of DNA of the respective cluster isolates and delivered either no signal or very low signals with 10,000 copies of DNA of non-cluster strains. Additionally, the 16S rRNA reaction amplified DNA of all three bacterial species. Implementation of the PCR components on the microfluidic chip and validation of the final POCT system comprising analyzer and microfluidic chip is currently in progress.

Discussion: We expect that the whole workflow from SNP detection to chip application will take around 2 ½ weeks. Once established, the POCT system will deliver results directly from swab samples within 1 h with little hands-on time. Thus, the system can be applied as time saving, easy-to-use, and cost-efficient method to complement WGS efforts during screening for outbreak strains.

PRHYP 300

Integrated molecular surveillance identifies clusters of methicillin-resistant *Staphylococcus aureus* (MRSA) causing bloodstream infection, Rhineland-Palatinate, 2018-2021

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Questions: Variant analysis using the core-genome (SNP or allele based) have proven useful in identifying previously unrecognized transmission of methicillin-resistant *Staphylococcus aureus* (MRSA), mainly in the hospital setting [1]. Population-based statutory infectious diseases surveillance systems have not been assessed as sampling frames in this context, although research from diagnostic laboratories in England covering a larger population indicates their potential usefulness for population-level surveillance of transmission [2].

Methods: From 06/2018 to 12/2021, we obtained 103 isolates of 188 notified MRSA blood-stream infections, performed next-generation sequencing on Illumina MiSeq, and used allele-based complex types as well as a phylogenetic based approach (<16 SNP

threshold in a core genome of 1,861 genes) to cluster theses isolates. We retrospectively analyzed statutory surveillance data according to epidemiologic indicators for a common source of infection.

Results: Among 103 isolates available for typing, we identified 14 isolates in 6 clusters, each consisting of 2-3 isolates of a common cgMLST cluster type. All 14 clustering isolates were isolated from independent case individuals. However, in 5 of 6 clusters, we found that the patients were treated in the same hospital and that in 3/3 clusters with ward information available, patients had been treated on the same ward. District of residency was identical in 4/6 clusters, but post-codes in 2/6 clusters only, supporting hospital- over community-transmission as the more relevant mechanism. The median time between the date of notification of the first and last case within a given cluster was 20 months (range 10 to 30), suggesting unobserved links in the chain of transmission. We estimated that 3,9% to 13,6% of notified BSI are due to nosocomial transmission, converting into 0.02 to 0.24 cases per 100.000 persons per year during the period and population underlying this study.

Conclusions: We found evidence for nosocomial MRSA transmission causing BSI in Rhineland-Palatinate, at a low to moderate level. Evidence from metadata suggests unobserved transmission events between invasive, notifiable infection, e.g. via contaminated surfaces and/or colonized subjects in the nosocomial setting, providing an opportunity for preventive intervention. Molecular surveillance integrated into statutory infectious diseases surveillance on the sub-national level has the potential to reduce invasive MRSA infection by guiding targeted intervention. Prospective studies seem warranted.

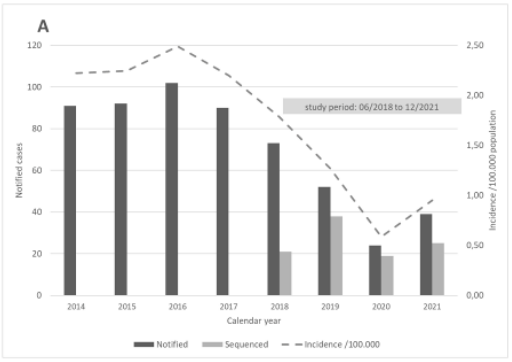
References

[1] Mellmann A, et al. (2016) Real-Time Genome Sequencing of Resistant Bacteria Provides Precision Infection Control in an Institutional Setting. *J Clin Microbiol* **54**:2874-2881

[2] Coll F, et al. (2017) Longitudinal genomic surveillance of MRSA in the UK reveals transmission patterns in hospitals and the community. *Sci Transl Med* **25**:9(413)

Fig. 1

Figure: Invasive MRSA-infections - statutory notifications Rhineland-Palatinate, 2014-2021



MRSA=methicillin-resistant *Staphylococcus aureus;* Sequenced=proportion obtained for whole-genome sequencing for this study (please note, in 2018, study was active from June to December only!)

Fig. 2

Integrated molecular surveillance of MRSA causing blood-stream infection identifies nosocomial transmission, Rhineland-Palatinate, 2018-2021

Integrated Molecular Surveillance (IMS)					Epidemiological context (person, place and time)									
					Patient		Place				Time of BSI			
Cluster‖	ID	MLST	cgMLST	ΔSNPs	Age	Sex	District of residency#	Postal Code†	Hospital / Clinic*	Common Ward*	Month	Year	Δt‖
A	2018009	5	26340	6	68	m	A	1	O1	A	08	2018	11
	2019028				79	f	A				07	2017	
B	2021011	225	26318	13	87	m	B	II	M2	n.a.	05	2021	30
	2018019				81	m	C: adjacent to B	III	N1		11	2018	
C	2019033	225	26287	12-14	77	m	D	IV		B	08	2019	17
	2020006				75	m	F: adjacent to D	V	R1	n.s.	03	2020	
	2018016				78	w	E: between D and F	VI		B	10	2018	
D	2019038	225	26285	2-15	88	m	E	VII	Z1	n.s.	11	2019	
	2021008				93	w	E	VIII	Y1	n.a.	04	2021	26
	2019007				89	m	G: adjacent to E	IX	Z1	n.s.	02	2019	
E	2019031	225	26295	12	90	m	H	X	H1	n.s.	07	2019	10
	2020012				60	w	H				05	2020	
F	2020003	225	26319	11	60	m	I	XI	O2	C	01	2020	23
	2021031				82	m	I	XII			12	2021	

‖ for each cluster strains were notified by the same diagnostic laboratory

out of 35 different districts; provided as part of notification by district public health authority;

* out of 47 different hospitals submitting overall 88 isolates/notifications (hospital names of 15 isolates/notifications missing); on average one hospital submitted 1,87 isolates [median 1; IQR 1-2; range 1-10];

‖ time elapsed between first and last isolate notified within given cluster

n.s. = not specified, i.e. missing data

n.a. = not applicable, i.e. different hospital

PRHYP 301

Validation of an *ex-situ* model for the analysis of biofilms from different One Health areas

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[2]*Rheinische Friedrich-Wilhelms Universität, Bonn, Germany*

[3]*Cardiff University, Cardiff, United Kingdom*

Introduction: Biofilms are a common way of bacterial growth and associated with antimicrobial resistance (Balcázar *et al*., 2015). However, few biofilm models are based on complex biofilms. Here, we aim to use a realistic sink trap model (Ledwoch *et al.*, 2020) for cultivation and testing of biofilms from different One Health areas. For analysis of biofilm compositions, sequencing of the 16S gene represents the most common way (Welsh and McLean, 2007), but High Resolution Melting Analysis (HRMA) has been proved as a fast and easy screening tool for distinction or clustering of microbial communities (Hjelmsø *et al.*, 2014).

In this study, the reproducibility of our biofilm model for a diverse set of biofilms from different countries and sampling sites was evaluated. Furthermore, the use of HRMA, in screening for changes in bacterial communities, was assessed by comparison of HRMA and 16S gene sequencing data.

Method: Biofilm preparations from different One Health areas (households and hospitals) were enriched and cultivated after initial inoculation in a trap model simulating different sample sections of a U-bend (figure 1). After 6 days of cultivation, biofilm DNA was extracted. 16S sequencing of the bacterial V3-V4 region of the DNA was done and sequencing data combined with meta data of the samples were analyzed and plotted in a Principle Component Analysis using R Studio. HRMA was performed as described by Andini *et al.* (2017). After qPCR of bacterial ITS gene followed by HRMA, data were normalized, plotted and compared to results of 16S sequencing.

Figure 1: Schematic presentation of the biofilm trap model simulating a U-bend

Results: Although the diversity of samples clearly decreased in the enrichment step, sequencing data and meta data show a clustering of biofilms both by their country of origin and their sampling site (figure 2). No clear differences between the bacterial composition of the sampling sections were observed. Strongly different biofilm compositions could also be distinguished by HRMA data, which further supports its use as a screening tool for differences in biofilms.

Figure 2: Clustering of biofilm samples in a PCA based on their original location (A) or their country origin (B)

Discussion: Our results provide a first insight into the reproducibility of the proposed sink trap model, which represents a far more realistic approach compared to current biofilm models. For future prospects, this complex biofilm model provides the possibility for efficacy testing of disinfectants used in different One Health areas. Based on bacterial number, genetic resistance marker prevalence and phenotypic resistance profiles of untreated controls, treated and regrown biofilms, it will be possible to evaluate which products pose a low risk of resistance selection and transmission.

Fig. 1

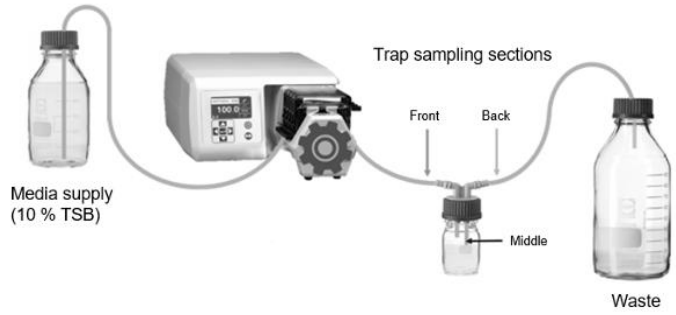
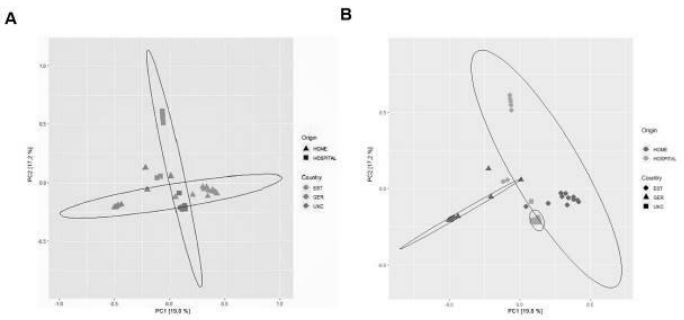


Fig. 2



PRHYP 302

Adherence to antibiotic prescription guidelines in four community hospitals in Germany and its impact on clinical and economic consequences

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Introduction: Urinary tract infections (UTIs) and bloodstream infections (BSIs) represent significant burdens on healthcare systems worldwide and are a common reason in healthcare facilities to administer antibiotic agents. The appropriate and evidence-based prescription of antibiotic agents is crucial to ensure effective treatment outcomes and reduce the emergence of antimicrobial resistance. To ensure evidence-based administration of antibiotics antibiotic-stewardship programs (ABS) can have a great impact on clinical outcome, reduction of antimicrobial resistance and economic performance. Commonly available tools for antibiotic therapy are national guidelines. This study aimed to evaluate guideline adherence and the impact on antibiotic treatment costs in the management of UTIs and BSIs at four German community hospitals from January 2019 to December 2020.

Materials and methods: We conducted a retrospective analysis of records of 586 patients in four German community hospitals from January 2019 to December 2020 using electronic clinical, microbiological and laboratory data. The data on the clinical diagnosis, the completed diagnostics and the antibiotic therapy were compared to the national guidelines for the management of UTIs and BSIs. The total costs of the antibiotic Treatment used were estimated considering local price charts and comparing them with the costs of a treatment based on established guidelines, also considering the local prices.

Results: 58 % of the patients were diagnosed with UTIs, 31 % with BSIs and 11 % were misdiagnosed. About 98 % of the patients received antibiotic treatment. However, only 61 % received microbiological diagnostics, which would have been important for guiding appropriate antibiotic agents. Patients with cultures taken showed germ growth in 86 % of cases, with Escherichia coli being the leading pathogen. A list of the germ spectra is shown in the figures. The treatment was intravenously administered in 63 % of cases, with Ceftriaxon as the leading antibiotic agent in both diseases. A de-escalation of antibiotic therapy occurred in 19 % and an escalation was considered necessary in 10 % of the cases. The adherence to guidelines was found to be rather low (33 %). The overall cost of antibiotic agents used differed from guideline adherent treatment with a median cost of EUR 34 (IQR: 5 – 53) and EUR 17 (IQR: 3 – 43) based on non-guideline adherence treatment ($p = 0.003$).

Discussion: Our findings indicate low guideline adherence with respect to antibiotic treatment in almost all cases, mainly administered intravenously although oral options were also available. These results highlight the need for improved adherence to guidelines, rational use of antibiotics, and consideration of alternative administration routes. Strategies should focus on education, clinical decision support systems, and audits to enhance prescribing practices.

Figure 1: Germ spectra in BSI (n)

Figure 2: Germ spectra in UTI (n)

Fig. 1

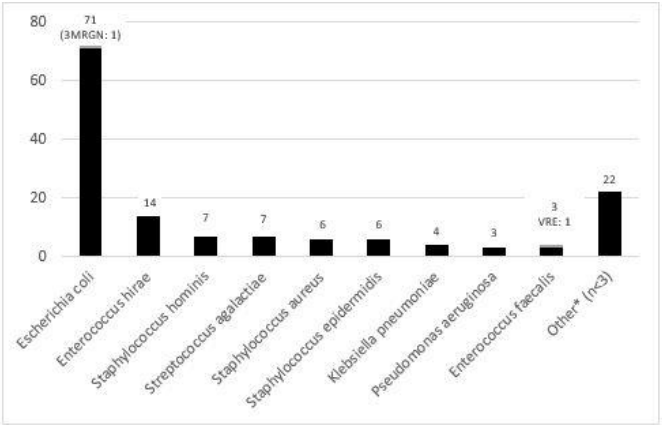
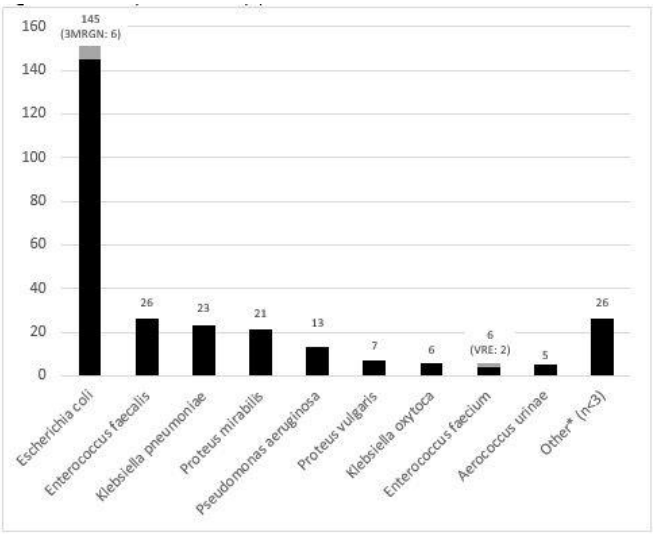


Fig. 2



PRHYP 303

Assessing the resolution of antibiogram similarity in molecular transmission analysis of multidrug-resistant pathogen by utilizing cgMLST as the benchmark

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Question: In this study, we investigate the power of antibiograms in the context of molecular transmission analysis of multidrug-resistant pathogens. Specifically, we aim to determine if comparisons of antibiograms between two isolates can provide sufficient information to exclude transmission events or to at least qualify them as highly unlikely.

Methods: Between 2015 and 2021, all vancomycin-resistant enterococci (VRE), methicillin-resistant Staphylococcus aureus (MRSA), and multidrug-resistant Escherichia coli (MDR *E. coli*) isolates found in inpatients were sequenced using either the the Illumina MiSeq platform (Illumina Inc., San Diego, USA) or the Pacific Biosciences Sequel II platform (Pacific Biosciences Inc., Menlo Park, CA, USA) and subsequently typed using cgMLST. The cgMLST distances were calculated between all paired isolates within each species and stored for future analysis. Comprehensive antibiotic susceptibility testing was conducted for all isolates, covering a range of antibiotics such as Amikacin, Amoxicillin, Ampicillin, Aztreonam, Cefepime, Cefotaxim, Cefoxitin, Cefpodoxim, Ceftazidim, Ceftriaxon, Ciprofloxacin, Ertapenem, Gentamicin, Imipenem, Levofloxacin, Meropenem, Moxifloxacin, Piperacillin-Tazobactam, Tetracycline, Tigecycline, and Trimethoprim, depending on the species. The number of discrepant antibiotic susceptibility testing results between all paired isolates within a given species was calculated and compared to the corresponding cgMLST distance.

Results: Our analysis comprised a total of 3350 MRSA, 2337 VRE, and 1492 MDR E. coli isolates. When comparing isolates with the same results in antibiotic susceptibility testing, the cgMLST distances exhibited a range of 0 to 1731 for MRSA, 0 to 472 for VRE, and 0 to 2218 for MDR E. coli. Notably, as the number of different antibiotic testing results increased, both the average and minimum cgMLST distances also increased. For MRSA, the minimum cgMLST distance reached 6 when there were at least 9 differing antibiotic testing results. Similarly, for VRE, the minimum cgMLST distance rose to 6 when there were at least 6 differing antibiotic testing results. In the case of MDR E. coli, a minimum cgMLST distance of 8 was observed when there were at least 6 differing antibiotic testing results.

Discussion: Comparing antibiograms of pathogens can provide valuable insights in the context of outbreak or transmission analyses. However, when the antibiograms are identical or show no significant differences, little information is gained. This is because even if the antibiograms differ in 3 or 4 results, the cgMLST distances for the investigated pathogens can still be very small. On the other hand, if the antibiograms exhibit significant differences (typically in 6 to 9 results, depending on the pathogen), the cgMLST distances exceed 5, indicating distinct clonal origins, at least in the context of an outbreak investigation.

PRHYP 304

Surveillance of Health care infections and multidrug resistant organisms in outpatient intensive care facilities

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Introduction (background, relevance and question of the work): An increasing number of people with chronic respiratory insufficiency receive intensive care outside a hospital. Since 2018, the Infection Protection Act in Germany obliges outpatient intensive care (OIC) facilities to implement measures to prevent health care associated infections (HAI) and the further spread of pathogens. However surveillance, as an important tool for implementing modern hygiene management, has been lacking in this area so far. And also the number of HAI in this setting is unknown.

Therefore, we investigated whether and how an infection surveillance system can work in OIC-facilities. In addition, information should be gained on the number of infections and multi drug resistant organisms (MDROs).

Material/method (patient collective, method, statistical procedures): The project addressed facilities that treated at least two adult OIC patients with tracheostomy tubes. A surveillance system was developed, introduced and evaluated. The participating nursing services collected patient-related data on HAI and MDRO and entered them anonymously in an online database. The collected cumulative data was reflected to all participants as "reference data" together with their own data. The participating services were thus able to optain an overview of their facilities infection rates with those of other facilities and, if necessary, adjust preventive measures accordingly.

Results (results with data and statistics): From November 2020 to April 2022, 20 providers of OIC with 80 care facilities nationwide took part in the surveillance project. In total, 712 patients with tracheostomy tubes were cared for in the OIC-facilities (187,227 patient days) during the project. Surveillance was able to detect 783 infections. About 33% of the infections were urinary tract infections, 28% respiratory tract infections, 9% wound infections and 30% other not specified infections.

Discussion (Relevance of the work presented and conclusion): Implementing a surveillance also seems to be possible in the setting of OIC - although the effort greatly depends on the organizational structure of the nursing service. The obtained data on infection frequencies and MRDO can provide valuable information about the effectiveness of the infection prevention strategies in the respective facility. Participants have already reported positive effects on patient care as a consequence of the study.

PRHYP 305

Detection of *Pseudomonas aeruginosa* in clinical samples upon hospital admission a risk factor based guide for selection of anti pseudomonal antibiotics-based on a retrospective cohort study

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Introduction (background, relevance and question of the work): In Germany, empiric antimicrobial treatments frequently include coverage of *Pseudomonas aeruginosa*. Anti pseudomonal antibiotics are frequently use in patients admitted to hospitals. Many of these substances are classified as a reserve or watch status by the WHO. Inappropriate risk assessment of invasive detection of Pseudomonas aeruginosa is one reason for overuse of antipseudomonal antibiotics, further increasing selection pressure in patients.

The objective of this study was to identify risk factors for invasive P. aeruginosa in patients upon hospital admission.

Material/method (patient collective, method, statistical procedures): All patients 18years of age and older with a detection of *P. aeruginosa* and/or enterobacterales in clinical samples taken within 48h of admission to Charité University hospitals between 2015 and 2020 were included into this retrospective cohort study.

Multivariable logistic regression analysis was performed to identify independent risk factor for identification of P. aeruginosa in clinical samples.

Results (results with data and statistics): Overall we included a total of 27,710 patients. In 3,764 (13.6%) patients *P. aeruginosa* was detected in clinical samples within 48h after admission. *E. coli* was detected in 14,142 (51%) patients followed by *Klebsiella spp.* in 4,432 (16%) patients.

Regression analysis identified that male patients had an increased risk for detection of *P. aeruginosa* (OR 1.60; 95% CI 1.46-1.75). Prior colonisation with a multi drug resistant *P. aeruginosa* or detection of a *P. aeruginosa* in clinical samples during a previous hospitalisation increased the risk (OR 39.41; 95% CI 28.54-54.39) and OR 7.87, 95% CI 6.60-9.38) respectively. Admission to a specialised ward for patients with cystic fibrosis was associated with an increased risk (OR 26.99; 95% CI 20.48-35.54).

Presence of chronic pulmonary disease (OR 2.05; 95% CI 1.85-2.26), hemiplegia (OR 2.16; 95% CI1.90-2.45) were associated with an modest increase in risk for presence of.

Interestingly we identified colonisation with MDRGN other than *P. aeruginosa* to be an independent protective factor for detection of *P. aeruginosa* in clinical samples.

Discussion (Relevance of the work presented and conclusion): *P. aeruginosa* is frequently cultured from clinical samples taken from patients within 48h after admission. Newly identified and verification of well established risk factors could help to identify patients at risk for an infection with *P. aeruginosa*. Future studies should investigate the potential of such risk factor analysis in clinical routine for antibiotic prescriptions.

MVP 306

Molecular cross-talk between Sa3int phages and their *Staphylococcus aureus* host

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As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity, but is also a leading cause of life-threatening acute and chronic infections. *S. aureus* strains can carry up to four temperate phages, many of which possess accessory genes coding for staphylococcal virulence factors. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3int phages, which integrate as prophages into the bacterial *hlb* gene thus disrupting the expression of the sphingomyelinase Hlb, an important virulence factor under certain infection conditions. The virulence factor-encoding genes carried by the Sa3-phages are all highly human-specific and probably essential for bacterial survival in the human host. Thus, both insertion of the prophages into and excision from the bacterial genome have the potential to confer a fitness advantage to *S. aureus*. However, how the *S. aureus* host modulates the life cycle of its temperate phages remains largely unknown (1). Our data suggest that the bacterial factors supposedly involved in the interaction of the bacterial host with its phages are strain specific, with certain *S. aureus* strains being more prone than others to support either a lysogenic or a lytic life cycle (2). We constructed and integrated Sa3int phages into different phage-cured *S. aureus* strains and found significant differences in phage transfer rates between different strains. Based on this finding, strains were grouped into low and high transfer strains. Indicating that in low transfer strains, the phages are more directed towards lysogeny. To get a more precise picture of the regulatory circuits we constructed replication deficient mutants, performed differential RNAseq to determine the transcriptional units and analysed a set of mutant strains. By transcriptional start site prediction we identified promoter-regions within the structural module of prophage Φ13 that are differentially active in high and low transfer strains and are a tool to identify regulators by measuring promoter-fusion constructs in different mutant backgrounds.

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MVP 307

GMP-compliant production of phages

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Question: Multidrug resistance (MDR) in bacteria is increasing dramatically and is likely to become a serious health problem in the coming years unless new therapeutic options are found [1]. The use of bacteriophages could be a promising approach, as these viruses specifically infect and lyse bacteria. So far, no clinical trial has been completed to Western standards with evidence of phage efficacy [2]. At present, production under GMP conditions is mandatory in Germany for phages to be used in a clinical trial or after magistral preparation in humans [3].

Methods: After selection of phages with therapeutic potential and bacterial host that assume an efficient production, host strains were grown in 5-10 L-culture bags and infected with its designated

phages. At the end of cultivation, phages were harvested by filtration and cell-free lysates were further purified by 1-2 chromatography steps (depending on the anticipated application). Optimizing the purification of a phage was performed stepwise by taking different process parameters into consideration, e. g. binding of phages to column, washing to remove impurities like host cell proteins and endotoxins, elution of phages with optimized elution buffer, capacity of the column and phage recovery.

Results: A platform-like manufacturing process has been established, in which essential process steps (cultivation (USP, upstream processing) and purification (DSP, downstream processing)) must only be adapted to the individual phage in a few parameters to ensure successful production. This platform provides the basis for pharmaceutical scale and quality phage production. Requirements for phage IMPs and APIs in terms of process, specifications and analytics were defined with the relevant authorities. After their release, the first phage-IMPs/-APIs were delivered to the clinical partners to be used in a clinical trial or after magistral preparation.

Conclusions: The requirements for GMP production are currently not substantially different for production of an IMP or API and are comparable to those for other drug-classes. Unlike most other drugs, phage therapy benefits the use of a mixture of different phages. Due to their high specificity, the production of an enormous number of phages would therefore be necessary to be able to treat many patients, which would not be economically feasible at the given requirements. After safety and efficacy have been demonstrated, it will therefore be necessary to adapt the regulations to allow the exchange of phages in fixed cocktails, their approval, and the requirements for production, to take a step further towards practicability of phage therapy.

Literature

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MVP 308

Four new *Staphylococcus epidermidis bacteriophage*s with cross species activity

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Increasingly implant systems are used for organ, joint and bone prostheses in the human body in order to increase the life quality. However, these implants can be colonized by bacteria and, thus, lead to severe complications. In particular, foreign-body associated colonizations with coagulase-negative staphylococci and biofilms thereof are a major concern since they lead to periimplantitis. Conventional therapies with antibiotics may encounter their limits due to antibiotic resistance, biofilm formation, and non-replicative bacteria. Bacteriophage therapy is considered a possible supplement or alternative to antibiotics. In the context of personalized medicine, specific bacteriophages can be isolated and used in a targeted approach; alternatively, broadly active bacteriophages or cocktails thereof can be applied. In this project, four bacteriophages were isolated from sewage water using Staphylococcus (S.) epidermidis. Three of these bacteriophages had a moderate host range of 15-29 % of the clinical S. epidermidis isolates, 0-38 % of the S. lugdunensis isolates, and for 4-16 % of the S. hominis isolates. The fourth bacteriophage was able to lyse 71 % of the S. epidermidis, 82 % of the S. lugdunensis, and 24 % of the S. hominis isolates tested. None of the isolated bacteriophages were able to lyse any of the S. aureus isolates investigated. Further characterizations and functional tests are required. These preliminary results demonstrate that broadly active bacteriophages can lyse related bacteria across species borders and may be suitable for therapeutic applications.

MVP 309

Enrichment, purification and validation of bacteriophages against *Pseudomonas aeruginosa* for therapeutic applications

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Increasing resistance rates of bacteria against antibiotics call for therapeutic alternatives to overcome this worldwide problem. Bacteriophage therapy could provide such an opportunity. For any medical application, it is essential that the drug is free of endotoxin and other contaminants which otherwise might lead to a severe reactions in the patients. We focussed on methods to purify Pseudomonas (P.) aeruginosa bacteriophages. First, high titer phage lysates of P. aeruginosa were concentrated by membrane ultrafiltration. This was followed by affinity chromatography in order to remove endotoxins from the bacteriophage samples. Afterwards, the eluted samples were further purified by treatment either with organic solvents or with detergents. In both methods, a phase separation was achieved and the aqueous phase containing the purified phage suspension was further examined. Different tests for detection of endotoxins were used for this purpose: first, the limulus-amebocyte lysate test based on the blood of horseshoe crabs; second, the EndoLISA® test based on a genetically engineered protein, which is activated by endotoxin and produces a fluorescent signal; and third, the monocyte activation test which allows not only the detection of endotoxins but also the full range of pyrogens, including non-endotoxin pyrogens such as lipoteichoic acid from Gram-positive bacteria. In this test, the human monocytic cells are activated by pyrogens and produce interleukin-6, which is detected by ELISA. The endotoxin levels of our initially high-titered lysates were reduced drastically by these methods. The monocyte activation test confirmed that the purified bacteriophage samples were free of measurable endotoxins and other bacterial toxins. However, the treatment of the samples reduced the bacteriophage titers at the end of our procedures. The findings on strongly reduced endotoxin levels through chromatography, organic solvent and detergent treatments are an important step on the way to applicable bacteriophage therapy. However, further investigations are needed to increase sample quantities.

MVP 310

Quantitative analysis of crAssphage and the indicator bacterium *Phocaeicola dorei* along with two antibiotic resistance genes in sewage and reduction capacity by a retention soil filter

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Introduction: Combined sewer overflows (CSO) are often loaded with lots of harmful substances which are released into the environment posing a potential risk for human health. For instance, bacteria with antibiotic resistances could be selected for when encountering antibiotics in the water. Therefore, wastewater treatment plant (WWTP) uses retention soil filters to minimize or reduce environmental pollution from CSO. Different target

organisms have been used as potential indicators of pollution and clearing capacities. Here, we enumerated the predominant gut bacteriophage crAssphage (gene CPQ56), the 16S rRNA gene of the indicator bacterium *Phocaeicola dorei* and two antibiotic resistance genes (*sul1* and *tetW*) affecting tetracycline and sulfonamide in initial CSO and evaluated the reduction capacity of the retention soil filter (RSF).

Methods: At three different time points 7 biological RSF replicates each were collected from in the WWTP Aachen-Soers, Germany before and after the use of the RSF. DNA for all samples was obtained with standard extraction procedures and, for comparison with and without prior DNase treatment. Target genes were subsequently analysed by ddPCR.

Results: The amount of all tested genes varied between the different time points but also between the biological replicates. No significant differences were observed between the samples treated with or without DNase, indicating that intact organisms (viruses and bacteria) were detected rather than free DNA. On average, the crAssphage occurred in the sewer overflows with 1866 phage particles/mL (range of 155 to 6719 particles/mL). In contrast, only an average of 83 cells/mL of *Phocaeicola dorei* were detected (range of 155 to 6719 cells/mL). We furthermore found 11480 bacterial cells/mL (range of 522 to 40263 cells/mL) carrying the sulfonamide resistance gene, but only 504 bacterial cells/mL (range 0 to 1555 cells/mL) carrying the tetracycline resistance gene. Using Spearman"s rank-order correlation a weak but significant positive correlation was found between the crAssphage and *Phocaeicola dorei* (n=21, rs=0.49, p=0.026). The RSF achieved a reduction of 99,5% ± 0,5% of the crAssphage and a similar high reduction of sulfonamide resistance gene carrying bacteria. The reduction of bacteria with the tetracycline resistance gene was with 98,4% ± 1,1% a little bit less effective and even less effective with *Phocaeicola dorei* (88,6% ± 14,9%)

Discussion: The amount of crAssphage in wastewater was around 100 times higher than that of *Phocaeicola dorei*. This aspect, along with the previous finding that the crAssphage correlates with antimicrobial resistance genes in wastewater makes this phage a sensitive human-specific pollutant indicator. The high amounts of sulfonamide resistance genes are in line with previous findings and likely indicates the broad and massive use of sulfonamides.

MVP 311

Single and combined measures for *Campylobacter* mitigation in a One-Health approach targeting commercial chicken meat production - bacteriophages, curcumin and organic acids

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Introduction: Campylobacteriosis is one of the most frequently reported human gastrointestinal diseases in the European Union (EU), and associated with rare but severe long term sequelae. Transmission to humans can occur via contaminated poultry meat. Being under discussion for reducing the prevalence and concentration of *Campylobacter* in poultry products, combined intervention measures as part of a multiple hurdle approach were investigated in this study.

Materials and methods: In our study, we applied bacteriophages (phages), organic acids (sorbic, benzoic, propionic and acetic acid) and the plant extract curcumin as pre-harvest intervention measures in commercial broiler fattening plants in Germany. These feed additives are known to effect several bacteria of the chicken microbiome. *Campylobacter* counts in chicken flocks with single or combined measure application were examined and compared to controls. *Campylobacter* load after and during the experiment was determined in fecal and cecal samples, using the DIN EN ISO 10272-2:2017 methodology. Furthermore, samples were analyzed for phage concentrations and phages susceptibility of bacterial isolates towards.

Results: In the experimental group receiving a combination of organic acids, phages (both via water) and curcumin (via feed), reductions of up to 1.11 log units were detected in fecal samples 34 days post hatch (dph). Significant reductions of up to 0.76 log units were detected in cecal samples 35 dph. After the second phage application (40 dph), a significant reduction of 1.39 log units occurred in fecal samples compared to the control.

Single application of an organic acid blend resulted in a significant *Campylobacter* reduction of up to 4.85 log units 33 days post hatch. Subsequently, significant reductions by up to 1.04 log units were observed in cecal samples 42 dph. After single application of a phage mixture 33 dph, a 1.08 log unit reduction was observed 34 dph. Inconsistent, non-significant reductions were detected in the experimental group receiving the plant extract curcumin via feed.

Discussion: The significant reductions after combined application of phages, organic acids and curcumin at particular times of the fattening indicate that the investigation of combined intervention strategies against *Campylobacter* is a promising approach. However, no synergistic effects did occur in our study as mitigation results were similar after single measure application. Although the most meaningful reduction was observed after application of organic acids, poor solubility limits the practical usability of this measure. Further studies are needed to investigate whether the organic acid blend can be improved by interval usage, change of application route, lower concentrations of the acids or if other measure combinations not tested during this study do show synergistic effects.

PWP 312

Challenges in GMP-conform-Analysis of fecal microbiota for stool transplantation

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Background: Ulcerative colitis is a chronic inflammatory bowel disease and difficult to treat with standard therapy. Fecal microbiota transfer is considered as an effective therapy against this disease [1]. For patient safety, it is to ensure that the donor of the stool is healthy and the encapsulated multi-donor stool is free of pathogens [2]. However, this treatment is classified as medication, therefore the Arzneimittelgesetz (AMG) and the Arzneimittel- und Wirkstoffherstellungsverordnung (AMWHV) is applied and we have to operate with good manufacturing practice (GMP).

Methods/Results: To screen the stool donors we had to use several culture-based, molecular, serological and microscopic methods. In addition, a multiplex real-time PCR system with a gastrointestinal panel that tests for 22 common gastrointestinal pathogens including viruses, bacteria and protozoa is used to analyze the encapsulated multi-donor stool. We have qualified and validated this amount of different values extensively and found reasonable quality assurance. A Change control processes is required for every change in processes, reagents or rooms. It is necessary to backup also raw data and ensure that these data files, which are mostly in company-

owned formats, are readable for many years. We store retained samples and all technical and organizational notes have to be very detailed and forgery-proofed. In addition the complex and innovative character of the project challenges the necessary formal approval process by the local governmental supervisory bodies.

Conclusions: Diverse microbiological, virological and serological analysis are necessary to ensure the safety of the patients before a microbiota transfer. Disproportionate costs arise by increased material and human recourses due to the AMG and AMWHV conform handling of these analysis. If the legislative organs are not able to simplify the handling for such advanced new methods, it will be hardly possible to apply such methods into wide clinical use.

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PWP 313

Competitivity of *Streptococcus mitis/oralis* strains from CF patients and non-CF patients against *Pseudomonas aeruginosa*

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Introduction (background, relevance and question of the work): CFTR gene mutation in cystic fibrosis (CF) leads to a higher viscosity and different composition of mucus which favours airway infection with pathogens, such as *Pseudomonas aeruginos*a. Infection with *P. aeruginosa* is associated with a decrease in lung function and disease progression. One approach to counter *P. aeruginosa* infection is to use respiratory commensal strains as probiotics. Previous trials raised the hypothesis, that respiratory commensal strains from CF patients are less competitive against *P. aeruginosa* than from non-CF patients. To validate this hypothesis, we compared CF to non-CF strains of *Streptococcus mitis/oralis* in terms of their competitivity against *P. aeruginosa*.

Material/methods (patient collective, method, statistical procedures): S. *mitis/oralis* strains were collected from airway samples and, depending on the sample donor, assigned to the CF-cohort (17 strains), or the non-CF cohort. The non-CF cohort includes samples from patients without CF (16 strains) and from healthy persons (4 strains). Two different competition assays have been performed. In a Direct Droplet Assay, the *S. mitis/oralis* strains were suspended to 0.5 McF and dropped on an agar plate previously inoculated with PAO-1. A reduction in PAO-1 growth density on these spots was considered as an inhibitory effect. In a Coculture Assay, PAO-1 was cocultured with *S. mitis/oralis* in liquid media for 15-23 hours. PAO-1 growth in this coculture was then quantified by counting the colony forming units on selective media.

Results (Results with data and statistics): In the Direct Droplet Assay, 4 out of 20 *S. mitis/oralis* strains of the non-CF cohort had inhibitory effects on the PAO-1 growth, 14 did not have an inhibitory effect, while the results for 2 strains are inconclusive. In the CF-cohort, 6 out of 17 *S. mitis/oralis* strains inhibited PAO-1 growth, while 10 strains were not inhibiting and 1 was inconclusive.

In the Coculture Assay, 3 out of 20 *S. mitis/oralis* strains of the non-CF cohort were constantly inhibiting PAO-1 growth in 3 biological replicates, while 1 strain was constantly increasing it and 16 strains had variable effects. The three inhibitory strains were also inhibiting in the Direct Droplet Assay. In the CF-cohort, 7 out of 17 *S. mitis/oralis* strains were constantly inhibiting PAO-1 growth, while 1 strain was constantly increasing it and 9 strains had variable effects. 3 of the 7 inhibitory strains were also inhibiting in the Direct Droplet Assay.

Discussion (Importance of the work and conclusion): Although the initial hypothesis cannot be validated, we found several *S. mitis/oralis* strains that can inhibit *P. aeruginosa* growth. This effect seems to be strain-specific and not associated with CF. The mechanism underlying the inhibitory effect is currently examined using whole genome sequencing, comparing inhibitory and non-inhibitory strains.

PWP 314

National Research Data Infrastructure for the Research of Microbiota (NFDI4Microbiota) - Democratize access to microbiota data and high-end computational analyzes

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Introduction: Recent technologies like high-throughput molecular sequencing lead to the generation of large amounts of data. However, (re-)use of these data has failed to exploit its full potential. The NFDI (National Research Data Infrastructure) will change this by developing comprehensive research data management. NFDI4Microbiota aims to facilitate digital transformation in the microbiological community (including bacteriology, virology, protistology, mycology and parasitology), by providing access to data, analysis services, training, and standards.

Methods: The German microbial research will be engaged through training, community-building activities, and by creating a cloud-based system that will make the storage, integration, and analysis of microbial data - especially -omics data - consistent, reproducible, and accessible. Therefore, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable, and Re-usable) principles and Open Science. Central for the consortium is the development and provision of the computational infrastructure and analytical workflows required to store, access, process, and interpret various microbiology-related data types. Here, NFDI4Microbiota works on developing and implementing software and standardized workflows for users to analyze their own data (i.e. for quality control, data processing, statistical analysis, and visualizations of different data types and results).

Results: NFDI4Microbiota consists of ten well-established partner institutions and is supported by five professional societies (including DGHM) and more than 50 participants. Several workshops and training events were already performed and further will take place frequently. Moreover, the consortium launched an ambassador program to connect with the participants, thereby helping to identify the needs of the community. Technical solutions are developed, tested, and refined in several Use Cases from different fields of microbiology. The recently published

Knowledge Base with materials on research data management etc. is accessible via the web portal nfdi4microbiota.de. All information and specific services are and will be made available there as well.

Conclusions: NFDI4Microbiota helps the microbiological research community by providing technical solutions and a comprehensive training program. This combined approaches of education and services will ensure that microbial research in Germany is synergistic and efficient. Producers and users of data will benefit from FAIR data more likely to be cited and integrated into a wider microbial inquiry. The current data parasitism would shift to a future data mutualism helping all partners. The NFDI4Microbiota will support the whole community through this process with an elaborate training program and other services.

PWP 315

***Pseudomonas aeruginosa* changes the activity of its H2 type VI secretion system at body temperature**

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Introduction: *Pseudomonas aeruginosa* is an opportunistic human pathogen that successfully colonizes niches at a wide temperature range. It has three different types of type VI secretion systems (referred to as H1-T6SS, H2-T6SS and H3-T6SS), each with a specific set of effectors. These T6SS effectors enable *P. aeruginosa* to kill competing bacteria and mediates virulence. How temperature affects T6SS-mediated bacterial killing during *P. aeruginosa* colonization of the human body is little understood. Our goal is to better understand the activity of the three different types of T6SSs during changes from room temperature to body temperature.

Methods: To test the effect of temperature on T6SS-mediated bacterial killing, we performed spot assays. For that purpose, two fluorescently labelled strains deficient in the effectors Tse5 or TseT and their corresponding immunity proteins were exposed to the wild-type strain at 22°C and 37°C. Fluorescent microscopy of the macrocolonies and the quantification of bacteria by flow cytometry were used to assess changes in the relative abundance of competing strains in the mixed community. To measure T6SS activation at different temperatures, we analysed T6SS gene expression by RT-qPCR and Hcp protein expression by Western blot at 22°C and 37°C.

Results: We found similar levels of killing by the H1-T6SS effector Tse5 at 22°C and 37°C. In contrast, a big competitive advantage in bacterial competition was observed by H2-T6SS effector TseT at 22°C but not at 37°C. The absence of the TseT-mediated advantage at 37°C could be explained by negative regulation of the H2-T6SS, since we observed less Hcp2 expression at this temperature. Host temperature did not affect protein expression of the H1- and H3-T6SS.

Discussion: Temperature dependence of the TseT-mediated competitive advantage led us to the finding of atemperature-dependent expression of H2-T6SS proteins. These results confirm recent, independently acquired results by Rudzite and colleagues that demonstrate a clear advantage in bacterial competition mediated by all H2-T6SS toxins at 25°C but not at 37°C. The temporary suppression of H2-T6SS expression at 37°C may enable the redistribution of bacterial resources in harsh conditions of the host environment. For example, Wurtzel et al. had shown that genes involved in virulence such as type III secretion system, exoproteins and flagella are upregulated at 37°C. The study of factors contributing to the activation or suppression of the T6SS in the process of adaptation to the host environment is important for understanding the dynamics of chronic infection and preventing the dominance of *P. aeruginosa* in the pulmonary microbiome in predisposed patients.

PWP 316

Changes in the bacterial gut microbiome of goats with chronic granulomatous enteritis - Paratuberculosis

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Introduction: The intestinal microbiota plays an important role in immunity and the maintenance of overall health in humans and animals. To date, few studies have investigated the gut microbiome during the chronic enteric inflammatory disease, paratuberculosis (Ptb), which affects ruminants and is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The aim of this study was to uncover characteristic changes in the composition of the bacterial microbiome in the gut of animals with Ptb and to identify bacterial factors that may be involved in the pathogenesis of this disease. In addition, the study intends to better understand the molecular mechanisms of microbiome-host interactions.

Material/method: Goats of the breed "Thüringer Wald Ziege" from farms in Thuringia with and without a history of Ptb were sampled. The contents of 7 intestinal compartments of goats with Ptb and faeces of MAP-positive goats were examined by 16S rRNA gene sequencing, and the results were compared with the data obtained from healthy and MAP-negative goats. Health status of animals was assessed by macroscopy, histology, microbiology and parasitology after autopsy or slaughter for meat production. Microbiome analysis was performed using the microbiome explorer "Namco" (https://exbio.wzw.tum.de/namco/).

Results: A higher α-diversity of the microbiome was found in the large intestine in healthy goats compared to goats with Ptb, and also in the faeces of MAP-negative compared to MAP-positive goats. Furthermore, the β-diversity of the microbiome differed between healthy and diseased goats in the small and large intestine and between MAP-negative and MAP-positive goats in faeces. Differential analysis revealed various changes in the abundance of different taxa in the microbiome of goats with Ptb compared to that of healthy goats. At the phylum level, there was an increase in *Firmicutes* and a decrease in *Bacteroidota* and *Proteobacteria* ($p<0.01$) in the small intestine; an increase in *Actinobacteria* ($p<0.05$), and a decrease in *Desulfobacterota* ($p<0.01$) in the large intestine. At the family level the abundance of many taxa was increased or decreased in goats with Ptb compared to healthy animals, and differences were observed between the microbiota composition in the small and large intestine.

Discussion: As expected and known for diseases that cause dysbiosis in the gut microbiome, α-diversity was reduced and ß-diversity was different in goats with Ptb compared to healthy goats. Comparing the present results with the few studies on cattle in the same field have not revealed any specific signature for the bacterial composition of the gut contents or faeces of MAP-infected animals or animals with Ptb. However, a study of the mucosal microbiome along the intestine and transcriptome analyses will follow to uncover relevant microbial metabolic activities and products that may play a role in the onset and progression of Ptb, e.g. by interacting with the immune system.

PWP 317

Antibiotic resistance and resistomes of microbiota along the gut-lung axis

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Introduction: Lower respiratory tract infections are a major cause of death worldwide and increasing antimicrobial resistance (AMR) in relevant pathogens makes the treatment of these infections more challenging. In this sense, the resistome of the microbiome of the human body may serve as a reservoir for antimicrobial resistance pattern development. As both the microbiome of the lung and the gut are influencing the course of infectious lung diseases, we aim to understand how microbial communities along the gut-lung axis may contribute to AMR spread to respiratory pathogens. We do this by combining culturomics efforts with sequencing-based techniques to uncover AMR on the genetic and phenotypical level.

Material and Methods: Bronchoalveolar lavage samples (BAL) and rectal swabs (RS) as well as blood and pharyngeal swabs (PS) are collected from patients receiving a diagnostic bronchoscopy at the pulmonology department of the university hospital. All materials are transported to the lab in less than 30min. Microbes from the BAL and RS are cultured, identified and stored in a cryobank for composition and phenotypical data. DNA is isolated from the BAL, RS and PS native materials and the meta-genomes are sequenced for composition and resistome data. Patient data from questionnaires and clinical data is collected and analyzed.

Results: We have isolated a total of 296 and 410 isolates from respiratory samples and gut samples respectively. In an optimized culturomics trial, we ran 7 complete sets of samples performing high throughput cultivation efforts on various conditions in aerobic and anaerobic environments. The preliminary culture-based results show a high degree of dissimilarity between the niches. Though, a small species overlap from lung and gut microbiota in the patient collective consisting mostly of Streptococci is present. Antibiotic susceptibility testing of the first lung-gut isolate pairs indicates potential strain overlap between niches. While we are continuing our culturomics efforts, metagenomic analysis of the patients´ samples and genome sequencing of respective isolates will uncover genetic links between the lung and the gut in respect of AMR.

Discussion: Only few species have the ability to grow in the two niches of the lung and gut which differ in their physiology. However, presence of same species in the lung and the gut allows insights into the dynamics of bacterial dispersion and their potential role in spreading AMR along the gut-lung axis. Combining results from culturomics and metagenome sequencing will strengthen our understanding of occurrence and spread of AMR along the gut-lung axis and provide us with new insights on the potential role of the microbiome in this process. Bacterial isolates of interest with their genetic and phenotypic information will aid us in experimentally addressing the role of the microbiome in the development and spread of AMR.

PWP 318

Intraspecific diversity of type VI secretion system effectors and their combinations in *Pseudomonas aeruginosa*

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Bacteria use type VI secretions systems (T6SSs) to deliver effector proteins into neighboring cells and the extracellular space. How a bacterium benefits from its T6SSs depends on the effectors, which enable bacteria to kill other microbes, manipulate eukaryotic cells or obtain nutrients. Most of what we know about effectors, and thus about the function of T6SSs in *Pseudomonas aeruginosa*, is based on few reference strains. Little is known about effector diversity within the species. Here, we use comparative genomics to systematically test for the intraspecific diversity of T6SS effectors and their combinations in roughly 2000 phylogenetically distinct *P. aeruginosa* strains.

We found core effectors, which are omnipresent and conserved across the species, and accessory effectors, which vary between strains. The combination of core with accessory effectors results in an effector set. We observed a tremendous diversity of effector sets within the species. Mode of transport and effector target were identified to be possible drivers and constraints of effector diversity. Ancestral reconstruction of effector sets reveals putative loss and exchange of effector-encoding genes in the evolutionary history of some strains.

We show that the core effector TseT contributes to virulence *in vivo*, which indicates a role of the T6SS in pathogenicity across strains, whereas accessory effectors with known anti-eukaryotic activity contribute to strain-level variation of virulence. Accessory effectors with anti-prokaryotic activity provide the genetic basis for intraspecific killing and could provide a competitive advantage in mixed microbial communities.

These findings show the distinct contribution of T6SS effectors to the intraspecific diversity of a bacterial pathogen.

PWP 319

The acne microbiome and possible probiotic-based therapeutic intervention

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Background: Human skin is populated by trillions of microbes collectively called the skin microbiome. *Cutibacterium acnes* and coagulase-negative staphylococci are among the most abundant members of this ecosystem, with described roles in skin health and disease. However, knowledge regarding health-beneficial and disease-associated effects of these ubiquitous skin residents is still limited.

Objectives: Aims of this study were the characterization of the skin microbiome and its dysbiosis in acne vulgaris patients before and after treatment as well as the interrogation of the skin microbiome for health-beneficial functionality.

Methods: Culture-dependent and -independent methods were used. *C. acnes* and staphylococcal landscapes across two different skin sites of healthy individuals and acne patients were profiled with amplicon-based next-generation sequencing, and microbial interferences were assessed by antagonistic assays and transcriptomics.

Results: Healthy skin sites were colonized with multiple staphylococcal species, dominated by *S. epidermidis*, followed by *S. capitis* and *S. saccharolyticus*. Distinct *C. acnes* phylotypes were identified, spanning the 10 known single-locus sequence typing (SLST) classes. Acne-affected skin was characterized by a loss of *C. acnes* phylotype diversity, an overrepresentation of A-type *C. acnes* as well as a shift of the staphylococcal population. Relative abundance profiles indicated the existence of phylotype-specific co-existence and exclusion scenarios. Health-associated staphylococcal strains with anti-*C. acnes* activities were identified and further characterized to evaluate their suitability as skin probiotics.

Conclusions: Findings highlight the importance of skin-resident staphylococci and suggest that selective microbial interference is a contributor to skin homeostasis.

EKMPP 321

Comparative proteomics of vesicles essential for the egress of *Plasmodium falciparum* gametocytes from red blood cells

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Question: Transmission of malaria parasites to the mosquito is mediated by sexual precursor cells, the gametocytes. Upon entering the mosquito midgut, the gametocytes egress from the enveloping erythrocyte while passing through gametogenesis. Egress follows an inside-out mode during which the membrane of the parasitophorous vacuole ruptures prior to the erythrocyte membrane. Membrane rupture requires the exocytosis of specialized secretory vesicles of the parasites; i.e. the osmiophilic bodies (OBs) involved in rupturing the parasitophorous vacuole membrane, and vesicles (here termed P-EVs) that harbour the perforin-like protein PPLP2 required for erythrocyte lysis. To date, only some OB proteins are known, like G377 and MDV1/Peg3, and protein composition of the P-EVs remains unclear.

Methods: We used high-resolution imaging and BioID methods to study the two types of egress vesicles, OBs and P-EVs, in *Plasmodium falciparum* gametocytes in order to unveil their proteomes and modes of action.

Results: We show that OB exocytosis precedes discharge of the P-EVs and that exocytosis of the P-EVs, but not of the OBs, is calcium-sensitive. Further, the two types of vesicles exhibit distinct proteomes with the majority of proteins being assigned to the OBs. In addition to known egress-related proteins, our analyses revealed novel components of OBs and P-EVs, including proteins involved in vesicle trafficking.

Conclusions: Our data provide insight into the immense molecular machinery required for the inside-out egress of *P. falciparum* gametocytes.

EKMPP 322

The role of putative mTORC-related kinases in translational regulation during *Plasmodium falciparum* transmission

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Introduction: During human-to-mosquito transmission the malaria parasite *Plasmodium falciparum* quickly needs to adapt to a new environment. Among others, a specific protein repertoire must be synthesized for host cell egress and the completion of the sexual reproduction phase in the mosquito midgut. Transcripts coding for some of these proteins are already synthesized in the human host, while translation is initiated only after transmission. We recently identified the protein 7-Helix-1 as a crucial component in the process of translational re-initiation. 7-Helix-1 was shown to interact with known ribonucleoproteins and form a complex with repressed mRNAs. In silico analyses further demonstrated that 7-Helix-1 is homologous to the human stress regulator hLanCL2, which among others is involved in the mTORC-pathway. Although most of the mTORC-components are not present in *P. falciparum*, we hypothesize that 7-Helix-1 regulates translation via an mTORC-like signaling cascade.

Methods: In order to decipher the potential link between 7-Helix-1, mTORC-related components and translation, we will characterize the downstream kinases of the mTORC-pathway, KIN, PI3K and S6K, which are present in *P. falciparum*. Gene and protein expression of the kinases in the gametocyte stages is analysed by RT-PCR and IFA, respectively. To investigate the role of the three kinases during translational regulation, knockout (KO) parasite lines are generated and characterized. Further, the KO studies will be complemented with chemical inhibition/activation studies using commercial inhibitors and activators.

Results: RT-PCRs revealed transcript expression for all three kinases that might be involved in an mTORC-like pathway in mature and activated gametocytes of *P. falciparum*. For S6K, a granular localization in gametocytes could be demonstrated, similar to the expression pattern of 7-Helix-1. Potential co-localization and interaction of 7-Helix-1 with the kinases will be investigated in future experiments. Further, an S6K-KO parasite line has been generated which will be phenotypically characterized. The KO will be used to confirm the phosphorylation activity of S6K and to investigate the role of S6K in translation re-initiation during transmission.

Discussion: The mTORC pathway is one of the most important signaling cascades in mammalian cells integrating nutrient availability and regulating protein translation. Although *Plasmodium* has lost most of the mTORC components, three downstream kinases, KIN, PI3K and S6K, are present. In this project, we will investigate the potential role of these proteins in translational regulation during transmission of the parasite. Data gained in this project will help us understand how the parasite is able to specifically regulate protein synthesis depending on the environment.
