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ABSTRACTS

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DEUTSCHE GESELLSCHAFT FÜR HYGIENE UND MIKROBIOLOGIE E.V.

ABSTRACTS

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Workshop 1
Eukaryotic Pathogens (FG EK)
05. Sep 2022 • 13:30–15:00

001/EKV
The role of *Candida albicans* zinc acquisition and filamentation-associated genes in intestinal epithelial cell interaction

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The opportunistic fungal pathogen *Candida albicans* thrives on human mucosal surfaces as a harmless commensal. However, translocation across the intestinal barrier into the bloodstream by intestinal-colonizing *C. albicans* cells can cause disseminated candidiasis. The mechanisms required for this process remain unclear.
We showed that the fungal cytolytic peptide toxin, candidalysin (CaL), is essential for damage of enterocytes and subsequent fungal translocation using an *in vitro* model of intestinal epithelial cells grown in a transwell system. However, invasion and low-level translocation also occurred in a CaL -independent manner, suggesting additional mechanisms.
To shed more light on these processes, we performed dual-RNA sequencing to identify *C. albicans* and host genes differentially expressed during infection as compared to growth in culture medium. Focusing on the fungal side, we observed altered expression of genes involved in Zn-homeostasis. In contrast, genes of the core filamentation response (CFR) network, *IHD1*, *HGT2*, *DCK1*, *RBT1*, *ECE1*, *HWP1*, *ALS3* and orf19.2457, mostly showed similar expression patterns during infection and in hypha-inducing culture medium. While some of these genes, like *ALS3* and *HWP1*, are well-characterized regarding their function in *Candida*-epithelial cell interaction, the function of other CFR genes is unknown. Strains lacking CFR genes as well as Zn-uptake and -storage genes were therefore investigated during enterocyte infections to elucidate their role for intestinal invasion and translocation.
Our data suggests that genes required for Zn-uptake and -storage may play an important role in the context of these processes, since mutants lacking these genes were attenuated in their ability to cause damage and translocate when starved of Zn prior to infection. Interestingly, their damage potential was rescued by addition of Zn to the medium. By staining intracellular Zn in *C. albicans* hyphae during invasion of enterocytes, we observed an increased Zn-starvation response in a mutant lacking CaL compared to the parental wild type. Intestinal damage cause by *C. albicans* may therefore be essential to sequester host Zn during infection.
Furthermore, we confirmed previous observations by showing reduced adhesion and damage of mutants lacking adhesin-encoding genes *ALS3* and *HWP1*, as well as reduced epithelial damage and translocation by a mutant lacking CaL.
Of note, we monitored decreased adhesion and translocation for a mutant lacking the CFR gene *RBT1*. Rbt1 may therefore represent a new fungal factor involved in translocation trough the intestinal epithelial barrier in a CaL -independent way.

002/EKV
Proteomic analysis reveals that *Aspergillus fumigatus* flippase Rta1 is involved in Amphotericin B resistance

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Question: The filamentous fungus *Aspergillus fumigatus* is an opportunistic human pathogen, which can cause mycoses and allergies in susceptible individuals. Invasive pulmonary aspergillosis (IPA) is the most severe form of infection, most often seen in patients with immunosuppression. IPA is characterized by a high mortality rate ranging from 25-90%. With regard to therapy, the polyene drug amphotericin B (AmB), despite its toxicity, is still frequently used to treat IPA. This is due to the fact that AMB shows broad spectrum antifungal activity and is also effective against azole-resistant Aspergillus strains. AmB binds to ergosterol in the fungal membrane, but its mode of action has not been completely elucidated. To get further insights into the drug mechanisms of AmB, we investigated the proteomic profile of *A. fumigatus* in response to AmB and its lipososomal formulation.
Methods: We compared the proteomes of *A. fumigatus* cultivated in the presence of sublethal concentrations of AmB and liposomal AMB with untreated control cultures using liquid chromatography-tandem mass spectrometry. Selected proteins with significant increase in abundance upon AmB exposure were characterized further by the construction and characterization of single-gene deletion mutants in *A. fumigatus*.
Results: Proteomic analyses revealed a significant increase (≥ 4-fold) in abundance of 65 different proteins in response to AmB and liposomal AmB, while the level of 5 and 21 proteins decreased, respectively. In particular, the level of proteins anchored to the membrane, involved in catabolic processes or secondary metabolism increased prominently. Of particular note was the more than 300-fold increase of a RTA1 domain-containing protein after AmB treatment. Fungal RTA-like proteins represent lipid-translocating exporters, which are characterized by multiple transmembrane regions and often confer resistance to toxic chemicals. Indeed, the deletion of the corresponding gene in *A. fumigatus* led to an increased susceptibility to AmB and other antifungal polyenes.
Conclusions: The *A. fumigatus* flippase Rta1 is involved in mediating resistance to the antifungal drug AmB, most likely by maintaining lipid homeostasis and membrane stability.

003/EKV
Ergosterol biosynthesis in *Aspergillus fumigatus* – intricating differences

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The ergosterol biosynthesis pathway of fungi is a major target for current antifungals. Of the few antifungal classes currently available to treat fungal infections in human, the azoles, the allylamines and the morpholines specifically inhibit enzymes in this pathway. We studied the functional roles of several key ergosterol biosynthesis enzymes for growth and viability of the opportunistic fungal pathogen *Aspergillus fumigatus*. This mold causes severe life-threatening invasive infections in immunocompromised patients which are associated with extraordinarily high mortality rates. Our results indicate that inhibition/inactivation of these enzymes has different consequences for the viability and unexpected effects on the antifungal susceptibilities of this pathogen. These results will help to understand the antifungal activities of key agents used to treat infections caused by this mold and highlight the potential of other ergosterol biosynthetic enzymes as antifungal targets.

004/EKV

Influence of *Giardia duodenalis* attachment on infection-induced epithelial barrier breakdown in organoid-derived monolayers (ODMs)

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Introduction: As a widespread protozoal parasite, *Giardia duodenalis* is a common cause for abdominal pain, malabsorption and diarrhea. Intestinal epithelial barrier defects are frequently observed in patients affected by the multifactorial disease giardiasis, which is characterized by "leaky" barriers due to the disturbance of the tight junction complex. While the exact mechanisms leading to epithelial barrier breakdown remain unknown, suggested triggers include the secretion of virulence factors, proteases or caspases as well as mechanosensing from the host side due to parasite attachment. We aim to elucidate the influence of epithelial *Giardia* attachment on barrier integrity during infection of human intestinal organoids with transgenic *G. duodenalis* parasites.
Material/Methods: Targeting different proteins of the adhesive disc, genetically modified *Giardia* parasites with varying attachment abilities were generated. Using human organoid-derived monolayers (ODMs) cultured in a compartmentalized transwell system, infection dynamics were monitored via transepithelial electrical resistance (TEER) measurements, which serve as an indicator of monolayer integrity and, thus, epithelial barrier breakdown. In addition to infection with transgenic parasites, attachment was also hampered through physical barriers as well as formononetin administration.
Results: Preliminary experiments revealed that infection of ODMs with attachment-impaired *Giardia* trophozoites results in slightly reduced TEER loss compared to the wildtype strain. Consistent with these findings, inhibition of attachment with chemical factor formononetin resulted in epithelial barrier disruption comparable to non-restricted infection setups.
Discussion: Prior experiments revealed that treatment with *Giardia* lysates could not induce and protease/caspase inhibition could not prevent epithelial barrier breakdown in human ODMs. Thus, parasitic attachment was speculated to be the initial trigger leading to barrier disruption. Our results suggest a rather minor influence of attachment on *Giardia*-induced barrier breakdown. Instead, the mere parasite presence seems to be sufficient as a trigger – potentially due to production and secretion of virulence factors or withdrawal of nutrients and/or other factors from the host.

005/EKV

The intricated ways of the Ypd1-mediated signaling in *Aspergillus fumigatus*

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Introduction: The HOG pathway is a crucial stress pathway in *A. fumigatus* that consists of a multistep-phosphorelay and a MAP kinase cascade. This study is largely focused on the former part that consists of three elements: the group III hybrid histidine kinase (HHK) TcsC, the phosphotransfer protein (Hpt) Ypd1 and the two response regulators SskA and Skn7. A suitable stress situation will activate TcsC that is then assumed to transfer the signal via Ypd1 to the two stress regulators.
Methods: To scrutinize the role of the elements participating in this signal transduction pathway, we created various mutants that either lack components of the HOG pathway or express them in a controlled manner. Furthermore, we expressed various GFP fusion proteins to analyze the localizations and dynamics of HOG pathway components.
Results: Apart from environmental stress conditions, TcsC can also be artificially activated using agents such as the *Pseudomonas*-derived compound pyrrolnitrin or its chemical derivative fludioxonil. This pharmacological activation is permanent and lethal for the fungus and results in cellular swelling and lysis. We could recently show that this antifungal activity relies largely on the response regulator Skn7, whereas SskA is of minor importance. The fludioxonil-induced interplay between TcsC, Ypd1 and Skn7 is still an open issue. In recent work, we have shown that Ypd1 Is an essential protein for *A. fumigatus* and that the histidine residue at position 89, which is well-conserved in other HPt proteins, is crucial for the ability of Ypd1 to transfer phosphoryl groups to Skn7 (and SskA), which is supposed to keep them in a resting state. GFP localization experiments show a distinct localization, but for certain components also a controlled shuttling between different cellular compartments.
Discussion: Two questions arise from our data, namely – what is the precise function of TcsC in the multistep-phosphorelay and is a phosphorylated Skn7 protein indeed inactive. In recent time, we have mainly concentrated on TcsC. We will provide evidence that sheds new light on the function of TcsC and furthermore demonstrates that the localization of the different elements of the multistep-phosphorelay is a key factor that has to be taken into consideration in order to understand the function of this unique and fascinating signaling module.

006/EKV

Filamentation is not always essential for pathogenesis in systemic candidiasis

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The ability to undergo the yeast-to-hypha transition is believed to be a key virulence factor of *Candida albicans*. Hyphae facilitate active penetration of host cells, host cell damage, and escape from immune cells. In addition, *C. albicans* mutants locked in the yeast morphology have been shown to be avirulent or attenuated in systemic infections further emphasizing the importance of hyphae for virulence.
The *C. Albicans eed1*Δ/Δ mutant is able to initiate germ tube formation but fails to elongate these into hypha and eventually switches back to yeast cell growth. Despite being attenuated in damaging epithelial cells and macrophages *in vitro* and in virulence in an intraperitoneal infection model *in vivo*, the *eed1*Δ/Δ mutant was, to our surprise, as virulent as the WT in systemic candidiasis. Mice that succumbed to infection with the filament-deficient yeasts showed no differences to WT infected mice regarding severity of clinical symptoms, systemic inflammation, kidney dysfunction and injury. However, retained virulence was associated with rapid yeast proliferation leading to high organ fungal loads especially in the kidney. Massive proliferation was likely the result of metabolic adaptation and improved fitness as the mutant showed enhanced growth on physiologically relevant carbon sources and increased replication in macrophages.
Although yeast fungal burden increased early after systemic infection, renal cytokine production was delayed in response to the *eed1*Δ/Δ mutant, whereas increased immune cell infiltration was observed at later time points. Together with slower progression of systemic candidiasis caused by the *eed1*Δ/Δ mutant in immunosuppressed mice, this suggests that differences in immunopathology contribute to the virulence of this yeast.
These findings demonstrate that lack of filamentation is not necessarily associated with reduced virulence in systemic candidiasis by *C. albicans*.

Workshop 2
Clinical Cases in Medical Microbiology/TED (StAG KM)
05. Sep 2022 • 13:30–15:00

007/KMV

Ulceroglandular form of tularemia after squirrel bite – a case report

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Introduction: Tularemia, also called "rabbit fever", is a rare, but potentially severe zoonosis caused by *Francisella tularensis*. The disease has been described for more than 250 animal species, including mammals, birds, amphibians, fish, and invertebrates, which can potentially transmit the bacterium to humans. Infection of humans can also occur through mosquito or tick bites, contaminated food or water. Human-to-human transmission has not been reported yet. Because of the low prevalence of the disease in Germany, physicians might be unaware or underestimate tularemia and in consequence, the diagnosis might be quite late in the course of disease leading to a delay in the specific treatment.
Objectives: Here, we present the case of a 59-year-old female, who was admitted to the hospital seven days after a squirrel bite with an ulcerative lesion at the digit IV of her right hand and lymphadenitis in her right elbow with persistent fevers, myalgia, and headache.
Material & methods: The diagnosis of tularemia was based on a specific real-time PCR for *F. tularensis* and on cultivation of *Francisella* from the wound swab. Laboratory tests on blood, cerebrospinal fluid and urine (e.g. blood count, C-reactive protein [CRP], procalcitonin) were performed. X-rays of the thorax, the digit and a native Computed Tomography (CT) scan of the brain were done. Serum was checked for specific antibodies and DNA from the isolated bacterial strain (*Fth* A-1825/1), from the patient"s wound swab, has been sequenced.
Results: A patient with an ulcerative lesion at the digit, lymphadenitis in the elbow and ongoing symptoms despite a consequent antibiotic therapy with cefuroxime (500 mg twice a day) seven days after having been bitten by a wild squirrel (*Sciurus vulgaris*) was admitted to the hospital. Laboratory tests showed an increase in CRP, no leukocytosis, and procalcitonin was negative. Samples of cerebrospinal fluid and urine were normal. After another unresponsive seven days of therapy with piperacillin/tazobactam, tularemia was confirmed by real-time polymerase chain reaction (PCR) on day 14. Immediately, antibiotic treatment was changed to orally advised ciprofloxacin (500 mg twice a day) and the patient recovered rapidly. On day 14 after the bite, antibodies against the lipopolysaccharid of *F. tularensis* increased.
Conclusion: The risk to be bitten and/or infected by a wild red squirrel is low. On the other hand, squirrels represent a reservoir of *F. tularensis* and an increase of tularemia cases in humans and animals could be observed in Europe during the past decades. Therefore, tularemia should be considered as a diagnosis after an animal bite, when presented together with headache, growing pains, lymphadenitis and fever in combination with impaired wound healing. Specific real-time PCR analysis from wound swabs and antibody detection by e.g. ELISA are suitable methods for the identification of the pathogen.

008/KMV

Case of anisakiasis after consumption of raw fish in Germany

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Introduction: Anisakiasis is a zoonosis caused by anisakid nematodes, after oral ingestion of third-stage larvae in raw sea fish (1,2).
Case Report: A 52-year-old female patient presented to our gastroenterology department with a 24 hours history of epigastric pain. She reported having vomited once with bloody tinges. Additionally, she described a feeling of fullness and loss of appetite. She reported eating raw fish two days before the onset of symptoms. In a subsequent gastroscopy, multiple worm-like structures were seen, some of which were attached to the gastric mucosa. For further diagnosis, a parasite was gastroscopically recovered and presented as a 3-4cm large and approx. 2 mm thick roundworm-like structure (Figure 1). Repeated microscopy of stool (n=3) did not reveal worm eggs and a multiplex PCR for helminths (including *Hymenolepis* spp., *Ascaris* spp., *Taenia* spp., *Trichuris trichiura, Ancylostoma* spp., *Enterobius vermicularis*, *Necator americanus*, *Strongyloides* spp., and *Enterocytozoon* spp.) remained negative. Based on the macroscopic appearance and the patient's history, the parasite was identified as an anisakid larva and the diagnosis of anisakiasis was made.
Background: The incidence of anisakiasis worldwide is estimated at 0.32/100,000, with countries in the far-east, like Japan, accounting for the largest number of cases. In Europe, the number of cases is estimated to be less than 20 per country per year (3). The natural reproductive cycle of this parasite begins in large marine mammals, such as dolphins, in which the adult worm grows and secretes eggs. The eggs are excreted in the faeces and the hatched larvae are ingested by small crustaceans, which in turn are eaten by sea fish. If marine mammals eat the fish, the cycle is completed. Humans are incidental hosts and the larvae cannot develop or reproduce, but *Anisakis* spp. is able to penetrate gastric mucosa, causing gastrointestinal symptoms. Affected patients describe abdominal pain in a time interval of a few days after eating raw fish and the diagnosis can be made by gastroenteroscopy. Therapeutically, the parasite can be extracted mechanically, an anthelminthic therapy with albendazole may be attempted (1,2).
Conclusion: As a rare zoonosis, anisakiasis should also be considered in Europe in cases of sudden onset gastrointestinal pain associated with a history of eating raw fish.

References

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Figure 1. Third-stage larva causing anisakiasis

009/KMV

Atypical case of chronic sternal wound infection with *Coxiella burnettii*

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Patient case: A 74-year-old male patient was admitted to the University Hospital with a parasternal fistula following sternotomy several years prior for replacement of the aortic valve and ascending aorta with an aortic valved conduit due to aortic valve stenosis and an aortic aneurysm. On admission, the patient reported fatigue, hip pains radiating to the left knee and dyspnea. Comorbidities included a long history of cardiovascular disease, chronic heart failure and metabolic syndrome (diabetes mellitus type 2, hypertonus, hyperlipidemia). During the resection of the fistula and wound debridement, several intraoperative samples were taken for microbiological diagnostics. The patient was discharged after an uncomplicated clinical course and successful wound healing.

While culture-based microbiology detected *Staphylococcus epidermidis* from two intraoperative swabs and one retrosternal biopsy, 16S PCR and sequencing revealed DNA of *Coxiella burnetii* in an additional retrosternal tissue specimen. As the patient had already been discharged, the diagnosis was confirmed by serology in an external laboratory with positive IgM and IgG > 500 IU/ml. Subsequently, the patient was readmitted for further diagnostics and treatment.

Clinical course: On admission, the patient reported progressive dyspnea and fatigue but no fevers or myalgia. The targeted medical anamnesis revealed that he had tended sheep two years prior. Inflammatory parameters (e.g. CRP) were slightly elevated. A PET-CT confirmed a hypermetabolic infection of the pre-described fistulous tract as well as a graft infection of the ascending aorta. Surgical intervention was declined due to the risk associated with multiple preexisting illnesses and according to the patient"s wish. Therapy with doxycycline and hydroxychloroquine to treat *C. burnetii* infection was started and planned to be continued for a minimum of 18 months. The suspected S. epidermidis tissue infection was initially treated with vancomycin followed by daptomycin for four weeks but was discontinued due to severe side effects and the general palliative approach. Unfortunately, the patient died of acute heart failure approximately 2 months after the diagnosis. A connection between hydroxychloroquine therapy and the progressing heart failure could not be ruled out.

Conclusion: Wound and tissue infection by *C. burnettii* is a rare event. The detection of *C. burnetii* in a restrosternal tissue specimen shows the value of 16S PCR not only in culture negative endocarditis, but also in other primarily sterile patient samples. Infections of vascular grafts due to *C. burnetii* are probably underdiagnosed due to a lack of specific symptoms and the awareness of attending physicians for this pathogen.

010/KMV

Endocarditis caused by *Salmonella enterica* serotype Choleraesuis variant Kunzendorf in a patient with an aortic valve prosthesis

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Background: *Salmonella enterica* serovar Choleraesuis is a facultative anaerobic Gram-negative bacterium, which has been characterized as highly invasive pathogen among the non-typhoidal Salmonella (NTS). Invasive infections include sepsis and extra-intestinal infections such as infective endocarditis (IE). Case reports and case series in the previous literature report on patients in Asia or on patients in Europe with a travel history.

Case report: We report on a 63-year-old male patient who developed fever three months after receiving a biological aortic valve prosthesis. The patient reported no travel history. Transesophageal echocardiography (TEE) showed undulating vegetations on the prosthetic leaflets and paravalvular abscesses. C-reactive protein and procalcitonin levels were 129.8 mg/L and 6.98 ng/mL, respectively. The patient underwent surgical replacement of the infected prosthetic valve and aortic trunc. Blood cultures and microbiological cultures of the prosthetic valve tissue revealed *Salmonella spp.* based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) identification. Antimicrobial susceptibility testing of the isolate was performed with the Microscan Walk Away system revealing minimal inhibitory concentrations of (in µg/mL) ceftriaxone ≤1 and ciprofloxacin ≤0.06, besides others. Due to inconclusive preliminary agglutination, the isolate was forwarded to the National Reference Centre and subsequently identified as *Salmonella enterica* serotype Choleraesuis variant Kunzendorf.

Antibiotic treatment was initiated with ceftriaxone und daptomycin and later tailored to ceftriaxone. Ceftriaxone was recommended at discharge to be continued for six weeks. Five weeks after replacement of the prosthetic valve, the patient developed fever again. TEE showed a dehiscence in the area of the aortic trunc, which necessitated the re-replacement of the aortic valve prosthesis. Antibiotic treatment was started with ceftriaxone, daptomycin, ciprofloxacin and gentamicin. Ciprofloxacin was discontinued after day 9 and gentamicin was replaced by oral rifampicin. The patient"s condition improved substantially, and he was discharged eight weeks after the second valve re-replacement. Outpatient antibiotic treatment was recommended for eight weeks and included rifampicin, doxycycline as well as ciprofloxacin. The patient remained afebrile and satisfied on follow-up visits to the cardio thoracic outpatient clinic up until 3 months after the second IE episode.

Discussion: A complete microbiological diagnostic workup is essential to identify NTS as unusual pathogen in patients with IE even if no travel history is reported. Predisposing factors for IE due to NTS include cardiac prostheses and endovascular grafts. Multidisciplinary teamwork between microbiologists, cardiac thoracic surgeons and clinical infectious diseases specialists is essential to successfully treat patients with NTS endocarditis.

Workshop 3
Infection Immunology (FG II)
05. Sep 2022 • 13:30–15:00

011/IIV

The gut microbiota prime systemic antiviral immunity via the cGAS-STING-IFN-I axis

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Introduction: The microbiota are vital for immune homeostasis and provide a competitive barrier to bacterial and fungal pathogens. The innate immune receptors involved in host sensing of the microbiota and type I interferon (IFN-I) priming remain elusive. Moreover, how these obligate extracellular microbes at host barrier surfaces communicate with distal immune cells to mediate systemic immune modulation is unresolved. Therefore, we investigated how these commensal bacteria communicate with and modulate immune cells, and how this impacts viral infections.

Material/methods: By employing the in vivo imaging system (IVIS), we monitored in real-time the basal IFN-I levels in *Ifnb1* luciferase reporter mice, proficient or deficient in innate immune pathways induced by Toll-like receptors, Rig-I-like receptors and/or cGAS-STING after oral antibiotic treatment. Additionally, we analysed the transcriptional changes by RNA-seq in different

organs. We performed 16S rDNA sequencing of DNA isolated from bacterial membrane vesicles (MVs) from mouse serum and compared it with DNA isolated from fecal pellets to investigate how the gut microbiota communicate with distal sites/organs. Using different *in vivo* mouse models of infection, we investigated the impact of the microbiota and bacterial MVs on infections with the RNA virus, vesicular stomatitis virus (VSV), and the DNA virus, herpes simplex virus type 1 (HSV-1) and determined the relevance of the microbiota-driven tonic activation of the cGAS-STING-IFN-I axis for efficient viral clearance.
Results: Antibiotic suppression of the gut microbiota in mice reduces tonic type I interferon (IFN-I) level, resulting in decreased clearance of HSV-1 or VSV. We demonstrate that microbiota-driven antiviral priming is due to activation of the cGAS-STING-IFN-I axis, as its disruption dampens the tonic IFN-I-response and increases the viral replication in control but not antibiotic-treated animals. Mechanistically, priming does not require direct host-bacteria interactions but involves MV-mediated delivery of bacterial DNA into distal host cells. We found that DNA-containing MVs from the gut microbiota circulate systemically through blood and that purified MVs can prime the cGAS-STING-IFN-I axis thereby promoting the clearance of both DNA and RNA viruses.
Discussion: This study establishes the importance of the microbiota in host resistance to viral infections and highlights an unanticipated role of the cGAS-STING pathway in remote sensing of the microbiota. Moreover, it uncovers an underappreciated risk of antibiotic use during viral infections (*IMMUNITY*; May 10, 2022; 55 (5)5: 847-861).

012/IIV
Non-hematopoietic IL-33 negatively regulates IFNg- and IL-22 dependent antibacterial immune response in pneumonia
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Introduction: The innate immune response to infections is vital for preserving lung function during pneumonia as it fights the invading microbes. An unrestricted inflammation, however, can also lead to excessive tissue damage. In this study, we tested the hypothesis that alarmins are released during *Streptococcus pneumoniae* infection to regulate immune responses during pneumonia.
Material/method: An established mouse model of transnasal infection with *S. pneumoniae* was used to understand the role of the IL-33/IL1RL1 axis in pneumococcal pneumonia. To find correlations between human single nucleotide polymorphisms (SNPs) and hospitalizations with pneumococcal pneumonia, DNA samples provided by CAPNETZ Stiftung were screened for SNPs in the genes *IL33* and *IL1RL1* and frequencies were compared to age- and sex- matched controls.
Results: Our data demonstrate that *S. pneumoniae* infection leads to the release of several alarmins including uric acid, ATP and IL-33. Experiments with specific knock-out mice, inhibitors and degrading enzymes revealed that IL-33, but not uric acid or ATP, negatively regulates antibacterial immunity through its receptor IL1RL1. Bone-marrow chimera and scRNAseq experiments identified non-hematopoietic cells - most likely type 2 alveolar epithelial cells (AEC2) - as the relevant source of IL-33. IL-33 negatively regulates IFN-g and IL-22 production, resulting in impaired control of pneumococcal infection. We could also show that this mechanism is independent of IL-4/IL-13 signaling. Moreover, single nucleotide polymorphisms in *IL33* and *IL1RL1* were found to be associated with pneumococcal community-acquired pneumonia.
Discussion: Collectively, we show that upon *S. pneumoniae* infection, epithelial IL-33 negatively regulates bacterial defense by suppressing IFN-g and IL-22 production. Ongoing work aims to further unravel the cellular and molecular mechanisms of (i) how IL-33 controls IFN-g and IL-22 production and (ii) how these mediators affect the course of pneumococcal pneumonia.

013/IIV
Low pH found in cutaneous *Leishmania* lesions dampens NO-dependent antileishmanial macrophage function
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While information on local tissue acidosis and its effects on anti-tumor immunity is well investigated, data on tissue pH levels in infected tissues and their impact on antimicrobial activity are sparse. By studying the effects of cutaneous leishmaniasis, we found pH levels in Leishmania lesions to be decreased to 6.7, thereby indicating that Leishmania major-infected skin lesions are acidic. With the decreased extracellular pH the ability of lipopolysaccharide (LPS) and interferon γ (IFN-γ)-coactivated macrophages to produce leishmanicidal NO and thereby to fight the protozoan parasite Leishmania major was diminished significantly. NOS2 expression was similar in low and normal pH conditions and a shortage of reducing equivalents (NAPDH) or substrate (L-arginine) was excluded as well. We found the reason behind decreased NO levels to be a direct, pH-mediated inhibition of NOS2 enzyme activity. Reconstitution of physiological intercellular conditions significantly increased NO production and antiparasitic activity of macrophages even in an acidic extracellular microenvironment. In summary these findings point to a negative effect of low local tissue pH on NO production and leishmanicidal activity of macrophages. Modification of tissue pH holds a great potential to ameliorate antileishmanial defenses.

014/IIV
Expression of Arginase 1 during *Leishmania mexicana* infection affects the differentiation of monocytes and thereby causes chronicity of disease
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Introduction: Control of *Leishmania* (*L.*) parasites require IFNγ-dependent type 2 nitric oxide synthase (NOS2), an enzyme that converts L-arginine into citrulline and nitric oxide (NO). NOS2 activity is counteracted by arginase (ARG) 1 and 2, both of which are induced by Th2 cytokines and cleave L-arginine into urea and ornithine, a precursor of polyamines necessary for cell proliferation. Recently, we observed that the expression of ARG1 and ARG2 steadily increased in *L. mexicana*-infected BALB/c and C57BL/6 mice during disease manifestation. Here, we studied the functional role of host cell arginases during *L. mexicana* induced chronic cutaneous leishmaniasis (CL).

Methods: C57BL/6 wild-type (WT), germ-line knockout ($Arg2^{-/-}$) or cell-specific conditional knockout mice ($Arg1^{\Delta Tek}$, $Arg1^{\Delta Tek}Arg2^{-/-}$, $Arg1^{\Delta Cx3cr1}$, $Il10^{\Delta Cd4}$) were infected into the skin with 3×10^6 stationary-phase *L. mexicana* promastigotes. Infected skin tissue was processed for mRNA/protein expression, metabolites and scRNAseq analyses at different time-points post infection.
Results: C57BL/6 WT mice developed non-healing chronic CL, whereas $Arg2^{-/-}$ mice showed progressive disease with a delayed onset. In contrast, mice with a deletion of Arg1 in hematopoietic and endothelial cells ($Arg1^{\Delta Tek}$) and $Arg1^{\Delta Tek}Arg2^{-/-}$ double-deficient mice exhibited a strongly reduced pathology and ultimately resolved their skin lesions despite parasite persistence. A similar phenotype was observed in mice deficient for Arg1 in monocytes and macrophages ($Arg1^{\Delta Cx3cr1}$), suggesting that myeloid Arg1 accounts for chronic CL. Mice lacking IL-10 in $CD4^+$ T cells ($Il10^{\Delta Cd4}$) revealed IL-10 as a factor inducing ARG1 during infection. LC-MS metabolomics verified that high amounts of ARG1 caused depletion of L-arginine and a significant rise in polyamines in the infected WT skin and draining lymph node tissue. To unravel the mechanism further, we analyzed mice at 40 days p.i. when Arg1 mRNA was already upregulated in WT mice, but disease manifestation, parasite burden and the metabolic profile were still comparable between WT and Arg1-deficient mice. First, we checked whether increased ARG1 expression impeded NOS2 activity. Surprisingly, similar levels of NO were detected in WT and ARG1-deficient mice, although the expression of NOS2 protein in the infected skin was much higher in WT mice compared to ARG1-deficient mice. ScRNASeq analysis of viable skin lesion cells revealed distinct myeloid subpopulations that were enriched in WT mice, including a prominent $Arg1^+NOS2^+$ cluster of inflammatory macrophages. Trajectory analysis pointed to an altered differentiation of recruited monocytes in the presence of ARG1.
Conclusions: We propose that ARG1-dependent signals change the recruitment and the differentiation of monocytes in the skin of *L. mexicana*-infected mice. This leads to an enhanced pro-inflammatory immunopathology and provides cellular niches that favor parasite replication due to ARG1/NOS2 substrate competition.

015/IIV

Macrophages inhibit *Coxiella burnetii* by the ACOD1-itaconate pathway for containment of Q fever

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Infection with the intracellular bacterium *Coxiella (C.) burnetii* can cause chronic Q fever with severe complications and limited treatment options. Here, we identify the enzyme cis-aconitate decarboxylase 1 (ACOD1 or IRG1) and its product itaconate as protective host immune pathway in Q fever. Infection of mice with *C. burnetii* induced expression of several anti-microbial candidate genes, including *Acod1*. In macrophages, *Acod1* was essential for restricting *C. burnetii* replication, while other antimicrobial pathways were dispensable. Intratracheal or intraperitoneal infection of *Acod1-/-* mice caused increased *C. burnetii* burden, significant weight loss and stronger inflammatory gene expression. Exogenously added itaconate restored pathogen control in *Acod1-/-* mouse macrophages and blocked replication in human macrophages. In axenic cultures, itaconate directly inhibited growth of *C. burnetii*. Finally, treatment of infected *Acod1-/-* mice with itaconate efficiently reduced the tissue pathogen load. Thus, ACOD1-derived itaconate is a key factor in the macrophage-mediated defense against *C. burnetii* and may be exploited for novel therapeutic approaches in chronic Q fever.

016/IIV

The deubiquitinating enzyme OTUB2 fosters macrophage function during listeriosis and upon Poly(I:C) stimulation

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Introduction: The dynamic process of ubiquitination and deubiquitination plays a key role in the regulation of host cell responses in bacterial and viral infections. Ubiquitin molecules are covalently linked to target proteins, either by one of its seven lysine residues or by its N terminus methionine in a process known as ubiquitination. Ubiquitination is catalyzed by three different ubiquitinating enzymes, namely E1, E2 and E3, and regulates various cellular processes by modulating protein stability, protein trafficking and protein-protein interactions. Deubiquitinating enzymes (DUBs) antagonize this process by cleaving ubiquitin residues from substrates. Among the target molecules are proteins of the NF-κB pathway and MAP kinases, signaling cascades, which are important for the initiation and regulation of innate and adaptive immune responses. The DUB Otubain-2 (OTUB2) is a cysteine protease with substrate specificity to K11, K48 and K63 linked ubiquitin chains. It is known that OTUB2 deubiquitinates TRAF3 and TRAF6 and thereby inhibits Sendai virus-induced NF-κB activation. However, the role of OTUB2 in bacterial infections is unknown.
Material/Methods: To study the macrophage-specific role of OTUB2 in bacterial infections, we generated a novel conditional mouse strain lacking OTUB2 in myelomonocytic cells (LysM-Cre $OTUB2^{fl/fl}$) and infected WT and LysM-Cre $OTUB2^{fl/fl}$ mice with *Listeria monocytogenes*.
Results: Our preliminary data show that LysM-Cre $OTUB2^{fl/fl}$ mice harbored significantly higher bacterial loads in the liver at day 3 p.i. compared to the WT mice. *In vitro*, the activation of the NF-κB pathway and MAP kinases was reduced in OTUB2-deficient *Listeria*-infected bone marrow-derived macrophages (BMDM) compared to WT macrophages. In good agreement with the Sendai virus experiments, we could also detect a reduced activation of p-IRF3 and p-p65 in OTUB2-deficient BMDM upon stimulation with Poly(I:C), which mimics viral infection.
Discussion: Taken together, our data show that OTUB2 contributes to the control of *Listeria* in infected macrophages by augmenting protective NF-κB and MAP kinase signaling.

017/IIV

***Streptococcus pneumoniae* affects human dendritic cell function in a pneumolysin dependent manner**

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Introduction: Dendritic cells (DCs) are important antigen presenting cells of the innate immune system. They are widely distributed throughout the human body including mucosal surfaces of the respiratory tract. DCs are able to undergo a maturation process in response to bacterial as well as viral infections including Influenza A Virus (IAV), *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*). Once activated, DCs upregulate the expression of peptide-presenting molecules, co-stimulatory receptors and release cytokines to shape activation and differentiation of T cells.
Material/Methods: In our experiments, human monocytes were isolated and subsequently stimulated to obtain primary monocyte-derived-DCs (moDCs). We infected these moDCs with the mentioned pathogens in single as well as viral-bacterial co-infections and investigated their impact on DC function. Overall, we analyzed DC viability, expression of activation markers including CD80, CD86, CD40, MHCI and MHCII and cytokine production via flow cytometry.
Results: We observed that irrespective of single or co-infection *S. aureus* and IAV lead to an activation of moDCs and subsequently T cells. In contrast, pneumococci failed to induce DC maturation.

Pneumococci-infected DCs were characterized by low expression of the co-stimulatory molecules CD80 and CD86 and an additional downregulation of CD40. In addition, pneumococci infected DCs exclusively activate CD8+ and double negative T-cells but not CD4+ T-cells. These effects were not present when we used a mutant deficient for pneumolysin.

Discussion: This study highlights the impact of the well known pneumococcal virulence factor pneumolysin on human DC maturation and function. We show that in pneumococcal infections expression of different DC activation markers were suppressed by pneumolysin and that infected DCs failed to induce a CD4+ T-cell response [1].

1. Paulikat, A.D., et al., *Streptococcus pneumoniae* Impairs Maturation of Human Dendritic Cells and Consequent Activation of CD4+ T Cells via Pneumolysin. Journal of Innate Immunity, 2022.

018/IIV

Mitochondrial signaling modulates cytokine secretion and DNA-damage during *Helicobacter pylori* infection

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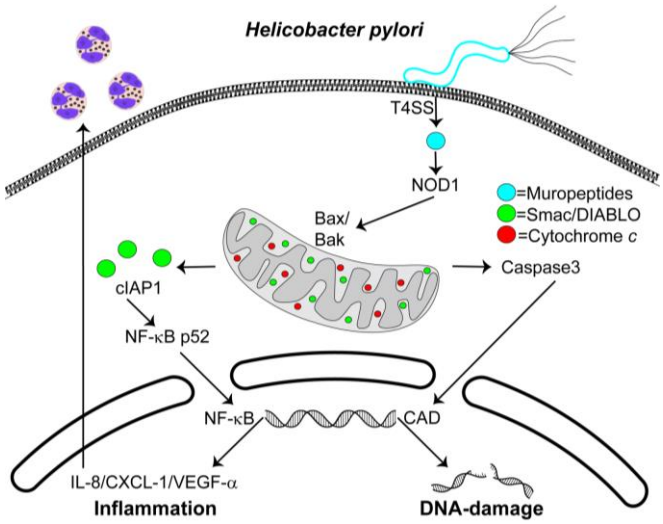
Introduction: *Helicobacter pylori* frequently colonizes the human stomach and is the major risk factor for stomach adenocarcinoma. To date, pathophysiologic mechanisms remain unclear in substantial parts. Many environmental stressors including infections may affect mitochondrial function and structure,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

Methods: We generated several human epithelial cell lines with specific defects in components of the apoptosis-system or depleted of their mitochondrial DNA to investigate their potential contribution to DNA-damage and mitochondrial modulation induced by *H. pylori*. Mitochondrial DNA sequencing and oxidative stress assays were used to assess the infection impact on mitochondrial heteroplasmy and viability. *H. pylori* strains deficient in various virulence factors were used to define the ones that interact with the mitochondrial apoptosis system.

Results: We found that *H. pylori* induced DNA-damage in cell culture as reported previously, which was dependent on a low-level activation of the mitochondrial apoptosis system and the caspase-dependent DNAse (CAD) and occurred in the absence of cell death. Depleting the cells from mitochondrial DNA had similar impact on DNA-damage. Inflammatory cytokine secretion during *H. pylori* infection was partly dependent on low-level apoptosis activation. Mitochondrial DNA depletion attenuated the mitochondrial sublethal apoptosis signaling as well as cytokine production. No difference in mitochondrial mass, membrane potential or heteroplasmy levels were detected upon infection. *H. pylori* triggered oxidative stress distinct by deregulation of antioxidants and an increase in mitochondrial superoxides. Low-level mitochondrial apoptosis activation and alteration of mitochondrial dynamics can therefore be a part of the immune reaction to *H. pylori*-infection.

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

Fig. 1



019/IIV

There is more than one way to die upon *S. aureus* infection

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The human pathogen *Staphylococcus aureus* is the leading cause of hospital-acquired infections and its multidrug resistance makes a successful treatment increasingly difficult. During infection, *S. aureus* can invade into host cells and later escapes from the vacuole into the cytoplasm. In response, the host cell activates several defence mechanisms including cell death signalling, such as apoptosis, necroptosis, and pyroptosis to clear the infection. Apoptosis is a form of programmed cell death (PCD), which can be divided into intrinsic and extrinsic apoptosis. The latter is often induced via the activation of Fas or the TNFα receptor and is characterised by the cleavage of caspase-8 and, subsequently, the activation of caspase-3/7. The intrinsic form of apoptosis is mostly induced after mitochondrial damage, leading to the cleavage of caspase-9, followed by caspase-3/7 activation. Necroptosis is a programmed form of necrosis induced after bacterial stimulation. It requires the interaction of RIPK3 and its substrate MLKL, leading to the formation of plasma membrane pores. In contrast, pyroptosis is an inflammatory form of PCD, often characterised by caspase-1- or caspase-11-mediated cleavage and activation of gasdermins, leading to pore formation and ultimately, the release of interleukins IL-1ß and IL-18. The collective activation of pyroptosis, apoptosis, and necroptosis is called PANoptosis and defines a newly described inflammatory PCD. The master regulator of PANoptosis is the PANoptosome, a multimeric cytoplasmic protein complex consisting of proteins involved in all three PCDs.

To investigate the impact of *S. aureus* infection on host cell fate, we performed RNA-sequencing of *S. aureus*-infected fibroblasts. The expression and activation of cell death proteins were analysed by immunoblotting and flow cytometry. Cytokine release was assessed by ELISA. Cell death was monitored by flow cytometry.

We found an elevated transcription of inflammatory and cell death-associated genes during *S. aureus* infection. For instance, we observed an increased TNFα, Fas and FasL expression. The caspases-8 and -3 were cleaved upon infection, indicating apoptotic cell death. Apoptosis was also confirmed by flow cytometric analysis using different cell death assays. Interestingly, neither caspase- nor TNFα-inhibition was able to block cell death caused by *S. aureus* infection. We further showed activation of caspase-1 accompanied by the release of IL-1ß, which characterises pyroptosis.

In summary, our analysis confirmed that intracellular *S. aureus* results in different forms of cell death, including apoptosis and pyroptosis. This may suggest, that different kinds of cell death occur in the same cell population during *S. aureus* infection.

Workshop 4
Bacterial strategies to survive in hostile environments (FG MP)
05. Sep 2022 • 13:30–15:00

020/MPV
The *Staphylococcus epidermidis* transcriptional profile during carriage

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Introduction: *Staphylococcus epidermidis* is an important component of the human skin microbiota, where it contributes to skin barrier homeostasis and the prevention of colonization by pathogens. However, *S. epidermidis* also possesses pathogenic potential to cause nosocomial infections associated with implanted medical devices. Its impact on the pathogenic lifestyle was the main subject of research, while only limited information is available on the factors that allow the bacterium to colonize its natural habitats. To gain insights into the commensal lifestyle we performed *in vivo* transcriptional analysis during human *S. epidermidis* colonization considering its two major ecological niches: the nose and the skin.
Methods: We used qPCR to profile *S. epidermidis* gene expression *in vivo* in nose and skin swabs of 11 healthy individuals.
Results: While heterogeneity in expression levels was observed for some genes, in several cases the expression level was the same in the nose or skin or both niches in all 11 individual donors. For example, expression of the *S. epidermidis* regulator *sar*A was found similarly in the nose and on the skin of all individuals. Also, genes encoding colonization and immune evasion factors (*sdr*G, *cap*C and *dlt*A), as well as the sphingomyelinase encoding gene *sph*, were expressed at both anatomical sites. In contrast, expression of the global regulator *agr* was almost inactive in the nose, but readily present on the skin. A similar site-specific expression profile was also identified for the putative chitinase-encoding SE0760. In contrast, expression of the autolysine-encoding gene *sce*D, and the wall teichoic acid (WTA) biosynthesis gene *tag*B were more pronounced in the nose as compared to the skin.
Discussion: In summary, our analysis identifies site-specific gene expression patterns of *S. epidermidis* during colonization. In addition, the observed expression signature was significantly different from growth *in vitro*. Interestingly, the strong transcription of sphingomyelinase together with the low expression of genes encoding the tricarboxylic acid cycle suggests very good nutrient supply in both anatomical niches, even on the skin where one might have suspected a rather lower nutrient supply compared to the nose. The differential gene expression patterns related to defined body sites provide important novel insights into the tremendous flexibility *S. epidermidis* employs to cope with varying environmental conditions. These insights could provide crucial information necessary to define novel therapeutic approaches to maintain human skin homeostasis.

021/MPV
Cell death during nutrient starvation in *Staphylococcus aureus* cells lacking (p)ppGpp is linked to disturbed GTP homeostasis

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In *S. aureus*, the alarmones guanosine pentaphosphate and tetraphosphate, collectively named (p)ppGpp are synthesized upon amino acid limitation or in response to cell-wall stress by the alarmone synthetases Rel*Sau*, or RelP and RelQ, respectively. (p)ppGpp is important for bacterial survival, virulence and persistence (1-3). Upon synthesis of (p)ppGpp, GTP levels decrease sharply via consumption of GTP and inhibition of enzymes involved in GTP synthesis (1, 5).
S. aureus wild type and isogenic (p)ppGpp^0 mutants show similar growth rates and final yields when optical densities were followed throughout growth. However, in the stationary phase (p)ppGpp^0 mutants show a significantly decreased ability to form colonies. This indicates that induction of the stringent response either prevents cell death and/or supports escape from a "viable but non-culturable" state. Accordingly, we observed a stringent response-like transcription profile (reduction in the expression of *rpsL* and an increase in *psmα*) (4) or *rsaD* expression. *rsaD* is indirectly regulated by GTP levels through derepression by the GTP-responsive transcriptional factor CodY. However, (p)ppGpp dependent survival is independent of *codY* as shown by the analysis of *codY* negative strains. Metabolome analysis further confirm dysregulation of GTP and ATP metabolism in the (p)ppGpp^0 strain during starvation. When we compared the growth of guanine-auxotrophic mutants (*guaAB*) in wildtype and (p)ppGpp^0 strains, no difference in survival in late stationary phase could be observed. This indicates that the uncontrolled increase of GTP in the (p)ppGpp^0 strain is sufficient to promote cell death under starving conditions. Cell membrane staining and analysis of membrane potential with the voltage-sensitive probe $DiOC_2(3)$ reveals alterations in membrane architecture and function in the (p)ppGpp^0 strain. Under anaerobic conditions no difference in survival between wildtype and the (p)ppGpp0 strain can be observed. Thus, the increased formation of reactive oxygen species under aerobic growth probably contributes to stationary phase cell death of (p)ppGpp^0 mutants.
(1) Geiger T., et al. (2014) J Bacteriol. 2014 Feb;196(4):894-902.
(2) Geiger T., et al. (2012) PLoS Pathog 2012; 8(11): e1003016
(3) Salzer A, et al. (2020) Front. Microbiol. 2020; 11
(4) Horvatek P., et al. (2020) PLoS Genet 2020; 16(12): e1009282
(5) Kriel A., et al. (2012) Molecular Cell 2012; 48(2): 231–41

022/MPV
The loss of the *lsr* operon in *Escherichia coli* of the phylogroup B2 may lead to a fitness advantage during long-term bladder colonisation

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Question: Gram-positive and Gram-negative bacteria shed and react to inter-species quorum sensing molecules called autoinducer-2 (AI-2). In *Escherichia coli* (*E. coli*), AI-2 is incorporated and processed by proteins encoded by the *lsr* operon. Interestingly, most *E. coli* strains of the phylogroup B2 have lost the *lsr* operon during evolution. Epidemiological studies have shown that *E. coli* strains that cause extraintestinal diseases, such as urinary tract infections, are frequently part of the phylogroup B2. Also, the B2 strain 83972, that was isolated from an asymptomatic bacteriuria (ABU) case, lacks the *lsr* operon. We therefore hypothesize here that the loss of AI-2 dependent quorum sensing has led to an extraintestinal fitness gain in *E. coli* of the phylogroup B2, e.g., during long-term bladder colonization.
Methods: We have analyzed all published genome sequences of *E. coli* strains with respect to the presence or absence of a full-length *lsr* operon. Next, we have complemented the *E. coli* strain 83972 with a full-length *lsr* operon from the *E. coli* K-12 strain MG1655 using the Red/ET recombineering method. Afterward, we analyzed phenotypes that are (i) regulated by AI-2 dependent quorum sensing and (ii) crucial for efficient long-term bladder colonization, namely growth characteristics and hydrogen peroxide (H_2O_2) resistance.
Results: We analyzed the presence of a full-length *lsr* operon concerning the different *E. coli* phylogroups. We saw that within phylogroup B2, > 90 %, while within the other phylogroups, only < 10 % of the *E. coli* strains lack a full-length *lsr* operon. Next, we

tested the growth behavior of *E. coli* 83972 and the *lsr* positive *E. coli* 83972 *attB*::*lsr* in lysogeny broth (LB). The *lsr* positive strain showed a significantly longer lag phase while both strains reached the same optical density after 23 h of growth. The resistance against H_2O_2 was tested during the mid-logarithmic growth phase in LB. The *lsr* positive strain was significantly less resistant to 20 mM H_2O_2 than the wild type strain.

Discussion: Amongst others, also due to the frequent void of urine and the attack by neutrophils that use reactive oxygen species to combat bacterial infections, the human bladder is a harsh environment for long-term colonization. It is interesting that the loss of the *lsr* operon is apparently widespread amongst *E. coli* phylogroup B2, which comprises many uropathogenic or ABU *E. coli* isolates, and may thus be related to increased extraintestinal fitness. Indeed, we saw phenotypes that may be relevant for the colonization of the extraintestinal ecological niche. The extended lag-phase of *E. coli* 83972 *attB*::*lsr* may be a disadvantage for rapid bladder colonization upon voiding. Also, the lower resistance to H_2O_2 would be expected to be disadvantageous concerning neutrophil attacks. Further experiments are necessary to clarify if the loss of the *lsr* operon in *E. coli* may indeed result in an evolutionary advantage for bladder colonization.

023/MPV

Neisseria meningitidis increases S1P levels in brain endothelial cells through sphingosine kinase 1 activation

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Question: *Neisseria meningitidis* (*Nm*) is an obligate human pathogen capable of crossing the meningeal blood cerebrospinal fluid barrier (mBCSFB) to eventually cause meningitis. Recent data showed that *Nm* modulates the sphingolipid metabolism of brain endothelial cells (BECs) to mediate uptake by activation and translocation of acid sphingomyelinase [1,2] . Furthermore, analyses of RNAseq data from infected BECs showed differential regulation of the sphingolipid salvage pathway enzymes, including upregulation of sphingosine kinase 1 (SphK1), which generates bioactive sphingosine 1-phosphate (S1P). S1P plays an important role in maintaining endothelial barrier properties via S1P receptors (S1PRs). We hypothesize that *Nm* manipulates the SphK/S1P/S1PR axis in order to enter BECs.

Methods: BEC cell line hCMEC/D3 was infected with *Nm* serogroup B strain MC58. LC-MS/MS was used for the sensitive, specific and simultaneous quantification of sphingolipid metabolites in BECs over 8 hour infection time. SphK enzymatic activity was measured in BECs either infected with wildtype strain MC58 or isogenic mutant strains (lacking capsule (*siaD)*, outer membrane proteins (*opcA, opa*) or Type IV pili (Tfp) (*pilE*) expression). In addition, purified Tfp were applied. Changes in expression of SphK1/2 or S1PR1/2/3 were measured using qPCR. The roles of SphKs and S1PRs in *Nm* adherence and invasion were determined using gentamicin protection assays, immunofluorescence microscopy and flow cytometry analyses in the presence of SphK inhibitors or S1PR antagonists.

Results: LC-MS/MS revealed a significant increase of intra- and extracellular S1P levels in BECs infected with *Nm* MC58. In line with this finding, infection of BECs with *Nm* MC58 led to an upregulation of SphK enzymatic activity 4h post-infection (p.i.). Infection of BECs with *Nm* MC58 and isogenic mutants or purified Tfp revealed that the Tfp significantly contribute to SphK activation. In addition, SphK1 expression was transiently increased after *Nm* infection and reached a maximum at 4h p.i., whereas SphK2 expression was not altered. Pharmacological inhibition of SphK with PF-543, K145 or SLM6031434 or siRNA-induced knockdown of both SphK1 and SphK2 significantly reduced the number of viable intracellular *Nm* without affecting bacterial adherence. Moreover, application of S1PR1+3 antagonist FTY720 similarly reduced the numbers of viable intracellular *Nm.*

Conclusions: In summary, in this study, we demonstrate a comprehensive measurement of the sphingolipid profile in hCMEC/D3 infected with *N. meningitidis* and revealed a significant impact of the pathogen on the salvage pathway. In particular, our data show that *N. meningitidis* leads to activation of SphK1, resulting in a significant increase in S1P levels and interference with S1PR diminishes bacterial uptake suggesting a role of the SphK/S1P/S1PR axis in bacterial invasion.

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024/MPV

Studying the interaction between *Bartonella* adhesin A and fibronectin as a novel therapeutic concept

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Introduction: Bacterial adhesion to the host is the most decisive step in infections. Trimeric autotransporter adhesins (TAAs) are important pathogenicity factors of Gram-negative bacteria. The prototypic TAA, *Bartonella* adhesin A (BadA), mediates *B. henselae* adhesion to endothelial cells (ECs) presumably via interaction with extracellular matrix (ECM) proteins, e.g. fibronectin (FN). This research aimed to study the interaction between BadA and FN in the context of bacterial host cell adhesion.

Material and methods: FN knockout ECs generated using LentiCRISPR were exposed to *B. henselae* strains (wild type or BadA deficient) using *in vitro* infection models. Whole-cell bacteria and FN were chemically crosslinked and analysed using mass spectrometry (XL-MS). The binding capacity of *B. henselae* strains to proteolytic FN fragments was evaluated using ELISA assays. Binding inhibition assays were performed using bacteria and a competitor for FN binding in an ELISA setup.

Results: BadA and FN interaction demonstrated to be important for *B. henselae* adhesion as bacterial binding to ECs was reduced under BadA- or FN-deficient conditions. Furthermore, the exact peptide-peptide interactions occurring within BadA and FN were identified using XL-MS analysis. The interacting regions supporting BadA binding were functionally localized within the heparin-binding domains of FN, using ELISA assays. Bacterial binding to FN was significantly reduced in heparin-competition assays.

Discussion: We determined the interaction between BadA and FN to be essential for *B. henselae* host cell adhesion. BadA interactions occur within the heparin-binding domains of FN and the exact binding sites were identified. The BadA-FN interactions described here are intended for the design of "anti-ligands" as a new class of anti-infectives. Interactions between TAAs and the ECM might represent the key step for host-cell adherence of human pathogenic Gram-negative bacteria.

025/MPV

Host compartment specific proteome signature of *Streptococcus pneumoniae* 19F during *in vivo* colonisation

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Background: Pneumococci encounter various host niches and hence, the proteome of pneumococci mirrors the specific adaptation to host compartments. By employing an *in vivo* pathoproteomics approach we recently identified pneumococcal antigens essential for pneumococcal meningitis (Schmidt *et al*., 2019 PLoS Pathog 15: e1007987). Here, we deciphered the *in vivo* proteome and adaptation of pneumococci during colonization of mice.
Methods: Mice were intranasally infected with GFP-expressing *S. pneumoniae* serotype 19F, strain EF3030. Three and 5 days post infection pneumococci were recovered from the nasopharyngeal compartment and sorted by fluorescence-activated cell sorting. After tryptic on-filter digestion peptides were measured using LC-MS/MS in a data-independent acquisition mode (DIA) and the generated data were analysed with the SpectronautTM software.
Results: The *in vivo* pathoproteome analysis of the 19F samples with 1.0 – 4.4 million sorted pneumococci resulted in the identification of approximately 1000 bacterial proteins per sample, reflecting approximately 50% of the theoretical total proteome. The *in vivo* proteome profiles of strain 19F were quantitatively compared to *in vitro* proteome profiles of 19F cultured in a complex medium or chemically defined medium. *S. pneumoniae* 19F showed host-compartment and media specific-proteome profiles as demonstrated by principal component analysis. The *in vivo* proteome signatures suggested a stringent adaptation to the nasopharynx mainly by optimizing the physiology. Virulence factor expression was only slighty altered during colonization in comparison to growth media. Pneumococcal proteins in higher abundance such as galactose-6-phosphate isomerase subunit LacA (EF3030_0540) or proteins of the arginine deiminase system (ArcT, arginine deiminase and carbamate kinase) and bacteriocin immunity protein (EF3030_0560) are indicative of an optimize physiology during *in vivo* colonization. In contrast, competence seems to be downregulated (lower abundance of ComB) and cell wall synthesis as well as suggested by the lower abundance of penicillin-binding protein (EF3030_10350).
Conclusion: The selective pressure and availability of nutrients results in a host-compartment specific proteome to foster adaption and pneumococcal fitness.

Workshop 5
Infection control interventions (StAG HY/FG PR)
06. Sep 2022 • 08:30–10:00

026/HYPRV
Reaching sustainability in hand hygiene compliance improvement in emergency medicine – a single centre experience

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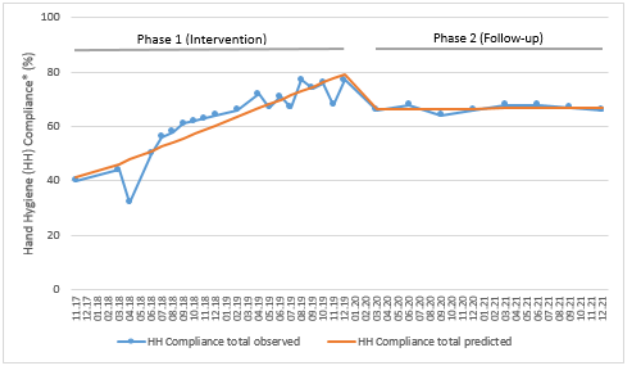
Background: Containment of pathogens" spread and the reduction of healthcare-associated infections require sufficient infection prevention and control (IPC) measures in healthcare. So far, IPC activities in hospitals have been mainly focusing on inpatients and invasive procedures rather than on emergency care. To provably strengthen IPC competencies and preparedness in this setting, a multimodal strategy for hand hygiene (HH) compliance improvement was realized over a 24-month period. Consecutive audits were realized to support sustainability and to show compliance development in the further course.
Methods: Implementation of HH was conducted in an ED in a tertiary care hospital with 50,000 annual visits as a prospective before-and-after study between 01/2018 and 12/2021.
The main intervention in phase 1 (01/2018-12/2019) was created in accordance with the WHO recommendations for multimodal strategies (Storr, 2017) as the implementation of i.) system change, ii.) monitoring of practices, iii.) improved communication, and iv.) culture change by fostering a safe climate. Monthly HH compliance data were obtained according to WHO's 5 moments of HH. Compliance was determined by dividing the number of observed indicated HH actions performed by the total number of observed HH opportunities (HHO). ED healthcare workers (HCW) received i.) immediate feedback and ii.) structured interdisciplinary participatory feedback during monthly meetings guided by leading medical and nursing staff. In phase 2 (01/2020 – 12/2021) quarterly, follow-up audits of HCWs" HH compliance were carried out.
The development of HH compliance was analyzed by regression models using an interrupted time series analysis approach.
Results: In total, 5,349 HHO were observed (nurses: 3,441; physicians: 1,908) in 28 observation periods. In phase 1 data on HH compliance and concrete measures for further improvement were discussed and concretized during 20 feedback meetings within a median of 10 participants. Baseline HH compliance was 40 % (nurses: 44 %; physicians: 40 %) and raised significantly to 77 % at the end of phase 1 (nurses: 86 %; physicians: 62 %). According to the regression model, the monthly compliance increase was 1.56 % (95 % confidence interval (CI95) 1.26 to 1.88). After phase 1 compliance significantly decreased by 13.05 % (CI95 21.78 to 4.34) but remained constant during phase 2 (figure 1). This trend was observable in nurses and physicians.
Conclusion: The data shows that the implementation of HH as the key IPC measure is feasible in emergency departments. The achieved increase in compliance can be kept at a slightly lower level for at least 24 months by regular HH audits.

Figure 1: Data of Hand Hygiene Compliance over time during and after the intervention



*"My 5 Moments of Hand Hygiene", WHO 2009

Figure 1: Data of Hand Hygiene Compliance over time during and after the intervention

027/HYPRV
News on the current antiseptic bathing practices in German intensive care units – a cross-sectional survey

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Introduction: In 2016, 29% of intensive care units (ICUs) included in our survey reported routine antiseptic patient bathing on their wards. At that time, multicenter randomized controlled trials (RCTs) on the benefits and risks of routine antiseptic bathing in German ICUs were lacking. We conducted a three-armed cluster-randomised controlled trial (cRCT) in 72 adult intensive care units to investigate the effect of daily patient bathing with chlorhexidine, octenidine or routine care with water and soap (control) on central-line associated bloodstream infection (CLABSI) rates [1]. This trial found no preventive effect of antiseptic bathing neither with chlorhexidine nor with octenidine when comparing CLABSI rates of the intervention groups with the control group [1]. The post hoc before-after analysis, however, suggests that ICUs with CLABSI rates ≥0.8 CLABSI per 1000 central-line (CL) days might benefit from bathing with 2% chlorhexidine-impregnated cloths (manuscript under revision). This

study aimed to clarify, what the current routine bathing practice in German ICUs is and whether routine use of antiseptic bathing in German ICUs has changed since our first survey in 2016.

Methods: We conducted a cross-sectional survey between September and November 2021. All ICUs in the German national surveillance system for nosocomial infections in intensive care units (ICU-KISS) were invited by e-mail. Data were collected using LimeSurvey. Results were compared with our previous survey from 2016. In the descriptive analysis, numbers with percentages were reported. P-values were calculated by Chi-quare or Fisher"s exact test.

Results: Among 1.508 ICUs contacted, 691 (45.8%) responded to our survey. Similar to our first survey, one third of responders (231 of 691 ICUs) have been using routine antiseptic bathing ($p = 0.268$). Antiseptic substances applied were octenidine (73.8 % in 2016 versus 68 % in 2021, $p = 0.337$), polyhexanide (15.4 % in 2016 versus 4 % in 2021, $p < 0.001$), chlorhexidine gluconate (4.6 % in 2016 versus 22.0 % in 2021, $p = 0.001$) and others (6.2 % in 2016 versus 6.5 % in 2021, $p = 0.927$).

Discussion: From 2016 to 2021, the proportion of ICUs with routine antiseptic bathing did not significantly change. Even though effectiveness of octenidine for CLABSI prevention could not be shown by our cRCT, octenidine is still the most frequent substance used for antiseptic patient bathing in German ICUs. Application of antiseptic bathing should be critically reviewed by ICUs and if necessary adjusted to ward-specific infection rates, current scientific evidence as well as patients" and healthcare workers" needs.

References

1. Denkel, L.A., et al., *Effect of antiseptic bathing with chlorhexidine or octenidine on central-line associated bloodstream infections in intensive care patients: a cluster-randomised controlled trial.* Clin Microbiol Infect, 2022.

028/HYPRV

Contact precaution for Vancomycin resistant enterococci (VRE) patients has no preventive effect

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Background: Vancomycin-resistant enterococci (VRE) are easily transmissible and are increasingly detected in connection with severe infections in hospitals. To prevent transmission, the KRINKO (1) recommends bundled measures that include strict contact precaution for VRE patients. However, the effect of this measure is controversial. The present study therefore aimed to investigate whether strict contat precaution is actually suitable for preventing VRE transmissions in a hospital setting.

Method: In a prospective, two-phase monocentric cohort study on a geriatric ward of a total duration of 8 months, all patients underwent VRE admission and discharge screening. In the first phase of 4 months, identified VRE patients were strictly isolated, and staff members had to wear protective clothing in case of direct contact. In the second phase of the study, only good standard hygiene was recommended; all barrier measures were omitted. For the duration of the study, cleaning compliance on the ward, hand hygiene compliance according to the criteria of the Clean Hands Campaign and the use of antibiotics were observed and documented. All VRE strains were characterised by whole genome sequencing and core genome MLST evaluation.

Results: The admission and discharge incidences of VRE during the two phases of the study were 1.8 / 1.5 and 4.19 and 5.00, respectively; there were no differences between phase 1 and 2 with regard the incidences. MLST typing / whole genome sequencing identified 3 transmissions in the first phase of the study (strict isolation) and 2 transmissions in the second phase (standard hygiene), reproduction indices R0 of about 1.0 for the respective hygiene regimes were calculated. The majority of the patients who were identified as VRE carriers at discharge showed isolated MLST types without any connection to transmission. Of note was the fact that all VRE patients (100 %) had received antibiotics during their stay, while the antibiotic use rate for non-VRE patients was only 40%. Cleaning compliance was > 80 % in both phases, hand hygiene compliance (hand disinfections / patient day) was determined with 45 ml and 48 ml, respectively, and was also comparable.

Conclusion: Adherence to strict contact precautions had no preventive effect on the transmission of VRE. MLST typing was necessary to identify transmission; the majority of VRE detections at patient discharge was not due to transmission, rather one must assume selection of VRE by antibiotic use.

References:

1. Hygienemaßnahmen zur Prävention der Infektion durch Enterokokken mit speziellen Antibiotikaresistenzen (rki.de) https://www.rki.de/DE/Content/Infekt/Krankenhaushygiene/Kommission/Downloads/Enterokokken_Rili.pdf?__blob=publicationFile

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029/HYPRV

Effect of daily antiseptic body wash with octenidine on ICU-acquired bacteremia and ICU-acquired multidrug-resistant organisms (MDRO) in intensive care units (ICU) – a multicentre, cluster-randomised, double-blind, cross-over study

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Introduction: The risks of developing bacteremia or acquiring an MDRO during treatment on an ICU are considerable, especially for multimorbid patients. As these healthcare-acquired complications are serious in nature and can increase morbidity, mortality and treatment costs, their prevention is highly relevant. In order to reduce these risks and positively influence patient safety, antiseptic bathing has garnered attention in an effort to reduce hospital-acquired infections. Previous studies have shown the efficacy of antiseptic bathing in high-risk environments using chlorhexidine; we aimed to evaluate octenidine"s effectiveness as a potential alternative in this regard.

Materials/Methods: We compared the rates of both nosocomial primary bacteremia and MDRO acquisition in a controlled, cluster-randomized, double-blind, cross-over study using octenidine-impregnated and placebo wash mitts. On 44 ICUs in 23 hospitals throughout Germany, we compared individual ICUs with themselves over a time period of 30 months (3 month wash-out phase, followed by 12-month intervention and observation phase for each type of wash mitt); randomization concerned only the order in which the octenidine wash mitts and placebo wash mitts were used. The two co-primary endpoints were ICU-acquired primary bacteremia and ICU-acquired MDRO, with all essential information obtained digitally via hospital information systems through individual ward-movement data and microbiological test results.

Results: After filtering 104,039 ICU episodes from 93,438 patients with 1,128,777 microbiological test results for informative episodes with an ICU stay of more than two calendar days, a statistically highly significant intervention effect favoring the octenidine wash mitts was detected in regard to ICU-acquired primary bacteremia. A risk reduction of 17% was seen across all participating ICUs, with a hazard ratio of 0.828, a 95% confidence interval of [0.748 ; 0.918] and a corresponding p-value of 0.00032. As for MDROs, however, no intervention effect was seen using the octenidine-impregnated wash mitts; the heterogeneity across all ICUs was substantial in this regard, resulting in a hazard ratio of 0.976 and a 95% confidence interval of [0.83 ; 1.148].

Discussion: Daily use of octenidine-impregnated wash mitts reduces the risk of ICU-acquired primary bacteremia by 17%,

whereas this intervention shows no effect on ICU-acquired MDRO colonization or infection. As far as MDROs are concerned, the substantial heterogeneity in the intervention"s effect suggests that mainly external influences drive the rate of colonization and/or infection on ICUs. Octenidine has emerged as a viable solution for preventing primary bacteremia in a high-risk setting with a vulnerable patient population.
Funded by DFG No. CH1525/1-2
DRKS-ID: DRKS00011282

030/HYPRV
Clonal- and plasmid-mediated spread of OXA-162-producing Enterobacterales in a German hospital
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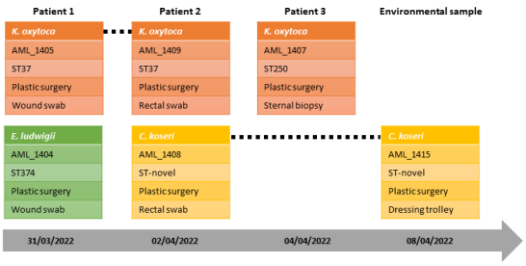
Introduction: Mobile genetic elements are the most efficient intra- and interspecies transfer mechanism of antimicrobial resistance determinants in bacteria. The plasmid- or chromosome-encoded OXA-48 is the most common carbapenemase in Enterobacterales in Germany. The aim of the present study was to investigate the molecular epidemiology and spread of OXA-48-like in Enterobacterales in our University Hospital.
Materials/Methods: Between March and April 2022, five isolates, i.e., 2 *Citrobacter koseri*, 2 *Klebsiella oxytoca* and 1 *Enterobacter ludwigii* were obtained from three patients and from the hospital environment in a German hospital. WGS was performed on MiSeq and MinION sequencer platforms. Velvet and Unicycler assemblies were used for species identification (JSpeciesWS), core genome MLST, and 7-loci MLST. The resistome and plasmidome of the isolates were analysed using ResFinder and PlasmidFinder, respectively. Plasmids were annotated using the tool Bakta.
Results: A *bla*$_{OXA162}$-positive *E. ludwigii* (AML_1404) and a *K. oxytoca* (AML_1405) were recovered from a wound swab from patient 1. Patient 2, who shared a room with patient 1, was found to be colonized with a *bla*$_{OXA162}$-positive *K. oxytoca* (AML_1409) identical to AML_1405, suggesting transmission between the two patients. Furthermore, patient 2 was also colonized with a *bla*$_{OXA162}$-positive *C. koseri* (AML_1408). Another *bla*$_{OXA162}$-positive *K. oxytoca* (AML_1407) was found in patient 3, who was hospitalized simultaneously in the same ward as patients 1 and 2. AML_1407 and AML_1405 differed by 2523 alleles according to cgMLST and were therefore considered epidemiologically unrelated. Finally, a *bla*$_{OXA162}$-positive *C. koseri* (AML_1415) was recovered from a dressing trolley, differing in only one allele from AML_1408 and indicating a transmission event with patient 2 (Fig. 1). In all investigated isolates *bla*$_{OXA162}$ was embedded in a *Tn1999*-like transposon that was the only antibiotic resistance determinant harboured by a 63 kb IncL plasmid. Interestingly, in September 2021, an *Enterobacter hormaechei* subsp. *steigerwaltii*, a *Klebsiella pneumoniae*, and a *Citrobacter freundii* were found in a surgical patient, all carrying a very similar IncL plasmid to patients 1, 2, and 3, suggesting that the plasmid was already circulating in the hospital.
Conclusion: The present study has shown that both clonal expansion and horizontal gene transfer of an IncL plasmid among Enterobacterales species within the same patient were the cause of the *bla*$_{OXA-162}$ spread in our hospital. These data highlight that active surveillance systems should not only track clonal expansion of a single species but also the horizontal spread of antibiotic resistance determinants into other genera mediated by mobile genetic elements.
Figure 1. Timeline of the OXA-162 multispecies outbreak. The boxes are coloured by species (*E. ludwigii*; green, *K. oxytoca*; orange, *C. koseri*; yellow). The dashed line represents transmission events.



Workshop 6
Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens (FG MS/FG ZO)
06. Sep 2022 • 08:30–10:00

031/MSZOV
Phylogenetic and genotypic profiling of linezolid-resistant MRSA from food and livestock
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Introduction: Resistance of MRSA from food and livestock to last resort antibiotics is highly concerning, since treatment options for infections in humans might be diminished. Linezolid belongs to the oxazolidinone group and is used as a critically important antibiotic to treat MRSA infections in humans. Known mechanisms of linezolid resistance include point mutations in the 23S rRNA gene and acquisition of the *cfr*, *optrA* or *poxtA* gene. Monitoring of MRSA from different niches is of importance to evaluate the antimicrobial resistance (AMR) potential.
Materials and Methods: In total, 41 linezolid-resistant MRSA (MIC ≥8 mg/L) from the strain collection of the National Reference Laboratory for coagulase-positive Staphylococci including *Staphylococcus aureus* at the German Federal Institute for Risk Assessment were analyzed by whole-genome sequencing (WGS). The isolates originated from livestock and food and were obtained from 2012 to 2018. AMR genes were determined by an in-house bioinformatics pipeline based on AMRfinder. In addition, point mutations in the 23S rRNA gene were analyzed using ResFinder. Phylogenetic relationships among MRSA isolates were investigated using SNP and cgMLST analyses. In addition, susceptibility to 19 antimicrobial substances was determined by broth microdilution.
Results: Phylogenetic analyses revealed a clear separation by large allelic differences between isolates associated to sequence type (ST) 9 and 398. Two ST9 isolates from turkey skin and turkey meat originating from the years 2012 and 2014 clustered closely together. Moreover, two ST398 isolates originating from chicken meat from 2012 and turkey meat from 2013 showed a close relationship. All isolates harbored a high number of AMR genes against several antimicrobial classes and accordingly expressed phenotypic resistance to various antimicrobial substances. AMR resistance was determined against 4 to 12 of the tested substances. Two ST398 isolates harbored the *cfr* gene and exhibited increased resistance to linezolid as well as chloramphenicol. Moreover, point mutations in the 23S rRNA gene were detected in 24/41 isolates.
Discussion: The study illustrates that linezolid-resistant MRSA are occasionally found in livestock and food samples. These isolates might cause difficult-to-treat infections in animals such as mastitis in dairy herds. Moreover, MRSA isolates might find their way to the community and hospitals by contaminated food or livestock handling. The close genetic relationship of some MRSA isolates from different origins indicates the transmission potential of these strains across various matrices. The lack of linezolid resistance associated genes in some isolates might be due to unknown mechanisms, sequence variabilities or loss of plasmids. Since the

genetic background of AMR in MRSA is not completely understood and transmission processes play a significant role, research in these areas is of high importance.

032/MSZOV

The transmission risk of multidrug-resistant organisms between pets and humans – preliminary results of an exploratory case control study

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Background: This project aims to assess the relevance of pet husbandry in the colonization of multidrug-resistant organisms (MDROs) of hospital patients. Contact between pets and owners is often very close and the transmission of pathogens, including MDROs, is possible. Currently, the potential role of pets as reservoirs of MDROs is unclear. The project focusses on the most common MDROs in hospital patients, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), 3rd generation cephalosporin-resistant Enterobacteriaceae (3GCRE) and carbapenem-resistant Enterobacteriaceae (CRE).
Materials/methods: To assess the contact to household pets as a risk factor for the colonization with one of the above named pathogens, we perform an exploratory, unmatched case-control-study. Among questions about well-known risk factors, study participants are queried regarding their contact to dogs and cats. This includes information about the number of pets in the household, the closeness of contact as well as medical history and feeding habits of pets. To assess the genetic relatedness of the human and pet MDROs, we collect nasal and rectal swabs of the participants and their pets to test them for MDROs. Phenotypically matching MDROs in the samples of participants and their pets will be tested for genetic relatedness using whole genome sequencing (WGS). The analysis will soon be extended with a long read sequencing technique that allows to examine mobile genetic elements of MDROs. The sample size will comprise about 4,000 human participants. The study is funded by the Federal Department of Health (BMG).
Results: Our first preliminary analysis comprises 3,287 participants so far, 28% (930/3,287) tested positive for MDROs. 55% (1,806/3,287) of participants were male and the mean age was 61 years (18-98 years). 22% of all participants (712/3,287) stated to own at least one pet dog or cat (range 1-12). Among the first 483 returned and analyzed pet samples, 11% (53/483) were positive for MDROs. In four cases MDROs of pet and owner were phenotypically matching. The matching pathogens were two pairs of VRE and 3GCRE each. WGS analyses revealed that one pair of 3GCRE and one pair of VRE were also genotypically matching. The transmission rate between pets and owners was 0.4% (2/483).
Conclusion: So far, our results show a low rate of MDRO transmission between pets and owners. However, the overall risk has to be evaluated in a final analysis. Our analysis will include long read sequencing data to get a better understanding of transmission mechanisms, also considering transmission via mobile genetic elements. The presentation at the DGHM 2022 will include detailed statistical analysis of risk factors for MDRO acquisition for pet owners as well as pets based on our preliminary data set.

033/MSZOV

Impact of biocides on the conjugative transfer in *E. coli* field isolates

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Introduction: Plasmids largely contribute to the resistome of *E. coli.* Mobilization of plasmids via conjugation is one key mechanism enabling the spread of antibiotic resistance. Biotic and abiotic factors may influence conjugation efficiency. Antibiotics at low concentrations, for instance, increase conjugation frequency in *E. coli*. However, our knowledge is scarce about the influence of biocides on conjugation efficiency.
Material and Methods: A subset of 40 *E. coli* isolates from livestock, food and humans was investigated in our study. Isolates were sequenced via short and long-read sequencing. Plasmid sequences were analyzed including the characterization of the Inc-Group, mobility elements and resistance genes. Conjugation frequencies (CF) were determined for suitable donor-recipient combinations in MH bouillon using liquid mating (3 hours incubation at room temperature) and subsequent enumeration of recipients and transconjugants on selective agar plates: $CF = cfu/ml_{transconjugant} : cfu/ml_{recipient}$. Plasmid transfer was proven by plasmid profile analysis of selected transconjugants. Influence of strain variability on conjugation frequency was determined by comparative analysis of several donor-recipient combinations. The effect of biocides added to the mating mix in subinhibitory concentrations was tested for benzalkonium chloride and chlorhexidine dihydrochloride. Concentrations were individually chosen based on minimal inhibitory concentrations of donor and recipient.
Results: Mating experiments were conducted with two different donor strains: D1 (plasmid size: 40 kb Inc-Group: IncN, resistance gene: bla-CTX-M1) and D2 (51 kb, IncFII, bla-CTX-M14). Conjugation frequencies were determined for D1 and five recipients as well as for D2 and two recipients. Recipient candidates R1-R5 either contained one plasmid with an Inc-Group different from the respective donor (n=4) or did not carry plasmids (n=1). Chromosomally encoded tetracycline resistance (R1-R3) or fluoroquinolone resistance with MIC values >8 mg/L (R4, R5) served as selective markers for recipients. Overall, CF of tested mating pairs ranged between $1x10^{-4}$ - $1x10^{-8}$ with or without biocide supplementation. Conjugation frequency was similar for plasmid IncFII in mating pairs D2 + R1 and D2 + R2 (1-5x10-4). In contrast, conjugation frequency for mating experiments with D1 and five different recipients varied between $8x10^{-5}$ - $1x10^{-8}$.
Discussion: External stressors such as biocides in subinhibitory concentrations have the potential to influence conjugation frequency. Our findings point out a high degree of strain specific variability independent of subinhibitory biocide exposure. Hence, our study justifies investigation of conjugation frequencies in field isolates to better understand the impact of sublethal biocide exposure on bacterial resistance development.

034/MSZOV

Molecular epidemiology and antimicrobial resistance of *Clostridioide*s (*Clostridium*) *difficile* isolates from diverse faecally contaminated environmental sources

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Clostridioides difficile is the most common pathogen causing antimicrobial-associated intestinal disease in humans and some animal species but can be present also in various environments outside the hospital. Little is known about antimicrobial resistance and epidemiology of "environmental" *C. difficile* strains. Therefore, the objective of this study was to investigate the presence and the molecular characteristics of toxin genes, genotypic antimicrobial resistance, and ribotype diversity of *C. difficile* isolates detected in different environmental samples.

C. difficile spores were recovered from selective enrichment of different environmental samples (e.g. sewage, sewage sludge and calf feces) which collected from Ostfriesland region. The obtained isolates were confirmed as *C. difficile* via amplification of the triose phosphate isomerase (*tpi*) gene by PCR. Toxin genes (*tcdA, tcdB* and cdtAB), antimicrobial resistance genes (ARGs), conjugative transposons, and ribotyping were determined via PCR. Furthermore, 23 *C. difficile* isolates were subjected to whole genome sequencing (WGS) for (core genome[cg]) multilocus sequence typing (MLST). Antimicrobial susceptibility of vancomycin, metronidazole, moxifloxacin, tetracycline, clindamycin, ciprofloxacin, clarithromycin, and rifampicin was determined by E-test or disk diffusion method.
Out of 75 *C. difficile* isolates, 69 (92%) were toxigenic and six (8%) were non-toxigenic. The most common RTs in calf feces isolates were RT126 (n=8 isolates) and RT127 (16). Within wastewater samples, RT127, RT014, RT126 and RT012/RT020/RT070/RT258 were most common (8, 5, 4, and 3, respectively). All isolates were susceptible to vancomycin and metronidazole, whereas a considerable number of isolates were resistant to clindamycin, fluoroquinolones, rifampicin, and macrolides. Seven isolates (9.3%) displayed resistance to three or more of the tested antimicrobial agents. MLST identified 17 distinct sequence types (STs). The majority of analysed genomes belonged to MLSTs of clade 1 followed by clade 3, 5, and 4. Molecular markers conferring resistance to fluoroquinolones (*gyrA* and/or *gyrB*) were the most frequent ones in *C. difficile* isolates. Six strains carried ARGs [*aac(6')-Ie-APH(2'')-Ia, ermB, ANT(6)-Ib, tetM, tet40, aph(3')-IIIa, ANT(6)-Ia* or *sat-4*), which conferred resistance to aminoglycoside, tetracycline or macrolide-lincomycin-streptogramin B (MLSB) antibiotics. Tetracycline resistance gene (*tetM*) associated with Tn*916-like* transposon was detected in 23 isolates.
In conclusion, *C. difficile* strains are commonly present in various faecally contaminated environmental sources, which could be serve as a potential source of community-associated *C. difficile* infection. WGS is an influential tool to characterize virulence-associated factors, mobile genetic elements and genotypical antimicrobial resistance. The study of epidemiological relatedness between clinical, animal and environmental origin is required.

035/MSZOV

Salmonella central carbon metabolism enhances bactericidal killing by fluoroquinolone antibiotics

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Introduction: Perisisters are drug-sensitive bacteria, which can survive prolonged exposure to high concentrations of antibiotics [1]. In general, persistence is non-inheritable, after removal of the antibiotic, drug-treated bacteria remain as susceptible as the original population. Various mechanisms have been described which allow bacteria to survive treatment with antibacterial agents, including activation of stress responses and antitoxin/toxin modules, increased efflux activity and reduced ATP concentrations [2-5].
Material/method: Here we investigated an Δ*atp* operon mutant of *Salmonella* Typhimurium and the corresponding wild type by exposing the bacteria during exponential growth to fourfold the MIC of ciprofloxacin. We determined reactive oxygen species (ROS) during antibiotic treatment and performed killing assays under ROS-diminishing conditions. In addition, we determined the NADH content and tested the survival of various metabolic mutants.
Results: Interestingly, the deletion of the ATP synthase resulted in an increased susceptibility to ciprofloxacin, despite having less ATP than the wild type [6]. However, drug treatment under anaerobic conditions or by addition of ROS quenchers during aerobic growth, led to heightened persistence of the Δ*atp* operon mutant. Furthermore, a detailed investigation revealed high levels of NADH in the mutant as well as increased formation of hydroxyl radicals during ciprofloxacin treatment. Additional deletions of metabolic genes, which are known to participate in the NADH metabolism or ROS formation, further increased the survival of the mutant after drug treatment.
Discussion: We conclude that the deletion of the ATP synthase forces the mutant to generate its energy via substrate phosphorylation, resulting in over-production of NADH, which in turn stimulates the formation of deleterious hydroxyl radicals.

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036/MSZOV

Genome-wide profiling and comparison of antibiotic resistances in *Campylobacter jejuni* and *Campylobacter coli* from poultry in Germany and Vietnam

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Introduction: *Campylobacter* spp. is the leading cause of bacterial food-borne gastroenteritis. The majority of *Campylobacter* infections are self-limiting, yet antimicrobial treatment was required in 30 % of the reported cases in Germany. The goal of this study was to compare antibiotic resistances of German (DE) and Vietnamese (VN) *Campylobacter* isolates in order to identify crucial resistance determinants, which might challenge global food safety.
Material/method: A total of 494 *Campylobacter* isolates of poultry from Vietnam and Germany were systematically analyzed. Phenotypic resistance was characterized by microdilution using the European standardized EUCAMP2 and customized plate formats. In-house pipelines based on Abricate and the NCBI AMRFinderPlus database were used to analyze whole genome sequencing data. In case of discrepancy between geno- and phenotype, strains were re-analyzed and additional web tools and raw read mapping via the Geneious software was performed.
Results: VN *Campylobacter* isolates showed more resistances against antimicrobials than DE isolates. The resistance determinants, *ermB* for macrolide, *catA* genes for chloramphenicol, *fexA* and *optrA* for florfenicol and *lnuC* for lincomycin resistance were exclusively present in VN strains. The gene *aph(2'')-li1*, conferring gentamicin resistance was only present in one DE strain. Different variants of the *tetO* gene and further tetracycline genes *tetL* and *tetW* as well as streptomycin and kanamycin resistance determinants were differently distributed among DE and VN *Campylobacter* populations. In contrast to previous postulations, the point mutation in the 50S rRNA L22 protein did not lead to macrolide resistance. Resistance against nourseothricin was conferred by the *sat*4 gene, mainly present in DE strains in operon structure with other resistance genes.
Most geno- and phenotypic results were concordant. However, several differences in prediction of resistances by genotype were

observed due to i), lack of gene variants in the AMRFinderPlus database and, thus, loss of identification due to homology or coverage thresholds (streptomycin, tetracycline), ii) low level of resistance (ampicillin, lincomycin and florfenicol), iii) unknown resistance mechanisms (one gentamicin resistant strain and ciprofloxacin resistant but nalidixic acid sensitive strains) and iv) partial genes or genes with mutations leading to truncated proteins (chloramphenicol or streptomycin sensitive strains).
Discussion: Some of the resistance determinants were putatively located on plasmids or in operon structures on the chromosome. Hence, their spread might be enhanced by co-transfer during natural transformation and by conjugative plasmid transfer. Prediction of resistance by genotype can be improved by consideration of mosaic genes, other missing gene variants in databases, mutations leading to truncated genes and by deciphering yet unknown resistance mechanisms in thermotolerant *Campylobacter* spp..

Workshop 7
Molecular insights into host-microbe crosstalk (FG PW)
06. Sep 2022 • 08:30–10:00

037/PWV
Acinetobacter baumannii targets mitochondria in infected human host cells

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Introduction: *Acinetobacter baumannii* is one of the most important highly antibiotic-resistant pathogen that causes a multitude of nosocomial infections, but the pathogenicity mechanisms remain largely unclear. Therefore, virulence traits of *A. baumannii* clinical isolates of the worldwide most prevalent international clonal lineage IC2 were determined in *in vitro* and *in vivo* infection models.
Material and Methods: Whole genome sequencing (WGS) was carried out using Oxford Nanopore and Illumina technology to generate hybrid assembly sequence data for fifteen carbapenem-resistant clinical *A. baumannii* isolates of IC2. WGS data were screened for virulence genes using an in-house script. For quantification of virulence, *in vitro* assays (e.g., Annexin V/propidium iodide staining, JC-1 mitochondrial fragmentation, host cell invasion) with infected human umbilical cord vein endothelial cells (HUVEC) and the *Galleria mellonella in vivo* infection model were employed. Complexome profiling of HUVECs was done by combining blue native gel electrophoresis (BNE) with quantitative mass spectrometry to analyze the host cell response. ATP production rate in infected HUVEC was measured using a Seahorse XFe 96 extracellular flux analyzer.
Results: All analyzed *A. baumannii* clinical isolates displayed high sequence similarity of ≥95% on nucleotide level and the presence of virulence genes was almost similar (n=73-76). However, induction of apoptosis in HUVEC and the median lethal dose in the *G. mellonella* model differed significantly, indicating different virulence properties. AB_2778 was the most virulent isolate and AB_1372 the least virulent isolate *in vitro* and *in vivo*. Mitochondrial membrane potential of HUVEC was severely decreased upon infection with AB_2778 in contrast to AB_1372. Further, TOMM20 staining and induction of caspase-9 indicate the impact on mitochondria. Complexome data uncovered the abundance of multiple protein complexes in an isolate dependent manner, whereas infection with AB_2778 led to the reduction of complexes of the respiratory chain, alteration of mitochondrial dynamics and induction of mitophagy. Measurement of the ATP production rate showed a significant decrease of ATP produced via the respiratory chain after infection with AB_2778 compared to AB_1372 and the uninfected control. Taken together the dramatically decreased mitochondrial membrane potential after infection with AB_2778 indicates that mitochondria are a potential target of virulent *A. baumannii* in human host cells.
Discussion: Complexome profiling indicated mitochondria as important targets of *A. baumannii* during infection. The characterization of host pathogen interaction and identification of targets are crucial to identify novel therapy options particularly for pathogens with limited options for antibiotic treatment.

038/PWV
Modeling *Neisseria meningitidis* interaction with the meningeal blood-CSF barrier using iPSC and multi-cell culture approaches

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Introduction: *Neisseria meningitidis* (*Nm*, meningococcus) is a human-specific bacterium that can cause meningitis by crossing the meningeal blood-cerebrospinal fluid barrier (mBCSFB) and infecting the leptomeninges. Previous research on this host-pathogen interaction has mostly been done on immortalized brain endothelial cells (BECs) alone, which do not retain certain physiological barrier properties *in vitro*, and has not yet included leptomeningeal cells (LMC) to model the physiological microenvironment. Here, we report on the development and application of physiologically relevant BEC-LMC co-culture models to examine Nm interaction at the mBCSFB.
Methods: We used BEC-like cells derived from induced pluripotent stem cells (iBECs) or hCMEC/D3 cells in co-culture with meningioma derived LMCs. Transmission electron microscopy, confocal and super-resolution microscopy techniques were applied for model characterization and detection of interacting bacteria. Tightness and integrity of the cell barriers was analyzed via transendothelial electrical resistance (TEER) and sodium fluorescein permeability. We used Gentamicin protection and transmigration assays to determine bacterial interaction and penetration of the barrier models and examined changes in host gene expression in response to infection via qPCR.
Results: We observed characteristic expression of BEC markers including tight junction proteins in iBEC, which remained distinct from LMCs in co-culture. LMC co-culture improved barrier tightness and stability of iBEC layers for seven days. BEC response to infection was generally not altered by LMC co-culture. Interestingly, we discovered bacteria already traversing in small numbers 6 h post-challenge, when barrier integrity was still high, suggesting a transcellular route. By 30 h, deterioration of the barrier properties has been detected, including loss of TEER and reduced expression of cell-junction components. We found proinflammatory activation of BECs upon Nm infection, especially in hCMEC/D3 models.
Discussion: Our work highlights the usefulness of advanced physiologically accurate *in vitro* models to study Nm interaction with the mBCSFB. These models can also be favorable tools to study modulation and penetration of blood-CNS barriers by other meningeal pathogens such as *Streptococcus pneumoniae* or *S. agalactiae* as well as pharmacological intervention.

039/PWV
Genome placement of alpha-hemolysin cluster is associated with host, virulence-associated factor profile and alpha-hemolysin sequence variation of *Escherichia coli*

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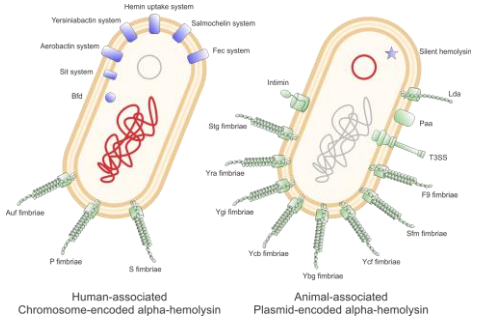
Introduction: Since the discovery of hemolysis, many studies focused on a deeper understanding of this phenotype in *E. coli* and its association with other virulence genes, diseases and pathogenic attributes in the host. So far, alpha-hemolysin as a hemolysis factor was mainly analysed in the context of humans and urinary tract infections. The aim of this study was to explore genomic signatures of host adaptation, virulence-associated factor prevalence and alpha-hemolysin sequence variation in alpha-hemolysin-encoding *E. coli*.
Methods: The study was based on a collection of 3000 genomes comprising in-house sequenced or RefSeq database strains. The bioinformatic analysis included phylotyping, serotyping, MLST typing, virulence factors profiling, pathotyping, GWAS, BAPS, phylogeny and alpha-hemolysin genome placement and sequence variation. Additionally, a new dataset of adhesins, toxins and iron acquisition genes was generated for in-depth analysis of these virulence factors.
Results: Virulence-associated factor profiling and genome-wide association analysis of hemolytic and nonhemolytic *E. coli* unveiled a high prevalence of adhesins, iron acquisition genes and toxins in hemolytic bacteria. Alpha-hemolysin encoded on the chromosome was associated with humans and plasmid-encoded alpha-hemolysin was found in farm animal isolates. Strains with different hemolysin genome placement and host origin had distinct adhesin and iron acquisition repertoire. Human *E. coli* with chromosome-encoded alpha-hemolysin had a high frequency of P, S, Auf fimbriae and multiple iron acquisition systems such as aerobactin, yersiniabactin, salmochelin, Fec, Sit, Bfd and hemin uptake systems. Animal *E. coli* with plasmid-encoded alpha-hemolysin had a similar adhesin profile to nonpathogenic *E. coli*, with a high prevalence of Stg, Yra, Ygi, Ycb, Ybg, Ycf, Sfm, F9 fimbriae, Paa, Lda, intimin and type 3 secretion system encoding genes. Pathotyping of alpha-hemolysin-positive *E. coli* revealed that the vast majority of human chromosome-encoded strains belonged to UPEC. Animal strains with plasmid-encoded hemolysin had no typical pathotype-characteristic virulence factors or belonged to aEPEC or ETEC. Analysis of HlyCABD sequence variation revealed the presence of variants associated with genome placement and pathotype.
Discussion: Taking into consideration that all investigations concerning the role of alpha-hemolysin were conducted using human *E. coli* isolates with chromosome-encoded hemolysin, this is the first study to examine *E. coli* genomes with plasmid-encoded hemolysin. We found that strains with plasmid-encoded hemolysin, which originate from animals, differ considerably in comparison to bacteria with chromosome-encoded hemolysin. Our results indicate that *E. coli* with plasmid-encoded alpha-hemolysin requires further attention with a focus on phenotypic characterization of host specific toxicity of alpha-hemolysin variants.

Fig. 1



040/PWV

Escherichia coli alpha-hemolysin HlyA induces intestinal epithelial barrier dysfunction and cell polarity change via PTEN-dependent regulation of cell junctions

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Introduction: *Escherichia coli* B2 phylotype reside the intestines of animals and humans. The pathogenicity factor α-hemolysin (HlyA) can induce intestinal epithelial leaks. In this study, we addressed the question, which host cell processes, dysregulated by *E. coli* HlyA, lead to impairment of epithelial barrier function and cell polarity and may potentiate intestinal diseases.
Method: Colon carcinoma Caco-2 cells were infected by HlyA$^+$ *E. coli*. Cell polarity regulation was visualized by live cell imaging in confocal microscopy for the phosphatidylinositol-4,5-bisphosphate (PIP2) abundance. Transepithelial electrical resistance (TER) was measured for characterization of barrier function in Caco-2 monolayers. Cell proliferation and separation of tight junctions and adherence junctions was analyzed by confocal microscopy. Cell signaling was analyzed by RNA-Seq and bioinformatics Ingenuity Pathway Analysis (IPA) software.
Results: Toxinogenicity from *E. coli* HlyA in the colon carcinoma cell line Caco-2 was characterized by PIP2 reduction at the host cell membrane, which was paralleled by inhibition of PTEN (phosphatase and tensin homolog), leading to the cell polarity changes. Epithelial barrier dysfunction followed these polarity changes by disruption of tight junctions with a reduction of TER to 50% within 3 h. Epithelial cell detachment was shown to increase and the separated Caco-2 cells in the culture supernatant remained viable (with 27±5 viable separated cells, shedded from Caco-2 monolayer in *E. coli* HlyA$^+$ infection versus 5±2 viable shedded cells in infection with the isogenetic *E. coli* HlyA-deficient mutant; $P<0.01$, n= 6–12, Student's *t*-test). This phenomenon of cell shedding could contribute of the induction of *focal leaks*. The affected cell signaling pathways by HlyA, e.g. the canonical pathways *PTEN signaling* or *colorectal cancer metastasis signaling* were identified by RNA-Seq and bioinformatics calculations in IPA.
Discussion: *Escherichia coli* HlyA affects host cell polarity and induces epithelial barrier disruption by retraction of tight junction proteins and induction of *focal leak* contributing to the diarrhea and antigen influx mechanism of the *leaky gut*. Signaling analysis revealed a broader insight into regulation mechanisms in this infection.

041/PWV

Bacterial factors influencing the exit of uropathogenic *Escherichia coli* from intracellular bacterial communities in bladder epithelial cells

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Introduction Uropathogenic *Escherichia coli* (UPEC) account for the majority of urinary tract infections (UTI), an increasingly

global disease burden prevalently among women. Despite antibiotic treatment, recurrence of infection is often reported. Recurrent UTI is usually treated with long-term, low-dose antibiotic further enhancing the risk of resistance development. One reason of recurrent UTI is the formation of intracellular bacterial communities (IBCs) by UPEC. Intracellular UPEC are protected from the host immune system and are more resistant to antimicrobial agents. Reservoir formation and subsequent reactivation and exit of UPEC from infected cells contributes to recurrent UTI. Our aim is to unravel bacterial factors involved in the intracellular life cycle of UPEC focusing on the exit from infected cells.

Materials & Methods We use an *in vitro* bladder epithelial model employing RT-112 bladder epithelial cells. RT-112 cells were infected with UPEC bacteria and subsequently the invasion, intracellular survival and the escape of the bacteria from the cells are quantified. The formation of IBCs and the localization of bacteria inside the cells was monitored by fluorescence microscopy. By means of recombineering we constructed specific deletion mutants of UPEC strains to test the impact of the corresponding genes on the bacterial phenotype.

Results We showed the development of IBCs as a prerequisite for further studies. We also observed the typical filamentation of bacteria, which is a strong indicator of bacteria exiting the cells. Our results show that different UPEC strains can invade and exit bladder epithelial cells to varying degrees. Investigation of several mutant strains showed that UPEC strain CFT073 deleted in the pore-forming toxin α-hemolysin is impaired not only in the invasion of bladder epithelial cells but also to much greater extent in the exit of cells. The use of either autophagy inducer or inhibitor did not influence invasion and exit of bacteria whereas the inhibition of the acidification of lysosomes in bladder epithelial cells showed a huge impact on invasion as well as on exit of bacteria from cells. The lack of colocalization of bacteria with endosomal or lysosomal markers indicates a rapid escape of bacteria to the cytosol of the cells.

Discussion Our results show clearly that the intracellular life-cycle of UPEC can be influenced by interfering with the endosomal/lysosomal pathway. The mechanism by which the cells and/or the bacteria are affected remains to be elucidated. Using several deletion mutants of UPEC strain CFT073 showed that the pore-forming toxin α-hemolysin has a major impact on the exit of bacteria from the cells. Further studies are needed to explain the mode of action of α-hemolysin during exit of bacteria from the cells.

Workshop 8
Enteric infections and epithelial barrier interaction (FG GI)
06. Sep 2022 • 08:30–10:00

042/GIV
Gut epithelial barrier dysfunction caused by *Campylobacter jejuni* is alleviated by vitamin D

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Introduction: The zoonotic foodborne bacterium *Campylobacter jejuni* (*C. jejuni*) is among the most frequent causes of bacterial gastroenteritis worldwide. Our study aims on a characterization of the relationship between epithelial barrier disruption, mucosal immune activation and vitamin D (VD) treatment in *C. jejuni* infection.

Method: A mouse model and human intestinal epithelial HT-29/B6 cells were used to study the interaction of *C. jejuni* with the VD signaling pathway and the efficiency of VD treatment (200 nM calcitriol) to improve epithelial barrier dysfunction during *C. jejuni* infection. Ussing chamber experiments were performed for assessment of epithelial electrical resistance measurements and paracellular marker fluxes. In parallel, molecular analysis was done by Western blotting and immunostainings for confocal microscopy. RNA-seq of human colon biopsies was performed prior to the investigation (four patients with campylobacteriosis versus six controls (GEO accession number GSE88710 to utilize the data to a cell signaling prediction software; Ingenuity Pathway Analysis).

Results: RNA-Seq datasets from colonic biopsies from patients with campylobacteriosis revealed an inhibition of VD receptor (VDR) downstream targets, related to signaling showing suppression of immune function. To verify this, the interference of *C. jejuni* with the VDR pathway was shown via VDR/retinoid X receptor (RXR) interaction by confocal microscopy. To overcome this inhibition by *C. jejuni*, treatment with VD was performed, which showed intestinal barrier-preserving effects under *C. jejuni* infection in the IL-10$^{-/-}$ mouse model as well as in human epithelial HT-29/B6 cells. Functional measurements of paracellular leakage of the *C. jejuni*-infected epithelia showed its molecular correlate in tight junction (TJ) protein redistribution off the TJ domain and induction of apoptosis. Moreover, the transmigration of *C. jejuni* through the cell monolayers was reduced by VD treatment. The supplementation with VD reversed barrier disruption and prevented the inhibition of the VDR pathway. This was paralleled by the functional recovery of transepithelial electrical resistance and permeability for fluorescein (332 Da; $P_{fluorescein}$: $0.35\pm0.04 \cdot 10^{-6}$ cm/s in control versus $0.70\pm0.03 \cdot 10^{-6}$ cm/s under *C. jejuni* ($P<0.05$) versus $0.30\pm0.03 \cdot 10^{-6}$ cm/s under VD treatment and *C. jejuni* ($P<0.01$), one-way ANOVA with Bonferroni correction, n = 6 – 7).

Discussion: In conclusion, VD treatment restores gut epithelial barrier function and decreases bacterial transmigration. VD supplementation might be a promising additional compound for multimodal treatment in humans and animals against *C. jejuni*.

043/GIV
Human gastric organoids reveal *Helicobacter pylori* tropism to highly differentiated pit cells

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Introduction: The human gastric gland is highly structured and containing different subtypes of cells. *Helicobacter pylori* (*H. pylori*) bacterium is known as carcinogenic pathogen capable to colonise the human gastric niche. The bacterium can attach to host cells and translocate its virulence factor CagA, which interacts with a variety of host factors and alters cellular pathways. A possible cellular tropism of *H.pylori* has not been elucidated.

Material/method: We used human gastric organoids and organoid-derived monolayers to recapitulate complex cellular heterogeneity of stomach epithelium. The model systems and infection of the monolayers were characterized using single-cell RNA-seq and results were verified using a range of molecular biology methods. Directed differentiation of organoids was used to

enrich the cultures for specific cell populations. Candidate genes were deleted using CRISPR/Cas9 in human gastric organoids.
Results: RNA sequencing demonstrated that organoid-derived monolayers rather resemble the pit region, while organoids represent the gland region of the gastric units. Further analysis highlighted several markers for highly differentiated pit cells, targeted by *H. pylori*. Directed differentiation of the monolayers lead to enrichment of the target cell population and confirmed preferential attachment. Attachment was independent of MUC5AC expression.
Discussion: Gastric organoids and organoid-derived monolayers represent distinct regions of the gastric gland such as deep gland and pit region, respectively, and each is a useful model to study interactions with distinct heterogenic cellular populations. Subsequent studies will have to identify the importance of the preferential target cell binding of *H. pylori*.

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Identification of antibiotics that do not cause Shiga-toxin mediated renal disease in *C. rodentium φstx2dact*-infected mice

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Enterohaemorrhagic *Escherichia coli* (EHEC) is a food-borne pathogen that causes disease ranging from watery diarrhoea to acute renal failure. Disease progression upon infection is attributed to the production and release of Shiga toxins (Stx). The treatment of EHEC infections is currently supportive and the use of antibiotics is highly controversial due to reports that antibiotics induce Shiga toxin expression and may, therefore, increase the risk of HUS development. While Stx induction has been assessed *in vitro* with a plethora of antibiotics and a variety of EHEC strains, *in vivo* confirmation has been hampered by the lack of an adequate mouse model. In 2012, Mallick et al[1] introduced the lysogenic phage encoding *stx2d* from EHEC into the mouse pathogen *C. rodentium*, creating a strain that mimics a human STEC infection in mice.
Here, we assessed a number of commercially available antibiotics for their ability to induce the production and release of Shiga toxin *in vitro* and *in vivo*. We infected mice with *C. rodentium φstx2d* and determined whether any antibiotics could clear the infection without detrimental effects to the host. Using the fluoroquinolone antibiotic enrofloxacin as an example for a known Shiga toxin-inducing antibiotic, we observed that the mice showed rapid weight loss after treatment-onset even though the infection was cleared. Kidney histology and serology for the kidney injury marker BUN (blood urea nitrogen) showed kidney damage comparable to that observed for infected but untreated mice. A number of tested antibiotics were, however, able to clear the infection without resulting in kidney damage. Interestingly, rifampicin, a protein synthesis inhibitor, was unable to clear infection at the concentrations used in this study. Rifampicin did not, however, induce Stx-mediated kidney damage, supporting in vitro data suggesting that rifampicin inhibits Shiga toxin production. This data suggests that while some antibiotics induce the SOS response and increase Stx production and release, other antibiotics may well be used as treatment options, especially during outbreaks.
[1]Mallick EM et al. (2012). J Clin Invest. 2012 Nov;122(11).

045/GIV

Enzymatic consumption of L-arginine promotes intestinal dysbiosis and colitis

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Question: The metabolism of the semi-essential amino acid L-arginine (L-arg) is complex due to the interactions of multiple enzymes that compete for L-arg as common substrate. For example, L-arg is interconvertible with other amino acids such as glutamate or proline. Furthermore, it serves as a precursor for the conversion into agmatine, creatine, nitric oxide, and urea or for the synthesis of proteins. Particularly, the dichotomy between the two L-arg consuming enzymes, arginase 1 (Arg1) and the inducible nitrix oxide synthase (NOS2), on the function of macrophages has been intensively investigated in the past. However, L-arg exhibits also pleiotropic effects on various other hematopoetic and non-hematopoetic cell populations and on microbial growth, which have not been studied in detail. Furthermore, the effects of L-arg consumption on the composition of commensal microbiota and the maintenance of intestinal immune homeostasis are not well understood. Therefore we started to investigate the role of Arg1 and NOS2 in the pathogenesis of experimental colitis and inflammatory bowel disease (IBD).
Methods: The expression of Arg1 and of NOS2, the composition of the intestinal microbiota, the intraluminal metabolome and the severity of intestinal inflammation was assessed in IBD patients and in mouse models of acute and chronic dextran sodium sulfate (DSS) and oxazolone-induced colitis using immunohistochemistry, qPCR, 16S rRNA sequencing, high resolution endoscopy, liquid chromatography/mass spectrometry and different conditional Arg1-knockout mice.
Results: The expression and enzymatic activity of Arg1 and of NOS2 correlated with the degree of intestinal inflammation in (sub-) mucosal tissue samples of IBD patients. Unexpectedly, Arg1-expressing hematopoietic and endothelial cells, similar as a global NOS2 expression, hampered the resolution of intestinal inflammation in both experimental colitis models. Indeed, disease progression correlated with compositional changes in the intestinal microbiota and an altered metabolome including a reduced availability of intraluminal L-arginine. Accordingly, arginine-free chow accelerated colitis while a dietary supplementation of L-arginine ameliorated disease. Fecal microbiota transplants (FMTs) from wild-type litters supplemented with L-arginine restored the protective, anti-inflammatory phenotype in recipient mice, similar as FMTs from control chow fed Arg1- or NOS2-knockout donors, suggesting that protection from colitis is related to an enhanced availability of L-arginine and the expansion of an anti-inflammatory microbiota.
Conclusions: Our data support the novel concept that Arg1 and NOS2 prevent the resolution of intestinal inflammation due to the consumption of intraluminal L-arg. Subsequently, L-arg metabolism and the dietary supplementation of L-arg represent promising diagnostic and therapeutic targets for clinical intervention in IBD.

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The pleiotropic actions of probiotic *Escherichia coli* Nissle 1917 promotes fitness against enteropathogenic *E. coli* in the surrogate insect host *Galleria mellonella*

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Introduction: The probiotic *Escherichia coli* strain Nissle 1917 (EcN) confers a health benefit to humans suffering from acute and persistent diarrheal diseases. Despite the widespread medical application of EcN, the underlying mechanisms of communication between the probiotic and its hosts remain largely elusive. EcN

secretes antimicrobial peptides (microcins M and M47) to limit growth of competing *Enterobacteriaceae*. We established the greater wax moth *Galleria mellonella* as a new *in vivo* model host for elucidating the role of microcins for competition between probiotic EcN and enteropathogenic *E. coli* (EPEC) during gut colonization. We aim to unravel novel functions of microcins in terms of (i) increased EcN fitness in the gut and (ii) chromatin modifications of host gene expression.

Materials and methods: We compared the microcin M and M47 mutant SK22D and a di-guanylate cyclase mutant EcNΔ*yegE* (exhibiting reduced biofilm formation) with the wild-type strain EcN and analyzed probiotic effects in the *G. mellonella* intestinal infection model at 37°C. The larvae were force-fed first with the wild type strain EcN or the mutants EcNΔyegE or EcN SK22D, and after 24 h with the EPEC strain E2348/69.

The larvae were homogenized in lysogeny broth (LB) 1, 24, 48, 72, and 96 h post-oral infection with EPEC to examine the competitive fitness of EcN wild type and its mutants during competition with EPEC in the *G. mellonella* gut. The homogenates were plated onto LB plates complemented with the appropriate antibiotic, and colonies were counted after incubation at 37°C for 24 h.

The foregut, midgut, and hindgut were dissected from larvae 24 h after EPEC feeding as mentioned above. DNA, RNA, and histones from the foregut, midgut, and hindgut were isolated using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany), phenol-chloroform, and EpiQuik total histone extraction kit (EpiGentek, Farmingdale, NY, USA) respectively. The measurements of DNA methylation, histone acetylation, and gene expression were performed by ELISA and RT-PCR.

Results: We report that EcN colonization in the gut protect larvae from EPEC infection by direct antagonism and inducing host antimicrobial peptide (AMP) gene expression. We suggest that EcN colonization in the gut is promoted by the YegE, while microcins regulated the probiotic effects through reducing pathogen survival. In addition, EcN wild type colonization induced expression of genes encoding antibacterial peptides as well as chromatin remodeling of the gut epithelial cells.

Discussion: We show that EcN plays a dual role by directly eliminating EPEC from the gut epithelial surface and sensitizing innate immunity for faster pathogen clearance and chromatin modifications.

047/GIV

A phage lysis cassette contributes to the virulence of *Yersinia enterocolitica* towards *Galleria mellonella*

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Questions: The human pathogen *Yersinia enterocolitica* strain W22703 exhibits insecticidal and nematocidal activity. This phenotype is caused by proteins of the toxin complex (Tc) encoded by the pathogenicity island Tc-PAI*Ye*. The island genes code for two regulators, TcaR1 and TcaR2, and for the toxin subunits. They are expressed at environmental, but silenced at body temperature. The three subunits form a tripartite ABC-type toxin complex, with subunit C causing cell death through its ADP-ribosyltransferase activity. Molecular and pathophysiological details of insect larvae infection and killing by strain W22703, however, have not been dissected. In particular, the mechanism by which the Tc proteins are translocated across the bacterial membranes still lacks disclosure.

Methods: Using larvae of the Greater waxmoth, *Galleria mellonella*, we established an oral model of infection that allowed us to follow the natural route of infection. For this purpose, larvae were infected with strain W22703 and its *tc*-gene mutants, and the survival rate of the insect were determined in correlation with *Y. enterocolitica* cell numbers. In addition, we isolated W22703 cells via immunomagnetic separation at two time points after infection.

Results: Time course experiments allowed the dissection of distinct *Y. enterocolitica* infection stages starting with gut colonisation followed by invasion of the hemolymph, where the pathogen strongly proliferated. The transcriptome of strain W22703 is mainly characterized by a drastic reprogramming of the energy, amino acid and carbohydrate metabolism, by an increase of motility and signaling molecules, and by cell membrane rearrangements. Several phenotypes including penetration of the gut epithelium depend on the presence of the Tc proteins. Strikingly, a mutant lacking a phage-related holin/endolysin (HE) cassette, which is located within Tc-PAI*Ye*, resembled the phenotypes of W22703 D*tcaA*.

Conclusions: Our data demonstrate the pivotal role of the Tc proteins in the infection and killing of insect larvae, the specific transcriptional adaptation of *Y. enterocolitica* to the invertebrate host, and the function of the dual lysis cassette for the release of a bacterial toxin.

Workshop 9
Bacteriophages today - from basic research to patient treatment (FG MV)
06. Sep 2022 • 08:30–10:00

048/MVV

Potential of giant phage muc347 for the treatment of infections with multidrug- resistant *Klebsiella pneumoniae*

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Questions: Despite of an increased awareness for the need of antibiotic stewardship in veterinary and human medicine, the development of antimicrobial resistance (AMR) in bacteria with human and animal hosts is still on a rise. The multidrug-resistant (MDR) bacteria of the ESKAPE group represent a major threat to human health due to their potential to acquire and exchange AMR genes. As antibiotic-based therapies become less effective, phages are rediscovered as promising adjuvant and last-line treatment options against MDR bacteria in humans.

Here, three distinct *K. pneumoniae* (Kp) strains causing recurrent osteomyelitis in a patient in 2020/21 were used to isolate and characterize lytic phages and their suitability for adjuvant treatment.

Methods: Screening for lytic phages was performed by plaque activity assay using samples from a wastewater treatment plant (WWTP) in Munich collected in 2021. After purification (single plaque isolation) and propagation (high-titer lysates, incl. CsCl-gradient purification), the biological properties of the phages were analysed *in vitro*. Genome data on Illumina WGS was used for *in depth* analysis of isolated phages and human Kp strains

Results: Three MDR *K. pneumoniae* isolates recovered from repeatedly occurring human infections showed distinct AMR profiles including loss of sensitivity to different carbapenemases. Multilocus sequence typing and SNP phylogeny revealed a close genetic relationship of two isolates, while the third exhibited substantial differences. Highly active phages against the Kp-isolates were recovered from samples of the WWTP. Subsequent microbiological/molecular characterization revealed one phage with a genome of >300 kb belonging to a yet underreported group of giant viruses (myovirus). Microbiological and genetic analyses of the genome suggest it is a promising candidate for therapeutic use.

Conclusion: Wastewater represents a promising source for genetically diverse *Klebsiella* phages including giant phages suitable for the treatment of MDR *K. pneumoniae* causing human infections.

049/MVV

Effects of phage-antibiotic combinations on differential gene expression of a *S. aureus*/*P. aeruginosa* co-culture

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Introduction: So far, most studies have focused on phage-bacterial interactions in mono-species systems. However, many infections (e.g., wounds) are polymicrobial and little is known about the interactions between different phages. In this study the effect of a simultaneous challenge of *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) by the phages Sb-1 and NP-3 with or without co-addition of meropenem (MP) was investigated at mRNA level.
Material/Methods: A co-culture of a multidrug resistant clinical isolate of SA- and PA-strain were challenged with single to triple combinations of the antimicrobials followed by transcriptome analysis after 1 hour of incubation, using RNAseq and the Illumina sequencing platform, along with CFU determination. Experiments were performed in LB-medium and on pig skin tissue samples.
Results: In LB-medium, combined phages led to an at least 2-fold downregulation in up to 15% of phage genes. Conversely, 25% upregulated phage genes were observed with co-addition of MP. When challenged with single Sb-1 or single MP, down regulation dominated over upregulation in SA which was even more pronounced with the Sb-1/NP-3 combination (205 genes/ 7%). Upregulation of SA-genes prevailed, when PA was challenged by single NP-3 but strongest gene expression occurred with the triple combination (488 genes/ 16%). PA generally responded by upregulation of genes (23 – 113 genes/ 1-2%), again with the highest number of genes being affected with the triple combination (190 genes/ 3%). Differential expression of genes by at least factor 8 were mainly those involved in anaerobic respiration. Unlike PA, SA also upregulated genes involved in protein biosynthesis. A significant bacterial reduction was only achieved with the triple combinatory approach. The analysis of the skin tissue samples is nearly completed and will also be presented.
Discussion: The combination of two phages without MP resulted in general downregulation of phage genes slowing down phage propagation. However, the addition of MP to the phages not only reversed this effect but the enhanced upregulation of bacterial and phage gene expression resulted in bacterial decline already one hour after incubation. The comparison between standard laboratory conditions, i.e., LB-medium, with more realistic conditions, i.e., skin tissue samples, will provide insights into the stability or universality of phage-phage and phage-antibiotic interactions under different environmental conditions.

050/MVV

Interrogating COVID-19, the lung microbiota, and therapeutic phages to mitigate secondary lung infection and inflammation

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The Coronavirus 2019-nCoV pandemic greatly burdens both society and the healthcare system. Like other respiratory viruses, the mortality rate due to acute bacterial pneumonia caused by co-infection with multi-resistant bacteria in COVID-19 patients is estimated to be up to 16%. Using an interdisciplinary approach, we systematically identified the co-infecting multi-resistant bacteria in COVID-19 patients, then isolated multiple virulent phages against these bacteria through culture-dependent and culture-independent methods. We next developed a multispecies phage cocktail consisting of five highly efficient phage isolates, with a broad interspecies host range, against the most common sources of bacterial pneumonia in COVID-19 patients, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. We examined the efficacy of the cocktail against these bacteria through *in vitro* tissue culturing and *in vivo* using wax moth larva, as well as evaluating its anti-biofilm effect. Going forward, we will further assess the safety and efficacy of the cocktail in animal models, which will pave the ground for the future clinical trial of the designed cocktail against bacterial pneumonia.

051/MVV

Predicting the clone-specific host range of *Staphylococcus aureus* phages based on computational clustering of receptor-binding proteins

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Staphylococcus aureus poses a high risk of life-threatening, often antibiotic-resistant infections, and its phages might become helpful for diagnostics and disease treatment. The most important factor determining the host range of *S. aureus* phages is the species-specific structure of wall teichoic acids (WTA), which is the only know phage receptor in *S. aureus*. While most other *Staphylococcus* species carry a WTA composed of a glycerol-phosphate backbone, nearly all *S. aureus* strains possess a ribitol-phosphate WTA with GlcNAc-glycosylation in different conformations. These cell wall polymers cannot mutate as easily as proteinaceous phage-receptors of other bacteria such as Gram-negatives. Due to this slow-evolving nature of the phage-receptors, we aimed to investigate the degree of conservation, structure and function of the corresponding receptor-binding proteins (RBPs).
Through bioinformatic analysis of *S. aureus* phage genomes, we discovered various RBPs necessary for adsorption to the host. With the help of multi genome analysis of over 300 *S. aureus* phage genomes, we found that *S. aureus* ribitol-phosphate binding phages cluster in 10 different groups based on their predicted RBPs. To confirm the binding capabilities of these receptors, protein fusion constructs were created by addition of a fluorescent N-terminus to the phage RBPs. These constructs were then used to investigate the specific binding of these proteins to the host cells, which matched the in-vivo behavior of the corresponding phages. The created phage clusters will allow us to predict the success of phage adsorption to different WTA glycosylation types during the initiation of phage infection and may be useful for gaining more insights into the host range of both known and novel phages.

052/MVV

Enterococcus faecium-specific bacteriophages and their effect on biofilm cultures

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Introduction: *Enterococcus (E.) faecium* does not only belong to the commensal intestine flora of humans and animals but also causes infections such as urinary tract infections, endocarditis, sepsis, and dental root canal infections as a facultative pathogen. Due to the formation of biofilms and the increasing relevance of resistance against vancomycin, *E. faecium* has developed into a therapeutic challenge. Bacterial biofilm formation leads to a decrease in sensitivity to anti-infective drugs. Bacteriophages might be useful for the treatment of bacterial biofilms, even in the case of resistant strains.
Material/Methods: We isolated nine *E. faecium* phages from sewage water and tested their ability for bacterial lysis on different (partly vancomycin resistant) clinical *E. faecium* isolates. Based on their host range, the best three phages were further characterized by determining the adsorption rates on bacteria and the one step replication kinetics. Additionally, these phages were used for testing their ability to prevent and inhibit enterococcal biofilms.

Results: The nine phages lysed 74 out of 82 tested *E. faecium* clinical isolates, with 60 of the 82 strains showing vancomycin resistance. Additionally, 30 out of 50 tested *E. faecalis* isolates could be lysed. By means of transmission electron microscopy, two phages were characterized as myoviruses, one as a syphovirus, and five as podoviridae. Three tested phages successfully prevented the biofilm formation of two clinical isolates in microtiter plates, using crystal violet staining. Furthermore, using the viability stain PrestoBlue, the reduction in the number of living cells in 24 h old biofilms caused by two of the isolated phages was detected.
Conclusions: Based on the host spectrum and their effects on biofilm formation, three of the tested phages might qualify as best candidates for further clinical testing. The application of bacteriophages could improve the anti-infective therapy repertoire and might reduce the amount of antibiotics used.

053/MVV
Artilysin® represents a promising antibacterial agent against *Acinetobacter baumannii* infections
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Introduction: The widespread emergence of antimicrobial infections with multidrug-resistant (MDR) Gram-negative bacteria such as *Acinetobacter baumannii* is a severe public health threat. The designed phage-derived endolysin Artilysin® Art-Top3 represent an alternative to conventional antibiotics. Artilysin® molecules pass the outer membrane of Gram-negative bacteria, resulting in cell wall destabilization and subsequent bacterial death from osmotic pressure.
Material/method: The antibacterial activity of engineered Art-Top3 (Lysando Innovations Lab GmbH) against clinical isolates of carbapenem-resistant Enterobacterales (CRE) and *A. baumannii* was determined by broth microdilution, *in vitro* activity assays and time-kill kinetics. Biofilm assays were performed under static and dynamic conditions in order to determine the impact of Art-Top3 on mature biofilms and biofilm growth. Activity of Art-Top3 against *A. baumannii* was determined in human serum as a matrix. Cytotoxicity of Art-Top3 was evaluated in human cell lines using the MTT assay. Art-Top3 influence on human erythrocytes was examined *in vitro*. The *Galleria mellonella* (larvae of the greater wax moth) infection model was employed to assess the antibacterial activity of Art-Top3 *in vivo*.
Results: Art-Top3 showed a three-fold higher antibacterial activity (mean MIC: 0.25 µM) against carbapenem-resistant *A. baumannii* compared to other CRE such as *Enterobacter cloacae and Klebsiella pneumoniae* (mean MIC: 0.76 µM) isolates. The biomass of preformed, mature *A. baumannii* biofilms was strongly reduced (≥ 50%) by 10xMIC Art-Top3 treatment. The simultaneous incubation of bacteria and Art-Top3 almost completely prevented biofilm formation of *A. baumannii*. Art-Top3 showed high activity in up to 100% human serum after 24 h of incubation. No cytotoxicity of Art-Top3 against *A. baumannii* in human cells was detected even at a high dose of 25 µM. Human erythrocytes were not lysed after incubation with high Art-Top3 concentrations (2 mg/ml). After 72 h, Art-Top3 rescued 40% of *Galleria mellonella* from a lethal *A. baumannii* infection.
Discussion: Art-Top3 exhibited a high antibacterial activity towards carbapenem-resistant *A. baumannii* in time-kill kinetics, anti-biofilm assays and *Galleria mellonella* infection experiments. Antibacterial activity of Art-Top3 was shown also in several other Gram-negative species, and therefore, these results provide a promising basis for novel therapeutic strategies of infections caused by MDR Gram-negative species.

Workshop 10
SARS-CoV-2: lessons learned for IPC (StAG HY/FG PR)
06. Sep 2022 • 14:45–16:15

054/HYPRV
Divergent dynamics of humoral and T cell immunity after two and three BNT162b2 vaccinations in adults aged >80 years
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Question: How are the humoral and T cell responses after a 3rd compared to a 2nd BTN162b2 vaccination?
Methods: Quantifying SARS-CoV-2-specific antibodies, performing SARS-CoV-2 neutralization tests, and assessing antigen-reactive T cells (identified as $CD40L^+IFN\gamma^+CD4^+$T cells after overnight stimulation of PBMC with SARS-CoV-2 spike peptides) by Flow Cytometry, we compared the specific humoral and cellular response after 3rd vs. 2nd vaccination with BNT162b2 in a cohort of adults aged >80 years.
Results: RBD-specific IgG (fig 1B) and SARS-CoV-2-neutralizing antibody (fig 1F) titers were substantially increased (fig 1D) after 3 compared to 2 doses, while marginally higher spike S1-specific blood IgG concentrations were observed 2 weeks after 3rd than after 2nd vaccination (fig 1D,E). Concentrations of S1- specific IgG and neutralizing antibodies declined from the acute responses at week 5 and week 26, but at a lower rate and with extended half-life after 3rd (W40) compared to 2nd (W24) vaccination (Fig 2).
Spike-specific CD4 T cell frequencies reached similar levels after 2 and 3 doses (fig 1C,D). After the respective acute response, frequencies returned to approximately pre-third vaccination levels with no significant differences in the rate of decline after 2nd and 3rd vaccination (fig 1C). Quantified cytoplasmic expression of IFNγ indicated functional enhancement of spike-specific T cells upon 2nd but not further upon 3rd vaccination, while more cytoplasmic IFNγ was found in spike-specific CD4 T cells from senior adults recovered from COVID-19 (fig 1G).
Conclusions: A third vaccination against SARS-CoV-2 in older adults induces a durably escalated humoral response in the bulk of vaccinees for at least three months, indicating longer lasting humoral immunity. Peak virus-specific T cell frequencies were not further increased after a 3rd vaccination and average per-cell production of IFNγ remained not only unaltered but also remarkably lower than in recovered donors of similar age. Thus, even a 3rd BNT162b2 dose failed to induce durably enhanced quantities of spike-specific T cells and a functional quality reached after natural infection.
Legend
Fig 1. Study overview (A). SARS-CoV-2 RBD-specific serum IgG levels (B) and frequencies of SARS-CoV-2 spike-specific T cells (C) were analyzed. Log2 of the fold change in peak response values for spike (S1 subunit)- and RBD-specific IgG, neutralizing antibody titers, and spike-specific T cell frequencies in participants after 2nd and 3rd vaccination (D). Analysis of S1-specific serum IgG levels (E) and serum titers of 100% virus neutralization for SARS-CoV-2 (F). Comparison of the median staining intensity (MSI) of IFNγ of spike-specific T cells of vaccinated participants and of >80 year old donors recovered from COVID-19 (G). Each symbol represents data of one donor. Horizontal lines indicate median values. The dotted horizontal line indicates the cutoff for antibody positivity at 7.1 BAU/mL (B), 35.2 BAU/mL (B) and the lower detection limit for the VNT 100 assay at a reciprocal titer of 8 (C). *P* values were determined by two-tailed Wilcoxon matched-pairs signed rank test throughout except when comparing

vaccinated (W26) vs recovered groups (F, two-tailed Mann-Whitney test).

Fig. 1

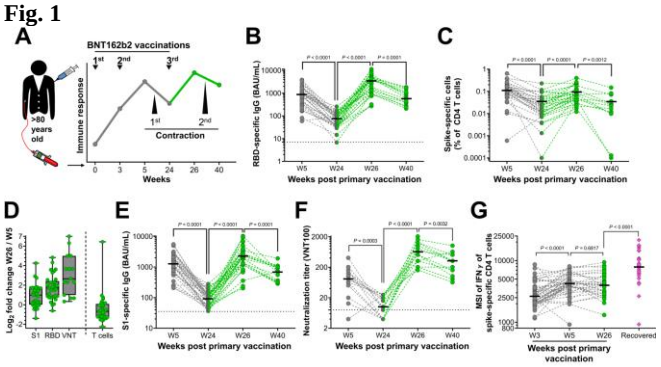
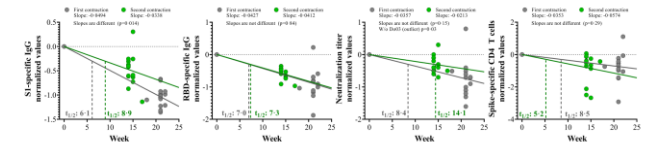


Fig. 2



055/HYPRV

Inability to work and side effects of the first, second and third dose of COVID-19 vaccination in healthcare workers

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Question: Viable healthcare workers play an essential role in the critical infrastructure needed to cope with the ongoing COVID-19 pandemic and its consequences on availability of public healthcare due to the increasing burden of disease. COVID-19 vaccinations became a central strategy to reduce the spread and the severity of SARS-CoV-2 infections, especially among healthcare workers. However, side effects and the consequent inability to work could overstrain public health and need to be taken into consideration. The aim of our study was to examine the number of sick days and the frequency of side effects after the first, second and third dose of COVID-19 vaccination of healthcare workers vaccinated with BNT162b2mRNA (Comirnaty BioNTech/Pfizer), mRNA-1273 (Spikevax, Moderna), ChAdOx1-S (VaxZevria, AstraZeneca) as well as Ad26.COV2-S (COVID-19 vaccine Janssen).

Methods: 1,797 study participants were recruited who met the following inclusion criteria: age ≥ 18 years, vaccination against SARS-CoV-2, working in healthcare. Data on side effects were collected by an electronic questionnaire.

Results: A total number of 588 healthcare workers (32.72%) were on sick leave due to side effects of at least one COVID-19 vaccination leading to a total number of 1,777 sick days. The maximum absolute number of sick days was taken after the second vaccination (785/1,777) with an average of 2.14 sick days per respondent. 498 of 1,406 persons with a third dose of COVID-19 vaccination took on average 1.92 days to recover. While only 5.65% of the participants took a sick leave after their first administration (mostly after receiving a vector vaccine), 20.66% took a sick leave after the second dose and 27.31% after the third one (Figure 1).

Figure 1: Probability to report sick after COVID-19 vaccination

Median length of sick leave was 2 days and lasted up to 47 days. While there was no significant difference in sick leave after the second dose between the vaccines BNT162b2mRNA and mRNA-1273, sick leave after the third COVID-19 vaccination was significantly longer in case of mRNA-1273 administration (Figure 2).

Figure 2: Length of sick leave after COVID-19 vaccination by dose and vaccine

The relative number of self-reported side effects decreased from first over second to the third vaccination dose whereas the number of reported effects was significantly different between the vaccines of BNT162b2mRNA and mRNA-1273.

Conclusions: A considerable number of healthcare workers took sick days after the COVID-19 vaccination. In mRNA vaccines sick leave occurred mostly after the second and third vaccine administration. As recommendations changed to heterologous vaccine regimes after a vector vaccine in Germany, no data could be reported for sick leave after vector vaccine as second or third dose. Side effects and consequent inability to work of healthcare workers due to COVID-19 vaccination are not negligible and should be further investigated.

Fig. 1

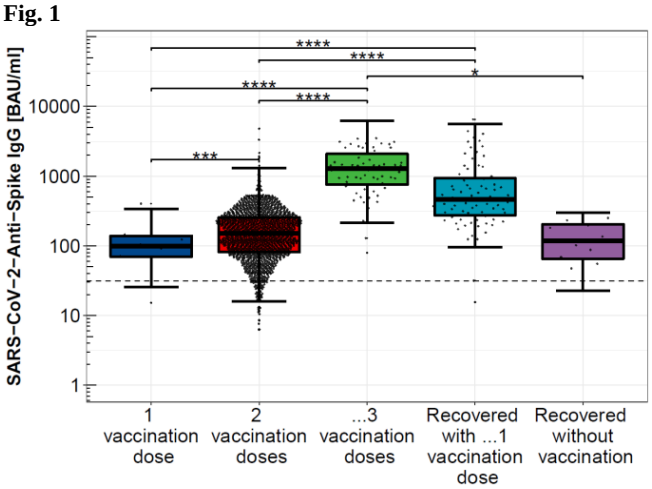
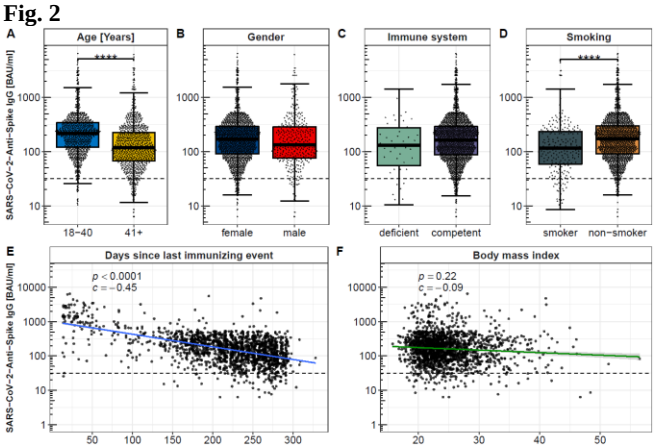


Fig. 2



056/HYPRV

Diminished immune responses following SARS-CoV-2 vaccination in a longitudinal cohort of dialysis patients

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Introduction: Patients undergoing hemodialysis were among the first to be vaccinated against SARS-CoV-2 due to their increased risk for severe disease and high case-fatality rates. As impaired cellular and humoral responses towards severe viral vaccinations have been identified within this group, we established a longitudinal cohort to assess antibody production and decay dynamics within this population.
Material and Methods: 51 individuals undergoing chronic hemodialysis were followed for 10 months, with blood and saliva samples collected 3 weeks post 2nd dose, 16 weeks post 2nd dose, 3 weeks post 3rd dose, 20 weeks post 3rd dose and 3 weeks post 4th dose. Cohorts of healthcare workers and healthy individuals with similar vaccine regimens were used as controls. Antibody titers in blood and saliva and ACE2 binding inhibition as a proxy for neutralization were assessed with multiplex bead-based immunoassays, while T-cell responses were assessed by IGRA.
Results: Following 2 doses, dialysis patients exhibited detectable but variable cellular and humoral immune responses against SARS-CoV-2. Although vaccination-induced antibodies were detectable in both saliva and plasma, antibody titer, ACE2 binding inhibition and T-cell mediated interferon γ release were all significantly reduced ($p<0.001$) compared to a control population. When the same cohorts were examined 3 months later, antibody titers had decreased significantly by 75% within the dialysis cohort ($p<0.001$), with 20% of the dialysis cohort and 0% of the control cohort classified as seronegative (value below assay cut-off). T-cell responses were also reduced for dialysis patients (651mIU/mL control, 370mIU/mL dialysis) with nearly twice as many being non-responsive (21% control, 40% dialysis). Following boosting, antibody titers, ACE2 binding inhibition and T-cell responses all increased within both groups but were significantly lower in dialysis patients ($p<0.001$). 84% of the dialysis cohort showed ACE2 binding inhibition response against WT, although this was reduced to 39% towards the Omicron BA1 variant. 3 months later, antibody titers and ACE2 binding inhibition once again significantly decreased in dialysis patients, with only 60% considered to have inhibitory antibodies against WT and only 10% against Omicron. A 4th dose strongly enhanced humoral immunity, with a significant increase ($p<0.001$) in ACE2 binding inhibition towards WT and an increase in response towards Omicron from 10% to 83%.
Discussion: Humoral and cellular responses are significantly boosted following admission of a 4th dose for patients undergoing dialysis, including against the Omicron variant. By contrast, the same level of response appears to be achieved by control patients following a 3rd dose. Further monitoring of this cohort will be required to assess the rate of antibody decay following the 4th dose as compared to the decay following the 2nd and 3rd doses respectively, and whether a 5th dose will be required.

057/HYPRV

From wild type to omicron – changes in in-hospital SARS-CoV-2 cluster characteristics and dynamics

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Introduction: SARS-CoV-2 in-hospital clusters have been described extensively. Often as single incidences on hospital level or as regional or national analyses. To what extent characteristics and dynamics of SARS-CoV-2 clusters varied over time was analysed here, for a German university hospital. This work emphasizes on virus variants and infection prevention and control (IPC) counter measures.
Methods: Clusters from 10/20 to 04/22 were included. A cluster was defined as an epidemiological linkage, validated by the infection prevention and control (IPC) team, between i) at least two patients or ii) at least three healthcare workers (HCW) or iii) at least one HCW and one patient. Clusters were grouped in accordance to virus variants into i.) clusters with supposedly predominant wild type, alpha and delta (WAD) SARS-COV-2 variants and ii.) clusters with predominantly omicron subtype cases. Since sequencing capabilities were restricted, cluster affiliation to either group was based on the primarily (>90%) circulating local variant. All cases were displayed longitudinally in relation to regional case counts and landmarks of IPC counter measures. Both groups were compared for specific characteristics e.g. duration and cases as well as dynamics, such as accumulated epi curves. For comparison Wilcoxon rank sum test and chi square test were used.
Results: Forty-two SARS-CoV-2 clusters with 528 cases were analysed. WAD-clusters were detected within 57 weeks from 10/20-11/21, whereas omicron clusters were detected within 11 weeks from 01/22–04/22. Twenty-one clusters with 297 cases were attributed to the WAD group and 21 clusters with 231 cases to the omicron-group. In terms of cluster characteristics, there were no significant differences in median size (8 vs. 8 cases, $p=0.94$) or median duration (14 vs. 12 days; $p=0.48$), nor in the proportion of involved HCWs (46.8% vs. 50.2%; $p= 0.48$). Patients in the WAD group were older (median 75 vs. 68 years; $p=<0.05$). Clusters mirrored the regional incidences. A longitudinal depiction of the cluster related cases showed an amplitude of cluster-related omicron cases, which did not exceed the amplitude(s) of cluster related WAD cases, whereas IPC measures were ever increasing. Both cumulated epi curves showed that time spans from onset to each cases" onset of infection were significantly shorter for the omicron group (median 6 vs. 11 days; $p=<0.05$).
Conclusions: SARS CoV-2 infection dynamics varied during the pandemic, depending on the predominant circulating virus variant. In-hospital clusters reflected the outside incidences, although the omicron clusters did not follow the excessive public case counts. Nonetheless, omicron clusters showed a sped up dynamic, forcing all involved parties to adapt. Consecutive counter measures and a possible increase of HCWs" IPC-competence therefore supposedly minimized the impact of omicron on the development of in-hospital clusters.

058/HYPRV

Encounters with uncertainty and complexity – reflecting on infection control in Aotearoa New Zealand during the COVID-19 pandemic

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The disruptiveness of the COVID-19 pandemic created the need for rapid responses in health systems already under pressure and challenged Infection Prevention and Control (IPC) experts in Aotearoa New Zealand with levels of uncertainty and complexity not previously encountered. Traditional health sector solutions to change in the external environment are often driven top-down, though when faced with uncertainty and unpredictable situations, collaboration and shared responsibility may be more effective (Figueria et al., 2019; Hammond, 2000).
Question: What insights do theoretical concepts such as "complex adaptive systems" and "collective competence" offer to assist in understanding how IPC experts navigated difficulties posed by decision-making under uncertainty and dealing with intrinsic cognitive and collaborative complexities during the COVID-19 pandemic response?
Methods: An exploratory case study was conducted using modified interviews based on the speed-dating technique to explore collaborative decision-making processes during the COVID-19 response by IPC experts (Infectious Diseases Physicians n=3; Public Health Physicians n=2; Microbiologist n=1; IPC Nursing Director n=1). Theoretical concepts of "complex adaptive systems" and "collective competence" underpinned data analysis of reflective discussions.
Results: Progress towards decisions was non-linear. As IPC experts collectively sought to make sense of the rapidly evolving international pandemic crisis and provide sound advice, they encountered obstacles such as conflicting opinions, knowledge deficits, physical and cognitive fatigue, and the general burden of responsibility. This led to back-tracking, redirecting and reframing of problems. On the other hand, group performance benefited from

a sense of interdependency by "being in this together" and the ability to rely on each other's knowledge and expertise.

Conclusion: Theoretical frameworks assisted in illuminating the Canterbury IPC response to COVID-19 by highlighting how an effective response to this health service challenge was facilitated by leveraging off existing networks, promoting multi-stakeholder engagement and sharing knowledge across disciplines with a collaborative approach.

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Workshop 11
Molecular infection epidemiology and prediction of antimicrobial resistance (FG MS)
06. Sep 2022 • 14:45–16:15

059/MSV

Carriage rate of *Neisseria meningitidis* DNA among men who have sex with men as a potential cause for urethritis by sexual transmission

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Introduction: *Neisseria meningitidis (N. meningitidis)* is a Gram-negative bacterium that can cause meningitis and sepsis. Approximately 5-10 % of people harbour *N. meningitidis* in their nose and throat without any symptoms. In 1939 E.G.D. Murray implied *N. meningitidis* as a cause for infections of the male urogenital tract that might be confused with gonorrhoea. In 2019 a French research group indicated orogenital transmission of *N. meningitidis* as a cause for urethritis in men who have sex with men (MSM). This "new" capsule-free variant adheres to the mucosal surface of the human body and cannot be detected by PCRs targeting the capsule-linked ctrA gene which is the target for the meningitis causing pathogen. We investigated orogenital, rectal and urine samples of MSM by qPCR targeting the sodC gene (Cu, Zn superoxide dismutase gene) which is specific for *N. meningitidis* and which is not capsule linked. The aim was to reveal the carriage rate of *N. menigitidis* among MSM as a potential cause for urethritis by sexual transmission.

Material/methods: 270 samples (107 pooled oral and rectal swabs, 22 rectal swabs, 135 urines, 6 without known origin) of 163 male patients (median age 37 years) of an HIV outer clinic of the University Hospital Essen were investigated. Semi-automated DNA extraction was performed using aliquots of urine and of liquid transport medium carrying the swabs, respectively. Aliquots of the eluate were investigated by an in house qPCR targeting the sodC gene. A commercial primer-probe set served as inhibition control (Beta-actin control kit, Eurogentec). SodC positive samples were retested using an inhouse qPCR targeting the ctrA gene. Limit of detection (LOD) for sodC PCR was evaluated by using serial dilutions of a commercial DNA of *N. meningitides* (Vircell), of culture (DSM 10036) and of isolated DNA of the reference strain, respectively. LOD was 1fg/µl, 0.01cfu/ml and 1copy/µl, respectively (commercial DNA, culture, extracted DNA) corresponding with a ct value of 32. Thus, all samples with a ct below 32 were considered positive.

Results: Overall, 26% (n=71) of samples were sodC gene positive (63 swabs, 8 urines). Only 2% (n=6) of these were also positive for the ctrA gene (all swabs).

Conclusions: The significantly higher detection rate for the sodC gene compared with the detection rate for the ctrA gene clearly demonstrates a high prevalence of DNA from capsule-free *N. meningitidis* isolates among MSM. Due to the fact that most of the positive samples were pooled oral and rectal swabs (n=49) we cannot differentiate between colonization of the oral region of the carrier or sexual transmission to the rectum region of the carrier. Nevertheless, capsule-free strains of *N. meningitides* should be considered a potential cause of urethritis among MSM, especially when no other pathogen can be found.

060/MSV

Analysis of antibiotic resistance determinants in genome sequences from 23,267 *Clostridioides difficile* isolates

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Introduction: Recently, we have introduced a database for genome sequences from *Clostridioides difficile*, that is publicly available at the EnteroBase platform (https://enterobase.warwick.ac.uk/), and which greatly facilitates genome analyses and molecular epidemiological investigations on this important pathogen (Frentrup *et al*. 2021). This database currently (as of May 2022) holds 23,267 *C. difficile* genome sequence assemblies and associated metadata. Here, we report antibiotic resistance determinants in those genome sequences.

Material/Method: Genome sequences were downloaded from EnteroBase and scanned for antibiotic resistance genes and resistance-causing mutations in housekeeping genes by using the Resistance Gene Identifier tool and the Comprehensive Antibiotic Resistance Database (CARD). Additional resistance traits against therapeutically relevant antibiotics (metronidazole, fidaxomicin) were screened for by using BLASTn and tBLASTx. Phenotypic susceptibilities to metronidazole and fidaxomicin were tested by using E-test and broth-dilution, respectively.

Results: Numerous resistance determinants were detected, including genes and target mutations that may cause resistances against tetracyclines, fluoroquinolones, lincosamides, glycopeptides, and several other drug classes. The plasmid pCD-METRO, which was recently shown to confer resistance to metronidazole, was detected in *C. difficile* isolates recovered from humans, companion animals, livestock, and wild animals. While this plasmid-borne antibiotic resistance in *C. difficile* was discovered only recently, our results show that it had been carried by clinical isolates in Germany since at least 2008. Mutations causing reduced susceptibility to fidaxomicin have emerged several times independently in distinct geographic places, frequently in association with early clinical studies on this new drug. Furthermore, our susceptibility tests on *C. difficile* isolates confirmed that detected genetic markers indeed caused decreased phenotypic susceptibilities against therapeutically relevant antibiotics metronidazole and fidaxomicin, respectively.

Discussion: Our analysis of this large genome dataset provides unique insights into the emergence of antibiotic resistance in *C. difficile*. Implementation of automated detection of antibiotic resistance in EnteroBase will be highly useful.

Reference:

Frentrup *et al*. (2020) A publicly accessible database for *Clostridioides difficile* genome sequences supports tracing of transmission chains and epidemics. *Microbial Genomics* 6: e000410 [doi: 10.1099/mgen.0.000410]

061/MSV

Development and validation of a *Mycobacterium abscessus* core genome multilocus sequence typing scheme to facilitate molecular surveillance

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Introduction: *Mycobacterium abscessus* (Mab) is a multidrug-resistant non-tuberculous mycobacterium that is increasingly being reported as the etiological agent of severe respiratory, skin and mucosal infections. Mab is divided into three subspecies of which abscessus and massiliense are clinically the most relevant. Within these subspecies, multiple clusters of genetically closely related isolates have been identified, which are called dominant circulating clones (DCCs). These clones have been isolated from patients across the whole globe and have been associated with increased virulence, higher rates of resistance, and worse clinical outcomes. Yet, routes of Mab transmission remain largely unknown and standardized methods to unravel them are lacking. Therefore, we established a novel core genome multilocus sequence typing (cgMLST) scheme to facilitate molecular surveillance.
Methods: A set of 97 genetically diverse Mab genomes were used to create a cgMLST scheme consisting of 2904 loci. Genomes of 1797 isolates (evaluation set) covering all subspecies and DCCs were used to validate the scheme, re-analyse global population structure and compare cgMLST results with traditional 7-loci MLST analysis. Lastly, a set of 342 isolates (calibration set) including extra-pulmonary outbreak strains, strains suggested to be involved in indirect patient-to-patient transmission and sequential isolates from chronically infected cystic fibrosis patients, were used to set genetic distance thresholds for cluster detection.
Results: For 99.4% of evaluation datasets, more than 95% of the cgMLST targets were successfully identified, indicating a stable cgMLST scheme. Most DCCs previously classified using core genome single nucleotide polymorphism analysis (cgSNP) were confirmed by our cgMLST approach and also correlated well with traditional 7-loci MLST sequence types. Isolates with unknown taxonomy could be classified within a DCC using a threshold of 250 alleles compared to a set of reference genomes. 99% of pairwise comparisons between epidemiologically linked isolates were below 25 alleles and 90% below 10 alleles. Still, many isolates with no obvious epidemiological link also differed with less than 25 alleles.
Discussion: CgMLST is a powerful tool for standardized high-resolution molecular epidemiological investigations of bacterial pathogens. The scheme for Mab can be used to classify unknown isolates within the three known subspecies and within the recently described global clonal complexes. It also allows for early outbreak detection and identification of new transmission routes. The stable scheme (i.e. fixed loci), together with a harmonized expandable nomenclature (i.e. allele numbers) allows direct comparability of results by different laboratories and facilitates prospective global Mab surveillance. Therefore, we believe that it should be part of a strategy to tackle the growing public health treat of this emerging pathogen.

062/MSV
Molecular antibiotic resistance surveillance of *Neisseria gonorrhoeae* in Germany

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Introduction: Increasing antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* (NG) poses a major public health threat. Within the framework of the Gonococcal Resistance Surveillance (Go-Surv-AMR) at the Robert Koch-Institute, whole genome data, phenotypic, epidemiological and clinical data are combined to investigate the emergence and distribution of antimicrobial-resistance determinants in Germany.
Methods: Antimicrobial susceptibility (azithromycin, cefixime, ceftriaxone, ciprofloxacin, penicillin and tetracycline) of the NG isolates was determined using E-test. Minimum inhibitory concentrations (MICs) were interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (version 11.0). The Sequence types were assessed by *Neisseria gonorrhoeae multiantigen sequence typing* (NG-MAST). After extraction of DNA the genomes were sequenced using the Illumina technology. The web-based platform Pathogenwatch was used to predict resistance genes.
Results: Between 2016-2020, a total of 83 laboratories sent 2344 NG isolates. The majority of isolates were from men (88%). The median age was 33 years in men and 29 years in women. A total of 592 NG-MAST sequence types (STs) were identified, including 257 novel ones. In addition, phylogenetic groups were identified that are globally distributed and associated with decreased susceptibility to azithromycin, cefixime, or ceftriaxone. Among these, decreased susceptibility to azithromycin was predominantly clonal. The spread of ST12302 isolates correlated with the increase in azithromycin-resistant isolates in 2016-2018. Using whole-genome sequencing, this phenotype could be attributed to the mosaic-like *mtr* locus.
Discussion: Molecular AMR surveillance is an effective tool to determine resistance determinants of NG. Despite the high genetic variability of the pathogen, whole-genome analyses showed that the majority of azithromycin-resistant isolates were associated with a novel mosaic *mtr* locus acquired by recombination. Furthermore, an increase in isolates whose susceptibility to cephalosporins decreased was observed. These developments are being monitored in the current AMR surveillance.

063/MSV
A first genomic glance at the *Staphylococcus epidermidis* population in German health care facilities

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Background: For a long time, *Staphylococcus (S.) epidermidis* was considered a harmless skin commensal, that rarely caused infections and was mainly found as a contaminant in blood cultures. Over time, however, this pathogen has developed into a notorious nosocomial pathogen with an impressive spectrum of resistance properties. Genomic studies have shown that multi-resistant, hospital-adapted lineages of *S. epidermidis* have evolved and spread worldwide in recent decades. In this study we examined a set of multi-resistant *S. epidermidis* from Germany using phenotypic and genotypic analyses.
Materials: A total of 178 *S. epidermidis* isolates were included. They were sent to the NRC between 2017 and 2022, either for molecular typing with regard to a suspected outbreak (15 potential events) or due to striking resistance characteristics (especially multi-drug resistance). They originate from 25 locations in 11 federal states. At least 81 isolates were cultured from blood stream infections. All isolates were tested for antimicrobial susceptibility to 18 antibiotics according to EUCAST. In addition, linezolid-resistant isolates were tested for the presence of *cfr* by PCR. NGS data for all isolates were obtained by Illumina sequencing. Initial genomic analysis included extraction of multilocus sequence type (MLST) and detection of relevant resistance determinants within SeqSphere® (Ridom, Münster, Germany); in addition, an adhoc cgMLST scheme was used to determine the genetic relatedness of the isolates.
Results: About 90% of the isolates were methicillin-resistant and more than 75% showed elevated MIC values for more than 11 of the 18 antibiotics examined. While a large proportion of isolates exhibited resistance to rifampicin (76%) and linezolid (81%), only a few isolates showed elevated MICs for teicoplanin (10%), vancomycin (1%), tigecycline (1%) and daptomycin (0.6%). *Cfr* was detected in 20 of 144 linezolid-resistant isolates. More than 75% of the isolates belonged to ST2 and almost all of them were

highly multidrug resistant. Other sequence types represented several times were ST22 and ST5. For both ST2 and ST22, we were able to show a close relationship between the isolates in the adhoc cgMLST and respective isolates could be grouped into 16 clusters containing 2 to 54 isolates. Five ST2 clusters comprised strains that had been isolated in regions far away from each other, making the involvement of direct transmission events rather unlikely.

Discussion: Although the collection of isolates analyzed here is far from representative, the results suggest that hospital-adapted multidrug-resistant S. epidermidis are widespread in German hospitals. Presumably, a nationwide spread of such lineages is also associated with the increased detection of linezolid-resistant strains, which are increasingly isolated in outbreak situations. Further comparative genome analyses are planned to compare the isolates with hospital-adapted strains from around the world.

064/MSV

Genome-based prediction of antibiotic resistance in clinical strains of *Helicobacter pylori*

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Introduction: *Helicobacter pylori* infection is one of the most prevalent bacterial infections worldwide. Chronic infection leads to chronic active gastritis as well as to the development of other complications such as ulcers, MALT lymphoma or gastric adenocarcinoma in a subgroup of patients. Failure of standard eradication therapies is rising drastically due to the increased development of multi-resistant bacterial strains. In particular, primary resistance to clarithromycin has increased significantly in the last decade and the WHO classified *H. pylori* as a high priority organism for the development of novel antibiotics in 2017. Since the combined action of multiple antibiotics are required for successful treatments, the use of even a single antibiotic in other indications, such as respiratory diseases, will contribute to the emergence of resistant *H. pylori* strains and render ineffective future eradication attempts. Clinical guidelines for the diagnosis and treatment of *H. pylori* typically recommend antibiotic susceptibility testing only after unsuccessful second-line treatment options, which also contribute to increases in antibiotic resistance. The culture-based antibiotic susceptibility testing of *H. pylori* can take up to two weeks and thus empirical antibiotic therapies are typically started before testing.

Methods and Results: To address this fundamental issue, we assembled a curated collection of more than 400 clinical isolates of *H. pylori* with diverse antibiotic resistance patterns. Genome sequences were determined for all strains using Illumina MiSeq technology. Genomic variants were processed by feature selection methods and analyzed to develop whole-genome based classification models using machine-learning algorithms (penalized logistic regression, gradient-boosted tree) tuned via hyper-parameter optimization. Accurate models with an AUC > 0.8 were constructed for clarithromycin, levofloxacin and metronidazole. Those models will ultimately be implemented on an online genotype-to-phenotype platform accessible to physicians to predict antibiotic susceptibility using sequencing data generated locally in the hospital and subsequently select the optimal therapy.

Discussion: This approach will hopefully help optimize therapeutic efficiency and curtail the development of antibiotic resistance in *H. pylori.*

Workshop 12
Microbial mechanisms engaged to foster within host survival (FG MP)
06. Sep 2022 • 14:45–16:15

065/MPV

Outer membrane vesicles of *Chlamydia trachomatis* may transport the porin OmpA to mitochondria to block apoptosis

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Chlamydia trachomatis (Ctr) is the most common bacterial agent of sexually transmitted disease. *Ctr* is an obligate intracellular bacterium that replicates inside a membranous vacuole in the cytosol of epithelial cells. *Ctr* protects infected cells against mitochondrial apoptosis, and we have in the past provided evidence that this ability is important for the growth of the bacteria. We have recently mapped the anti-apoptotic activity of *Ctr*-infection to a step downstream of the activation of the mitochondrial effector proteins Bax and Bak. The infection disrupts some activation steps of these proteins. The downstream protein regulating Bax/Bak-activity is the voltage gated anion channel 2 (VDAC2), and deletion of VDAC2 removed some of the protective activity of *Ctr*-infection. VDAC2 is mitochondrial porin. Porins are transmembrane beta-barrel proteins in the outer membrane of both Gram-negative bacteria and mitochondria (which originated from bacteria). Intriguingly, the major outer membrane porin of *Ctr* (MOMP, here referred to as OmpA) has anti-apoptotic activity when expressed in uninfected Hela epithelial cells, which is molecularly indistinguishable from *Ctr*-infection, suggesting the possibility that OmpA is a major anti-apoptotic protein from *Ctr*.

This model requires that OmpA translocates from *Ctr* to mitochondria; how could that be achieved? We here focus on the possibility of outer membrane vesicles (OMVs) as transport vehicles. Gram-negative bacteria release OMVs that can cross membranes with astonishing ease and can target different loads to different organelles. We hypothesize that *Ctr* uses OMVs to translocate OmpA to the outer mitochondrial membrane. We have been able to isolate vesicles that are likely OMVs or similar from HeLa cells infected with *Ctr* and have identified OmpA in the vesicle fraction. Electron microscopy analysis is consistent with this interpretation and gives a size estimate of the vesicles. When uninfected HeLa cells were incubated with isolated vesicles, we observed a signal for OmpA by immunocytochemistry at mitochondria, and OmpA was identified in the mitochondrial fraction of these cells. We further used the dye rhodamin R-18, which de-quenches upon membrane insertion to test for membrane fusion. Rhodamin R-18-stained, *Ctr*-derived vesicles appear to fuse with mitochondrial membranes upon incubation with intact cells. Preliminary data further indicate that *Ctr*-derived vesicles can have anti-apoptotic activity in uninfected HeLa cells. We interpret out results as evidence for an intriguing model, where OmpA translocates from the outer chlamydial membrane to mitochondria of the infected host cell and inhibits apoptosis, enabling bacterial replication. This suggests that *Chlamydia* uses a porin with activity like the mitochondrial apoptosis-inhibiting porin VDAC2, and thus exploits its evolutionary relationship with mitochondria to inhibit apoptosis in an infected cell.

066/MPV

Live imaging of *Yersinia enterocolitica* translocon formation and immune recognition in host cells

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Introduction: The type 3 secretion system (T3SS) is a critical virulence factor of many important human pathogenic bacteria that serves to translocate effector proteins into host cells. Upon contact with the host cell, a translocon/pore complex is formed at the tip of the injectisome, which is required for translocation of effector proteins across the host cell membrane. During infection with *Yersininia enterocolitica*, the translocon is formed in a prephagosomal compartment that is connected with the extracellular space. As the phagosome matures, the translocon and the membrane damage it causes are recognized by the cell autonomous immune system.
Method: Here, we employed a novel method that allowed us to film the spatiotemporal dynamics of the *Yersinia* translocon by inserting a small peptide tag into the minor translocon protein YopD, which can be bound with high affinity by a corresponding fluorophore-tagged nanobody during the infection process. This enabled us to record the integration of YopD into translocons and its intracellular fate in living host cells.
Results: YopD was integrated into phosphatidylinositol-4,5-bisphosphate-enriched prephagosomal membranes approximately 2 minutes after bacterial uptake. The mean overall lifespan of translocon-associated fluorescence signals was determined to be about 27 minutes. Damage of the phagosomal membrane, as visualized by recruitment of GFP-labeled galectin-3, occurred on average about 14 minutes after translocon formation. Shortly after the recruitment of galectin-3, guanylate-binding protein 1 (GBP-1) was recruited to the phagosomes, which was accompanied by a decrease in the signal intensity of the translocons, suggesting their degradation or disassembly.
Discussion: In summary, we were able for the first time to record the spatiotemporal dynamics of *Yersinia* T3SS translocon formation and degradation and their recognition by components of the cell autonomous immune system. The approach described has the potential to provide insights into the T3SS of other bacterial species and is likely to be applicable to other secretion systems.

067/MPV
The secreted acid phosphatase SapS contributes to intramacrophage survival and virulence of *Staphylococcus aureus*
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Introduction: Secretion of bacterial signaling proteins and adaptation to the host, especially during infection, are processes that are often linked in pathogenic bacteria. The human pathogen *Staphylococcus aureus* is equipped with a large arsenal of immune-modulating factors, allowing it to subvert the host immune response. Recently, we showed that one of the low-molecular-weight protein tyrosine phosphatases produced by *S. aureus*, PtpA, is secreted during growth and contributes to virulence of this pathogen. *S. aureus* produces with the acid phosphatase SapS (aka SAcP) another secreted protein phosphatase, however, its impact on immune evasion and infectivity has not been determined yet.
Methods: A non-polar *sapS* deletion mutant was created in *S. aureus* strain SA564 by allelic exchange and its phenotype tested in intramacrophage and hydrogen peroxide survival assays, a *S. aureus*-based murine abscess model, and transcriptional studies focusing on genes whose products are involved in reactive oxygen species detoxification.
Results: We observed that deletion of *sapS* in *S. aureus* strain SA564 significantly reduced the capacity of the mutant to withstand intracellular killing by RAW 264.7 cells, and that SapS is secreted during macrophage infection. When injected into C57BL/6 mice, the SA564 D*sapS* mutant displayed markedly reduced bacterial loads in liver and kidney tissues at four days post infection when compared to the wild type. Analysis of the immune cell composition in blood of infected mice at four days post infection revealed significantly reduced cell numbers of neutrophils in mice infected with the *sapS* deletion mutant and a lower proportions of cd11b and cd11c on monocytes, suggesting a reduced inflammation in mice challenged with SA564 *ΔsapS*. Treatment of exponential growth phase cultures of the Δ*sapS* mutant with 50 mM hydrogen peroxide significantly reduced the number of CFU, when compared to the CFU rates obtained with hydrogen peroxide challenged cultures of the wild type. Our quantitative transcript analyses revealed furthermore that SapS affects the transcription of several genes involved in oxidative stress adaptation, such as *ahpC* (encoding alkyl hydroperoxide reductase), *katA* (encoding catalase A) and *sodM* (encoding superoxide dismutase M).
Conclusions: Our results suggest that SapS has a critical role during infection, probably by counteracting various host defense mechanisms.

068/MPV
Consequences of formyl-peptide receptor activation on the inflammation of skin
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The skin acts as a barrier with over 90% keratinocytes as the predominant cell type in the epidermis. Keratinocytes respond to pathogenic microorganisms and injury by producing antimicrobial peptides and cytokines that promote the immune responses and wound healing. Skin commensals and pathogens such as Staphylococcus aureus secrete high amounts of phenol-soluble modulin (PSM) peptides, agonists of FPR2. It is known that FPR2 is crucial for the recruitment of neutrophils in local infections and can influence inflammation. Although it has been shown that various epithelia cells express FPRs, the consequences of FPR activation regarding keratinocytes were unclear.
Since an inflammatory environment influences *S. aureus* skin colonization, e. g. on the skin of patients with atopic dermatitis (AD), we wanted to know, if FPRs influence bacteria induced skin inflammation. We hypothesized that FPRs influence bacterial colonization and inflammation of the skin. To verify this, we investigated FPR activation or inhibition in keratinocytes regarding the consequences on cytokine release, proliferation and apoptosis. Furthermore, we analyzed how FPR activation influence lipopeptide induced inflammation. In addition, we treated primary human keratinocytes with *S. aureus* and analyzed the influence of FPR2 inhibition on bacterial colonization. In an in vivo tape-stripping model mimicking atopic dermatitis using wild-type (WT) or Fpr2-/- mice we demonstrated that inflammation enhances the eradication of *S. aureus* from the skin in an FPR2 dependent manner.
We could thereby show that *S. aureus* uses FPR2 to establish skin colonization, which indicate that a better understanding of FPRs could be beneficial for patients suffering from AD.

069/MPV
***Staphylococcus aureus* synthesises multiple purine deoxyribonucleosides to kill phagocytes**
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Macrophages are crucial effector cells of the innate immune system that contribute to the clearance of a wide range of infectious agents. Nonetheless, successful pathogens including *Staphylococcus aureus*, a deadly microbe that causes invasive diseases in humans, have evolved refined strategies to kill phagocytes during infection to promote virulence and dissemination. By combining a chromatography-based approach and CRISPR-Cas9 screening in human immune cells, we here show that manipulation of macrophage responses by *S. aureus* involves excessive biosynthesis of deoxyguanosine (dGuo), a highly cytotoxic deoxyribonucleoside that targets the mammalian purine salvage pathway to activate apoptotic cell death. Since this molecular route is co-stimulated by staphylococcal deoxyadenosine (dAdo), another bioactive deoxyribonucleoside, we further demonstrate that synchronous targeting of the purine salvage pathway-apoptosis axis by dGuo and dAdo accelerates phagocyte cell death suggesting that *S. aureus* systematically generates a cocktail of immunomodulatory deoxyribonucleosides to boost its survival within foci of infection. Overall, our study identifies a sophisticated and immunologically silent virulence strategy used by *S. aureus* for immune evasion, ultimately aiding in the design of novel therapeutic or vaccine-based approaches for controlling infectious diseases caused by drug-resistant staphylococci or other Gram-positive pathogens.

070/MPV

D-Alanylation of lipoteichoic acids in *Streptococcus suis* reduces association with leukocytes in porcine blood

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Introduction: *Streptococcus suis (S. suis)* is a common swine pathogen but also poses a threat to human health in causing meningitis and severe cases of streptococcal toxic shock-like syndrome (STSLS). Therefore, it is crucial to understand how *S. suis* interacts with the host immune system during bacteremia. As *S. suis* has the ability to introduce D-alanine into its lipoteichoic acids (LTAs), we investigated the working hypothesis that cell wall modification by LTA D-alanylation influences the interaction of *S. suis* with porcine blood immune cells.
Methods: We conducted loss-of-function experiments for the D-alanine D-alanyl carrier ligase (DltA) modifying LTAs. Surface hydrophobicity, complement activation, phagocytosis, oxidative burst response of porcine granulocytes, survival of bacteria, the cytokine response and the association of bacteria with porcine blood monocytes were read out using various techniques. Association of fluorescent streptococci with blood leukocytes was analyzed by flow cytometry.
Results: D-alanylation of LTAs was associated with reduced phagocytosis of *S. suis* by porcine granulocytes, reduced deposition of complement factor C3 on the bacterial surface and increased hydrophobicity of streptococci. At the same time survival of *S. suis* was not increased by LTA D-alanylation in whole blood of conventional piglets with specific IgG. However, we found a distinct cytokine pattern as IL-1β but not TNF-α levels were significantly reduced in blood infected with the Δ*dltA* mutant. Especially in the absence of specific antibodies, the association of *S. suis* with porcine monocytes was reduced by D-alanylation of its LTAs. This *dltA* dependent phenotype was also observed with a non-encapsulated *dltA* double mutant. High antibody levels caused high levels of *S. suis* – monocyte – association followed by inflammatory cell death and strong production of both IL-1β and TNF-α, while the influence of LTA D-alanylation of the streptococci became less visible.
Discussion: Modifying the surface by attaching D-alanine to its LTAs enables *S. suis* to specifically modulate its association with blood leukocytes and interferes with cytokine induction in whole porcine blood. In contrast to TNF-α, activation and secretion of IL-1β is inflammasome-dependent, suggesting a possible influence of LTA D-alanylation on inflammasome regulation. While IL-1 signaling was shown to be beneficial to control and clear streptococcal burden, an exacerbated inflammatory response due to inflammasome activation is able to induce STSLS, making IL-1β induction a double-edged sword to the host.

Workshop 13
Competition in the microbiom and its relevance for the host (FG PW)
06. Sep 2022 • 14:45–16:15

071/PWV

The role of host cell-derived extracellular vesicles during *Aspergillus fumigatus* infection

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Introduction *Aspergillus fumigatus* is a ubiquitously distributed saprophytic fungus that mainly propagates through formation of asexual spores called conidia. Due to their small size, conidia can reach the lung alveoli upon inhalation, where they are rapidly cleared by the immune system in immunocompetent individuals. In patients with immunodeficiency, clearance is impaired allowing conidia to germinate and cause severe and often fatal invasive infections. Polymorphonuclear leukocytes are indispensable in fungal clearance and extracellular vesicles (EVs) produced by human neutrophils were shown to exhibit antifungal properties under certain conditions. EVs are nano-scaled, membrane bound entities secreted by most cell types. However, due to the small size and the lack of high throughput techniques to characterise and quantify EVs as well as the inability to genetically manipulate human primary neutrophils, the role of these host-derived EVs during *A. fumigatus* infection remains largely unexplored. The aim of our lab is to examine the role and mode of action of antifungal EVs during *A. fumigatus* infection.
Materials/Methods PLB-985 cells were differentiated to resemble neutrophil-like cells using dimethylformamide (DMF) and infected with *A. fumigatus* conidia *in vitro*. EVs were purified from cell culture medium from infected and non-infected cells by a combinatory filtration and differential centrifugation-based method. We investigated the concentration and size distribution of isolated EVs by nanoparticle tracking analysis, their antifungal activity by confocal laser scanning microscopy (CLSM) using an *A. fumigatus* reporter strain, and the proteomic cargo. In addition, we established a tool for the quantification and visualisation of EVs by genetically labelling the EV-associated tetraspanins CD63, CD81, and CD9 with a Nanoluciferase (Nluc) and a green or red fluorescent protein. Plasmids encoding for these fusion proteins were transiently transfected into the easy-to-transfect A549 lung epithelial cells.
Results/Conclusion By using the neutrophil-like cell line PLB-985, we established a model system for the investigation of neutrophil-derived EVs during *A. fumigatus* infection. We show that differentiated PLB-985 cells infected with *A. fumigatus* secrete EVs exhibiting antifungal activity *in vitro*. Moreover, transiently transfected A549 cells expressed the genetically labelled tetraspanins. Luminescence measurement in the cell culture media and isolated EV fractions of these cells indicated the association of the fusion proteins with EVs and allowed for their relative quantification. Addition of labelled EVs to *A. fumigatus* resulted in their successful visualisation by CLSM and showed a clear co-localisation of host cell-derived EVs with fungal hyphae after 24 hours. Transferring this system to PLB-985 cells might aid in understanding the role of neutrophil-derived EVs during fungal infections.

072/PWV

A salvaging strategy can enable stable metabolite provisioning among bacteria

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Introduction: Bacteria require a diverse set of metabolites to proliferate. While some bacteria fulfil their metabolic needs through *de novo* biosynthesis, other rely on uptake. Mechanisms enabling stable metabolite provisioning among free-living bacteria, i.e., in the absence of means for positive assortment, remain largely unclear. Particularly, since the production of "public goods" bares the inherent risk of overexploitation which may result in community deterioration and extinction.
Objectives: Metabolites may be obtained via degradation of environmental material, e.g., proteolysis. Yet, degradation is inherently limited to compound metabolites such as polypeptides and polysaccharides. Salvaging, the process of recycling and reuse, on the contrary is applicable to practically all classes of metabolites. Here, we investigate the salvaging of cobamides (vitamin B12-like enzyme cofactors) and explore its impact on bacterial population dynamics and stability.
Materials & methods: We used synthetic *Escherichia coli* co-cultures and metabolite profiling via high-performance liquid chromatography (HPLC) to investigate the salvaging of cobamides in multi-member bacterial populations.
Results: We observed that salvaged cobamides were readily released and provisioned to other population members. This unprompted release of metabolically valuable cobamides was surprising as it invites overexploitation by non-productive (i.e., non-salvaging) population members. We were able to show that salvagers were innately protected from overexploitation due to partial privatization of the salvaged cobamides. Due to its intracellular nature, salvagers were able to retain preferential access to cobamides even in the absence of means for positive assortment, such as growth in structured habitats in which non-productive members can be spatially excluded.
Conclusion: We found that salvaging of cobamides can lead to unprompted metabolite provisioning which is inherently protected from overexploitation. Our findings are likely transferable to other intracellular metabolites and may apply for salvaging as well as *de novo* biosynthesis. In sum, salvaging may represent on overlooked, yet prevalent, mode of metabolite acquisition and provisioning, particularly in environments that do not provide easy means for positive assortment (e.g., free-living bacterial communities).

073/PWV

Promoting gut decolonisation of multi-drug resistant bacteria via the microbiome

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Introduction: The fight against multi-drug resistant (MDR) Enterobacteriaceae has been declared as a high priority by the WHO. Colonization of the human gut with MDR Enterobacteriaceae, including MDR *E. coli*, is associated with an increased risk of infection and also dissemination within the community. Several experimental interventions have been explored to promote decolonization of the gut of MDR Enterobacteriaceae, for example treatment with antibiotics or fecal microbiota transplantation (FMT). However, both potentially exhibit adverse effects on the gut microbiota (for example diarrhea and loss of colonization resistance). In contrast, probiotics developed to selectively decolonize the microbiota of carriers from MDR strains are a promising alternative, specifically, if they achieve their aim without affecting health-promoting commensals. Previous studies showed that closely related commensal Enterobacteriaceae can compete against each other in the murine gut resulting in the displacement of the losing species from the ecosystem. We hypothesize that the human gut is a great resource for probiotics, which show the potential to selectively decolonize MDR Enterobacteriaceae.
Material and Methods: To identify commensal strains with protective properties we established an *ex vivo* assay, spiking candidate probiotics and a MDR *E. coli* strain into cecum content of mice or humans. This assay can be used as a universal screening-tool, which enables a screening of commensal isolates. To further verify protective effects, our goal was to implement competition experiments in mice.
Results: As a novel resource for the identification of potentially probiotic bacteria, a strain collection with nearly 400 strains was generated from 250 donors from three cohorts comprising individuals from different age groups and nationalities. We were able to show growth reduction of MDR *E. coli* after co-cultivation with specific commensal strains (42/ 232 tested strains). To demonstrate the probiotic effect of the respective bacterial strain, we show that SPF mice treated with ampicillin, were able to promote a 100 % clearance of MDR *E. coli*, after administration of a probiotic strain.
Discussion: For promising candidates we intend to identify their metabolic niche and potential cooperation partners as well as to gain mechanistic insights using loss-of-function genetic screens. On top of that, it should be verified that the probiotic strains fulfill all required characteristics. The strains need to show a protective effect, should have a GRAS status (generally-regarded-as-safe) and not express any virulence factors or antibiotic resistance genes.

074/PWV

Chemotaxis and autoinducer-2 signalling enhance gut colonisation and contribute to niche segregation of *Escherichia coli* strains in the mammalian gut

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Bacteria communicate and coordinate their behavior by producing and sensing extracellular small molecules called autoinducers. The astounding structural diversity of these molecules allows bacteria to synchronize their behavior on both intra- and interspecies levels. Autoinducer 2 (AI-2) is produced and detected by a variety of bacteria, thus principally allowing interspecies communication. Although AI-2 is a major autoinducer molecule present in the mammalian gut, its role in bacteria-bacteria and bacteria-host interactions remains elusive. Here, we show that chemotaxis and AI-2 signalling promote gut colonization by *Escherichia coli*, which is in turn connected to the ability of the bacteria to benefit from utilization of fructoselysine. We further show that the genomic diversity of *E. coli* strains in respect to AI-2 signaling allows ecological niche segregation and stable co-existence of different *E. coli* strains in the mammalian gut.

075/PWV

Commensal *Klebsiella oxytoca* strains exert antibacterial activity via secretion of the natural product tillimycin protecting mice from lethal *Salmonella* infection

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Question: The composition of the intestinal microbiota and their secreted natural compounds have a significant impact on the outcome of enteric infections. Strains of the *Klebsiella oxytoca* species complex (KoSC) including *K. oxytoca*, *K. michiganensis, and K. grimontii* produce chemically reactive small molecules,

namely tillimycin and tillivalin, which have been linked to the induction of intestinal inflammation through their activity as DNA damaging agent killing eukaryotic cell. Yet, whether these toxins encoded in commensal strain have beneficial effects for the host remains unknown. Hence, our aim was to study how widespread the presence of toxin-producing *Klebsiella* strains is in the healthy community and whether tillivaline and tillimycin exert antibacterial activity on enteropathogens such as *Salmonella* Typhimurium.

Methods: We characterized publicly available and de novo sequenced genomes of commensal and nosocomial KoSC strains from healthy and diseased individuals for the presence of the gene cluster responsible for toxin production. To complement the bioinformatic analyses, an *ex vivo* screening assay to assess the capacity of toxin producing and non-producing strains to supress *Salmonella* growth *ex vivo* was utilized. Candidate strains with strong antibacterial effect were further characterized and the toxin cluster was inactivated using a CRISPR/Cas9-based gene editing system. Mouse models were utilized to identify whether toxin production contributes to protection against *Salmonella* strains in different inflammatory and microbiota settings *in vivo.*

Results: Healthy children are more frequently colonized with KoSC strains than healthy adults and high proportions of all isolated strains encode the til gene cluster responsible to toxin production without showing any clinical symptoms. As *K. oxytoca* seems to be an important colonizer of the infant gut and *Salmonella* infections occur most often in children younger than 4, we assessed the capability of *K. oxytoca* to protect mice against this pathogen. We observed a strong protective effect of *K. oxytoca* in antibiotic naïve and antibiotic treated mice with a delayed onset of inflammation and significantly prolonged survival. In order to understand which factors are important to mediate protection against *Salmonella*, we screened different toxin-positive and toxin-negative isolates in an *ex vivo* spike-in assay and found that only toxin-positive strains are able to outcompete and actively inhibit *Salmonella*. *In vitro* and *in vivo* experiments with wildtype and toxin-deficient strains revealed that protection of *K. oxytoca* against *Salmonella* occurs via a novel, direct toxin-mediated, mechanism.

Discussion: KoSC strains are able to protect mice from lethal *Salmonella* infection via secretion of the natural products tillivaline and tillimycin. This study highlights the dual role of bacterial metabolites that can be both: beneficial and detrimental depending on the microbial microenvironment.

Workshop 14
Exit Strategies of Intracellular Pathogens (FG EK/FG MP)
06. Sep 2022 • 14:45–16:15

077/EKMPV
Deciphering the role of sphingolipids in bacterial egress

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Introduction: *Chlamydia psittaci* is a zoonotic pathogen of the genus *Chlamydia* which is a group of Gram-negative bacteria. They are characterized by an obligate intracellular development which includes host cell invasion followed by intracellular growth and replication and a final host cell exit. *Chlamydia* species can exit the host cell by lytic egress or by the so-called extrusion formation without host cell damage as non-lytic egress type. The aim of this project is to understand the exit pathways of *Chlamydia* species using *C. psittaci* as a model organism.

Material/method: In this study, the exit of *C. psittaci* was characterized with focus on non-lytic egress. As sphingolipids play an essential role in chlamydial infections, we hypothesized that sphingolipids could also control egress of *C. psittaci* from its host cell. We investigated the role of sphingolipids in chlamydial egress using knockout (KO) mutants of the ceramide transporter CERT and of the serine palmitoyl transferase (SPT) as host cell sphingolipid metabolism proteins. In addition, we examined the role of calcium signaling as regulator of *C. trachomatis* egress for the exit of *C. psittaci* and the non-lytic exit pathway of *C. psittaci* was characterized in contrast to cellular death.

Results: KO or chemical inhibition of CERT or SPT KO induced an early non-lytic release of *C. psittaci* and *C. trachomatis*. Similar to extrusion formation of *C. trachomatis*, the observed release mechanism of *C. psittaci* was calcium dependent. Our results indicated that the calcium sensor STIM1 was recruited to *C. psittaci* inclusions at CERT KO or chemical inhibited conditions. Furthermore, the chemical inhibition of STIM1 by SK&F 96365 induced this non-lytic release type. In addition, we observed an activation of the apoptopic executioner caspases 3 or 7 and a mitochondrial membrane depolarization during the non-lytic release of *C. psittaci*. Interestingly, we could not detect a SYTOX or propidium iodide staining during the non-lytic release as markers of the loss of plasma membrane integrity.

Discussion: Taken together, we identified the host cell sphingolipid metabolism and calcium signaling as regulators of non-lytic *C. psittaci* egress. In addition, we showed that the non-lytic release mechanism of *C. psittaci* shared characteristics of apoptotic cell death, but it seemed to lack the loss of plasma membrane integrity. The described novel chlamydial release mechanism will be further investigated to identify and to characterize bacterial exit regulators and to understand the role of sphingolipids as host cell regulators of chlamydial egress. We speculate that sphingolipids could also regulate exit pathways of other intracellular pathogens.

078/EKMPV
How a perturbation in fatty acid homeostasis affects mycobacterial phagosome escape

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Introduction: Tuberculosis, caused by *Mycobacterium tuberculosis* (*Mtb*), is the second deadliest infectious disease in the world. *Mtb* and other pathogenic mycobacteria cause major damage to the membrane of their vacuole to escape into the host cytosol. This bacterial translocation depends on the secreted pore forming peptide ESAT-6 that cooperates with phthiocerol dimycocerosate (PDIM), a complex mycobacterial membrane lipid, to generate pores and membrane damage. It was hypothesized that *Mtb* uses primarily host-derived fatty acids (FAs) as carbon and energy source but also as building blocks to synthesize membrane lipids during infection. To this end, bacterial fatty acyl-CoA synthetases activate imported FAs with coenzyme A. In total, the *Mtb* genome encodes 36 FA-activating enzymes, known as FadDs. One example is the outer membrane protein FadD6 (i.e. FACL6) that was described as a FA importer that is upregulated and involved in triacylglycerol synthesis upon *in vitro* dormancy. Strikingly, the function of FACL6 during infection is still poorly understood.

Methods: Using the powerful *Dictyostelium*/*M. marinum* system, this project aims to characterize the role of FACL6 in lipid acquisition, assimilation and phagosome escape of mycobacteria. To achieve those aims, we will map alterations in host-to-pathogen FA flows in the D*facl6* mutant with the help of clickable lipid probes combined with in-resin CLEM or by lipidomics.

Results: Infections of *Dictyostelium* with the *M. marinum* D*facl6* mutant resulted in greater MCV damage, visualized by cells expressing TrafE-GFP, an E3 ubiquitin ligase that is recruited to damaged endosomes and the MCV. Interestingly, the *M. marinum* D*facl6* mutant is highly attenuated in *Dictyostelium*, however, mycobacteria growth is restored in host cells lacking Atg1/ULK kinase indicating that D*facl6* bacteria are detected by xenophagy.

Indeed, when Perilipin and Ubiquitin were used to label cytosolic D*facl6* bacteria, a more efficient escape to the cytosol was observed. *In vitro* FACL6 was found to have broad substrate specificity. We observed that the growth of the D*facl6* mutant is inhibited when palmitate was used as single carbon source, but not when the medium was supplemented with oleate. This indicates that FACL6 is needed for the activation of C16-FAs that might be used for TAG synthesis during infection. TLCs also revealed that FACL6 deletion causes an accumulation of PDIMs likely to detoxify excessive FAs, which might explain the observed higher MCV escape efficiency. We are currently establishing a fluorescent reporter construct under the control of the *facl6* promotor to monitor the activity of FACL6 depending on the carbon source and on the subcellular localization of the bacteria.

Outlook: Interestingly, also host FA-activating enzymes might affect mycobacterial lipid acquisition. The pharmaceutical inhibition of host and bacteria FA-activating enzymes might consequently lead to fresh starting points for anti-Tb therapies.

079/EKMPV

Unveiling the mechanisms of vesicle discharge during gametocyte egress of *Plasmodium falciparum*

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Introduction: At the onset of gametogenesis, the activated *Plasmodium falciparum* gametocytes egress from their host erythrocytes to prepare for fertilization. Gametocyte egress follows a well-defined programme and involves the rupture of two membranes, the membrane of the parasitophorous vacuole (PVM) and the erythrocyte membrane (EM). Essential for gametocyte egress is the exocytosis of at least two types of specialized secretory vesicles. While the osmiophilic bodies (OB) are associated with PVM rupture, another subset of vesicular structures, here named g-exonemes, were observed to relocate to the parasite membrane shortly before EM rupture. While significant parts of the signalling pathway during gametocyte egress are known and some of the effector proteins already described, biogenesis and trafficking of the egress-related vesicles are still under-investigated.

Material/Method: We employed BioID analyses to unveil the interactomes of OBs and g-exonemes. BioID is a proximity labelling application for detecting putative protein-protein interactions. The method uses a promiscuous biotin ligase (BirA), fused to a bait protein that can be induced to biotinylate interacting and proximal proteins during a defined labelling period by biotin supplementation. TurboID represents an optimized version of the BioID, which uses a biotin ligase with higher activity and therefore decreases the labelling period dramatically. Biotinylated proteins can be isolated, analyzed via mass spectrometry (MS) and investigated as candidate interactors with the bait protein or as constituents within a subcellular domain.

Results: Transfectant lines were generated that express the g-exoneme-resident protein PPLP2 and the two OB-resident proteins MDV and G377 fused with a mutated biotin ligase BirA. The expression of the GFP-tagged BirA fusion proteins in gametocytes as well as the ability of the fusion proteins to biotinylate proximal proteins were confirmed by Western blot (WB) and indirect immunofluorescence assays (IFA). Mature gametocytes of each parasite line were isolated after biotin treatment and biotinylated proteins were purified by affinity chromatography using streptavidin beads. Following mass spectrometry analysis, significant hits were filtered according to selected parameters, thereby identifying, among others, members of the LCCL and PSOP families and selected proteases. For selected hits, tagged parasite lines were generated, using the pSLI-HA-*glmS* knockdown system, and expression of HA-tagged proteins was confirmed by WB and IFA, demonstrating that all proteins were highly expressed in the gametocyte stages. In addition, colocalization was verified by using antibodies directed against G377 and PPLP2.

Discussion: We were able to unveil the interactome of egress-related vesicles of *P. falciparum* gametocytes, providing the first indications of the immense molecular machinery required to mediate the egress of gametocytes from erythrocytes during gametogenesis.

080/EKMPV

Diverse strategies used by *Candida albicans* and *Candida glabrata* to exit from macrophages

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Phagocytes are essential for defense against infections with the human opportunistic pathogens *Candida albicans* and *C. glabrata*. Both fungal species are efficiently taken up by macrophages, but have evolved strategies to survive and proliferate inside the phagosome and ultimately exit from this hostile environment.

To characterize the interaction of *C. albicans* and *C. glabrata* with macrophages, we have established *in vitro* models based on human monocyte-derived macrophages. After phagocytosis, surviving *C. albicans* cells rapidly form hyphae, followed by hyphal extension and escape within hours. Our data suggest that hyphae support escape in at least three different ways: by damaging macrophage membranes *via* the toxin candidalysin, by inducing inflammatory host cell death *via* NLRP3-mediated pyroptosis, and by physical forces generated upon filament elongation. Intra-phagosomal growth requires metabolic adaptations towards utilization of alternative carbon sources and micronutrients such as biotin, but it is not clear how hyphal formation is triggered and how nutrients are acquired inside macrophages to sustain hyphal growth for hours.

Candida glabrata does not form filaments but replicates as yeast cells within macrophages, and one hypothesis posits that it might exist intracellularly for prolonged periods *in vivo*. We have established a long-term *in vitro* model in which *C. glabrata* can persist inside macrophages for up to 7 days without inducing substantial inflammatory immune responses or host cell death. Persisting *C. glabrata* adapt by expressing genes related to cellular quiescence regulation and nutrient starvation. However, we also observed that proliferating *C. glabrata* cells can damage macrophage membranes and exit from these immune cells at later stages, suggesting that also *C. glabrata* is equipped with specific exit strategies.

We aim to unravel the common and distinct strategies that *C. albicans* and *C. glabrata* use for intracellular proliferation, persistence, and finally exit from macrophages.

081/EKMPV

Cell exiting and reuptake – efferocytosis of *Salmonella*-infected enterocytes by enterocytes

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Introduction: The intestinal epithelium represents the first line of defense against invasive enteric pathogens. In the adult host, exfoliation of *Salmonella* infected enterocytes and shedding into the intestinal lumen represents an important mechanism to prevent invasive infection. In contrast, enterocyte exfoliation is much less observed in the neonatal intestine.

Question: One of the main questions need to be addressed first is how the neonatal intestine removes *Salmonella* infected enterocytes.

Methods: We are taking the advantages of our *Salmonella* infection model in neonatal murine host and organoids technology to investigate the removal mechanism of *Salmonella* infected enterocytes. The main techniques are organoids cultivation, fluorescence microscope, electron microscope and blocking assay.
Results: Instead of exfoliation, we observed efferocytosis of *Salmonella*-infected enterocytes by neighboring enterocytes in the small intestine of *Salmonella* infected neonate hosts *in vivo*. Engulfed cells appeared as endosomal compartments with Dapi positive chromatin material and exhibited the typical DNAse type II 5" OH DNA cleavage sites. We were able to replicate this observation using neonatal but not adult intestinal stem cell organoids confirming the age-dependent propensity of neonatal enterocytes for efferocytosis. Using blocking antibodies or preincubation with annexin V or C1q, we also identified the significant and synergistic involvement of phosphatidylserine and the C1q-CD91 and the thrombospondin-integrin alphaV/CD36 mechanism for cellular uptake.
Conclusions: Here, we can conclude that efferocytosis is an age-dependent process. Moreover, efferocytosis of infected enterocytes by neighboring enterocytes may therefore represent a previously unrecognized mechanism of the neonatal antimicrobial host defense.

082/EKMPV
Transdifferentiated macrophage-like human cells to investigate *Leishmania* exit mechanisms
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Leishmaniasis is a neglected tropical disease caused by the intracellular parasite *Leishmania (L.)*, which infects macrophages as its definitive host cell. The mechanism of parasite spreading from the initially infected cell to another cell remains poorly understood, partly due to limitations of genome editing methods in primary human macrophages. To circumvent this, we established a *Leishmania* infection model using BLaER1 cells that can be transdifferentiated into a macrophage-like phenotype. Following the generation of CRISPR/Cas9-mediated knockouts in the undifferentiated stage, we can investigate the *Leishmania* development and exit in transdifferentiated macrophage-like cells after infection. In order to proof that the human BlaER1 cell can be used as a suitable infection model for human leishmaniasis, we first examined the immunophenotype of BlaER1 cells by flow cytometry and found it to be comparable with M-CSF- or GM-CSF-derived human macrophages. Afterwards, we confirmed the susceptibility of BlaER1 for the infection with *Leishmania* promastigotes using confocal microscopy. When comparing infected BlaER1 and human macrophages, the infection rate, transformation of the promastigotes into amastigotes and cytokine responses were very similar between these cell types. We hypothesize that pyroptosis, a NLRP3 inflammasome-dependent type of cell death, could be involved in early spreading events in leishmaniasis. In a first step, we confirmed *Leishmania*-induced NLRP3 inflammasome activation as we found a stronger IL-1β secretion in *L. major*-infected wild type BlaER1 cells, compared to infected BlaER1 with knocked out components of the inflammasome. To proof a role of the inflammasome activation in parasite spreading, we induced a non-canonical inflammasome response by LPS stimulation of infected BlaER1 cells. This indeed led to amastigote exit from host cells. As non-canonical inflammasome activation does not occur in murine cells, results on *Leishmania* spreading gained from the various mouse models are not fully transferable to human infections. Thus, our finding underlines the importance of research on *Leishmania*-mediated inflammasome activation in human cells and demonstrates the suitability of BLaER1 cells as a model to investigate *Leishmania* exit mechanisms.

Workshop 15
Infektionsdiagnostik von COVID-19 (StAG DV/FG DKM) and Diagnostics and Clinics of Zoonotic Infections (FG ZO/ FG DKM)
06. Sep 2022 • 14:45–16:15

083/DKMV
Virus variant specific clinical performance assessment of SARS-CoV-2 rapid antigen tests in point-of-care use including Omicron VOC
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Question: Antigen rapid diagnostic tests (RDT) for SARS-CoV-2 are quick, widely available, and inexpensive. Consequently, RDT have been established as an alternative and additional diagnostic strategy to quantitative reverse transcription polymerase chain reaction (RT-qPCR). However, reliable SARS-CoV-2 virus variant of concern (VOC) specific clinical and large-scale performance data is limited, especially for Omicron VOC.
Methods: This single-centre prospective performance assessment compares RDT from three manufacturers (NADAL®, Panbio™, MEDsan®) with RT-qPCR including deduced standardised viral load from 35,479 oropharyngeal swabs for detection of SARS-CoV-2 in a clinical point-of-care setting.

Results: RDT sensitivity compared to RT-qPCR was 38.50% (95% CI 34.00%–43.20%) with an overall specificity of 99.67% (95% CI 99.60%–99.72%). RDT sensitivity depended clearly on viral load with decreasing sensitivity accompanied by descending viral load. VOC dependent sensitivity assessment showed 42.86% (95% CI 32.82%–53.52%) for wild-type SARS-CoV-2 , 43.42% (95% CI 32.86%–54.61%) for Alpha VOC, 37.67% (95% CI 30.22%–45.75%) for Delta VOC, and 33.67% (95% CI 25.09%–43.49%) for Omicron VOC. Sensitivity in samples with high viral loads was significantly lower in Omicron VOC (50.00%, 95% CI 36.12%–63.88%) compared to wild-type virus (79.31%, 95% CI 61.61%–90.15%, p=0.015, Fig. 1).

Fig. 1: Spike protein variant depending on antigen rapid diagnostic test sensitivity

RDT sensitivity compared to RT-qPCR as reference standard by spike protein variant. ***Fig. 1A*** *included 257 samples with molecularly confirmed spike protein variant,* ***Fig. 1B*** *included 407 samples with either molecularly confirmed spike protein variant or epidemiologically assigned spike protein variant (in case no spike protein was determined molecularly, and the variant of the infection source was known, or a VOC was responsible for more than 90% of all COVID-19 cases in Germany at the time of sampling).* ***Fig. 1C*** *included 218 samples with a viral load < 10^6 SARS-CoV-2 RNA-copies per ml and an either molecularly or epidemiologically assigned spike protein variant wild-type, Alpha VOC, Delta VOC, or Omicron VOC.* ***Fig. 1D*** *included 184 samples with a viral load ≥ 10^6 SARS-CoV-2 RNA-copies per ml and an either molecularly or epidemiologically assigned spike protein variant wild-type, Alpha VOC, Delta VOC, or Omicron VOC. *: p<0.05*

Conclusions: RDT sensitivity for detection of the Omicron VOC is reduced in infectious individuals with high viral load, which curtails the effectiveness of RDT (Fig.2).

Fig. 2: Schematic antigen rapid diagnostic test reliability in the course of a SARS-CoV-2 infection

This aspect further limits RDT use although RDT are still an irreplaceable diagnostic tool for rapid, economic, point-of-care and extensive SARS-CoV-2 screening use.

Fig. 1

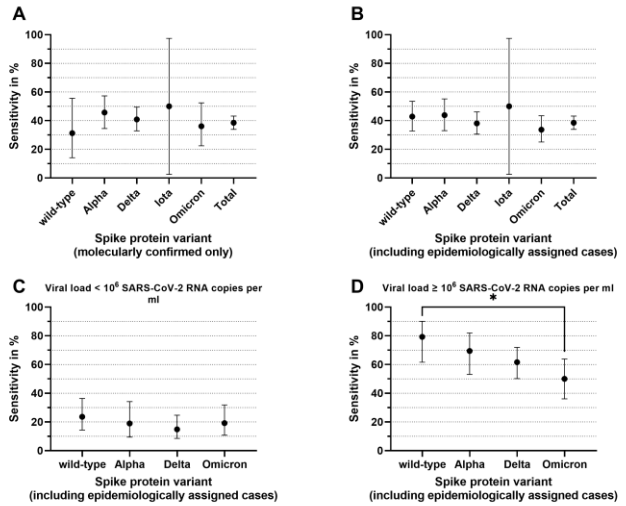
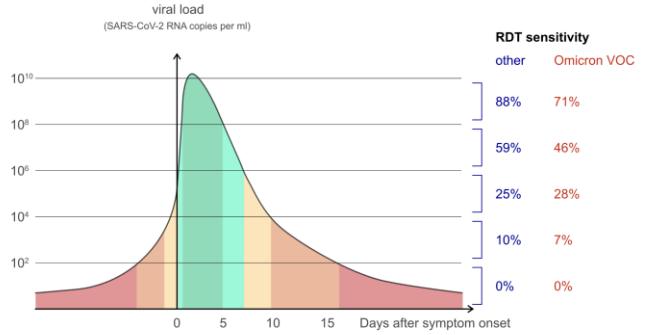


Fig. 2



084/DKMV

Use of clinical specimen panels for the decentralised technical evaluation of SARS-CoV-2 rapid antigen detection tests

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Introduction:During the ongoing COVID-19 pandemic, rapid antigen detection tests (RDTs) have been proving valuable for quick and easy detection of SARS-CoV-2 infection. However, since their introduction into the European market in 2020 until May of this year, certification (CE marking) of such tests has been the sole responsibility of manufacturers and has not been requiring independent evaluation of quality by third parties. Hence, we have established evaluation panels comprised of pooled clinical specimens to enable the systematic and decentralized technical evaluation of a large number of SARS-CoV-2 RDTs.

Material/Method: Upper respiratory specimens from SARS-CoV-2 PCR-positive individuals with similar viral loads were pooled and subsequently diluted in negative matrices comprised of upper respiratory specimens from SARS-CoV-2 PCR-negative individuals in phosphate-buffered saline to obtain defined RNA concentrations. Quantitative reference material was used to determine viral nucleic acid concentrations by PCR. Viral cultures were used to determine replication competency of specimen pools. CE-marked RDTs were selected randomly and depending on their availability at the time of evaluation.

Results: So far, our clinical specimen panels have been used for the evaluation of >240 CE-marked SARS-CoV-2 RDTs by us and our partners. Of these, 204 have passed sensitivity criteria defined as the detection of at least 75% of specimen pools that have a Cq value ≤25. Previous studies have shown that a minimum of 10^6 genome copies per mL of SARS-CoV-2 specimen represents the amount of infectious virus particles required for virus propagation in cell culture (corresponding to a Cq value of 25 in our PCR assay). We confirmed the presence of replication-competent virus in some specimen pools with Cq values ≤25, while we were unable to propagate virus in specimen pools with Cq values >25. Using a random selection of RDTs to compare specimen pools and unpooled clinical specimens of comparable Cq values, we did not observe any discrepancy in detectability between pooled and fresh samples.

Discussion: Independent, systematic and reliable evaluation of RDT sensitivity is vital to ensure the quality of self-certified SARS-CoV-2 RDTs currently marketed in Europe. Here, we show that standardized evaluation panels consisting of pooled clinical specimens can provide a useful tool for the evaluation of SARS-CoV-2 RDTs. Taken together, using specimen panels we found that sensitivities across RDTs vary substantially; however, most RDTs reliably detected potentially infectious specimens, indicating that such RDTs can be used to identify contagious individuals.

085/DKMV

WGS-based SARS-CoV-2 surveillance reveals reinfection cases

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Introduction: During the Covid-19 pandemic, whole genome sequencing (wgs) became one of the key tools for molecular surveillance of viral lineages, new variants, and mutations of concern. Besides lineage classification and molecular outbreak investigations, potential reinfections can be confirmed or ruled out by wgs. For an overview, we analysed our large wgs-based surveillance dataset comprising SARS-CoV-2 genomes along with relevant metadata and quality metrics for occurrence of reinfections or viral persistence.
Material/method: From January 2021 until May 2022, we sequenced more than 10'300 SARS-CoV-2 positive patient samples for molecular surveillance using *artic* amplicon enrichment, Illumina sequencing, consensus genome generation with ivar (consensi published at gisaid.org) and lineage classification with pangolin. To investigate potential reinfections, SARS-CoV-2 genomes from same patients but different sampling dates were retrospectively reanalysed for potential reinfections or viral persistence by consistency in viral lineage and genetic distance using pangolin and SNP-based phylogenies.
Results: We identified 261 pairs of genomes from same patients but different time points. Among these, eight cases of reinfections could be confirmed by divergent viral lineages and distances of 46 – 94 SNPs. Timespans between first infection sampling and verified reinfection ranged from 59 – 400 days. Reinfections mostly occurred with Omicron lineages BA.2 (n=5), but also with BA.1 (n=2) and B.1.6172.2/Delta (n=1), including sublineages. In most of the 253 remaining cases, SNP distances between the first and second sample were zero (n=245) or low (n=6: 1 SNP; n=1: 2 SNPs; n=1: 7 SNPs). Among these, timespans between sampling were mostly shorter (0 - 65 days, median = 7 days), with only eight pairs of genomes with 20 days or more in between. However, in one pair of genomes, the detection of the same lineage and 0 SNPs distance indicated a persistent infection with the same strain even after 65 days.
Discussion: The dataset used was collected for standard molecular surveillance and primarily not for longitudinal studies in recurrent patients. Nonetheless, we were able to detect and confirm potential reinfections in eight cases using wgs. Our results show that in most cases the timespan between first and second infection is a valuable indication for or against the occurrence of a reinfection, which may then be confirmed by a divergent viral lineage. Interestingly, in case of a young adult, a reinfection with lineage BA.2 was detected only 59 days after infection with BA.1. In contrast, in a pair of genomes from an 89-year old woman, the same viral lineage was still detected with 0 SNPs distance after 65 days. Thus, only wgs of viral genomes can clearly resolve such issues. In the future, larger studies of different datasets can help to better understand occurrence, probability and genetic details of SARS-CoV-2 reinfections with different virus variants.

086/DKMV

TRAIL, IP-10, and CRP in predicting the progression of SARS-CoV-2 infection – a prospective, cohort study

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Background: Early prognostication of COVID-19 severity will potentially improve patient care. Biomarkers such as TNF-related apoptosis-inducing ligand (TRAIL), interferon gamma-induced protein-10 (IP-10), and C-reactive protein (CRP) might represent possible tools for point-of-care-testing and severity prediction.
Methods: In this prospective cohort study we analysed serum levels of TRAIL, IP-10, and CRP in COVID-19 patients, compared them to control subjects, and investigated association with disease severity.
Results: A total of 899 measurements were performed in 132 patients (mean age 64 years, 40.2% females). Among COVID-19 patients,TRAIL levels were lower (49.5 vs. 87 pg/mL p=0.0142), while IP-10 and CRP showed higher levels (667.5 vs. 127 pg/mL, p<0.001; 75.3 vs. 1.6 mg/L, p<0.001) as compared to healthy controls. TRAIL yielded an inverse correlation with length of hospital and ICU stay, SAPS II and NEWS scores, and IP-10 showed a positive correlation with disease severity. Multivariate regression revealed that obesity (adjusted odds ratio 5.434, 95% confidence interval 1.005-29.38), CRP (aOR 1.014, 95%CI 1.002-1.027), and peak IP-10 (aOR 1.001, 95% CI 1.00-1.002) were independent predictors of in-ICU mortality.
Conclusions: We demonstrated a correlation between COVID-19 severity and TRAIL, IP-10, and CRP. Multivariate regression showed a role for IP-10 in predicting unfavourable outcome, *i.e*. in-ICU mortality.

087/DKMV

Antibody binding and ACE2 binding inhibition is significantly reduced for both the BA.1 and BA.2 omicron variants

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Introduction: The rapid emergence of the omicron variant (B.1.1.529) and its large number of mutations led to its classification as a variant of concern (VOC) by the WHO. Subsequently, omicron evolved into distinct sublineages (e.g., BA.1 and BA.2), which as of May 2022 represent the majority of global infections. Initial studies into the neutralizing response towards BA.1 in convalescent and vaccinated individuals showed a substantial reduction.
Methods: We assessed antibody (IgG) binding, ACE2 (Angiotensin-Converting Enzyme 2) binding inhibition as a proxy for neutralization, and IgG binding dynamics for omicron BA.1 and BA.2 RBDs compared to WT, all VOCs and the lambda and mu RBDs, in a large cohort (n=352) of convalescent, vaccinated, and infected and subsequently vaccinated individuals.
Results: While omicron BA.1 and BA.2 RBDs were capable of efficiently binding to ACE2, antibodies elicited by infection or immunization showed reduced binding capacity (Figure 1). IgG binding and ACE2 binding inhibition towards BA.1 and BA.2 RBDs were significantly reduced (all p<0.001) compared to WT, with BA.1 IgG binding significantly reduced compared to BA.2 (p<0.001). Boosting with a third vaccine dose elicited increased IgG binding and ACE2 binding inhibition towards all VOCs (Figure 2). Infection with previously circulating variants did not affect antibody binding dynamics towards BA.1 or BA.2. There was no significant difference between adults and children in ACE2 binding inhibition (p=0.48), although children did have significantly reduced binding capacity towards omicron (p=0.01).
Conclusions: While ACE2 and vaccine/infection-elicited antibodies can efficiently bind BA.1 and BA.2 RBDs, the extent of the mutations within both variants appear too divergent to enable a strong inhibitory binding response. As a result, both omicron variants are able to evade recognition by pre-existing antibodies. Booster doses elicit a significant increase in antibody response, which correlates, with a significant increase in both IgG binding and ACE2 binding inhibition against omicron RBDs. Our data adds weight to a growing body of evidence that the continuous adaptation of vaccines towards novel highly contagious variants is required.
Figure 1 – Biolayer interferometry (BLI)-based affinity measurements. Biotinylated RBD WT, RBDo BA.1 and RBDo BA.2 were immobilized on streptavidin biosensors and treated with four concentrations of ACE2 (a-c). WT, BA.1 and BA.2 RBDs can

efficiently bind ACE2 (a-c) but vaccine/infection elicited antibodies show reduced binding to omicron BA.1 and BA.2 RBDs (d). Reproduced and adapted from Junker et al, 2022.
Figure 2 – A third vaccine dose (a) results in increased ACE2 binding inhibition against RBDs of all variants including omicron BA.1 and BA.2, compared to responses following the second dose (b). Individual samples are highlighted by connected lines with bars representing medians. Reproduced and adapted from Junker et al, 2022.

Fig. 1

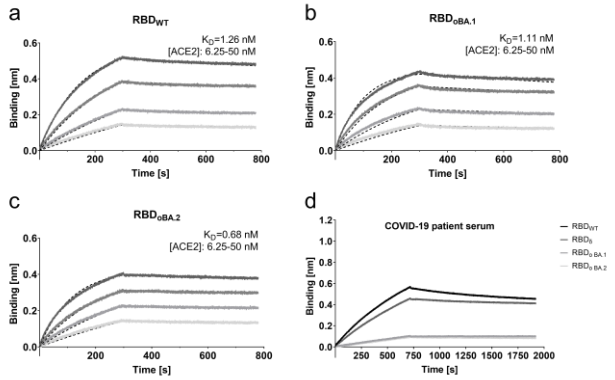
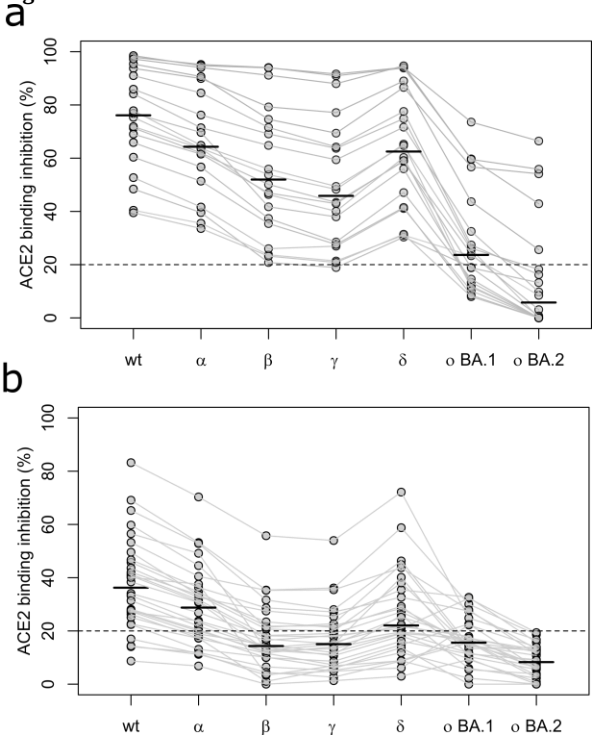


Fig. 2



088/DKMV
We know what our city did last summer – Usage of cost-free SARS-CoV-2 testing in a medium-sized city in Bavaria
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Introduction: Starting from March 2021, cost-free SARS-CoV-2 point-of-care antigen tests were offered to all German citizens without COVID-19 symptoms. The goal was to break chains of infection by detecting and isolating asymptomatic people, who might spread the virus. Analyses and publications exploring the usage patterns of such test offerings are rare.
Material and Methods: We analyzed (i) 12 weeks (over 25"000) [1] and (ii) over 50"000 pseudonymized datasets from eight test centers in a medium-sized city in Bavaria, regarding age, place of residence, and type of appointment. One test center was open for eight months, which allowed for analyses over time. In parallel, a survey with over 1"000 participants was conducted to investigate motivation and self-assessment of such SARS-CoV-2 test usage.
Results: The different test centers were visited by different groups of people. A comparable older age group (average 50 years) with mainly residence in the city (84%, 10% rural district) was attracted by a mobile bus test center, driving to parts of the city where only few other options were offered. At a "park and ride" location the ratio of people not living in the city or district (41%) and the ratio of spontaneous tests (71%) was highest. In contrast, the center at the central bus station showed a high ratio of scheduled tests (62%) and was visited by comparatively younger (average age 37 years). With daily opening hours except Sundays this test center was analyzed over eight months. The group of ~18 to 30 years was highly overrepresented compared to the demographic of the city. Over time with decreasing number of performed tests the ratio of spontaneous testing increased. Furthermore, residents from other federal states have been tested more frequently as soon as touristic activities were possible again and mainly linked to the holidays of the respective state. Overall, 77% of the visitors used these test centers just once compared to only 1% who were tested 10 times and more in the eight months study period. Those data contrasts with the survey, where almost one third claimed to use the tests regularly [2].
Discussion: Since SARS-CoV-2 tests at home, at work or at pharmacies can also be used, the number of regular tests should be underestimated in our dataset. Nevertheless, voluntary regular testing was not shown by this data as a usual habit of a big proportion of people even if testing is cost free. If a broad range of people should be attracted for voluntary testing, data suggests various forms of test options must be offered confirming the usage of easily accessible services and the importance of adjusted services for different user groups.
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[2] Epid Bull 2022;15:3-13, doi: 10.25646/9798

Workshop 16
Molecular pathogenesis of bacterial infections (FG MP)
07. Sep 2022 • 08:30–10:00

089/MPV
A bacterial porin inhibits apoptosis during infection by mimicking the function of a human mitochondrial porin
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Pathogens have evolved strategies to target apoptotic signaling pathways. *Chlamydia trachomatis* (*Ctr*) is an obligate intracellular bacterium that replicates in a vacuole in the cytosol of human epithelial cells and protects infected cells against apoptotic stimuli. We found that OmpA, the major porin of the outer membrane of *Ctr*, translocates during infection from the bacteria to host cell mitochondria. Expression of OmpA in uninfected HeLa cells protected against apoptosis at the exactly same step as did *Ctr*-infection, by interfering with the activation of pro-apoptotic Bax and Bak. Deletion of the endogenous Bax/Bak-inhibitor, the mitochondrial porin VDAC2 from Hela cells, had an apoptosis-sensitizing effect, and expression of OmpA could compensate for the loss of VDAC2, suggesting functional equivalence of the porins. Within the *Chlamydiales* order, only bacteria from the *Chlamydiaceae* (such as *Ctr*) and from the *Simkaniaceae* (*Simkania negevensis* is the only known species) can grow in human cells. All

members of the *Chlamydiaceae* have OmpA; we identified a related porin from *S. negevensis* and found that it could also inhibit apoptosis. *Parachlamydia acanthamoebae* is an obligate intracellular symbiont of free-living amoebae and cannot grow in human cells. *P. acanthamoebae* induced apoptosis in Hela cells; however, expression of *Ctr*-OmpA in Hela cells blocked apoptosis and permitted some replication of *P. acanthamoebae*. Mitochondria derive from bacteria and have, like Gram-negative bacteria, two membranes and porins. Our observation that chlamydial porins can inhibit apoptosis and promote growth of the bacteria in human cells suggests that a class of obligate intracellular organisms utilize the evolutionary relationship between Gram-negative bacteria and mitochondria to block apoptosis and thereby to secure their intracellular growth.

090/MPV

Identification of bacterial interaction partners of *Legionella pneumophila* mip

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Introduction: *Legionella pneumophila* is the main causative agent for Legionnaires" disease, which is a severe atypical pneumonia. The outer membrane protein Mip (Macrophage infectivity potentiator) is one of its major virulence factors. It consists of two domains that are connected with a 6.5 nm α-helix. While the N-terminal domain facilitates homodimerization, the C-terminal domain is closely related to the human FK506-binding protein 12 (FKBP12), and exhibits peptidyl-prolyl-*cis/trans*- isomerase (PPIase) activity. Although the contribution of Mip to pathogenesis manifold, and ranges from stress tolerance to initiation of cellular infection and systemic dissemination, until now no natural substrates are known.
Material, Methods: First, the identification of bacterial interaction partners from Mip was performed by crosslinking *in vivo*. Co-immunoprecipitation and coprecipitation with and/or without substance led to the validation of the results from mass spectrometry and the determination of the binding sites. AlphaFold v.2.0 was used to predict the structures of the proteins, which thereafter docked and simulated dynamically to predict the interaction sites bioinformatically. Furthermore, isolation of flagella and quantification of concentration from different *L. pneumophila* strains repeated and determined the statistical significance.
Results: Using *in vivo* cross-linking we identified stringent starvation protein SspB, hypothetical protein Lpc2061 and flagellin FlaA as interaction partners of Mip. *In vitro* interaction studies revealed that the FK506-binding domain, Y185 and dimerization regions are important for the Mip-Lpc2061 interaction. Furthermore, the binding between Mip and Lpc2061 was strengthened by the addition FlaA. The identified interaction partners were docked to Mip using deep learning based AlphaFold v2.0, and showed binding characteristics consistent to our *in vivo* and *in vitro* findings. Moreover, best poses of dockings were simulated dynamically showing an increase in stability through the tripartite interaction of Mip-FlaA-Lpc2061 similar to aforementioned experiments. In light of these results, we determined that Mip promotes flagellation of the bacteria, and that for this the PPIase activity as well as the dimerization of Mip are important.
Discussion: PPIases of pathogens are involved in a broad spectrum of phenotypes including virulence, metabolism, and multiple stress responses. Here, we present for the first time the identification of bacterial interaction partners of *L. pneumophila* Mip. To our knowledge, this is the first report in which a PPIase and its bacterial interaction partners were demonstrated to influence flagellation.

091/MPV

The uncharacterised protein YgfB contributes to β-lactam resistance in *Pseudomonas aeruginosa* by inhibiting the expression of the amidase AmpDh3

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Introduction: The uncharacterized gene *ygfB* contributes to β-lactam resistance in multi drug resistant (MDR) *Pseudomonas aeruginosa* (*Pa*) strains by increasing expression of the cephalosporinase AmpC [1], which mediates resistance to broad-spectrum 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) arising from the peptidoglycan pathway upon binding to the transcriptional regulator AmpR [3].
Material/Methods: By transcriptomic analysis, expression analysis of transcripts and proteins, promoter activity assays, antibiotic susceptibility testing, LC-MS analysis of peptidoglycan precursors and protein-protein/protein-DNA interaction assays we unraveled the mechanism how YgfB contributes to AmpC overproduction and β-lactam resistance.
Results: YgfB inhibits the expression of the amidase AmpDh3, which degrades the AmpR activating anhMurNAc-peptides in the cytoplasm, resulting in increased AmpC production. YgfB binds directly to an essential transcriptional activator of AmpDh3, called AlpA, as well as to the binding site of AlpA on the *ampDh3*-promoter and thus inhibits AmpDh3 production. Since AlpA production can be induced by DNA-damage, for example by treatment with ciprofloxacin (CIP) [4], we investigated a potential synergism between ciprofloxacin and β-lactams in the MDR strain ID40. Combining ciprofloxacin with β-lactams in a *ygfB* deletion strain completely breaks resistance toward all β-lactams tested. Presence of *ygfB* however dampens this effect. YgfB thus limits a more pronounced synergy between ciprofloxacin and β-lactam antibiotics, explaining why synergism between CIP and β-lactams can only be observed in some *Pa* strains.
Discussion: We hypothesize that differences in the regulatory network modulating *ampDh3* expression in various *P. aeruginosa* strains might at least partially explain why the synergistic action of ciprofloxacin and β-lactam antibiotics shows strain-by-strain variations.
Taken together, we identified a novel contributor and mechanism contributing to the complex regulation network of MDR in *Pa*. Given the great plasticity of these regulation networks that finally cause MDR, we think that this is an important step toward a better understanding of MDR in *Pa*.

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092/MPV

Core and accessory effectors of the type VI secretion system mediate strain-specific behaviour and virulence of *Pseudomonas aeruginosa*

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Effectors of the type VI secretion system (T6SS) enable bacteria to kill other microbes, manipulate eukaryotic cells, and take up nutrients. Although multiple of these functions are generalisable across bacterial phyla, the T6SS can differ considerably even between strains of the same species. Little is known about the intraspecific diversity of T6SS effectors in bacterial populations of the environment and the clinic beyond laboratory reference strains. Here, we focus on the opportunistic pathogen *Pseudomonas aeruginosa* and combine comparative genomics with *in vitro* and *in vivo* experiments to determine the impact of genetic variation in T6SS effectors on strain-specific phenotypes.
Similar to the concept of a pangenome that consist of a core genome present in all strains of a species and an accessory genome of genes that vary between strains, we find T6SS core effectors in all analysed *P. aeruginosa* strains and accessory effectors that vary between strains in their enzymatic activity or by presence absence. Core effectors are known to kill bacteria, take up nutrients, and act on eukaryotic cells, which demonstrates the broad functionality of *P. aeruginosa* T6SSs across strains. Accessory effectors have the potential for intra-specific killing between strains and further enhance virulence, as we demonstrated on an effector-specific interaction with phagocytic cells during infection. By mix and match, combinations of core and accessory effectors form numerous effector sets that can be specific to individual strains and mostly differ from those of characterized laboratory strains.
In summary, we find tremendous intraspecific variation in some but not other T6SS effectors, which assemble diverse effector sets and advance our understanding of the T6SS, the intraspecific diversity of *P. aeruginosa*, and its relevance in clinical settings.

093/MPV

Identification of hemolysis-related pathogenicity factors of *Bartonella bacilliformis*

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Introduction: *Bartonella bacilliformis* is the causative agent of Carrión"s disease, a vector-borne biphasic illness restricted to the South American Andes. In the acute phase, bacteria infect erythrocytes causing a severe hemolytic anaemia with case-fatality rates as high as 90% in untreated patients. Erythrocyte infection in Carrion"s disease involves at least two steps: (i) binding of the bacteria to the erythrocyte surface and (ii) bacterial invasion through the erythrocyte membrane. Exact knowledge of these processes is crucial for a therapeutic drug development.
Material & 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 analyse whether direct cell contact is needed to cause hemolysis.
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.
Conclusion: By using a transposon library, markerless deletion and complementation mutants, 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* since the loss of at least one of the two factors prevents hemolysis, respectively. This hemolysis is contact-dependent and therefore requires bacterial adherence to erythrocytes. Ongoing work is aimed to inhibit the hemolytic activity by screening an inhibitor library for active compounds.

094/MPV

The expression and secretion of chitinase A (ChiA) – a novel virulence factor of *Salmonella Typhimurium*

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Question: *Salmonella enterica*, a foodborne-pathogen can cause serovar-specific gastroenteritis or systemic disease in humans. For the successful infection of the host, *Salmonella* expresses and secretes various virulence factors and toxins. In our work, we were interested in chitinase A (ChiA), a novel and not yet defined virulence factor of *S.* Typhimurium. First, we analyzed the expression of ChiA in contact to different human cell types. Next, we focused on the contribution of ChiA to the host cell adhesion and invasion proficiency of *S.* Typhimurium. By BLAST sequence analyses, two potential regulators, a cell wall active muramidase and a holin could also be found within the *chiA* operon. As shown in *S.* Typhi, a muramidase and a holin are essential components for the secretion of typhoid toxin. Therefore, we examined whether these two factors may also play a significant role in the secretion process of ChiA in *S.* Typhimurium.
Methods: For host cell contact expression patterns we performed Western Blot analyses. With various cell types and cell lines, cell adhesion and cell invasion CFU assays were implemented to characterize ChiA as a potential virulence factor. To unravel regulation of ChiA expression, we used fluorescence reporter strains and Western blot analysis. To test, if ChiA secretion is muramidase/holin dependent, the TCA precipitated supernatants of wild-type and muramidase mutants were analyzed by Western Blot analyses.
Results: We show that ChiA expression is induced only in contact with polarized intestinal epithelial cells (IEC) (Caco-2 and HT29-MTX). In contrast, unpolarized IEC as well as non-intestinal epithelial cells (HeLa) do not induce ChiA expression. In line with the expression pattern, ChiA dependent adhesion and invasion occurs only in contact with polarized IEC. Further, the induction of ChiA expression completely depends on an inner membrane-bound transcriptional regulator found within the *chiA* operon. The secretion of ChiA from bacteria to the extracellular environment depends entirely on a muramidase/holin pair secretion mechanism.
Conclusion: Although characterized in other pathogenic bacteria, chitinases are unknown virulence factors in *S.* Typhimurium. Previous microarray studies found *chiA* upregulated in various host cells. In our study, we show that ChiA is not only tightly regulated and expressed, but also relevant for invasion and adhesion in contact with polarized IEC. The intestine cell type specificity as well as polarization dependency lead to further questions, which particular cellular targets are recognized by ChiA. ChiA as a hydrolytic enzyme breaks down glycosidic bonds, which can be found in many glycosylated proteins and lipids on the cell surface. The discovery of the muramidase/holin pair secretion mechanism for ChiA secretion confirms other studies that such secretion systems are widespread and can be found in various pathogens such as *S.* Typhimurium, *S.* Typhi and *Serratia marcescens*.

Workshop 17
Surveillance of nosocomial pathogens (StAG HY/FG PR)
07. Sep 2022 • 08:30–10:00

096/HYPRV
Transmitted or not – in-depth analysis of closely related multidrug resistant organisms and epidemiological links of their corresponding patients in a hospital

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Question: Identification of clusters and outbreaks of multidrug resistant organisms (MDRO) in a hospital is a crucial task in infection control. Using advanced typing techniques, we can nowadays detect single transmissions of MDRO between two patients. Here, we address the question to what extent it is possible to predict transmission based on typing and epidemiological information by analyzing seven years of data of the three most common MDRO (Methicillin-resistant *S. aureus* [MRSA], MDR *E. coli*, Vancomycin-resitant Enterococci [VRE]) in a hospital.
Method: All initial findings of MDRO of all inpatients were subjected to whole genome sequencing (WGS)-based cgMLST typing. We combined cgMLST allelic profile data of all typed isolates collected between 01.01.2015 and 31.12.2021 with the corresponding patient location data from the hospital information system. Each patient location data contained information about the clinic, ward, and room of a patient along with start and end time of the stay. After collecting all the necessary data, we reviewed, how many epidemiological links between two patients could be automatically identified base on the location data, if they had the same MDRO with a specific cgMLST distance.
Results: For MRSA, at cgMLST allelic distance 0 and 1, in more than half of the cases it was possible to identify an epidemiological link (58% and 51% respectively). At an allelic distance of ≥6, the number of links decreases to ≤15%. For MDR *E. coli*, at cgMLST distance 0 and 1, in >40% of the cases it was possible to identify an epidemiological link (47% and 51%), while at ≥3 allelic distance, it decreases to 12% and below. For VRE, at cgMLST distance 0 and 1, in >32% of the cases it was possible to identify an epidemiological link (39% and 32%), while at ≥3 allelic distance it decreases to ≤17%.
Conclusions: Independently of the analyzed species, we determined that the smaller the cgMLST distance the higher the probability to find an epidemiological link in the hospital. This observed pattern confirms what we intuitively expected, since we usually assume that two closely related isolates share a common origin. It was quite interesting to learn that in approx. 42% - 60% of the cases (depending on the species) of two even identically typed isolates (i. e. cgMLST allelic distance of 0) it was not possible to identify an epidemiological link based on the entries in the electronic health record. Coincidentally equally typing, uncovered epidemiological links, second-degree transmissions inside the hospital and transmissions outside of the hospital could be explanations for this unexpected high number.

097/HYPRV
Wild-type isolates as outbreak indicators detected by a phenotype-based algorithm

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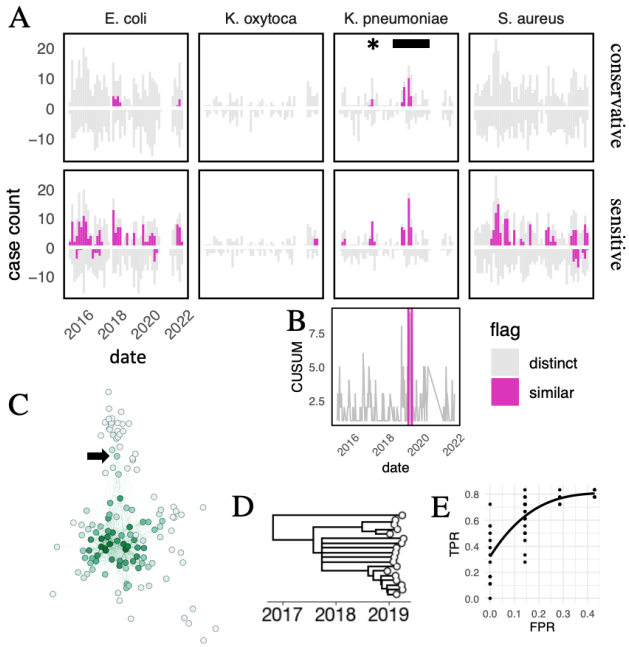
Introduction: Genomic sequencing has become a standard to investigate outbreaks of pathogens and their transmission routes. However, samples are usually arbitrarily selected for sequencing based on noticeable features like antimicrobial resistance patterns. Wild-type samples are mostly ignored, even though they are far more numerous. However, these samples offer many more opportunities to discover transmission events and infer hygiene problems.
Methods: We propose an algorithmic approach to select clusters of similar routine isolates for subsequent sequencing and outbreak investigation.
Results: By leveraging spatio-temporal and phenotype similarity, we identified outbreak samples with more than 80% sensitivity and an acceptable number of false positives, subject to algorithm calibration. This screening algorithm identified a previously unrecognized wild-type Klebsiella pneumoniae outbreak on a neonatal ward.
Discussion: Our approach provides an easy-to-implement method to screen routine samples for more all-encompassing outbreak monitoring and early identification at no extra cost.
Figure 1: Algorithmic selection of putative outbreak samples for subsequent sequencing. (A) Histogram of routine samples of four bacterial species across six years (grey) in a neonatal ward (counts >= 0) and sampled at random from all hospital records (counts < 0). Samples similar in space, time, and phenotype are flagged pink and constitute putative outbreaks. By chance, hardly any samples are flagged as suspicious. Two parameter sets are displayed, one conservative (upper row: t=14, p=1, d=1.5, |c|=5) and one to maximize sensitivity (lower row: t=14, p=1, d=2.5, |c| =4). The respective true and false-positive (TPR/ FPR) rates are 55.56%/ 0% and 83.33%/ 14.29%. We sequenced all 25 available isolates in a suspicious Klebsiella pneumoniae (Kp) cluster from 2019 irrespective of their label (black bar), detecting an unknown outbreak with a plausible origin in 2017 (asterisk). (B) Alternative algorithm to label intervals based on the cumulative sum of cases ("CUSUM") also identifies the suspicious Kp cluster in 2019 but not 2017. (C) Patient transfer network "G" as reconstructed from routine isolate records. In green is shown the degree of nodes, with many transfers to and from intensive care units (labels not shown, outbreak ward marked with arrow). (D) Phylogenomic tree reconstructed from 18/ 25 samples sequenced from the flagged cluster in 2019 (black bar in A). All isolates have fewer than 21 core SNVs, compatible with a within-hospital outbreak [1]. Two clades, deep branches, and an estimated most recent common ancestor (MRCA) from 2017 indicate clonal circulation over many months. (E) Receiver operating characteristic (ROC) to evaluate the performance of the proposed algorithm. The typical trade-off between sensitivity and false positives can be seen.
[1] David et al. 2019, Nature Microbiology 4 (11): 1919–29.

Fig. 1



098/HYPRV

Vancomycin-resistant Enterococci colonisation and infection in a German university clinic – first results of a retrospective cohort study from 2015 to 2017

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Introduction: Vancomycin-resistant Enterococci (VRE) have become a major infection control challenge in the last years in Germany and on a global scale. In case of (hospital-acquired) infections caused by VRE antibiotic therapy options are limited and clinical outcomes may be worsened. In order to better understand and to analyze the local epidemiology of VRE in our hospital, we conducted a retrospective cohort analysis including all patients with VRE from 2015 to 2017.
Materials and methods: We identified all patients with VRE using the microbiology laboratory information system and the local infection control software. Clinical and epidemiologic characteristics were collected from patient charts. Infections were classified using CDC criteria. Categorical parameters are shown as number and percentage. The local ethics committee approved the study (# 8987_BO_K_2020).
Results: Overall, there were 822 single VRE patients in the study period corresponding to 1492 VRE cases (each patient hospital stay is a case; a patient can have more than one stay). 607 of the VRE cases were female (40.7%) and 54 VRE cases (3.6%) were aged 18 and below. The mean age of the VRE cohort was 55 years. 40 of the VRE cases were *E. faecalis* (2.7%) and 1452 *E. faecium (*97.3%*)*. The incidence was 0.8 VRE cases per 100 patient hospital stays (i.e. 0.8% of all hospital stays). The incidence density was 1.1 VRE cases per 1000 patient days. In 528 (35.4%) of the 1492 VRE cases the acquisition was nosocomial.
In 107 cases (7.2%), a VRE infection was found. Most prevalent were bloodstream infections (in total n=41, 38.3%; n=25 of which were primary bloodstream infections), postoperative wound infections (n=30, 28.0%) and urinary tracts infections (n=18, 16.8%).
VRE cases had most frequently rectal/stool colonization (n=597 VRE cases, 40.0%). VRE cases were most often cared for in the following departments: gastroenterology (n=298, 20.0%), hematology and oncology (n= 262, 17.6%), visceral surgery (n=196, 13.1%), heart surgery (n=174, 11.7 %) and nephrology (n=168, 11.3%).
Discussion: With 1492 cases in the study period, VRE was a relevant burden at our hospital. About every third VRE case was hospital-acquired underlining the need for ongoing infection control and antibiotic stewardship efforts. 7.2% of the VRE cases had a VRE infection, which demonstrates the importance of VRE from the infectious diseases perspective as well. Medical specialties such as gastroenterology and hematology and oncology were especially affected.

099/HYPRV

Investigation of the prevalence and distribution of hypervirulent *Clostridioides difficile* strains in hospitals in Berlin and Brandenburg

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Introduction: *Clostridioides difficile* infections (CDI) are a growing concern worldwide as they are one of the most common heathcare associated infections. The emerge of hypervirulent strains poses an increasing threat to vulnerable populations (especially elderly people). Current surveillance systems for CDI in Germany partially record the incidence as well as the clinical course of the disease. However, there is no nationwide data collection on the frequency and distribution of *C. difficile* ribotypes and hypervirulent strains in hospitals in Germany. Therefore, the aim of this pilot study was inter alia to collect data regarding currently circulating *C. difficile* strains in hospitals in Berlin and Brandenburg. In particular, this study aimed to improve data availability on the occurrence and spread of the hypervirulent strain RT027.
Material/Method: Stool samples from patients with a laboratory confirmed diagnosis of CDI were collected from hospitals in Berlin and Brandenburg from February 2020 to November 2021. The samples were collected by the local microbiologic laboratory and forwarded to RKI for further analysis. Stool samples were analysed by means of selective culture to isolate *C. difficile*. Toxigenic strains were identified using an agglutination test and ELISA. Ribotyping was performed, antibiotic susceptibility was assessed, and toxin genes were analysed in collaboration with the German National Reference Laboratory for *C. difficile*.
Results: Clinical samples of hospitalized patients with CDI in Berlin (n= 19 hospitals) or Brandenburg (n= 3 hospitals) were enrolled in this study. Altogether, 496 samples were received from which 492 *C. difficile* isolates were obtained. 476 isolates were determined to be toxin producing by ELISA. Ribotyping was performed on all toxigenic isolates, with 65 different types being identified. The most frequent ribotypes were RT014 with 16% followed by RT027 (12.8%) and RT001 (7%). Analysis of toxin genes among the toxigenic isolates revealed the presence of *tcdA* & *tcdB* in 363/476 isolates, and of additional *cdtAB* in 113 isolates. The percentage of RT027 among the isolates obtained from each hospital ranged from 0 to 38%. The median age of the patients was 79 years. Of all included patients, 54% were female and 46% were male.
Discussion: These are the first systematically collected data in this region that assess the distribution of different ribotypes. RT027 was the second most abundant strain among CDI patients in 22 hospitals in Berlin and Brandenburg. The data also shows that RT027 is unevenly distributed across different facilities and remains a prominent threat especially to the vulnerable population. Comparing the results of the current study with previously collected data from Germany, an incline in the percentage of RT027 over the last 10 years can be observed, which again highlights the need for a more comprehensive and molecular surveillance of CDI on the national, regional and local level.

Workshop 18
National reference centres and consulting laboratories (StAG RK)
07. Sep 2022 • 08:30–10:00

100/RKV

National Reference and Consultant Laboratories in Germany

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The National Reference and Consultant Laboratories in Germany play a valuable role for the scientific progress in the field of medical microbiology. They cover the entire spectrum of infectious agents from prions, viruses and bacteria to parasites and contribute substantially to laboratory capacity in Germany. The continuous development of the reference laboratory structure is of key importance to ensure public health microbiology. This poster aims to inform the scientific community of the structure of the German National Reference and Consultant Laboratories and the activities in integrated molecular surveillance of pathogens in Germany.

101/RKV

Presumptive foodborne botulism in an infant

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Introduction: Infant botulism arises after colonisation of the immature infant gut by spores of botulinum neurotoxin (BoNT)-producing *Clostridium* spp. and affects infant <1 yr of age. Typically, it begins with a period (days to weeks) of poor feeding and reduced activity. Botulism is characterised as an afebrile descending symmetric paralysis starting at the facial muscles (mydriasis, diplopia, dysarthria, dysphagia), progressing into the extremities and eventually leading to death by respiratory failure. In contrast to infant botulism, foodborne botulism is not an infection but an intoxication caused by preformed BoNTs, which have been secreted by BoNT-producing clostridia under suitable condition in protein-rich foods of plant or zoonotic origin. Foodborne botulism is characterised by a rapid onset 12–72 hrs after uptake. In infants foodborne-botulism is extreme rare with not even a handful of documented cases worldwide. Infant botulism is frequently associated with honey consumption which is a known vehicle for spores. Botulism in humans is caused by serotypes A, B, E, F, and H produced by strains of *Clostridium botulinum* Groups I and II, *C. baratii*, *C. butyricum* or *C. sporogenes*.
Materials & Methods: Clinical suspected botulism is confirmed by the presence of BoNT in serum usually within 1-3 days of symptoms onset. Serum is injected *i.p.* into mice which are observed for typical botulism symptoms. Presence of BoNT is confirmed and serotyped by blockade with serotype-specific antitoxins. The producing *Clostridium* spp. can be identified from stool for about 2 weeks. Anaerobic culture as well as suspected colonies are tested by qPCR for one of the 8 BoNT genes. Sanger and NGS is used to identify the underlining species as well as the BoNTs" sero- and subtype.
Results and Discussion: A 5-month-old infant was presented to the paediatric emergency department with a one-day history of poor feeding, constipation, and development of generalised weakness. Contradictory to an initially suspected case of infant botulism, the boy had no history of honey feeding and had already been introduced to semi-solid foods, which usually renders the intestinal microflora environment resistant to colonisation by BoNT-producing clostridia. Furthermore, the boy developed fever and showed increased CRP levels indicating an acute infection. He had been fed with a home-made vegetable-meat-puree within 48 hrs prior to hospitalisation. Botulism could be confirmed by the identification of BoNT/B from serum and a *C. botulinum* Group I strain producing BoNT/B2 from stool as well as an unused portion of the puree. Additionally, *C. perfringens* and the produced cytotoxic enterotoxin (CPE) was detected in stool which might well explain the additional signs of an acute infection. The acute onset of botulism after the introduction of solid foods and the presence of the same BoNT/B2 strain in the food and stool are suggestive for an unusual case of foodborne botulism in an infant.

102/RKV
Taxonomic assignment of *Bacillus cereus* biovar *anthracis* – "there and back again"
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In 2001, an untypical bacterium was detected in chimpanzees which caused a deadly, anthrax-like disease. Based on initial findings, *Bacillus anthracis* was assumed as causative agent and the bacteria were assigned accordingly. Further analyses with bacterial isolates confirmed presence of the anthrax virulence plasmids, but revealed important differences compared to classic *B. anthracis*, which resulted in the more cautious designation "*B. anthracis*-like". At that time, the designation "*B. anthracis*" was reserved to a monophyletic cluster with bacteria possessing four specific prophage regions and a nonsense mutation in the *plcR* gene – features lacking in the chimpanzee strains. Therefore, the designation "*B. anthracis* sensu lato" was not accepted. Many experts in the field insisted on "*B. cereus*" due to the chromosomal background, neglecting the presence of anthrax virulence plasmids and disease. This finally resulted in the designation "*B. cereus* biovar *anthracis*" (*Bcbva*), which was not perfect, but an acceptable compromise.
In the last years, the number of genome sequences in the databases was exploding and more species of the *B. cereus* group were described. Taxonomic classification based on phenotype (and disease in the case of pathogens) was shifted to sequence-based taxonomy. Genomospecies were defined by Average Nucleotide Identity (ANI), raising the problem of variable thresholds. Based on ANI, *Bcbva* can be classified today as *B. anthracis*, together with classic *B. anthracis* (in Germany risk group 3) and some strains of *B. thuringiensis* (risk group 1). To overcome this confusing taxonomy, a new system was proposed in which anthrax toxin producing strains like *Bcbva* would be designated as *B. mosaicus* biovar Anthracis (or *B.* Anthracis), to reflect their clinical relevance. Nevertheless, the NCBI database contains strains designated as *B. anthracis* isolated from marine sediments, a hot spring or cypress bark, and these strains lack the virulence plasmids, the *plcR* mutation and the prophage regions. The fact that obviously harmless bacteria are called *B. anthracis* raises formal and legal problems which should be avoided by correct strain assignment.

103/RKV
Immunofluorescence analysis reveals no increased seroprevalence of anti-*Bartonella schoenbuchensis*-IgG antibodies in German forest workers
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Background: *Bartonella schoenbuchensis* is suspected to cause deer ked dermatitis and febrile diseases in humans. Deer keds (*Lipoptena cervi*) which infest cervids (e.g., roe deer, fallow deer) have been demonstrated to harbour *B. schoenbuchensis* DNA. In terms of a one health perspective, deer keds are discussed as potential vectors for *B. schoenbuchensis*.
Methods: We analysed the seroprevalence of anti-*B. schoenbuchensis*-IgG antibodies in sera of forest workers (FW; *n* = 82) compared to control sera of non-forest workers (NFW; n = 118) from North Rhine-Westphalia, Germany. For this purpose, an immunofluorescence assay (IFA) using Vero E6 cells infected with *B. schoenbuchensis* was established, and serum titres were assessed. Immunoblotting using *B. schoenbuchensis* whole cell lysates was performed to identify potential immunodominant target proteins.
Results: Using polyclonal rabbit anti-*B. schoenbuchensis*-IgG, the herein established IFA antigen was technically evaluated. When using human sera, 54.9% (n = 45/82) of FW were tested positive at a titre ≥320 whereas IFA reactivity was 66.1% (n = 78/118) in NFW. When the cut-off titre was set to ≥640, then 18,3% (n = 15/82) and 20,3% (n = 24/118) displayed seroreactivity, respectively. In immunoblot analysis, IFA-positive sera reacted with 18 different bands ranging from ca. 40-300 kDa.
Conclusions: Our data do not demonstrate elevated seroprevalence of anti-*B. schoenbuchensis*-IgG titres in FW which are regularly exposed to deer keds. Therefore, FW do not seem to have a higher risk to suffer from *B. schoenbuchensis* infections.

104/RKV
Epidemiology and antifungal drug susceptibility of German *Candida auris* isolates
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Introduction: The emerging yeast *Candida auris* quickly adapts to environmental stresses and can rapidly develop antifungal drug resistance. These abilities contribute to its success as a human pathogen. Besides, it can be easily transferred within medical care units, having led to hospital outbreaks in many countries. In contrast to the US, India or the UK, cases of *C. auris* infections are still a rare event in Germany. To provide an overview of current situation in Germany, we performed a comprehensive analysis of primary *C. auris* isolates which were obtained in German hospitals and reported to the German National Reference Center for Invasive Fungal Infections (NRZMyk) during a period of seven [AA1] years (2015-2021).
Methods and Materials: We performed whole genome sequencing (WGS) for consequent phylogenetic analysis and identification of resistance-associated mutations. Antifungal drug susceptibility testing (AFST) was performed with either custom-made or commercially available broth microdilution assays. In case of phenotypic resistance, *ERG* or *FKS* genes were amplified by PCR and sequenced to identify mutations.
Results: Between 2015 and 2021, the NRZMyk obtained 25 viable *C. auris* isolates from 26 reported cases. Isolates were obtained from various specimens, including urine (8/25), blood (4/25) and the ear (4/25). Three of the patients had a Covid19 infection prior to the detection of *C. auris*. According to WGS data, the strains could be grouped into clades I, II and III. AFST was performed in a custom-made broth microdilution approach in accordance with EUCAST. Commercially available YeastOne and Micronaute assays were added to compare minimal inhibitory concentrations (MIC) by using different test systems. A strain was defined phenotypically resistant or sensitive if the MICs were similar in at least two of three assays performed. Although most of the isolates (22/25) were resistant to fluconazole, many of them were still susceptible to other azoles, especially to itraconazole and posaconazole. On the contrary, elevated Amphotericin B MIC-values were found in nearly all cases. Echinocandin resistance was only observed in one case and could be associated to a S639Y mutation in Fks1. In total, we could find three isolates which were multidrug resistant against azoles and amphotericin B in at least two of the used AFST approaches.
Discussion: Compared to other countries, *C. auris* infections are still a rare event in German hospitals. During the analyzed time period (2015-2021) only one hospital outbreak occurred which could be kept to two patients only. Our findings support the usage of echinocandins as the first treatment option. According to the *in vitro* results, posaconazole seemed to be the most effective azole against *C. auris*. Although the number of cases is still low and multidrug resistance was rare, we recommend ongoing awareness of *C. auris* and tight monitoring in the case of infection to avoid outbreaks.

105/RKV
Changes in the epidemiology of invasive meningococcal diseases during the COVID-19 pandemic in Germany
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Question: The COVID-19 pandemic impacted Germany since March 2020. In order to reduce the spread, nation-wide containment measures such as social distancing were implemented by the German government. *Neisseria meningitidis* (Nm) is also transmitted by respiratory droplets and it has already been proven in the past, that Nm carriage is associated with the frequency of social contacts.
Methods: In this study, prevalence data of invasive Nm infections in the time frame of 2020 and 2021 during the containment measures in Germany were compared to the preceding year"s period in order to analyse changes in the epidemiology. IMD cases were analysed from April 2020 to December 2021 and compared to data beginning in 2001. Activities that correlate with the spread of diseases that are transmitted via respiratory droplets were furthermore analysed with the Google mobility data as well as the Oxford COVID-19 Government Response Tracker.
Results: Since calendar week 14 (beginning of a lockdown effect) there were only 51 cases of IMD in 2020 and only 69 in 2021 which was a significant decline compared to previous years (73% decline 2021 vs. 2019, Figure 1).
Figure 1: Cumulative invasive meningococcal disease (IMD) cases during the years 2009 - 2021
Despite a large variability in mobility since the beginning of the COVID-19 pandemic, IMD numbers remained low during all phases. In a multi-variate linear regression model the seasonal effect was still a highly correlating variable with the weekly number of IMD cases ($p<0.0001$) as well as to a lower extend the shopping mobility ($p=0.01$, Figure 2).
Figure 2: Weekly number of invasive meningococcal disease (IMD) cases during the COVID-19 pandemic compared to previous average numbers and Google mobility data
Conclusions: A strong decline in IMD numbers was observed throughout the pandemic, but seasonal effects were still observed. Analysing the Google mobility data shows a positive correlation of shopping mobility with IMD numbers.
Since invasive bacterial infections such as meningitis and sepsis are life threatening events and the coverage for Nm did not change significantly, a bias by unnotified cases seems unlikely. In light of these dynamics infection surveillance and epidemiologic analysis are important to monitor further developments as the pandemic is ongoing.

Fig. 1

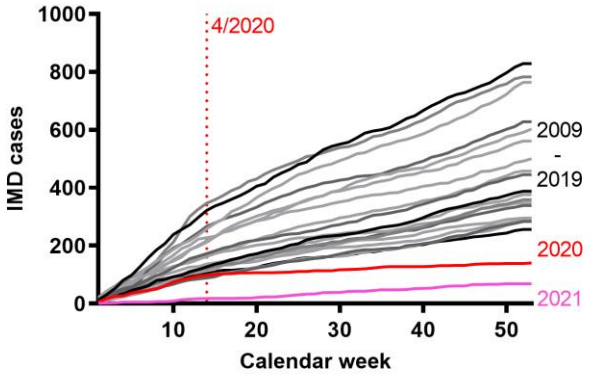
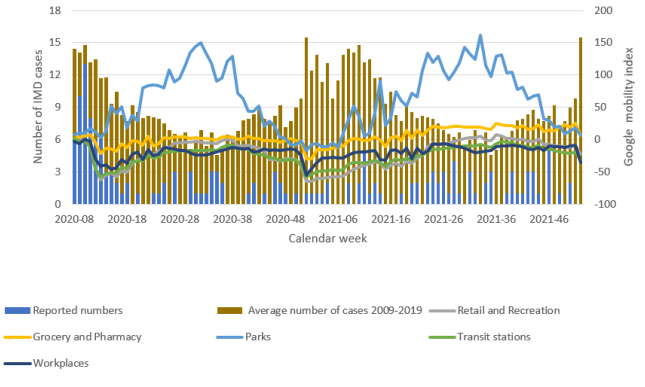


Fig. 2



106/RKV
Invasive streptococcal disease in Germany during the SARS-COV-2 pandemic
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Introduction: The German National Reference Center for Streptococci (GNRCS) monitors invasive streptococcal disease in

Germany. This includes *Streptococcus pneumoniae*, *Streptococcus pyogenes* (GAS), *Streptococcus agalactiae* (GBS), *Streptococcus dysgalactiae* (SD) and all other validly published streptococcal species. Here we report on the species-specific reduction of invasive streptococcal disease during SARS-CoV-2 pandemic in Germany.

Material/method: Species identification was performed using a combination of microbiological, biochemical, immunological and molecular-biological techniques, including hemolysis-assessment, catalase-test, optochin- and bile-susceptibility, pyrrolidonyl-arylamidase-test, leucine-aminopeptidase-test, Lancefield-typing, *emm*-typing, serotyping and multiple PCR-sequence analyses.

Results: SARS-CoV-2 reached Germany beginning of March 2020. A strong reduction in case numbers of invasive pneumococcal disease was reported shortly after. This reduction continued till October 2021 and amounted up to a reduction of 75%. A comparable reduction of 67% was observed for invasive GAS infections, accompanied with a disproportional decrease of *emm*1 (-85%) and *emm*12 (-73%). However, no effect was seen for GBS and SD. Among other streptococci, a 40% reduction was observed for oral-streptococci of the Mitis-, Salivarius- and Mutans-Group, while streptococcal species not commonly associated with the oral microbiome, showed no effect.

Discussion: The SARS-CoV-2 pandemic had a strong reducing effect on invasive streptococcal disease caused by *S. pneumoniae*, *S. pyogenes* and oral streptococci of the Mitis-, Salivarius- and Mutans-Group, while no effect was seen for invasive GBS and SD disease. These reductions are obviously limited to respiratorily transmitted streptococci, and therefore seem to be related to non-pharmaceutical interventions (face masks, social distancing, working from home, school closures) during the SARS-CoV-2 pandemic.

Workshop 19
Microbiome: Host-Microbiota studies and technological advances (FG PW)
07. Sep 2022 • 08:30–10:00

107/PWV
Evaluation of microbiome enrichment and host DNA depletion in human vaginal samples using Oxford Nanopore"s adaptive sequencing

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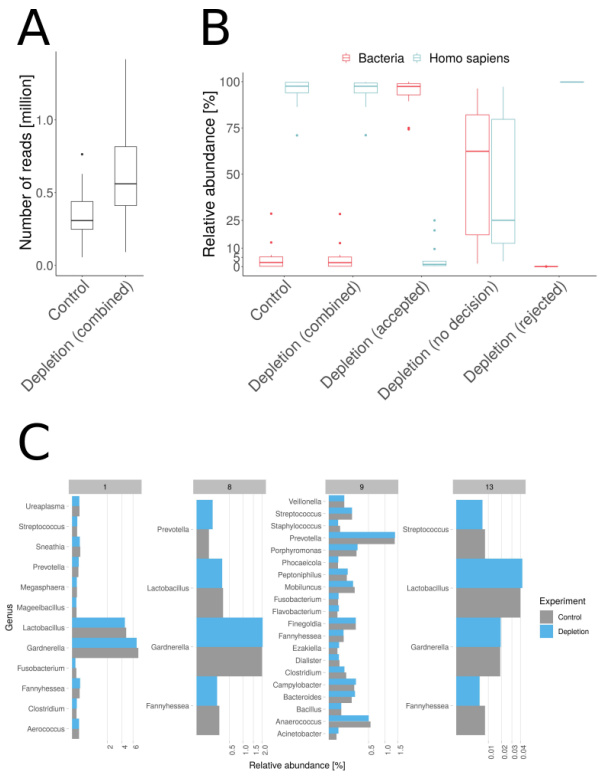
Introduction: Metagenomic sequencing is promising for clinical applications to study microbial composition concerning disease or patient outcomes. Alterations of the vaginal microbiome are associated with adverse pregnancy outcomes, like preterm premature rupture of membranes and preterm birth. Methodologically these samples often have to deal with low relative amounts of prokaryotic DNA and high amounts of host DNA (> 90%), decreasing the overall microbial resolution. Nanopore's adaptive sampling method offers selective DNA depletion or target enrichment to directly reject or accept DNA molecules during sequencing without specialized sample preparation.

Results: Here, we demonstrate how selective 'human host depletion' resulted in a 1.70 fold (± 0.27 fold) increase in total sequencing depth, thus providing higher taxonomic profiling sensitivity. At the same time, the microbial composition remains consistent with the control experiments. The complete removal of all human host sequences is not yet possible and should be considered as an ethical approval statement might still be necessary. Adaptive sampling increased microbial sequencing yield in all 15 sequenced clinical routine vaginal samples.

Methods: DNA of vaginal swabs was extracted via the ZymoBIOMICS DNA Miniprep kit and sequenced on the GridION (Oxford Nanopore). Adaptive sampling was enabled in the MinKNOW software. In "human depletion experiments", the human reference genome GCA_000001405.28_GRCh38.p13 was used for target depletion. The basecalled reads generated during adaptive sampling were extracted from the fastq files via samtools v1.19 and split based on the read_until.csv-file into three categories: 'accepted', 'rejected' and 'no decision'. The reads were taxonomically classified via centrifuge v1.0.4.

Conclusion: Our results demonstrated that Oxford Nanopore's unique adaptive sequencing feature has reliably increased the overall sequencing depth of bacterial sequences in clinical metagenomic samples via 'human depletion' without changing the microbial composition during sequencing. Therefore, more samples can be barcoded and sequenced simultaneously, reducing the total cost per sample in a diagnostic laboratory, making molecular monitoring of human reservoirs with low microbial concentrations and high host DNA loads more feasible. We strongly believe that adaptive sampling will prove exceptionally useful within clinical research and the individual microbiological and microbiological diagnostic approach in routine diagnostics.

Figure 1. Reduced result overview:
(A) Total number of sequenced reads for depletion and control experiments. (B) Human (blue) and bacterial (red) proportions for each sample of the control and depletion experiments. Depletion experiments were additionally split into the three decision categories: 'rejected', 'no decision', and 'accepted'. (C) Comparison of the relative abundance of bacterial genera for four of the 15 vaginal samples.
Fig. 1



108/PWV
The oral-gut axis – salivary and fecal dysbiosis in inflammatory bowel diseases (IBD)

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Introduction: The gastrointestinal tract traverses from mouth to anus and harbours diverse bacterial communities. Several sequencing-based studies have identified intestinal enrichment of oral-associated bacteria and their ability to induce gut inflammation in mice, suggesting that intestinal pathobionts, especially members of genus *Streptococcus*, originate from the oral cavity. This study aimed to assess the salivary and fecal microbiomes of inflammatory bowel disease (IBD) patients compared with healthy controls (HCs), and to investigate abundances and composition of shared bacterial genera among both niches.
Materials & methods: Metagenomic DNA was isolated from saliva and fecal samples of 14 IBD patients and 12 HCs. The V3–V4 regions of the 16S rRNA gene were targeted for sequencing on Illumina MiSeq. Raw reads were processed for sequence quality check, chimera filtering, and cluster formation using the IMNGS platform. For downstream analysis of generated OTUs, R pipelines Rhea and microeco were used.
Results: The salivary microbiome differed significantly among the IBD and HCs groups (p = 0.038). At genus level, *Veillonella* and *Prevotella* were highly abundant in IBD (median: 25.4% and 22.2%, respectively) compared to HCs (17.9% and 13.4, respectively). In contrast, *Neisseria* (12.7%), *Streptococcus* (12.5%), *Haemophilus* (5.4%), *Porphyromonas* (5.1%), and *Fusobacterium* (3.1%) were predominant in HCs and accounted for 38.8% of their salivary microbiome, while in IBD patients they accounted for only 18.9% (3%, 10.2%, 3%, 1%, and 1.7%, respectively). Ten KEGG pathways were significantly enriched in IBD, and 12 were associated with a healthy state. For the fecal microbiome, IBD patients had a significantly lower fecal microbial richness and diversity compared to HCs. The abundance of *Bifidobacterium* (3.9% vs. 5.4%), *Bacteroides* (2.5% vs. 5.0%), *Subdoligranulum* (2.1% vs. 4.1%), *Ruminococcus* (0.0% vs. 3.5%), and *Collinsella* (2.1% vs. 2.8%), was significantly lower in the IBD compared with HCs. *Blautia* (17.0% vs. 13.1%), *Anaerostipes* (2.0% vs. 1.8%), *Escherichia-Shigella* (0.5% vs. 0.0%), and *Streptococcus* (0.5% vs. 0.2%) were significantly higher in the IBD. The fecal microbiome of IBD patients showed significantly less richness in pathways of Vitamin B6, histidine, and retinol metabolism. Sequences of the genus *Streptococcus* were detected in both habitats and further identified using culture-based methods: 57 and 91 streptococcal strains were isolated from the saliva samples, while 40 and 31 strains were isolated from the fecal samples of HCs and IBD groups, respectively.
Discussion: These findings revealed the dysbiotic signature of the salivary microbiome in IBD patients, which can be used to develop biomarkers for screening and early diagnosis of IBD conditions.

109/PWV
Effect of a planetary health diet on the human gut microbiome – a descriptive analysis

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Introduction: In 2019, international researchers developed the so-called Planetary Health Diet, i.e. recommendations pertaining to healthy diets derived from sustainable food systems. Core elements of this diet are an increase in fiber uptake, less animal product consumption and a reduction in each person"s carbon footprint [1,2]. However, it is currently unknown whether and how such a diet impacts on the composition of the human intestinal microbiome. Here, we present preliminary data on the gut microbiome of healthy volunteers adhering to the Planetary Health Diet as opposed to other diets.
Material and Methods: We collected stool samples from 41 healthy volunteers aged between 19 and 59 years. Individuals opting to follow recommendations put forth by the Planetary Health Diet received detailed instructions and recipes, whereas individuals in the control group followed their habitual dietary pattern (omnivorous or vegetarian/vegan). All participants provided stool samples at inclusion and after 2, 4 and 12 weeks. Whole-genome DNA was extracted and sequenced via shotgun-metagenomic sequencing. Additionally, same stool samples were also subjected to conventional stool culture and identification of bacteria was carried out using MALDI-TOF mass spectrometry.
Results: 17 individuals followed the Planetary Health Diet recommendations, whereas the control group comprised 18 omnivorous and 7 vegetarian/vegan individuals. The α-diversity of the gut microbiome in the Planetary Health Diet group remained the same within the first four weeks. However, there was a considerable increase in *Blautia obeum* and *Bifidobacterium adolescentis*. In contrast, the microbiota composition of the control group remained unchanged in the course of 4 weeks.
Discussion: We observed an increase in specific gut microbiota species after 4 weeks of dieting according to the Planetary Health Diet, which was absent in the control group. However, additional analysis is required to elucidate the clinical significance of this finding. At the time of writing, the analysis of samples after 12 weeks is still ongoing.
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110/PWV
The bacterial and fungal gut microbiome in SARS-CoV-2-infected patients

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Introduction: The gut microbiota contributes to the maturation of the immune system, to regulate immune responses and to maintain health. Patients with severe COVID-19 illness are characterized by a dysregulated hyper-inflammatory immune response, but the role of SARS-CoV-2 on the gut microbiome and the relevance of the gut microbiome for the severity of disease needs to be clarified. We studied the bacterial and fungal gut microbiome early after admission to a tertiary care hospital. We analysed, whether a specific gut microbiome exists in SARS-CoV-2 infected patients and if the gut microbiome allows for discrimination of patients developing mild or severe COVID-19 disease.
Material/Methods: The bacterial gut microbiome was analysed from rectal swabs of 212 patients using 16S rRNA gene sequencing of the V3/V4 region (117 SARS-CoV-2 positive and 95 SARS-CoV-2 negative patients). The fungal gut microbiome was studied using sequencing of the ITS2-region from patients exhibiting positive amplicon PCR (n=53). In addition, inflammatory cytokines and immune cells were quantified from blood.
Results: The bacterial gut microbiome of SARS-CoV-2 positive patients was characterized by a lower bacterial richness (p=0.018), a higher relative abundance of the phyla Proteobacteria and Bacteroidetes and a lower abundance of Actinobacteria compared with SARS-CoV-2 negative patients. The relative abundance of several genera was lower in SARS-CoV-2 positive patients including *Bifidobacteria*, while the relative abundance of

Bacteroides and *Enterobacteriaceae* was increased compared with SARS-CoV-2 negative patients. Higher proinflammatory markers and a lower CD8+ T cell count characterized patients with severe COVID-19 illness. The relative abundance of antiinflammatory genera *Faecalibacterium* and *Roseburia* and the connectivity of a network of bacterial genera with anti-inflammatory properties was reduced in patients with severe COVID-19 illness. The fungal gut microbiome in patients with severe COVID-19 illness exhibited a reduced Shannon-diversity (p=0.0047), richness (p=0.0373) and evenness (p=0.0062) and a reduction in the relative abundance of the phylum Ascomycota.
Discussion: The dysbiosis of the bacterial and fungal gut microbiome might contribute to the hyperinflammatory immune response and the increased proinflammatory signature of severe COVID-19 illness. Further studies are needed to investigate whether the differences in severe COVID-19 illness are consequences of SARS-CoV-2 infection or a predisposing factor for critical illness.

111/PWV
Adaptive nanopore sequencing on miniature flow cell detects extensive antimicrobial resistance
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Introduction: Rapid screening of hospital admissions to detect asymptomatic carriers of resistant bacteria can prevent pathogen outbreaks. However, the resulting isolates rarely have their genome sequenced due to cost constraints and long turn-around times to get and process the data, limiting their usefulness to the practitioner.
Methods: Here we use real-time, on-device target enrichment ("adaptive") sequencing on a new type of low-cost nanopore flow cell as a highly multiplexed assay covering 1,147 antimicrobial resistance genes.
Results: Using this method, we detected three types of carbapenemase in a single isolate of Raoultella ornithinolytica (NDM, KPC, VIM). Further investigation revealed extensive horizontal gene transfer within the underlying microbial consortium, increasing the risk of resistance spreading. From a technical point of view, we identify two important variables that can increase the enrichment of target genes: higher nucleotide identity and shorter read length.
Discussion: Real-time sequencing could thus quickly inform how to monitor this case and its surroundings.

Figure 1: Real-time sequencing reveals extensive resistance load and horizontal gene transfer. (A) Genome reconstruction of a strain of R. ornithinolytica carrying nine plasmids and four carbapenemase genes. Color-coded coverage from 90x (black, e.g., chromosome) to 250x (red, e.g., plasmid carrying OXA-1). (B) Gene transfer of VIM-1 across three strains and four loci. The carbapenemase is flanked by multiple transposases (see annotation), which likely mediate its mobilization. Vertical lines indicate 100% sequence identity between corresponding genes. (C) Comparison of shared resistance genes between the enrichment sequencing run ("Flongle"), the R. ornithinolytica isolate, all four "isolates" combined and the "metagenome" assembly. Of all resistance genes identified in the metagenome, 79.7% were found in the isolates. Surprisingly, several resistance genes were not identified in the metagenome, among them several carbapenemase copies. In the R. ornithinolytica isolate genome, about two-thirds of resistance genes were also found using on-device target enrichment. All plasmid-encoded genes among them were detected, including all carbapenemases. (D) Pairwise shared sequences between isolates and metagenome-assembled genomes. Putative transfers were defined as loci with a minimum length of one kilobase and 99.9% sequence identity between each pair of loci. Extensive sequence transfer is observed between the three isolate genomes (and their corresponding bins from the metagenomic assembly). (E) Miniature, low-cost flow cell used for on-device target enrichment ("Flongle", Oxford Nanopore Technologies), with a one-cent coin placed on top as scale.

Figure 2: Effect of two variables during adaptive sequencing on enrichment efficiency compared to a standard nanopore sequencing run [...].

Fig. 1

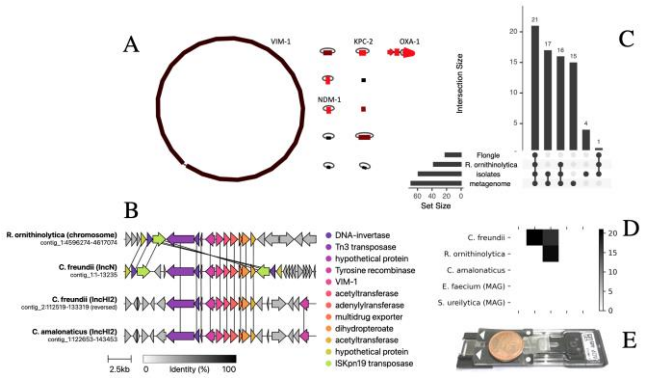
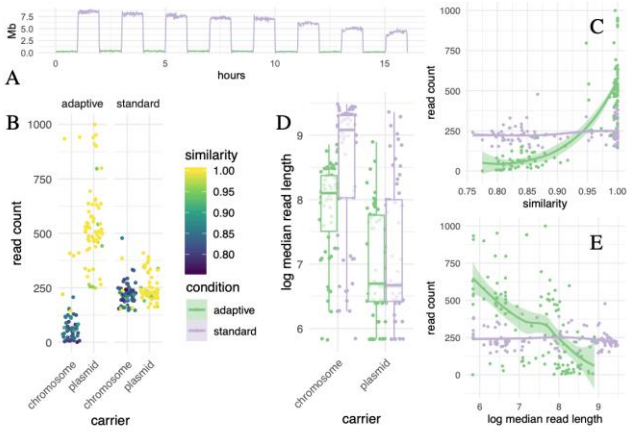


Fig. 2



Workshop 20
Update "Infektionsdiagnostik und Qualitätssicherung" (StAG DV/FG DKM)
07. Sep 2022 • 08:30–10:00

112/DKMV
Carbapenemases in *Proteus* spp – a challenge for the microbiology laboratory
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Introduction: *Proteus mirabilis* is the 3rd most common species causing urinary tract infections and the 5th most common cause of gram-negative bloodstream infections. Isolates are usually susceptible to most β-lactams including ampicillin. In contrast to other Enterobacterales, there is little data on carbapenemase

production in *P. mirabilis.*, which could be related to the difficult detection in this species.

Material/Methods: Eighty-three clinical isolates of *Proteus* spp. with elevated MICs to carbapenems, 3rd generation cephalosporins or evidence of carbapenemase production were included. Susceptibility testing for all isolates was performed by disk diffusion, Vitek2 and broth microdilution. Additionally, tests for carbapenemase production were evaluated, including CARBA NP, mCIM, sCIM, modified zCIM, faropenem disk test and growth on mSuperCarba agar. All isolates were subjected to OXA-23/-58 PCR and underwent whole genome sequencing (WGS) using Illumina technology.

Results: Among 83 isolates, 43 harboured carbapenemase genes (13 $bla_{\text{OXA-48-like}}$;12 $bla_{\text{OXA-23}}$;12 $bla_{\text{OXA-58-like}}$;2 bla_{NDM};2 bla_{VIM};1 bla_{IMP};1 bla_{KPC}). Carbapenemase-producing *Proteus* spp. (CPP) were highly susceptible to ertapenem (60% S, MIC50 0.5 mg/L) and meropenem (65% S, MIC50 1 mg/L); 60% were missed by the automated susceptibility testing system. Furthermore, inhibition zones of carbapenemase-producers and carbapenemase-negative isolates largely overlapped (FIG 1). In addition to low carbapenem MICs, CPP showed atypical susceptibility to other β-lactams; 21/43 isolates tested S by Vitek2 to piperacillin-tazobactam and 32/43 to ceftazidime.

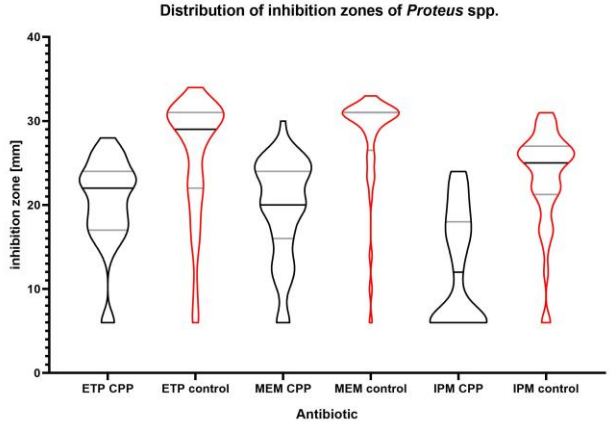
Sensitivity/specificity of phenotypic tests for carbapenemase production were 30%/40% for CARBA NP, 63%/85% for mCIM, 93%/95% for modified zCIM, 95%/13% for sCIM, 95%/85% for faropenem, 70%/83% for mSuperCarba. Most assays performed poorly for OXA-23/OXA-58 and these carbapenemases were well detected only by targeted PCR or WGS.

To facilitate detection in the routine laboratory, a new algorithm was developed for the prediction of carbapenemase production, taking into account inhibition zones of 6 antibiotic disks (ampicillin-sulbactam, ticarcillin-clavulanate, cefuroxime, ceftazidime-avibactam, faropenem, temocillin); sensitivity of the algorithm was 100%, specificity 92.5%.

Discussion: Carbapenemase-producing isolates in *Proteus* spp. have atypical susceptibility profiles and ertapenem/meropenem MICs are in the susceptible range in most isolates. Additionally, common confirmation tests such as CARBA NP perform poorly in this species and only the modified zCIM and molecular assays demonstrated a good performance. For this reason, carbapenemases are likely often missed in routine diagnostics and *Proteus* spp. might serve as a hidden reservoir for carbapenemases.

FIG 1 Violin plot of inhibition zones by disk diffusion testing. ETP=ertapenem, MEM=meropenem, IPM=imipenem, CPP=carbapenemase producing *Proteus* spp..

Fig. 1



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Evaluation of the UMIC Cefiderocol for antimicrobial susceptibility testing of cefiderocol

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Background: Multidrug-resistance in Gram-negative bacteria is a major challenge for healthcare systems worldwide. The continuous spread of various resistance mechanisms in clinically relevant species has drastic consequences in terms of therapeutic options. Consequently, novel antimicrobial agents are urgently needed. One new antibiotic is cefiderocol, which is a cephalosporin that is highly resistant to hydrolysis by beta-lactamases and enters the cell via a "trojan horse" mechanism with its siderophore characteristic. However, antimicrobial susceptibility testing of cefiderocol is extremely challenging and only few reliable commercial products exist. Here, we present a comprehensive evaluation of the UMIC Cefiderocol for MIC determination that was part of an international multicenter study.

Methods: The UMIC Cefiderocol was evaluated following the manufacturer"s instruction using nonduplicate clinical isolates from the strain collection of the German National Reference Centre for Multidrug-resistant Bacteria. Species were *Escherichia coli* (n = 20), *Klebsiella pneumoniae* (n = 15), *Enterobacter cloacae* complex (n = 10), other *Enterobacterales* (n = 15), *Acinetobacter baumannii* (n = 15), *Pseudomonas aeruginosa* (n = 20) and *Stenotrophomonas maltophilia* (n = 5). Briefly, strains were inoculated in 0.9 % NaCl and MIC determination with UMIC Cefiderocol strips was performed using iron-depleted cation-adjusted Mueller Hinton broth provided by the manufacturer. Pre-configured frozen microtiter plates with cefiderocol served as reference. MICs were read visually and interpreted using EUCAST and CLSI breakpoints.

Results: Essential agreement with the reference method was 88.8 % with three Very Major Errors (= false-susceptible results) and no Major Errors (= false-resistant results). MIC reading was complicated due to several unusual growth phenomena like skipped wells, cloddy growth or smearing in the wells.

Conclusion: In our evaluation, the UMIC Cefiderocol showed promising results and could be a valuable tool for clinical microbiology laboratories for antimicrobial susceptibility testing of cefiderocol. Hence, UMIC Cefiderocol is a user-friendly tool for routine laboratories, although MIC reading needs guidance on how to interpret the different growth phenomena.

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Method dependent discrepancy of susceptibility testing against Oxacillin in *Staphylococcus pettenkoferi*

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Introduction: *S. pettenkoferi* is a coagulase-negative staphylococcus first descripted by Trülzsch and colleagues [1]. This species is known as a facultative pathogenic bacterium. Bloodstream infections, osteomyelitis and native valve infections have been reported previously [2-4]. For treatment purposes it is of interest whether *S. pettenkoferi* is susceptible for oxacillin or not. Antimicrobial susceptibility testing for oxacillin provides contradictory results depending on the applied testing method.

Material/Methods: 23 clinical isolates of *S. pettenkoferi*, collected from blood culture, identified with MALDI Biotyper (Bruker Daltonics GmbH, Bremen, Germany) were inoculated on blood agar (COS, bioMérieux). An 0,5 McFarland suspension of each isolate was plated out on Mueller-Hinton agar (MHE, bioMérieux) for 30 µg cefoxitin disk test (Oxoid™) and oxacillin MIC test strip testing (Liofilchem®). Plates were incubated for 18±2h at 35±1°C. The results were interpreted following the EUCAST breakpoint table. Microdilution for oxacillin was carried out according to EUCAST protocol. For comparison, isolates were tested via VITEK® 2 (bioMérieux, Marcy-l"Étoile, France) using AST-P654. Real-time PCR was performed using the primers 5"-AAAATCGATGGTAAAGGTTGGC and 5" –

AGTTCTGCAGTACCGGATTTGC for amplification of mecA, 5" – TCACCAGGTTCAAC[Y]CAAAA and 5" – CCTGAATC[W]GCTAATAATATTTC for amplification of mecC. As control three fully sequenced isolates, of which two were confirmed mecA positive, and *S. pettenkoferi* DSMZ DSM 19554 reference isolate were investigated.

Results: VITEK® 2 cefoxitin screen was positive for 20 isolates, of which 18 showed susceptibility in disk diffusion. In oxacillin MIC measurements 15 isolates were susceptible in microdilution, but just 9 out of them showed a corresponding susceptible result in VITEK® 2 oxacillin testing and only 3 in the oxacillin MIC strip test. MecA / mecC could not be detected in all tested isolates via real-time PCR.

Discussion: Results show a method related discrepancy in the interpretation of the susceptibility of *S. pettenkoferi* against oxacillin. Both, phenotypic and genotypic tests show discrepant results.

Genotypic testing of the isolates for other oxacillin resistance mechanisms could shed light on the phenomenon described.

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115/DKMV

Prospective evaluation of the Qvella FAST™ System and the FAST PBC Prep Cartridge for species identification and antimicrobial resistance detection in positive blood cultures

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Introduction: Improved blood culture diagnostics are crucial for an accurate and rapid pathogen identification (ID) and antimicrobial susceptibility testing (AST). If conventional methods are used, this process may take up to 48 hours, but more recently developed methods allow for same-day results. Here, we evaluated the performance of the FAST™ system and FAST PBC Prep Cartridges (Qvella Corporation, Richmond Hill, Canada) on prospective residual positive blood cultures in comparison to our routine diagnostic workflow.

Material/method: Between March and May 2022, a total of 90 prospectively collected positive blood culture samples were assessed using the FAST™ system to compare ID and AST results to our routine diagnosis workflow (comprising ID by MALDI-TOF MS using Sepsityper technique and/or culture-grown colonies, AST by MicroScan WalkAway and disc diffusion (DD) (after direct inoculation of the blood culture). The FAST™ system generates a so-called "liquid colony" (LC), a suspension of bacterial cells isolated from the blood culture, which was further processed by MALDI-TOF MS and AST. Polymicrobial and false-positive samples (including unsuccessful or contaminated samples) were excluded. Categorical agreement was assessed for AST results, employing the clinical breakpoints guidelines put forth by EUCAST 2022.

Results: 74 samples (82.2%) fulfilled the inclusion criteria for the comparative ID and AST analysis. In comparison to culture-grown colonies, the ID concordance of the FAST System and Sepsityper were respectively 100% and 84.6% for Gram-positive pathogens, while both techniques were concordant at 100% for Gram-negative bacteria (Table 1). The categorical AST agreement of the FAST™ system in comparison to the routine workflow for Gram-positive bacteria reached 95.8% and 94.9%, respectively, for Microscan WalkAway and disc diffusion, which was slightly higher than for Gram-negative bacteria (94.8% and 90.8%, respectively). Most discrepancies were minor errors (Table 2).

Discussion and conclusion: This is the first study to assess the FAST™ system for ID and AST of pathogens recovered from blood cultures in comparison to ID by Sepsityper and AST by Microscan WalkAway. The FAST™ System generated accurate results and holds thus its promise for wider application in the future. At present, our study is still running and more samples will be included.

Fig. 1

Table 1. Percentage of concordance between MALDI-TOF MS identification results from the FAST™ system and the Sepsityper as compared to MALDI-TOF MS from culture-grown colonies.

% of concordance in comparison to culture grown colonies (reference)	FAST™	Sepsityper®
GRAM+	100% (52/52)	84.6% (44/52)
GRAM-	100% (22/22)	100% (22/22)
TOTAL	**100% (74/74)**	**89.2% (66/74)**

Table 2. Categorical agreement of AST results from MicroScan WalkAway and disc diffusion, as obtained by the FAST™ system in comparison to the routine workflow.

Gram-positive (N= 52)

	Routine Microscan				Routine DD					Microscan		%	DD	%
LC Microscan	S	I	R	Total	**LC DD**	S	I	R	Total	CA	526	95.8	240	94.9
S	339	4	5	379	S	157	1	10	194	VmajE	5	0.9	10	4.0
I	0	21	9	33	I	0	1	0	1	majE	5	0.9	2	0.8
R	5	0	166	181	R	2	0	82	89	minE	13	2.4	1	0.4
Total	361	27	186		Total	176	4	114						

Gram-negative (N= 17)

	Routine Microscan				Routine DD					Microscan		%	DD	%
LC Microscan	S	I	R	Total	**LC DD**	S	I	R	Total	CA	307	94.8	89	90.8
S	253	5	7	265	S	79	3	2	95	VmajE	7	2.2	2	2.0
I	0	14	4	20	I	1	4	0	5	majE	1	0.3	2	2.0
R	1	0	40	43	R	2	1	6	10	minE	9	2.8	5	5.1
Total	258	23	52		Total	89	8	10						

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Rapid bacterial identification from positive blood cultures by infrared spectroscopy using short-term incubation

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Introduction: Reducing the turnaround-time for bacterial identification from positive blood culture samples is vital to guide antimicrobial therapy. Short-term incubation on solid media was successfully implemented in most laboratories with standard identification systems. The aim of this study was to evaluate same-day identification from positive blood cultures with the new infrared-based IVD-certified identification system I-dOne (Alifax, Italy) using ATR-FTIR technology.

Methods: After some preliminary studies the following protocol was performed: Positive monomicrobial blood culture (BacT/ALERT FA/FN plus, bioMeriéux) samples were centrifuged at 375 x g for 4 min and 500 µl of the supernatant was inoculated on tryptic soy agar plates. Infrared identification (I-dOne, v.2.0.0.) was performed after 2, 3, 4, 5, 6 and 24 h of incubation. At least two spectra per culture were recorded and a quality score equal or above 2.25 (out of 5) was accepted. Additionally, culture purity and CFU count of the supernatant were determined on blood agar after overnight incubation. All blood culture isolates were identified by the reference method MALDI-ToF (VITEK MS Legacy, bioMeriéux).

Results: 68 blood cultures comprising 20 different bacterial species were included. After overnight incubation, all 68 isolates were correctly identified at the genus level and 65 at the species level.

Short-term incubation times of ≤ 2, ≤ 3, ≤ 4, ≤ 5 and ≤ 6 h yielded correct species identification in 0 %, 6.7 %, 13.3 %, 20 % and 33,3 % of *Staphylococcus* spp. (n = 15), 15.4 %, 38.5 %, 46.2 %, 84.6 %

and 92.3 % of *Enterococcus* spp. (n = 13), 12.1 %, 27.3 %, 45.5 %, 66.7 % and 81.8 % of Enterobacterales (n = 33), and 0 %, 50 %, 50 %, 100 % and 100 % of non-fermenters (n = 4), respectively. All three Streptococcus spp. isolates were not correctly identified within 6 hours. Overall same-day identification (≤6 h) of Gram-negative bacteria (n = 37) yielded better results compared to Gram-positive bacteria (n = 31) at the species level (83.8 % vs. 54.8 %) and at the genus level (94.6 % vs. 74.2 %), respectively.

Discussion: Infrared technology (ATR-FTIR) is a promising technology for standard bacterial identification in the microbiology laboratory. Moreover, same-day identification of the most common pathogens is feasible after short-term incubation (within 6 hours) of positive blood culture samples. We observed a better identification in Gram-negative bacteria than in Gram-positive bacteria within 6 hours in the log phase of bacterial growth. Variations within a certain species and between species may be due to surface structures, growth rates, delay in analysis, diagnostic algorithms, strain differences or differences in the blood culture matrix. Future studies should work on a fast and easy protocol to identify bacteria also directly from blood culture samples and demonstrate the implementation in the laboratory workflow.

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Improved sample preparation and library for identification of clinically relevant filamentous fungi by MALDI-TOF mass spectrometry

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Introduction: Rapid and reliable species identification is indispensable for the proper treatment of fungal infections. Slightly modified sample preparation techniques and an improved MALDI reference library were evaluated for suitability of identification of filamentous fungi in clinical routine laboratories.

Material / Methods: Three different sample preparation techniques were compared: An adopted "extended Direct Transfer (eDT)" protocol using a formic acid moistened toothpick to harvest and transfer the cell material, the regular tube-based "extraction from Agar plate (ExA)" and "Extraction from Liquid broth (ExL)". For the workflow validation, a set of 11 common filamentous fungi was analyzed at two clinical microbiology laboratories to confirm reproducibility by using the three sample preparation techniques. All samples were spotted in triplicate. In a second step, the three sample preparation techniques were applied on 255 prospective / retrospective clinical isolates. Isolates not identified by eDT / ExA were subjected to ExL and identified after a short over-night cultivation step. A curated MALDI reference library covering more than 200 species and adapted (and verified) log(score) values were used at the test sites.

Results: In the workflow validation study, 99 MALDI measurements and 99 identification log (scores) were carried out in each center, resulting in high identification rates of all 11 challenge isolates, FIG 1. The prospective study included 255 filamentous fungi isolates covering 89 different species / groups, which were selected by the two study sites For eDT alone a combined identification rate of 91% could be reached at the two test sites, compared to 61% for ExA. After solving the "no Identification" results by ExL a combined ID rate of 99% (eDT) and 95% (ExA) could be achieved (FIG 2).

Discussion: An improved, rapid sample preparation method based on a short on-target extraction was developed and applied at two clinical test sites. Most samples (>90%) could be identified successfully already after this extremely easy and short sample preparation technique. Less than 10% of all eDT samples required the more laborious ExL protocol for identification. The ExL sample preparation technique offers an easy possibility to identify fungal species which are growing deeply into the agar and are difficult to harvest. The general performance of ExA was even slightly lower (61%/95%) compared to eDT. An excellent identification functionality of the new MALDI reference library and method could be shown successfully since nearly all (99%) of the selected clinical samples could be identified correctly.

FIG 1: Identification rates of the tested filamentous fungi (cut-off ≥1.6 and ≥1.8 for low and high confidence identification, respectively) in the "workflow validation" study

High & Low Conf. ID log(score) ≥1.6			
Species	eDT (ID Rate %)	Extraction from agar (ID Rate %)	Extraction from liquid (ID Rate %)
Alternaria alternata	100	100	100
Aspergillus flavus	100	100	100
Aspergillus fumigatus	100	100	100
Aspergillus nidulans	100	100	100
Aspergillus niger	100	100	100
Fusarium oxysporum	92	72	100
Mucor circinelloides	63	67	100
Nannizzia incurvata	100	100	100
Penicillium chrysogenum	100	100	100
Scopulariopsis brevicaulis	100	100	100
Trichophyton rubrum	75	100	100
Total ID Rate (%)	**94**	**94**	**100**
Total number of spectra	264	198	198

High Conf. ID log(score) >1.8			
Species	eDT (ID Rate %)	Extraction from agar (ID Rate %)	Extraction from liquid (ID Rate %)
Alternaria alternata	83	100	100
Aspergillus flavus	100	72	100
Aspergillus fumigatus	100	100	100
Aspergillus nidulans	100	100	100
Aspergillus niger	96	100	100
Fusarium oxysporum	75	44	100
Mucor circinelloides	29	22	100
Nannizzia incurvata	88	100	100
Penicillium chrysogenum	100	100	100
Scopulariopsis brevicaulis	100	100	100
Trichophyton rubrum	42	94	100
Total ID Rate (%)	**83**	**86**	**100**
Total No. Spectra	264	198	198

FIG 2: Results of the 255 prospective / retrospective filamentous fungi isolates

	Test Site 1		Test Site 2		Total	
	eDT	Extraction from Agar	eDT	Extraction from Agar	eDT	Extraction from Agar
Successfully identified and identical to reference method	49 (98%)	36 (72%)	84 (85.%)	25 (50%)	133 (91%)	61 (61%)
Successfully identified with additional liquid extraction	1 (2%)	10 (20%)	14 (14%)	24 (48%)	14 (8%)	34 (34%)
Total identified rate with the workflow	100%	92%	99%	98%	148 (99%)	95 (95%)
No ID	0	4	1	1	1 (0.4%)	5 (2%)
No. of contaminations			3	3	3	3

118/DKMV

Evaluation of FT-IR Spectroscopy-based automated classification for typing of *Listeria monocytogenes* O-serogroups

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Background. *Listeria monocytogenes* (Lm) is the causative agent of listeriosis, an important food-borne zoonosis. Lm is an intracellular pathogen with several virulence factors and a unique potential to spread from cell to cell, thereby to cross blood–brain, intestinal and placental barriers. Listeriosis is characterized by septicaemia, encephalitis, meningitis, meningoencephalitis, abortion, stillbirth, perinatal infections and gastroenteritis. Though its occurrence is sporadic throughout the world, it can result in severe damage during an outbreak, and can be fatal for animals and humans. Lm shows somatic (O) and flagellar (H) antigens, which enable its differentiation into 8 serogroups and 14 serotypes. Among them, the 4b is responsible for the most severe clinical cases, followed by serogroup 1/2. Typing of Lm at serotype level is crucial for clinical and epidemiological investigations. Several methods are currently used for this purpose (serotyping, PCR and genome sequencing) which are laborious and cost intensive. In this study, we evaluated the accuracy and the discriminatory power of the FT-IR spectroscopy (FT-IRS) for typing of *L. monocytogenes* isolates at O-serogroup level. Machine learning was applied to develop an automated classifier, built with artificial neural networks (ANNs), and validated with an external dataset.

Materials and methods. N=59 well characterized Lm strains isolated from human invasive diseases, belonging to the serogroups 1/2 (n=20 1/2a, n=17 1/2b and n=3 1/2c) and 4 (n=19 4b) were analyzed by the FTIRS-based IR Biotyper system (IRBT - Bruker Daltonics, Germany). Strains were cultivated on sheep blood agar for 48±2 h at 37 °C. IR absorption spectra were acquired in 4 technical replicates from bacterial suspension in ethanol solution. IRBT classification was performed applying the *Listeria monocytogenes* O-groups classifier available in the IR Biotyper software V4.0. The classifier was built using artificial neural networks (ANNs) trained and validated with different isolates sets grown on different (selective and non-selective) solid culture media. The classifier delivers a result in terms of class and

reliability score (<1 highly reliable, 1 ≤ score ≤ 2 moderately reliable, >2 non-reliable), based on the spectral distance of the tested samples to the spectra used to train the ANNs.
Results. All strains were correctly classified by IRBT classifier (59/59), with high reliability score, and an accuracy (in terms of spectra) of 100%.
Discussion. In this study, FT-IR spectroscopy showed to be an innovative, promising and user-friendly approach for typing of *L. monocytogenes* isolates at O-serogroup level. Further investigations will be necessary to evaluate the discriminatory power of the system at serotype and strain level, to assess its potential role in routine and outbreak analysis.

119/DKMV
Biofilm infections of endobonchial valves in COPD patients after endoscopic lung volume reduction
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Introduction: Endoscopic lung volume reduction (ELVR) using endobronchial valves (EBV) is a treatment option for a subset of patients with severe chronic obstructive pulmonary disease (COPD) suffering from emphysema and hyperinflation. Although ELVR has been shown to improve lung function, quality of life and exercise capacity, some patients cannot sufficiently benefit from ELVR and valves must be removed. One of the most frequent indications for valve removal are recurrent exacerbations or pneumonia. Device-related infections represent a serious complication among patients with medical devices. Infections may bedue to the formation of bacterial biofilms, which evade host defenses and antibiotic therapy. However, studies about microbial biofilm infections of EBV and their impact on ELVR treatment outcome are lacking.
Methods: We analyzed four patients" cases with ELVR, who underwent valve removal. Clinical data was compared to the microbiological findings of conventional EBV culture. In addition, EBV were analysed by Fluorescence in situ hybridization (FISH) in combination with PCR and sequencing (FISHseq) for visualization of the microorganisms.
Results: EBV culture showed several microorganisms in all four patients including oropharyngeal bacteria flora, *Pseudomonas aeruginosa, Streptococcus parasanguinis, Streptoccocus* sp. and *Fusobacterium* sp. FISHseq revealed on three of four EBV a biofilm consisting of *P. aeruginosa* (case 1), oral flora (case 2) and streptococci (case 3). In case 4, FISHseq showed only single bacteria on the EBV. All patients presented with clinical symptoms, including pneumonia and recurrent exacerbations.
Discussion: These findings show that biofilm formation on EBV might be a potential cause of recurrent exacerbations or pneumonia leading to treatment failure in ELVR. Further studies are needed to evaluate the potential risk of biofilm formation on EBV and treatment options to avoid clinical consequences for patients with ELVR.

120/DKMV
Comparison of the automated plate assement system (APAS) to manual plate reading of clinical urine cultures
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Question/Background: Urine cultures are one of the most frequent cultures in clinical microbiology labs. Therefore, reading and processing of these plates account for a large part of daily work. The objective of this study was to evaluate the performance of the automated plate assement system (APAS) in comparison to manual read by experienced technicians.
Methods: Midstream urine samples from primary, secondary and tertiary care centers were randomly selected and prospectively evaluated. Urine samples were plated either manually or by an automated streaking system on brilliance ™ UTI clarity ™ and CNA media plates (Oxoid, Thermo Fisher). Plates were incubated 36ºC for a minimum of 16 hours. The APAS independance (Clever Culture Systems) is a standalone module with an AI software solution for reading and sorting incubated agar media plates. The interpretation followed current MiQ recommendations. Technicians read plates prior APAS. Results were evaluated independently and compared afterwards. Results were either graded as concordant, minor error (false positives) or major error (false negatives with a single overnight incubation).
Results: Overall 381 samples were included into this study. A total of 143 samples (37,4%) didn´t show significant growth. The APAS achieved a concordant result in 326 samples (85,4%). Overall sensitivity of the APAS was 97.0% (95%CI 94.0-98.8) and specificity was 68.5% (95%CI 60.2-76.0; Table 1). A total of 45 samples showed minor errors (11.8 %). In 7 samples (1.8%) major errors were detected. The APAS failed to detect growth of potential pathogens wit 10^3 (n=4); 10 to the 5 (n=1) and 10 to the 6 (n=2).
Conclusion: The APAS correctly read and identified the majority of plates, producing satisfactory results. False negatives could be reduced with identification after an additional overnight incubation as recommended in the MiQ. Implementation of the APAS independence potentially decreases the workload for technicians.
Caption

Table 1. Diagnostic performance of the automated plate assement system (APAS) with urine samples compared to manual reading and processing.

Statistic	Value	95% CI
Sensitivity	97.02%	93.96% to 98.79%
Specificity	68.53%	60.24% to 76.03%
Positive Likelihood Ratio	3.08	2.42 to 3.93
Negative Likelihood Ratio	0.04	0.02 to 0.09
Positive Predictive Value	83.52%	79.90% to 86.59%
Negative Predictive Value	93.33%	87.00% to 96.70%

Workshop 21
Breaking News in der Lebensmittelmikrobiologie (FG LM/FG ZO)
07. Sep 2022 • 08:30–10:00

121/LMZOV
Disease-elleviating effects of peroral activated charcoal treatment in acute murine campylobacteriosis
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Foodborne *Campylobacter jejuni* infections are on the rise and responsible for worldwide serious health issues. Increasing resistance of *C. jejuni* strains against antimicrobial treatments necessitates antibiotics-independent treatment options for acute campylobacteriosis. Activated charcoal (AC) constitutes a long-known and safe compound for the treatment of bacterial enteritis.
Methods: In this preclinical intervention study, we addressed potential anti-pathogenic and immune-modulatory effects of AC during acute experimental campylobacteriosis. Therefore, microbiota-depleted IL-$10^{-/-}$ mice were infected with *C. jejuni* strain 81-176 by gavage and challenged with either AC (daily dose of 2.5 g/kg body weight) or placebo via the drinking water starting on day 2 post-infection. The placebo cohort mice received autoclaved drinking water only.

Results: On day 6 post-infection, AC as compared to placebo-treated mice did not only harbor lower intestinal pathogen loads but also presented with alleviated *C. jejuni*-induced clinical signs such as diarrhea and wasting symptoms. The improved clinical outcome of AC-treated mice was accompanied by less colonic epithelial cell apoptosis and reduced pro-inflammatory immune responses in the intestinal tract. Notably, AC treatment did not only alleviate intestinal, but also extra-intestinal and systemic immune responses as indicated by dampened pro-inflammatory mediator secretion.
Conclusion: Given the anti-pathogenic and immune-modulatory properties of AC in this study, a short-term application of this non-toxic drug constitutes a promising antibiotics-independent option for the treatment of human campylobacteriosis.

122/LMZOV

Characterisation of the emerging Shiga toxin-producing *Escherichia coli* lineage of serotype O187:H28 from food

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Shiga toxin-producing *Escherichia coli* (STEC) can cause diarrhea or the haemolytic uremic syndrome (HUS) in humans. These infections are often linked to the consumption of contaminated food. STEC of serotype O157:H7 are well known; however, there is increasing evidence for non-O157 STEC as risk for human health. In Germany, serotype O187:H28 is one of the most frequently detected STEC serotypes in animal and food during the years 2015 and 2021. Here we provide insight into this emerging STEC lineage and its genetic characteristics.
We investigated 70 STEC isolates confirming the serotype and presence of genes coding for different Shiga toxin subtypes. Furthermore, whole genome sequencing (WGS) was applied for phylogenetic analyses using core-genome multi-locus sequence typing (cgMLST) and the determination of virulence associated genes (VAGs).
Isolates were derived from animals (n = 10), animal food products (n = 15) and plant based food (n = 45). Three isolates were originally determined as O175:H28 by serological analyses; however, WGS determined the genoserotype O187:H28. All isolates harboured genes for the Stx2g Shiga toxin subtype except for one strain, which had a combination of *stx2g* and *stx2a*-genes. None of the strains was positive for the Intimin encoding gene *eae*. In contrast, virulence genes associated with further pathogenic *E. coli* types like enterotoxigenic *E. coli* (ETEC) or enteroaggregative *E. coli* (EAEC) were detected. Phylogenetic analyses revealed a predominance of MLST ST200. No clustering according to origin or food matrix was observed.
STEC of O187:H28 were mainly isolated from flour and products thereof as well as game animals and game meat. A contamination of the plants on the field by game animals can be assumed. STEC of O187:H28 were assigned as so-called hybrid strains as additional virulence factors of ETEC and/or EAEC were determined by WGS. This highlights the risk for human health by these STEC strains, which was underlined by the publication of a Swedish O187:H28 STEC isolate derived from a human case.

123/LMZOV

The A-subunits of Subtilase and Shiga Toxin of Shiga Toxin-producing *E. coli* damage eukaryotic cells without their corresponding B-subunits

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Introduction: The major reservoir of Shiga toxin (Stx)-producing *E. coli* is the gastrointestinal tract of ruminants, wild ruminants, and many other mammals. Whereas most of the mammal hosts do not develop diseases, zoonotic transfer of STEC to humans can cause hemorrhagic colitis and the hemolytic uremic syndrome. The causative agents of these diseases are mostly referred as a subgroup of STEC, the enterohemorrhagic *E. coli* (EHEC). The major pathogenicity determinant of STEC is the production of one or more Shiga toxins, combined with adherence determinants allowing intimate attachment or aggregative adherence to the intestinal epithelia. In the group of STEC, isolates from several wild ruminants, and also foods, another toxin has been described, the subtilase cytotoxin. Both toxins are AB5 toxins composed of an enzymatically active A-subunit and a B-subunit pentamer. The subtilase genes occur in combination with s*tx*, mostly in STEC isolates lacking the locus of enterocyte effacement (LEE).
Materials and Methods: Toxin subunits have been expressed recombinantly and purified by FPLC. Cellular changes in eukaryotic cells have been investigated in cell culture tests.
Results: Molecular investigation of recombinantly expressed subunits of subtilase and Shiga toxin revealed an unexpected activity. Both, the A-subunits of subtilase and Stx are able to cause cellular changes in eukaryotic cells without their corresponding B-subunit. Although higher concentrations are necessary for this cytotoxicity than needed by the holotoxin.
Discussion: A new mode of uptake of the toxins is proposed and the classical paradigm of AB5 toxins will be discussed. The existence of toxic single A-subunits that have not found a B-pentamer for holotoxin assembly might improve the pathogenic potential of AB5 toxin-producing strains.

124/LMZOV

***Salmonella* spp. surrogates for assessing heat inactivation of pathogens during fruit juice production**

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Introduction: *Salmonella enterica* is a major bacterial foodborne pathogen causing gastroenteritis. To reduce the viable counts in food commodities, heat treatments such as pasteurization are applied. For an unbiased comparison of diverse treatments, surrogate strains are in need. These are nonpathogenic counterparts that mimic the behavior of pathogens under the sought technological conditions. As surrogates may not react identically under all conditions, there is a particular interest in identifying microorganisms to be used as surrogates for *Salmonella* spp. for heat inactivation in the fruit juice industry.
Material & Methods: In the present study, the heat resistance of five *Salmonella enterica* strains from serovars associated with fruit juice outbreaks, namely *S.* Senftenberg, *S.* Typhimurium, *S.* Saintpaul, and *S.* Enteritidis, as well as of a *Salmonella* cocktail was determined. Furthermore, three potential surrogate *Escherichia coli* strains were included in the study. Thermal inactivation was evaluated in biological triplicate and technical duplicate at 55°C, 57.5°C, 60°C, 65°C, and 72°C in phosphate buffered saline (PBS) and strawberry nectar (12° Brix).
Results: The D- and z-values of single *Salmonella* and potential surrogate strains as well as the *Salmonella* cocktail were calculated. The results show significant differences in PBS, while a different behavior was found in strawberry nectar. Thus, *E. coli* ATCC 11229 is proposed as a good surrogate candidate for *Salmonella* strains, as its heat resistance at 60°C, 65°C, and 72°C in strawberry nectar is higher than that of the examined pathogens. The results also show the differences in D-values in the tested matrices, which indicate a clear influence of the environment on heat resistance of bacteria, which may be caused, among others, by changes in heat transfer.
Discussion: This study highlights the importance of the food matrix in selecting surrogate strains for technological applications. In conclusion, *E. coli* ATCC 11229 is proposed as a surrogate strain for *Salmonella* spp., that can be used in the validation of

thermal as well as novel treatments in the production of strawberry nectar.

Workshop 22
Zoonoses (FG ZO)
07. Sep 2022 • 13:45–15:15

125/ZOV
Immune-modulatory effects upon oral application of cumin-essential-oil to mice suffering from acute campylobacteriosis

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Introduction: Human campylobacteriosis, commonly caused by *Campylobacter jejuni*, is a food-borne infection with rising prevalence responsible for significant health and socioeconomic burdens worldwide. Given the threat from emerging antimicrobial resistances, the treatment of infectious diseases with antibiotics-independent natural compounds is utmost appreciated. Since the health-beneficial effects of cumin essential oil (EO) have been known for centuries, its potential anti-pathogenic and immune-modulatory effects during acute experimental campylobacteriosis were addressed in the present study.
Methods: Therefore, microbiota-depleted IL-10-/- mice were infected with *C. jejuni* strain 81-176 by gavage on day 0 and 1 and received cumin-EO (200 mg/kg body weight) via the drinking water starting on day 2 until the end of the observation period. The placebo control mice received autoclaved tap water only.
Results: On day six post-infection, cumin-EO treated mice harbored lower ileal pathogen numbers and exhibited a better clinical outcome when compared to placebo controls. Furthermore, cumin-EO treatment alleviated enteropathogen-induced apoptotic cell responses in colonic epithelia. Whereas, on day 6 post-infection, a dampened secretion of pro-inflammatory mediators, including nitric oxide and IFN-γ to basal levels, could be assessed in mesenteric lymph nodes of cumin-EO treated mice, systemic MCP-1 concentrations were elevated in placebo counterparts only.
Conclusion: Our preclinical intervention study provides first evidence for promising immune-modulatory effects of cumin-EO in the combat of human campylobacteriosis. Future studies should address antimicrobial and immune-modulatory effects of natural compounds as adjunct antibiotics-independent treatment option for infectious diseases.

126/ZOV
The role of the RGD motif of streptococcal protein IdeC in *Streptococcus canis* infection

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Introduction: *Streptococcus canis* is a Lancefield group-G opportunistic pathogen that colonises the mucosal surfaces and skin of the host, predominantly affecting cats and dogs. *S. canis* is a zoonotic pathogen with human infections being observed more recently, mainly caused by contact with companion animals. This project is focused on the functional role of the *S. canis* protein IdeC. Specifically, what role this protein may play in mediating host-pathogen interactions.
Different virulence factors, e.g. proteases, enable colonization, survival and replication during pathogenesis of streptococci. *In silico* analysis has shown that the IgG-specific protease of *S. canis* termed IdeC is a secreted protein containing a signal sequence but no membrane anchor. IdeC is suggested to act with a similar mechanism to the IdeS protein of *Streptococcus pyogenes*, a secreted cysteine kinase that cleaves the IgG molecule between the hinge and CH2 region. The IdeC protein also has an Arg-Gly-Asp (RGD) motif, this motif is the minimal peptide sequence required to bind integrins. Bacterial proteins containing RGD motifs have been implicated in adhesion and invasion of host cells. This along with the ability of IdeC to bind back to the bacterial surface after secretion suggest a possible second function of IdeC in adhesion.
Methods: Fluorescent latex beads coated in recombinant IdeC protein are used to assess the interaction of IdeC with epithelial and endothelial cells. Further, a recombinant protein with RGD replaced with RGE has been produced to test the role of the motif in any interaction observed.
Results: Based on fluorescence microscopy analysis, IdeC coated latex beads displayed increased interaction with both epithelial and endothelial cells when compared to IdeC_RGE and BSA controls. Electron microscopy indicates that IdeC coated beads may be internalised however, a mechanism is yet to be determined.
Discussion: Here we present evidence that the streptococcal protein IdeC may have a second function, involving bacterial attachment and invasion into host cells during infection.

127/ZOV
Proteomics-based characterization of *Brucella canis* outer membrane vesicles identifies potential marker proteins for serodiagnosis and vaccine development

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Canine brucellosis is a worldwide emerging zoonotic disease spread through the close contact between dogs and humans. Its agent is *Brucella canis* that causes abortion, reproductive failure or lymphadenopathy in dogs. In humans, a *B. canis* infection may present with non-specific symptoms like fever, joint and muscle pain, fatigue or weakness. Especially professionals like breeders, veterinarians and laboratory workers are at risk, but infections of private dog holders are also known. The control and prevention of canine brucellosis, which causes noteworthy financial losses to breeders, is challenging. Diagnosing *B. canis* infections has pitfalls since serological methods developed to recognize *B. abortus* and *B. melitensis* infections do not detect anti-*B. canis* antibodies. Moreover, no commercial vaccines against canine brucellosis are available.
For potential vaccine development and the improvement of serological testing, we isolated outer membrane vesicles (OMVs) from *B. canis* and used an immunoproteomics approach to identify immunodominant proteins as antigen candidates. We performed two-dimensional gel electrophoresis with *B. canis* cell extracts followed by MALDI-TOF MS analysis and could correlate 182 protein spots with 82 *B. canis* proteins. Western Blot analysis of 2D SDS-PAGE gels using the sera from *B. canis*-infected dogs and sera from uninfected dogs, detected 50 immunoreactive *B. canis* proteins. Hence, 32 out of 82 identified proteins did not react with any sera. A total of 14 proteins, like GroEL and DnaK, were false-positive hits since they reacted with sera of *B. canis*-free dogs. However, 36 immunogenic proteins were detected by serum from bacteremic dogs and 16 out of these proteins also reacted with serum from infected but non-bacteremic dogs. Some of these immunoreactive proteins seem to be specific for *B. canis* and have not yet been described as immunodominant in *Brucella*.
Several immunodominant proteins were also found in OMVs isolated from supernatants of *B. canis* liquid cultures. The diameter

of these OMVs ranged between 30 and 70 nm according to electron microscopic examinations. We identified 103 proteins in the OMV preparations by liquid chromatography-mass spectrometry (LC-MS) analysis. Some outer membrane proteins of *B. canis* were enriched in the OMVs, like Omp31, which is a well-known immunogenic *Brucella* protein. Six of the OMV-associated proteins had been detected with the serum of infected dogs but are also immunoreactive proteins of other *Brucella*. We identified one immunoreactive *B. canis* protein in the OMVs, previously not described as immunodominant protein of any *Brucella* spp. that may trigger an immune reaction specific for *B. canis* in infected dogs.
Our study describes several immunoreactive proteins for the detection of acute and chronic canine brucellosis and shows that OMVs might serve as suitable vaccination platform.

128/ZOV

Analysing the association of the unique STEC subtilase cytotoxin with OMVs and its impact on host cells

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The main cause of the life-threatening hemolytic-uremic syndrome is infection with enterohemorrhagic *Escherichia coli* (EHEC), a highly human-pathogenic subgroup of Shiga toxin (Stx)-producing *E. coli* (STEC). EHEC can encode various exotoxins besides the cardinal virulence factor Stx. Some of these exotoxins, are not only freely secreted into the adjacent environment of the bacteria but can also be associated with outer membrane vesicles (OMVs), allowing EHEC to target cells distal from their attachment site.
Whereas the association of some EHEC exotoxins with OMVs is well-characterized, this is unclear in the case of the subtilase (SubAB) cytotoxin. SubAB is an AB5 cytotoxin like Stx targeting the host ER chaperone BiP and is usually encoded by non-classical EHEC serovars. Thus, our aim was to investigate whether SubAB is associated with OMVs and how OMV-associated SubAB impacts host cells in comparison to freely secreted SubAB, since cytopathic effects can be similar as with Stx or different as shown e.g. for another exotoxin, the EHEC-hemolysin.
We prepared the potentially SubAB-positive OMVs following an established multi-step ultracentrifugation protocol including a density gradient. To determine the association of SubAB with OMVs we used Western blotting using the bacterial outer membrane marker OmpA. The OMVs were further characterized in size and concentration using nanoparticle tracking analysis. The dependency of SubAB-positive OMV effects on the SubAB receptor *N*-glycolyl neuraminic acid (Neu5Gc) was tested with cytotoxicity assays using non-human vs. human cell lines growing in serum-free vs. serum-containing media. Cytopathic effects of SubAB-positive cells were again tested by Western blotting using the cleavage of host BiP and activation of diverse ER stress pathways via phosphorylation as markers.
Using the described methods we were able to show that SubAB is associated with OMVs. Interestingly, these OMVs consist of subpopulations with different sizes, i.e. ca. 70 nm and 120 nm. Furthermore, the SubAB-positive OMV effects on host cells are similar to Stx dependent on the Neu5Gc receptor, which cannot be synthesized by human cells and is only supplied by diet. Moreover, similar to free SubAB OMV-associated SubAB is able to cleave BiP and induce the phosphorylation of different ER stress signaling pathway proteins.
These results clearly show that SubAB is an additional OMV-associated EHEC exotoxin which emphasizes the importance of profiling virulence factors also from unusual EHEC serovars.

129/ZOV

An inactivation of *rpoS* results in enhanced virulence gene expression in *Escherichia coli* O104:H4

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Introduction: *Escherichia coli* (*E. coli*) O104:H4, which caused a severe outbreak in Germany in 2011, is a hybrid of enterohemorrhagic (EHEC) and enteroaggregative *E. coli* (EAEC) with respect to its virulence gene content. The outbreak strain is characterized by the production of the cardinal EHEC virulence factor Shiga toxin (subtype 2a, Stx2a) and the EAEC-specific aggregative adherence fimbriae (type I, AAF/I). A main aim of our research is to investigate the factors and mechanisms contributing to the exceptional pathogenicity of *E. coli* O104:H4. Here, we describe an inactivation by a spontaneous mutation of *rpoS* coding for the alternative RNA polymerase sigma factor S (RpoS), which resulted in enhanced virulence gene expression in *E. coli* O104:H4 *in vitro*.
Methods: Genomic DNA was sequenced with the PacBio® Sequel IIe system. High-fidelity reads were assembled with the SMRT® Link software using the *E. coli* O104:H4 genome GCF_000299455.1 as a reference sequence. Whole genome comparison and single nucleotide polymorphism (SNP) analysis was performed with Ridom SeqSphere+. Protein levels during growth in lysogeny broth (LB) medium were detected with semi-quantitative Western blot. Directed mutagenesis (deletion) of *rpoS* in *E. coli* O104:H4 *Δstx2* was performed using Red recombineering system.
Results: During a routine long-read whole genome re-sequencing of EHEC strains in our laboratory we discovered an *E. coli* O104:H4 isolate that had acquired a spontaneous mutation of the *rpoS* gene. A SNP (ATG/ATA) in the start codon of *rpoS* was detected, which was expected to result in a significant reduction (97 to 99.9%) in its translation efficiency. Indeed, Western blot analysis confirmed the dramatic decrease of RpoS in *E. coli* O104:H4 *rpoS* ATG/ATA strain in comparison to the wild type isolate. Whole genome sequence comparison revealed no further differences within the coding regions of these two strains. Interestingly, the *E. coli* O104:H4 *rpoS* ATG/ATA isolate exhibited a significantly enhanced production of EAEC-specific virulence factors, e.g. AAF/I and the serine protease SepA during growth in LB medium. Moreover, the RpoS spontaneous mutant was characterized by an increased synthesis of flagellin, which besides functioning in virulence-related motility and chemotaxis, serves as a pro-inflammatory factor of *E. coli* O104:H4. Phenotypic characterization of an *rpoS* deletion mutant generated by directed mutagenesis (*E. coli* O104:H4 *ΔrpoS*) further confirmed the *rpoS*-dependent control of these virulence genes in *E. coli* O104:H4.
Conclusion: Our data indicate that RpoS functions as a negative regulator of *E. coli* O104:H4 virulence gene expression. A spontaneous mutation in the start codon of *rpoS*, as well as the deletion of *rpoS* resulted in the increased production of EAEC-specific virulence factors of *E. coli* O104:H4 *in vitro*. If the inactivation of *rpoS* also results in an overall increase in *E. coli* O104:H4 pathogenicity *in vivo* remains to be further elucidated.

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Mapping and characterisation of *Escherichia coli* O104:H4 small regulatory RNAs

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Introduction: *Escherichia coli* (*E. coli*) O104:H4 is a Shiga toxin-producing serotype that caused a large gastroenteritis outbreak in Germany in 2011. Notably, more than 850 of the cases progressed to hemolytic uremic syndrome (HUS), which is to date the highest incidence of *E. coli*-related HUS worldwide. Despite previous genome and transcriptome analysis, there is limited understanding of the regulatory features mediating the exceptional pathogenicity of *E. coli* O104:H4. Here, we aimed to map and characterize small non-coding RNAs (sRNAs) involved in the post-transcriptional control of *E. coli* O104:H4 virulence, adaption and stress response.
Methods: Differential RNA sequencing (dRNA-seq) was performed on genomic DNA-free total RNA from *E. coli* O104:H4 grown to mid-logarithmic and early stationary phase using the Terminator 5´-Phosphate-Dependent Exonuclease (TEX) treatment protocol. Complementary DNA libraries were sequenced on an Illumina platform. Raw reads were mapped to the *E. coli* O104:H4 genome using READemption and sRNA candidates were annotated using the ANNOgesic analysis pipeline. Directed mutagenesis of candidate sRNAs was performed using the phage lambda (λ) Red recombineering system. The sRNA mutants (sRNA_52, sRNA_71, sRNA_196, sRNA_175, sRNA_316) were phenotypically characterized with respect to their growth kinetics, motility, biofilm formation and resistance to oxidative stress.
Results: We annotated 412 sRNA candidates in *E. coli* O104:H4 using dRNA-seq. The sRNAs were both chromosome- and plasmid-encoded, with 47% of them mapping antisense to open reading frames. About 20% of the mapped sRNAs were found to be homologous to previously experimentally validated sRNAs in *E. coli* K-12 MG1655. A computational target prediction suggested several sRNA candidates to be involved in the regulation of virulence or stress response-related genes of *E. coli* O104:H4. Mutants of the selected sRNAs were characterized with no significant difference in the growth kinetics in lysogeny broth and minimal medium in comparison to the wild-type strain. Nevertheless, mutants of sRNA_52 and sRNA_71 showed increased motility and reduced biofilm formation as compared to the wild type. Moreover, strains lacking sRNA_175, sRNA_196 and sRNA_316 were characterized by decreased hydrogen peroxide (H2O2) resistance, which was also confirmed by H2O2 spot assay.
Conclusion: In summary, this study provides a comprehensive global mapping of sRNAs in *E. coli* O104:H4 and further enhances our understanding of its virulence-related gene expression and regulation. Phenotypic assessment of sRNA mutants suggested the involvement of regulatory sRNAs in controlling *E. coli* O104:H4 motility, biofilm formation, and oxidative stress response. Ongoing experiments will address the direct contribution of the mapped sRNAs to the described phenotypes.

Workshop 23
Infektiologie und Labor (StAG DV/FG DKM)
07. Sep 2022 • 13:45–15:15

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Next- and third-generation sequencing outperforms culture-based methods in diagnosis of ascitic fluid bacterial infections of ICU patient

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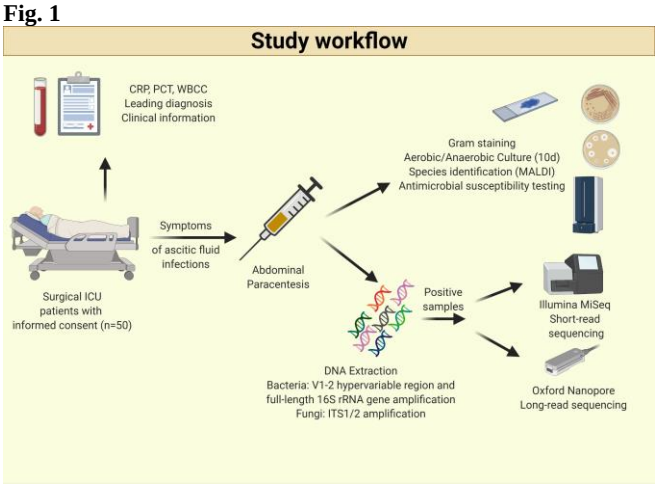
Objectives: Infections of the ascitic fluid are serious conditions that require rapid diagnosis and treatment. Ascites is often accompanied by other critical pathologies such as gastrointestinal bleeding and bowel perforation, and infection increases the risk of mortality in intensive care patients. Due to a relatively low success rate of conventional culture methods in identifying the responsible pathogens, new methods may be helpful to guide antimicrobial therapy and to refine empirical regimens. In our study, we aimed to explore the association of the clinical characteristics of critically ill patients with ascitic fluid infections and evaluate the comparative performance of standard microbiology diagnostic culture methods with short-read Illumina and long-read nanopore sequencing methods.
Methods: During the time between October 2019 and March 2021, we prospectively collected 50-ascitic fluid samples from ICU patients with suspected infection. Beside standard culture-based microbiology methods, excess fluid underwent DNA isolation and amplification of the V1-2 region or the full length of the 16S rDNA gene, which were analysed by next- and third-generation sequencing (NGS) methods.
Results: NGS-based methods had higher sensitivity in detecting additional pathogenic bacteria such as *E. faecalis* and *Klebsiella* in 33 of 50 (66%) ascitic fluid samples compared with culture-based methods (26%). Anaerobic bacteria were especially identified by sequencing-based methods in 28 samples (56%), in comparison with only three samples in culture. When comparing Illumina short-read with nanopore long-read results, the quality of the nanopore-sequenced reads was lower in comparison with Illumina, yet the longer read length enabled a comparable bacterial identification. Analysis of clinical data showed a correlation between sequencing results and various clinical parameters such as peritonitis and hospitalization outcomes.
Conclusions: Our results show that in ascitic fluid infections, NGS-based methods have a higher sensitivity for the identification of clinically relevant pathogens than standard microbiological culture diagnostics, especially in detecting hard-to-culture anaerobic bacteria. Patients with such infections may benefit from the use of NGS-methods by the possibility of earlier and better targeted antimicrobial therapy, which has the potential to lower the high morbidity and mortality in critically ill patients with ascitic bacterial infection.

Keywords
Ascitic fluid infections; Intensive care unit; Next-generation sequencing; Nanopore; Anaerobic bacteria; Full length 16S rRNA sequencing; Molecular diagnostics; Metagenomics.

Fig. 1



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Whole genome sequencing of *Staphylococcus aureus* isolates from blood culture with and without *S. aureus* bacteriuria

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Question: *Staphylococcus aureus* bacteremia (SAB) has an incidence of 22-32/100,000/year and is associated with a mortality between 14.6-29.2%. At the University Hospital Münster (UKM), *S. aureus* is identified in 6.8-8% bacteraemia cases (n=90-104 per year). Many patients (7.8%-39%) have concomitant *S. aureus* bacteriuria (SABU) in addition to SAB. The mechanisms of SAB-associated SABU are largely unexplained. The objective of this

study is to compare genomic characteristics of *S. aureus* from SAB with and without SABU.

Methods: During a prospective cohort study (February 2020-June 2021), all *S. aureus* isolates from blood and urine cultures of adult patients admitted to the UKM were collected. Exclusion criteria were the presence of risk factors for SABU at the time of urine sampling (indwelling urinary catheters, hydronephrosis, kidney stones and/or pregnancy). Data on demographics, clinical history, laboratory findings and treatment were also collected. Whole genome sequencing (WGS) of all *S. aureus* isolates from blood and urine (if applicable) was performed. Isolate relatedness was determined using core genome multilocus sequence typing (cgMLST). In addition, isolates were analysed for protein A (*spa*) type and virulence factors.

Results: In total, 62 patients were included (median age: 68 years, 35 (57%) were male). Three (5%) had infections with methicillin-resistant *S. aureus*, and 16 (26%) had a positive concomitant urine culture with *S. aureus*. The most common sources of infection were vascular catheters (n=14, 23%), and skin and soft tissue infections (n=11, 18%). Patients with SABU were less likely to have received antibiotics prior to urine culture collection (25% vs. 59%, p=0.04) and had a higher mortality than patients who did not have a positive urine culture (80% vs. 57%, p=0.17). Isolates belonged to 19 different sequence types (STs). The most common STs were ST7 (n=8), ST45 (n=8), ST5 (n=7); other STs (n=33) and in six isolates ST could not be determined. The most common *spa* types were t084 (n=7) and t091 (n=6). Interestingly, ST45 *S. aureus* isolates (n=8) were only isolated from blood cultures and could not be detected in any of the associated urine samples during our study. Sequence-based characterization showed high genetic relatedness of *S. aureus* isolates from blood and urine in patients presenting with SABU. Paired *S. aureus* isolate from urine and blood were similar and differed mainly in only 0-2 alleles of the cgMLST scheme. One pair differed in 3 and one pair in 6 alleles. We screened the genomes for 120 different virulence factors but were unable to detect differences in the prevalence between both groups.

Conclusion: This study shows a high degree of relatedness between *S aureus* isolates from blood and urine. In our cohort, a cluster of *S. aureus* isolates with no detection in urine samples were identified as ST 45. However, for more detailed analysis of genomic differences in both groups further samples are necessary.

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Extensively drug-resistant *Klebsiella pneumoniae* rapidly compensates for fitness and virulence costs associated with acquired resistance

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Introduction: The control of outbreaks and life-threatening infections caused by extensively drug-resistant (XDR) *Klebsiella pneumoniae* has become a severe challenge for health care systems worldwide. Consequently, the World Health Organization (WHO) ranked these XDR enterobacterial representatives as one of the most critical priority pathogens for which new antibiotics are urgently needed. However, there is evidence that only a limited number of *K. pneumoniae* clonal lineages (e.g., sequence type (ST)307) are the primary drivers of this dynamic due to their successful combination of multidrug resistance with high-level virulence. These strains are notorious for recalcitrant and difficult-to-treat infections with high mortality rates. Understanding which mechanisms mediate rapid resistance acquisition to new antibiotics (and drug combinations) and counteracting associated fitness costs is essential for the development of novel therapeutic strategies. Here, we investigated whether and how an XDR ST307 *K. pneumoniae* strain acquired resistance against the drug combination ceftazidime-avibactam (CAZ-AVI) using experimental evolution (EE) and explored how it managed the accompanying fitness and virulence costs.

Materials and Methods: We selected resistant variants in a first EE approach by gradually increasing CAZ-AVI concentrations. Then, genomics, proteomics, homology modeling, and functional studies were combined to investigate the underlying resistance mechanisms. In addition, we explored the ability of the strains to counteract accompanying fitness costs, again based on EE and downstream analyses. The latter included a comprehensive set of *in vitro* and *in vivo* assays combined with genomic and transcriptomic analyses.

Results: The first EE revealed two CAZ-AVI-resistant variants. We verified that resistance was based on distinct mutations of the major nonselective porin OmpK36. Both OmpK36 mutations were accompanied by a significant decrease in fitness and virulence-associated features. However, when removing the antibiotic selection pressure and upon growth in two different laboratory media following second EE, the evolved variants recovered their fitness and virulence while keeping CAZ-AVI resistance. These rapid adaptations were based on genomic and transcriptomic changes driven by major bacterial regulators, including the envelope stress response regulator *rpoE* and associated genes.

Discussion: Our study proves that OmpK36 mutations caused CAZ-AVI resistance. More importantly, our findings shed light on the comprehensive genomic and transcriptomic changes that balance the pathogen's survival with fitness and virulence traits upon antibiotic resistance acquisition. Although the phylogenetic diversity of *K. pneumoniae* and the complexities of compensatory mechanisms limit clear predictions, this study contributes to the understanding of factors essential for the success of high-risk *K. pneumoniae* lineages.

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Emergence of small colony variant of metallo-beta-lactamase producing *Escherichia coli* following repeated cefiderocol exposure *in vitro*

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Introduction: Knowledge on resistance mechanisms towards cefiderocol, a novel siderophore-conjugated cephalosporin antibiotic, is still limited. Although the presence of New-Delhi metallo-beta-lactamase has been demonstrated to facilitate the resistance development towards cefiderocol in various Enterobacterales, the role of serine beta-lactamases has not been investigated. Our study aimed to study the effect of the presence of various b-lactamases, such as NDM, VIM, KPC and OXA-48, on the development of cefiderocol resistance in *Escherichia coli*.

Methods: We performed liquid mating to transfer the beta-lactamases of interest onto a defined K-12 *E. coli* background (J53) and exposed these transconjugants to increasing cefiderocol concentrations in a serial passage experiment. Disk diffusion was performed after each passage to detect reduces susceptibility. Cefiderocol-resistant isolates were genotyped by whole genome sequencing to investigate the underlying resistance mechanism.

Results: Cefiderocol resistance isolates only emerged in isolates producing VIM and NDM metallo-b-lactamase, but not in those producing the serine b-lactamases KPC and OXA-48. We observed two distinct morphological changes of the J53 *E. coli*exhibiting reduced colony size after insertions of transposable elements in the *tonB* gene leading to alterations in the TonB binding site and morphological changes consistent with the small colony variant (SCV) phenotype due to mutations in the *hemB* and *hemH* genes. Passaging experiments suggested that these phenotypes were highly plastic. SCV phenotype is attributed to immune evasion and decreased susceptibility towards antibiotics.
Conclusion: Our experiments confirms that the production of metallo-beta-lactamases facilitates the development of cefiderocol tolerance upon exposure in vitro. The emergence of SCV following cefiderocol exposure may have clinical implications on bacterial clearance and warrants further investigation.

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Detection and transmission of linezolid- and vancomycin-resistant *Enterococcus faecium* isolates harbouring *cfr(D)* in Germany

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Background: The *cfr* gene encodes a 23S rRNA methyltransferase that confers resistance to linezolid. Although the recently described variant *cfr*(D) was designated according to the nomenclature for linezolid resistance genes, the data about its contribution to linezolid resistance is limited and controversial, as it does not confer a resistant phenotype when expressed in *Enterococcus* spp..[1] So far, *cfr*(D)-positive linezolid- and vancomycin-resistant *Enterococcus faecium* (LVRE) clinical isolates co-harboured another Linezolid resistance gene, namely *optrA*.[1,2] Here, we report a cluster of *cfr*(D)-carrying, *optrA*-negative LVRE in a German university hospital.
Methods: Within a five-week time span, we identified four patients in the same ward infected and colonized with LVRE. Minimum inhibitory concentrations (MICs) were determined using the Vitek2 automated system (BioMérieux) and the E-test in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards. Whole genome sequencing (WGS) and subsequent bioinformatic analysis were performed using the Illumina MiSeq platform to investigate possible clonal relationships and identify the underlying mechanisms of linezolid resistance.
Results: The MICs of linezolid ranged from 12 mg/L to 32 mg/L. None of the patients had previous linezolid treatment. WGS and subsequent SNP analysis revealed that all isolates belonged to the same MLST (ST80) and shared the same clonal origin. All strains harboured the methyltransferases *cfr*(D), *erm*B, and *erm*T. Further resistance genes or other known mechanisms involved in linezolid resistance, including *optrA* and chromosomal mutations of 23S ribosomal RNA, were not detected.
Discussion: In contrast to previous reports, our results imply that the presence of *cfr*(D) without other accessory linezolid resistance genes, such as *optr-A*, may be sufficient to confer phenotypic resistance towards linezolid. This is the first report demonstrating the emergence of *cfr*(D)-carrying LVRE clinical isolates in Germany. We are currently performing conjugation and transformation experiments to determine the transferability of the resistance mechanism and the impact of *cfr*(D) alone on the resistant phenotype.

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2 Ruiz-Ripa, L. *et al.* Mechanisms of Linezolid Resistance Among Enterococci of Clinical Origin in Spain—Detection of optrA- and cfr(D)-Carrying E. faecalis. *Microorganisms* **8**, 1155 (2020).

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Evaluation of an automated system for the counting of microbial colonies

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Introduction: Counting of microbial colonies on agar plates is important for various areas, including clinical diagnostics and research. The counting procedure is simple, but laborious and very time consuming. To simplify the counting process, automated instruments have been proposed. In this study, we aimed to evaluate the performance of a commercially available automated colony counter in regard to its accuracy and potential time saving.
Material/methods: Twenty clinical strains of *Escherichia coli* were included in the evaluation. Bacterial suspensions were prepared from an overnight culture and adjusted to the turbidity standard of McFarland 0.5. Afterwards, the samples were serially diluted in 0.9 % NaCl to produce suspensions containing approximately 10^5, 10^4, 10^3 and 10^2 cfu/ml. Ten µl of each suspension were spread onto tryptic soy agar, expecting the growth of approximately 1000, 100, 10 and 1 colonies per plate, respectively. After overnight incubation, each plate was automatically counted by the UVP ColonyDoc-It Imaging Station (Analytik Jena US). Additionally, correction of this automatic counting was performed by the visual adjustment of results on a computer display. The results of manual counting were used as reference values.
Results: Overall, the mean difference of the automated count without visual correction and the automated count with visual correction was 55.3 % and 1.6 %, respectively, in comparison to the manual counts. The mean differences for the plates with expected colony numbers per plate of approximately 1000, 100, 10 and 1 were 78.2 %, 29.4 %, 20.3 %, and 93.3 % for the automated count without visual correction and 3.9 %, 2.0 %, 0.4 %, and 0.0 % for the automated count with visual correction, respectively. For automated count without visual correction, the proportion of overestimations/underestimations were 0 %/100 %, 0 %/100 %, 65 %/10 % and 60 %/0 % for the plates with colony numbers of approximately 1000, 100, 10 and 1, respectively. For automated count with visual correction, the proportion of overestimations/underestimations were 10 %/85 %, 5 %/65 % 0 %/5 % and 0 %/0 % for the same colony numbers, respectively. The correlation between manual and automatic counting without correction showed a low correlation coefficient of 0.76, while manual and machine counts with correction were highly correlated (r^2=0.99). The mean time needed for automated counting without visual correction, automated counting with visual correction and manual counting was 0 min 33 sec, 1 min 20 sec and 1 min 12 sec, respectively.
Discussion: The fully automatic counting showed low accuracy, especially for plates with very high or very low colony numbers. After the visual correction of automatically generated results, the correlation with manual counts was high, however there was no advantage in reading time. Further investigations should include more bacterial and fungal species with various colony morphologies, as well as different agar types.

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Introduction of a standardised flow system for quantitative biofilm measurement – a test system to measure the effect of antimicrobial substances in biofilms by FISH

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Background: Biofilm-associated infections are a significant risk for patients, since they tolerate higher concentrations to antibiotics than single, planktonic bacteria. Standardized test systems to measure the effect of antimicrobial substances on biofilms are lacking. Especially static devices are producing inhomogeneous biofilms leading to variable results.
We present an adaptation of the ibidi flow system to produce standardized, well-defined *Staphylococcus epidermidis* biofilms that allow analysis by fluorescence in situ hybridization (FISH) in combination with digital image analysis for biofilm quantification and activity measurement.
Methods: FISH is a culture-independent tool for in situ detection of microbial cells. The signal intensity of FISH correlates with the ribosome content of the bacteria indicating metabolic activity. In this study, we used the ibidi flow system to grow biofilms under controlled conditions and compared the results to a static microtiter model. FISH was used as a tool to visualize and quantify the biofilm thickness and activity of *in vitro* grown biofilms using digital analysis.
Results: We evaluated the robustness of the ibidi flow system for biofilm growth by analysis of the average thickness of the biofilms in separate channels in the flow chamber and in repeated independent runs under similar conditions. The biofilm profiles and the bacterial activity measurements indicated homogenous results in the ibidi flow system, especially compared to a static culture system that was used to grow biofilms in parallel. Overall intra- and interassay variability indicated high reproducibility in the ibidi flow system. First results of Daptomycin treated *Staphylococcus aureus* biofilms revealed differences in biofilm thickness compared to untreated biofilms.
Conclusion: The results demonstrate that the ibidi flow system in combination with FISH and digital image analysis is a reliable tool for antimicrobial susceptibility testing of biofilms.

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Specific PET-imaging of bacterial infections by the use of maltotriose

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Question: Life-threatening bacterial infections such as vascular graft infections or endocarditis often have a non-specific clinical presentation. However, the distinction between infectious and non-infectious conditions and the localization of bacteria in organs and tissues is important for the optimal treatment of patients. For this purpose, 2-deoxy-2-[^{18}F]fluoro-D-glucose positron emission tomography ([^{18}F] FDG-PET) is being used already clinically, but this imaging technique visualizes the immune response to infection, not the bacteria themselves. Therefore, we aimed to develop a novel diagnostic strategy for the direct detection of bacteria by designing a bacteria-specific ^{18}F-labeled imaging probe for PET based on maltotriose that is exclusively ingested by bacteria.
Methods: An ^{18}F-fluorinated maltotriose ([^{18}F]AAX90) was synthesized. To study bacterial uptake, both *S. aureus* (strain LS1) and *E. coli* (strain ATCC25922) were incubated in the exponential growth phase with 0.1 MBq/ml tracer for 0.5 to 3 hours, and tracer uptake was determined using a gamma counter. Bacteria were grown in tryptic soy broth without sugar or with 0.1 to 10 mM maltotriose or 5 and 10 mM glucose. The tracer was tested in PET biodistribution studies and validated in a subcutaneous infection model in which bacteria ($5x10^6$ cfu) were injected into the shoulder region. Injection of heat-inactivated bacteria into the contralateral shoulder served as a negative control. Three hours after infection, the tracer was administered via the tail vein and its uptake was analyzed by PET imaging.
Results: [^{18}F]AAX90 was taken up *in vitro* by both *S. aureus* and *E. coli*, with significantly higher uptake in *E. coli* than in *S. aureus*. Low concentrations of unlabelled maltotriose in the medium enhanced tracer uptake, whereas high concentrations inhibited it in both bacterial species. As little as 5 mM glucose in the medium completely abolished the uptake of [^{18}F]AAX90 in *S. aureus*, while there was no effect in *E. coli*. The tracer showed favourable pharmacokinetics in the mouse. *In vivo* evaluation of [^{18}F]AAX90 by PET imaging clearly showed accumulation of the tracer at the infection site, whereas no signal was seen in the other shoulder where heat-inactivated bacteria had been injected. Both bacterial species could be detected by PET imaging with [^{18}F]AAX90, but the imaging signal was very low in *S. aureus* infection.
Discussion: Here, we demonstrate the new PET tracer [^{18}F]AAX90 that allows specific imaging of bacterial infections. Our results from *in vitro* experiments as well as from an *in vivo* mouse model suggest that it is suitable for the detection of *E. coli* and possibly other *Enterobacterales*. Ongoing studies with different mouse models will show whether we are able to use [^{18}F]AAX90 to detect lower numbers of bacteria as well as at different stages of infection. In addition, translation to the clinic is currently in progress.

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A diagnostic stewardship initiative shows the impact of different staff training methods on blood culture diagnostics

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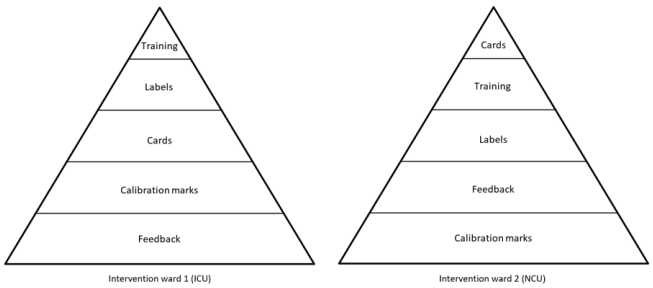
Introduction: Bloodstream infections have a high mortality and require rapid and adequate diagnostics. Bottle inoculation is usually performed in the clinic and preanalytical factors that influence blood culture (BC) results are often not widely known. BC diagnostics is an interdisciplinary issue that requires training of all staff members.
Material/Methods: A diagnostic stewardship (DS) initiative was conducted at two intervention (ICU, NCU) and control wards. The measurement had a total of three time periods, two three-month measurements, each with BC parameters such as filling volumes and pathogen identification, interspersed with a knowledge survey and staff training on indication, use and transport of BD BACTEC™ BCs. Methods implemented included: information cards, labels on BCs to identify the collection site, weekly feedback, and at ICU the BD Vacutainer® blood collection set. Subsequently, the use of the methods was evaluated as well as the subjective assessment.
Results: In total, 134 persons were trained, 92 (68.7%) nursing and 37 (27.6%) medical staff.
Before intervention, 32.7% of the knowledge questions were answered correctly. Afterwards, there was an improvement to 57.8%, at the end the result was 51.2%.
Bottle-related interventions were better accepted at ICU than at NCU with 91.5 vs 80.8% completed localisation labels and 76.7 vs 34.6% drawn calibration marks (Fig. 1). All interventions were generally rated well, but with a different ranking at the intervention wards and thus a dependent benefit of the intervention (Fig. 2).
Overall, it could be demonstrated that collection sites were more appropriate (shift from arterial to peripheral sampling by 30.4%) and contaminations decreased (13.3 to 7.9%).
Conclusions: The intervention wards rated the measures differently, but training, labels and information cards were mentioned as the best. Measures requiring active referral, such as weekly feedback, were rated well if used.
The ICU staff did not prefer the additional use of the BD Vacutainer® because of the subjectively assessed insufficient practicability compared to the BC collection by syringe. Among others, this was justified by the insufficient viewing window for observing the inflowing blood. Overall, responses showed that BC bottles were not perceived sufficiently practicable for everyday

clinical use. Particularly when using a filling device, the application of a filling mark is necessary to generate an optimal diagnostic blood volume, as to optimise the blood management as well. Improvements would be possible through a defined vacuum and optimised labelling.
Especially in BC diagnostics, a lot of knowledge is required in preanalytics. The data collected demonstrates that this cannot be assumed in a standardised way and that the tools used should be optimally designed. It was also shown that situation-adapted measures are the best accepted DS interventions.

Fig. 1

	Information cards used				Localisation labels completed				Feedback forwarded				Calibration marks for filling volume			
	Yes	Partly	No	Not specified	Yes	Partly	No	Not specified	Yes	Partly	No	Not specified	Yes	Partly	No	Not specified
ICU, N=47 (%)	6 (12.8)	18 (38.3)	21 (44.7)	2 (4.3)	41 (87.2)	2 (4.3)	2 (4.3)	2 (4.3)	2 (4.3)	13 (27.7)	29 (61.7)	3 (6.4)	27 (57.5)	9 (19.2)	11 (23.4)	0
NCU, N=26 (%)	9 (34.6)	9 (34.6)	6 (23.1)	2 (7.7)	17 (65.4)	4 (15.4)	3 (11.5)	2 (7.7)	20 (76.9)	4 (15.4)	0	2 (7.7)	6 (23.1)	3 (11.5)	16 (61.5)	1 (3.9)

Fig. 2



Workshop 24
Microbial pathogenesis of enteric Infections (FG MP/FG GI)
07. Sep 2022 • 13:45–15:15

140/MPGIV
Helicobacter pylori UvrC is essential for chromosomal micro-imports after natural transformation

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Introduction: *Helicobacter pylori* is a natural mutator due to a deficient DNA mismatch repair pathway, and naturally competent for transformation. As a result, it is one of the most genetically diverse human bacterial pathogens. *H. pylori* takes up exogenous DNA by the unique ComB apparatus and subsequently imports fragments into the chromosome by homologous recombination. The length of chromosomal imports in *H. pylori* follows an unusual bimodal distribution consisting of macro-imports with a mean length of 1,645 bp, and micro-imports with a mean length of 28 bp. The mechanisms responsible for this import pattern were unknown.
Methods: Here, we used a high-throughput whole-genome transformation assay to elucidate the role of nucleotide excision repair pathway (NER) components on import length distribution. Additionally, UvrC was also characterized using site-directed mutagenesis of functional domains and a UV tolerance assay.
Results: The data show that the integration of micro-imports depended on the activity of the UvrC endonuclease, while none of the other components of the NER pathway was required. Using *H. pylori* site-directed mutants, we showed that the widely conserved UvrC nuclease active sites, while essential for protection from UV light, one of the canonical NER functions, are not required for generating micro-imports. The widely conserved C-terminal DNA-binding region of UvrC has an atypical structure in *H. pylori* and did not contribute to UV tolerance or micro-imports, suggesting substantial functional divergence compared to other bacteria. A quantitative analysis of recombination patterns based on over 1000 imports from over 200 sequenced recombinant genomes showed that micro-imports occur frequently within clusters of multiple imports, strongly suggesting they derive from a single strand invasion event.
Discussion: We propose a hypothetical model of homologous recombination in *H. pylori*, involving a novel function of UvrC, that reconciles the available experimental data observations about recombination patterns in *H. pylori*.

141/MPGIV
Multiple new mechanisms how *Helicobacter pylori* can modulate LPS heptose biosynthesis and heptose-dependent host cell activation

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Background: Bacterial heptose metabolites, stemming from lipopolysaccharide (LPS) inner core biosynthesis, were recently defined as novel microbe-associated molecular patterns (MAMPs), having exquisite activity on various human cell types. The gastric pathogenic bacterium *Helicobacter pylori* produces heptose metabolites and is able to transport those into human epithelial and phagocytic cells a,b, with the help of its Cag type IV secretion system (T4SS).
Methods and Results: Using *H. pylori* as a model, we have now addressed the question, whether and how LPS heptose biosynthesis can be regulated by the bacteria in a pathogenic or host (cell) context. We have uncovered an unsurpassed strain variability in heptose biosynthesis traits and heptose metabolite effects on human cell activation. The core enzyme of LPS heptose biosynthesis, HldE, which shows strong protein and genetic variability between strains, was expressed in the bacteria in a strain-variable manner, as one determining factor of strain variation in product and pathway activity. Transcript regulation of genes in the *hldE* gene cluster was modulated by epigenetics, heptose supplementation, presence and absence of Cag T4SS activity, or the absence of the whole *cag* pathogenicity island.
In vitro reconstitution of the *H. pylori* LPS heptose biosynthesis pathway using purified enzymes, separate subdomains of HldE proteins, and HldE enzymes from different strains allowed us to uncover a further layer of variation in the strain-specific production of different heptose metabolites. Furthermore, we refined the biochemical characterization of novel active heptose metabolites and the activities of purified heptose biosynthesis pathway enzymes by nuclear magnetic resonance.
Conclusions: The present work will serve as a basis to reveal novel activities and pathway products of various bacteria which can produce distinct heptose metabolites which act as MAMPs. The present in-depth characterization of the heptose pathway also makes it amenable to be exploited as a novel therapeutic target against pathogenic and chronic inflammatory bacteria.

a) Faass L, Stein SC, Hauke M, Gapp M, Albanese M, Josenhans C. Contribution of Heptose Metabolites and the cag Pathogenicity Island to the Activation of Monocytes/Macrophages by *Helicobacter pylori*. Front Immunol. 2021, 12:632154. doi: 10.3389/fimmu.2021.
b) Stein SC, Faber E, Bats SH, Murillo T, Speidel Y, Coombs N, Josenhans C. *Helicobacter pylori* modulates host cell responses by CagT4SS-dependent translocation of an intermediate metabolite of LPS inner core heptose biosynthesis. PLoS Pathog. 2017, 13:e1006514. doi: 10.1371/journal.ppat.1006514.

142/MPGIV
Hfq RIL-seq in *Clostridioides difficile* reveals a network of sRNAs regulating sporulation initiation

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Background: Small regulatory RNAs (sRNAs) provide the posttranscriptional layer of gene regulation by base pairing with target mRNAs to regulate their translation or stability. The RNA-binding protein Hfq is a mediator of these regulatory RNA interactions in gram-negative bacteria but its role remains elusive in gram-positive bacteria.
Method: Here, using RIL-seq to capture the Hfq-mediated RNA-RNA interactome in the spore-forming Firmicute *Clostridioides difficile*, we identify Hfq as such a global RNA matchmaker and reveal a rich post-transcriptional regulatory network.
Results: We discover two novel sRNAs, which we name SpoX and SpoY, that target the master regulator of sporulation Spo0A thereby fine-tuning the formation of endospores in this important human pathogen. We show thatSpoX and SpoY base-pair with the 5" UTR and early coding sequence of Spo0A, respectively, to modulate Spo0A protein levels with opposite effects. Whereas SpoX increases spore frequency, the activity of SpoY leads to decreased/delayed sporulation.
Conclusions: Our work reveals an elaborate RNA-RNA interactome controlling the physiology and virulence of *C. difficile* and identifies a post-transcriptional regulatory mechanism in the production of *C. difficile* endospores which are critical for the pathogens resistance to traditional antibiotic therapies and for its transmission to new hosts.

143/MPGIV

Impact of strain-specific expression of conserved genes on phenotypic diversity within closely related *Escherichia coli* strains

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Introduction: *Escherichia coli* is an abundant and multifaceted bacterial species that can colonize various niches of warm-blooded organisms. While most *E. coli* strains live commensally with the host, others are pathogenic or probiotic. Interestingly, *E. coli* strains have been shown to co-evolve with the host from commensal to pathogenic with only few genomic modifications (mutations). One instance of this is observed amongst the three closely related (ANI > 99%) *E. coli* strains: CFT073 (uropathogen), 83972 (asymptomatic colonizer of the bladder), and EcN (probiotic). These strains were used to better understand the underlying mechanisms of bacterial diversification and the basis for pathogenicity or probiotic properties.
Material/Methods: To determine the orthologous genes amongst the three *E. coli* strains, pan-genome analysis was performed on NCBI Prokaryotic Genome Annotation Pipeline (PGAP) annotated closed genomes. For transcriptomic analyses, bacteria were either grown in pooled human urine or Dulbecco's Modified Eagle Medium (DMEM, Biochrom). After stopping the growth of the strains in mid-logarithmic phase, total RNA was isolated and the cDNA library was sequenced on Illumina NextSeq500. The processed bacterial reads were aligned to their corresponding reference genomes and the whole-genome differential expression analysis was performed.
Results: Overall, the three isolates are characterized by a different composition of virulence and fitness factors, with previously published observations that UPEC strain CFT073 has the greatest number of functional virulence factors and probiotic strain EcN the lowest, confirmed by our analysis of the three genomes. When grown in urine, the global gene expression profiles of the two urine isolates were more similar to each other than between the genetically more closely related strains CFT073 and 83972. However, when grown in DMEM, the transcriptional profiles of the two attenuated strains EcN and 83972 were more similar. Interestingly, the three strains exhibited different expression profiles of conserved genes in cell culture medium and pooled human urine. Based on their genome content and transcriptomes, we show that individual gene expression profiles, also of conserved genes, in all three strains may contribute to the different strategies they use to efficiently colonize and interact with their host.
Discussion: Our results impressively demonstrate how closely related isolates can differ in the expression profiles of their conserved genes. Using the three *E. coli* strains as an example, it can be well seen that besides the presence/absence of single determinants and deleterious mutations also significant global differences at the level of regulation of gene expression determines the different phenotypic manifestations of these strains.

144/MPGIV

Visualisation of type 3 secretion system components in *Yersinia enterocolitica* down to the molecular level by MINFLUX nanoscopy

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[3]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

Introduction: Type 3 secretion systems (T3SS) are essential virulence factors of numerous bacterial pathogens and inject immunosuppressive effector proteins into host cells. The needle-like T3SS machinery of *Yersinia enterocolitica* consists of more than 20 components, has a length of around 100 nm and its different sections along the length axis are up to ~30 nm broad. A temporally and spatially resolved visualization of the T3SS using fluorescence microscopy techniques has been challenging, as its intrabacterial components are highly dynamic and in permanent exchange with other bacterial structures. In addition, there is very few knowledge and information about the pore protein YopD. YopD is essential for forming a functioning pore complex in the host cell membrane during an infection and thus for effector translocation.
Material/Methods: We visualized and resolved the endogenously tagged sorting platform proteins YscL at the proximal end of T3SS and the pore protein YopD at the distal end of T3SS by STED, STORM and MINFLUX nanoscopy using self-labelling enzymes and nanobodies during cell infection.
Results: As a result, we show details of T3SS components with resolution down to the molecular level, and this is, according to the best of our knowledge, an unprecedented result by means of light microscopy.
In addition, 3D MINFLUX nanoscopy was essential to determine the precise three dimensional distribution of sorting platform protein YscL within a whole bacterium. Furthermore, preliminary results performing two-color MINFLUX nanoscopy allowed visualizing both YscL and YopD in parallel during a cell infection indicating the number of pore forming T3SS in relation to sorting platforms present intrabacterially.
Discussion: The work presented here is a prerequisite for further studies of microbiological infection processes at the molecular scale.
Continuation of this work will allow us to investigate T3SS structure, function and dynamics with unprecedented resolution and therefore gain new insights into the infection process of human pathogens in order to develop novel treatment and prevention strategies.

145/MPGIV

Single-cell RNA-sequencing reports growth-condition-specific global transcriptomes of individual bacteria

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RNA molecules are a very powerful proxy to report cellular identities and physiologies. The development of RNA-seq and dual-RNA-seq have opened a new window to decipher host-pathogen interactions. However, most of these methods have been averaging the signal of thousands of cells disregarding the heterogeneity of every individual infected cell. Recently the breakthrough of single-cell RNA-seq paved the way to understand

cell-by-cell variability notably the opportunity to discover cellular subpopulations that convey chronic infections. Also single cell analysis promises to reveal the variety of strategies used by both the pathogen and the eukaryotes to promote or control disease spread. . Also single-cell RNA-seq is a well-established technique for eukaryotic cells, it failed so far to capture the transcriptome of a single bacterium. Bacterial RNAs are extremely rare compared to their eukaryotic counterparts (one hundred less in abundance) and their transient nature in the order of few minutes has prevented easy adaptation of existing protocols. Here, using a poly(A)-independent single-cell RNA-seq, we report for the first time the development of a protocol that faithfully captures growth-dependent gene expression patterns in individual *Salmonella* bacteria, across all RNA classes and genomic regions. We analyzed *Salmonella* grown in three different conditions: (i) "stationary phase" after overnight growth reflecting mainly resting cells; (ii) "oxygen tension" mimicking the intestinal environment; and (iii) "salt stress" caused by increased sodium chloride concentration in the medium. Furthermore, principal components were identified bioinformatically, enabling us to differentiate between the different populations under the specific conditions. To validate our findings, we compared our results to previously published comprehensive bulk RNA-seq data. On this basis, we were able to conclusively relate the upregulated genes detected in our studies to the corresponding conditions in the bulk assay data. These transcriptomes provide important reference points for single-cell RNA-seq of bacterial species. Future applications could have the potential to elucidate insights of host-pathogen interactions and mixed microbial communities.

Fig. 1

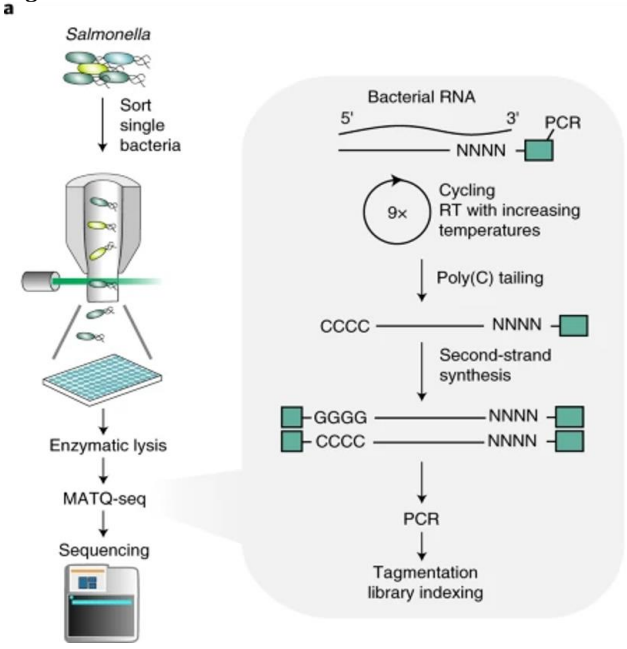
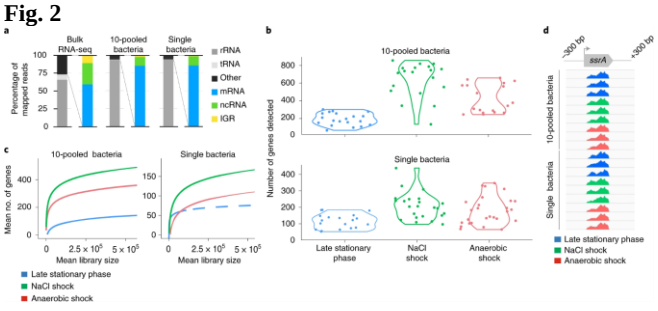


Fig. 2



Workshop 25
Technical- and water hygiene (StAG HY/FG PR)
07. Sep 2022 • 13:45–15:15

146/HYPRV

Rapid and cost-efficient point-of-care testing for multidrug-resistant *Acinetobacter baumannii* with a microfluidic, fully automated system

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[3]*microfluidic ChipShop GmbH, Jena, Germany*

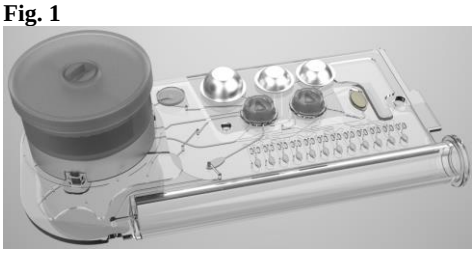
Introduction: Multidrug-resistant (MDR) *Acinetobacter baumannii* in hospitals cause death and high financial expenses due to extensive infection control procedures. Conventionally, species and resistance determination of MDR bacteria are performed by mass spectrometry and labor-intensive phenotypical assays in clinical microbiology laboratories. Some institutions also apply time-consuming and expensive whole genome sequencing for in-depth resistance and transmission analysis. In contrast, faster and more cost-efficient high-throughput MDR screening can be achieved by on-site application of real-time PCR-based point-of-care tests (POCTs) that target species- and resistance-specific genes.

Methods: To construct a real-time PCR-based POCT system for species and resistance determination of MDR *A. baumannii*, two species-specific genes (*rpoB*, *OXA-51-like*) and 18 resistance genes found in MDR *A. baumannii* (*OXA-23-like*, *OXA-24/40-like*, *OXA-51-like*, *OXA-58-like*, *OXA-143-like*, *mcr*, *ADC*, *NDM*, *GIM*, *VIM*, *IMP*, *GES*, *PER*, *TEM*, *SHV*, *VEB*, *CTX-M*, *KPC*) were selected as targets. For each gene, one to six sets of primers and fluorophore-labeled probes that amplify conserved genetic regions were designed and their functionality was tested *in vitro*. Parallel, a fully automated system comprising microfluidic chips (figure below) and a bench-top analyzer able to perform DNA extraction and real-time PCR starting from swab eluates were developed.

Results: Species-specific primers and probes were constructed to specifically identify *A. baumannii*-DNA. In addition, primers and probes for resistance detection were designed to amplify 41 *OXA-23-like*, 10 *OXA-24/40-like*, 321 *OXA-51-like*, 7 *OXA-58-like*, 8 *OXA-143-like*, 95 *mcr*, 211 *ADC*, 29 *NDM*, 2 *GIM*, 68 *VIM*, 82 *IMP*, 44 *GES*, 12 *PER*, 189 *TEM*, 181 *SHV*, 27 *VEB*, 225 *CTX-M*, and 63 *KPC* alleles. All oligonucleotides tested so far reliably detected 100 to 10,000 copies of DNA extracted from MDR bacteria in single- and multiplex experiments *in vitro*. First tests with the microfluidic system showed that lysis of different bacterial species could be accomplished via sonication on a membrane located on the microfluidic chip. In addition, chip implementation of all PCR reagents like lyophilized master mix as well as primers and probes was successful. So far, the system delivered detectable fluorescence signals within 1 h when performing test runs with bacteria eluted from swabs.

Discussion: Here, a real-time PCR-based POCT system for species and resistance determination of MDR *A. baumannii* directly from swabs was developed. In the next test phase, detection limits will be determined and analysis time will be optimized. A software for system control and result illustration is currently in development. For final evaluation, the POCT system will be head-to-head compared to conventional culture methods of clinical microbiology laboratories.

Fig. 1



147/HYPRV

Up the drain! Low transmission rate of multidrug resistant *Pseudomonas aeruginosa* from toilets in spite of long-term persistence in a haematology ward

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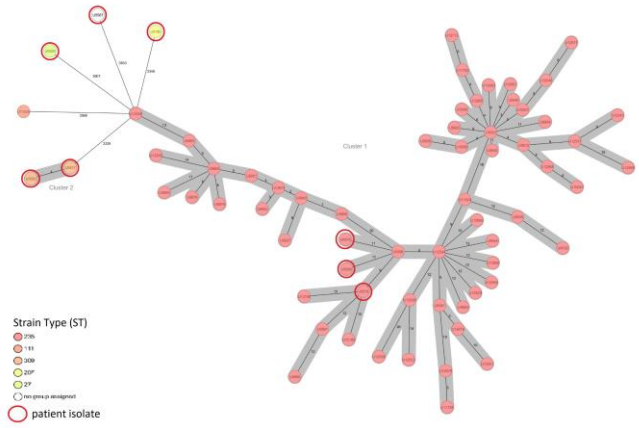
Objective: Toilet water serves as an important reservoir for bacteria such as *Pseudomonas aeruginosa* (PA), and can be a source of infection for vulnerable patients. When flushed with an open lid, little droplets contaminate a surface with a diameter of up to 2 meters around the toilet. This high contamination rate has repeatedly been a cause for transmission of multidrug-resistant (MDR) PA in hospitals. Our aim was to analyze retrospectively the transmission rate within a bone marrow transplant department with a long history of MDR PA toilet contamination.
Methods: The toilet water was treated with bromide every three weeks during the whole period, and water samples were screened for MDR PA every 3 months during the years 2016-2021. If MDR PA was detected, the toilets were disinfected repeatedly with an oxygen-releasing agent and sampled until no MDR PA could be detected. Simultaneously, all patients were instructed on matters of infection prevention in the bathroom (i.e. closing the lid before flushing the toilet, hand hygiene), then screened weekly by rectal and oral swabbing. Patient samples were effaced on selective agar for gram-negative bacteria. Microbiological investigations of the water samples were done according to DIN EN ISO 16266:2008. Antimicrobial resistance testing was done using the agar dilution test for all isolates. MDR PA isolates from both patient and toilet water samples were collected, and Whole Genome Sequencing (WGS) was performed for all available isolates. Data analysis was done with the Ridom SeqSphere+ software, and MLST, ad-hoc cgMLST (seeding genome: PAO1) and cgMLST were performed.
Results: During the 6-year period, each toilet was sampled 24 to 38 times. 78 toilet samples were positive for MDR PA during screening, and each room had at least one positive sample. 1 to 4 disinfections with oxygen-releasing agents per event were necessary to eliminate MDR PA, with an average of 1,43, and a median of 1 (95% CI 1 to 1) disinfections per positive screening result. 94 isolates from toilet water samples were available for sequencing, and 61 isolates were sequenced by now (work currently in progress). After WGS, 60 of 61 sequenced toilet isolates were assigned to ST235 and clustered (difference ≤ 20 alleles in pairwise comparison of 5656 alleles) according to ad-hoc cgMLST. During this period, only 12 patients had a hospital-acquired MDR PA. However, only 3 of 8 sequenced patient isolates matched the isolates found in toilet samples. Further, two patients had closely related isolates belonging to ST309, and are known to have stayed on the affected ward at the same time.
Conclusion: MDR PA shows low variability in toilets across the examined haematology ward over the study time, accordingly, its genome remains stable for long periods according to WGS. However, the use of bromide, closed toilet lids and toilet disinfection are efficient measures for preventing transmission of MDR PA from toilets to vulnerable patients.

Fig. 1



148/HYPRV

The current structure of German intensive care units – trends and obstacles for infection control measures

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Question: Multidrug resistant organisms (MDRO) and viruses with an airborne pathway such as SARS-CoV-2 are a worldwide threat to modern healthcare. Hospital design may play a role in preventing the spread of MDRO and airborne pathogens. The objective of our study was to get an overview about the situation in German ICU in the year 2021 and to evaluate the development during the last 30 years.
Methods: ICUs participating in the ICU component of the German national nosocomial surveillance system (KISS) were used for this survey, which was conducted between September and November 2021. The infection control practitioners responsible for ICU surveillance in the individual hospitals were invited by email. Data were collected on LimeSurvey. Answers were then stratified through year of building for each ICU (before 1990, between 1991 and 2000, between 2001 and 2010, after 2011). Differences were tested by Chi-Square or Wilcoxon Rank-Sum test.
Results: 542 German ICUs answered our survey. 78% of them have been built before 2011. The median number of ICU-beds increased from 10 (interquartile Range (IQR) 9-14) before 1990 to 14 (10-19) after 2011 ($p<0.001$) and the median number of single bedrooms increased from 3 (2-5) to 5 (2-8) ($p<0.001$). Anyway, due to the increase of the median total room number from 7 (6-10) to 10 (8-14) ($p<0.001$), the proportion of single bedrooms over the total room number didn't change. The median number of anterooms increased from 1 (0-2) to 2 (1-4) ($p<0.001$) and the median number of isolation rooms (single room with anteroom) increased from 1 (1-2) to 3 (2-4) ($p<0.001$). 80% of ICUs built before 1990 and 84.3% of those built after 2011 have an automatic ventilation system ($p=0.041$). Median room surface changed from 17m2 (15-20) to 20m2 (18-24) for single rooms and from 27.5m2 (21.7-34.5) to 33m2 (29.3-37.3) for multiple bedrooms ($p<0.001$). Just 73.8% of the ICUs have a hand rub dispenser placed close to the patient bed, with no significant change over time.
Conclusions: Many ICUs in Germany have been built longer than 10 years ago. There is a trend to more and bigger rooms, more isolation rooms and more automatic ventilation, but this happens at a still slow pace. The lack of single occupancy ICU rooms poses a limit to the management of a growing number of infected or colonized patients, and room surface still doesn't meet the requirements formulated by the German Society of interdisciplinary intensive care (DIVI) in 2010. About a third of the invited ICUs participated and we had to ask participants to choose one patient room as a model for answering the questions, so we could see only a part of the picture. Nevertheless, we can say that, in consideration of actual knowledge, there is an urgent need to renew the requirements for ICU-planning in Germany.

149/HYPRV

Long persistence of carbapenemase-producing *Enterobacterales* in hospital wastewater

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[1]*Universitätsklinikum Hamburg-Eppendorf, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Arbeitsbereich Krankenhaushygiene, Hamburg, Germany*
[2]*Heinrich-Pette-Institut, Virus Genomik, Hamburg, Germany*
[3]*Universitätsklinikum Hamburg-Eppendorf, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany*

Background Hospitals are considered a major reservoir of multidrug-resistant *Enterobacterales* (MRE) worldwide. Hospital wastewater can be a source of MRE input to municipal wastewater and subsequently to surface waters. In this study, we investigated the clonal relatedness of and presence of resistance genes in MRE at 14-month intervals in wastewater from a maximum care hospital.
Methods 24-h composite samples were collected over four days in November 2019 and a qualified random sample was taken from the main water line in January 2021. The presence of *Enterobacterales* with resistance to third-generation cephalosporins was analyzed by culturing on selective agar. The species of the isolates were determined by mass spectrometry (Biotyper, Bruker, Bremen, Germany) and the whole genome of representative isolates (30 β-glucuronidase and 30 β-glucosidase producing isolates per sample) was sequenced (n=300). Clonal relatedness of MRE was investigated by automated analysis of the core genome (cgMLST) using SeqSphere+ software (Ridom, Münster, Germany). Resistance gene analysis was performed by ResFinder software (Center for Genomic Epidemiology, Copenhagen, Denmark).
Results In β-glucuronidase-producing isolates, *Citrobacter freundii* (n=59) was identified at both time points in addition to *Escherichia coli* (n=81). Among 30 β-glucosidase-producing isolates, *Serratia marcescens* (n=71), *Enterobacter cloacae* (n=35), and *Klebsiella oxytoca* (n=35) were detected at both time points. *Klebsiella pneumoniae* (n=9) was detected only at the first time point. A wastewater-associated, carbapenemase-producing *E. coli* clone ST635 that was dominant at the first time point was not detected at the second time point, whereas one clone (ST73) with an SHV-12 betalactamase was dominant at the second time point. Overall, a carbapenemase gene was detected in 40.7% of *E. coli*, while a carbapenemase gene was observed in at least 88% of the other species. The relative proportion of carbapenemase-positive *Enterobacterales* was several times higher than in clinical third-generation cephalosporin-resistant isolates of the respective species. While different dominant clones were observed for *E. coli* at the two time points, consistently detectable clonal lineages were evident for *S. marcescens*, *C. freundii*, and *K. oxytoca*. A *K. pneumoniae* clone that occurred during an outbreak four years prior to the first sampling at the hospital was observed at the first but not at the second time point.
Conclusions The data collected show that single clonal lineages exist that persist permanently in hospital wastewater, resulting in a continuous release of these carbapenemase-positive clones into the municipal wastewater system. These strains can pose a health risk by spreading in the environment, transferring plasmids with resistance genes, and reintroducing resistant strains back into the healthcare system. Over time, only *E. coli* showed a strong change with different dominant clonal lineages.

150/HYPRV

Development of a *Legionella pneumophila*-specific qPCR to detect the virulence marker gene *lag-1* in environmental samples for a rapid risk assessment of water systems

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Question: Legionellae are water borne pathogens that can infect the human lung by inhaled legionella-containing aerosols. The most dominant species that is isolated from patients suffering from legionellosis is *Legionella pneumophila* that carry a virulence associated lipopolysaccharide marker on the membrane. Most cases occur sporadically making the identification of potential sources difficult. In a recent study, we identified a higher risk of infection by persons who live in buildings that harbour legionellae in the drinking water system. This risk drastically increased when the species was *L. pneumophila* carrying a virulence associated marker on the membrane.
In order to assess water systems rapidly for the risk of potential infection by these virulent legionellae clones, we used the corresponding genetic marker gene, *lag-1*, to design a specific qPCR.
Materials/Methods: Primers and probes that target the virulence associated gene *lag-1* were designed and tested *in silico* and *in vitro*. Two primer-probe sets were evaluated using an in-group consisting of isolates that harbour the *lag-1* gene and synthesize the specific LPS marker. Two out-groups were tested consisting of i) legionellae, that lack the LPS-specific virulence marker and ii) other water borne bacteria according to ISO TS 12869:2012. We analysed further 50 water samples in detail using the designed qPCR and the gold standard methods (cultivation and serotyping).
Results: The specificity of both sets is 99 % when it was tested against the in- and out-groups. Three isolates were tested false positive as they carry truncated versions of *lag-1*, and thereby lack the corresponding epitope on the membrane. There are no cross-reactivities with other bacteria.
Out of 50 water samples, 49 samples gave concordant results when the qPCR was compared with the gold standard method. One sample was under the limit of detection of 15 genomic units per reaction.
Conclusion: The current gold standard method to assess the risk of water systems with regard to legionellae relies on a technical alert value of 100 cfu of legionellae in 100 ml. This assessment does not differentiate between the *Legionella* species nor does it imply the analysis of virulence associated markers. In contrast, the here described qPCR enables the owner of a water system as well as public health authorities to rapidly asses the risk of water systems. This qPCR could also be a helpful additive tool in facilities with vulnerable people such as retirement homes.

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Eukaryotic Pathogens (FG EK)

151/EKP
Melanisation in human pathogenic melanized fungi is associated with altered cell wall constitution and biofilm formation capability

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Introduction: Melanization and biofilm formation are major virulence factors in pathogenic melanized fungi, making them capable of causing recurrent and chronic infections resistant to treatment and immune response. We here evaluated the interplay between melanization, cell wall constitution, and biofilm formation in melanized fungi.
Methods: Several biofilm-forming strains of *C. neoformans* (N=10), *A. fumigatus* (N=10), and *E. dermatitidis* (N=26), including melanin-lacking mutants and their corresponding wild types were examined for their biofilm formation capability using crystal violet assay. Confocal laser scan microscopy was performed for visualization. Furthermore, dihydroxyphenylalanine (L-DOPA[LK1]) as melanin inducer in *C. neoformans* and tricyclazole (TCA) as DHN-melanin biosynthesis pathway inhibitor in *E. dermatitidis* and *A. fumigatus* were added to cultivation. To determine the effect of melanization on cell wall constitution, we analyzed cell wall electronegativity via zeta potential measurements and cell surface hydrophobicity via microbial adhesion to hydrocarbons (MATH) assay. We assessed the effect of hydrophobicity and electronegativity on biofilm formation by performing adhesion assays on polystyrene surfaces with distinct hydrophobicity as well as distinct charges.
Results: Melanin-deficient mutants formed less biofilm compared to their corresponding wild types. While L-DOPA strengthened biofilm formation in *C. neoformans*, TCA inhibited biofilm formation in *E. dermatitidis* and less in *A. fumigatus*. Correlation analysis between biofilm formation capability and the relative hydrophobicity of the cell surface showed *A. fumigatus* and *C. neoformans* strains with higher hydrophobicity are more likely to form biofilms than those with less hydrophobicity. Adhesion assay revealed that, based on adherence to different hydrophobicity surfaces, *A. fumigatus* and *C. neoformans* strongly adhered to hydrophobic coated plates compared to the hydrophilic ones. Additionally based on adherence to different charged surfaces, strains formed more biofilms on negatively charged surfaces than on positively charged ones. Whereas melanin-lacking mutants and treatments inhibiting melanin synthesis showed the inverse results. Furthermore, zeta potential was found to be related to the capability to form biofilms.
Conclusion: The results here demonstrate that melanization in fungi increases the biofilm formation potential, and further, that biofilm formation of human pathogenic fungi is positively associated with electronegativity and hydrophobicity of cell surfaces.

52/EKP
Further insights into the occurrence and properties of the two different forms of Shiga Toxin 2a – nicked or unnnicked

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Question: Enterohemorrhagic *Escherichia coli* (EHEC) associated hemolytic uremic syndrome (eHUS) is one of the foremost causes of acute kidney injury in children. It is proposed that the progression of the disease is directly linked to the interaction and activation of complement by one of EHEC´s main virulence factors, Shiga toxin 2a (Stx2a). Recently it has been revealed that the biological properties and binding abilities of Stx2a, including its interaction with complement proteins may be determined by the structure of its A subunit (unnicked or nicked). Thus, assessment of complement regulation by Stx2a presenting different A subunit structures (different structural forms of Stx2a) is necessary for better understanding of the subsequent potential impact on eHUS pathogenesis.
Results: The effect of the different structural forms of Stx2a on the regulation of the three complement activating pathways was evaluated by enzyme-linked immunosorbent assays (ELISAs) determining qualitative and/or semi-quantitative functionality of the pathways and results will be presented at the meeting. We will also present some more details on the different cleavages which may occur and where they occur on the way of the toxin from the gut to its target organs kidney and brain.
Conclusion: The data are likely crucial for a better understanding of the biological properties of the different circulating Stx2a structural forms and their influence on eHUS pathogenesis.

153/EKP
An intestine-on-chip model to study *Candida albicans* commensalism and pathogenicity

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Background: The human intestine is the major reservoir of commensal *Candida albicans*. However, this intestinal *C. albicans* population is also the main source of systemic candidiasis upon dysbiosis of the microbiota and dysfunction of the immune system. In this highly complex niche, *C. albicans* interacts with several types of epithelial and immune cells, the microbiota, and a specific chemical environment. Traditional *in vitro* models often fail to fully recapitulate these features, which can result in an incomplete or biased understanding of host-pathogen interactions.
Methods: To study the role of the host and microbiota in the balance between commensalism and pathogenicity, we established an intestine-on-chip infection model, as a dynamic and more physiological approach. It includes a bloodstream-like compartment with endothelial cells, an intestinal epithelial barrier including resident innate immune cells, and displays organotypic microanatomical features with villi and crypts under perfused conditions. This model supports epithelial colonization with bacteria or addition of niche specific metabolites or proteins in each of the compartments. Besides, it allows more flexibility during infection and a wide range of readouts regarding both the host and the fungus, including microscopy.
Results: This model has been used to study how physiological or pharmacological features impact *C. albicans* infection. For example, we demonstrated that *Lactobacillus rhamnosus*, a representative of a healthy microbiota, can antagonize *C. albicans* pathogenicity in the intestine-on-chip model. We are also using the intestine-on-chip model to investigate and mimic the response of echinocandin-resistant and -sensitive *C. albicans* strains to caspofungin perfused vascularly.
The intestine-on-chip also allows us to dissect fundamental steps in *C. albicans* infection, in this case, translocation across the

epithelial barrier as a prerequisite to systemic disease. Microscopic images revealed that *C. albicans* forms microcolonies in the intestine during infection, similar to what has been reported for other mucosal tissues. We are applying image analysis tools to elucidate the interactions of these microcolonies with the host, their role on fungal translocation, and how the host microbiota or the immune system can impact their formation.
Discussion: Organ-on-chip models enable at the same time a more physiological and complex approach to study the infection at tissue level and the systematic dissection of the processes that take place, thus combining advantages of traditional *in vitro* and *in vivo* models. In this case, the intestine-on-chip model represents a platform to mimic *C. albicans* commensalism, and to study factors that either foster or disrupt colonization, leading to systemic candidiasis.

154/EKP

Interferon gamma-mediated *Toxoplasma gondii* control in intestinal organoids of the bank vole *Myodes glareolus* - how does it compare to lab mice?

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Question: The intracellular protozoan *Toxoplasma gondii* is one of the most prevalent parasitic species in the world. Interferon gamma (IFNg)-mediated control of so-called "virulent" *T. gondii* strains has been shown to differ between the laboratory mouse model and other wild-derived mouse strains, specifically due to differences and polymorphisms in host genes of the family of Immunity related GTPase (Irg). How these responses occur in rodent species that do not belong to the murine family but which are of ecological importance on the dissemination and maintenance of virulent strains in the environment, at least in Europe, is yet to be determined.
The intestinal epithelium is the first point of encounter of *T. gon*dii with its host. Yet, how dissemination occurs and what role IFNg and the regulation by the Irg system play in intestinal cells has not been reported in any host system.
Methods: We have established intestinal organoid-derived monolayers (ODMs) from the bank vole *Myodes glareolus*, a rodent species which has been assumed to be more resistant to *T. gondii*, and compared the responses in absence or presence of species-specific IFNg to the ODMs from the laboratory mouse strains C57/BL6 and the wild inbred strain CAST.
Results: Using this model, we have evaluated the events during the early phase of a *T. gondii* infection, focusing on the role of Irg-mediated *T. gondii* control in ODMS between different strains of lab mice and the bank vole *Myodes glareolus*. Our results show that stimulation with IFNg leads to reduced tachyzoite replication in *M. glareolus* ODMs compared to *C57/BL6* ODMs for both, type I RH and for type II Pru (Prugniaud) strain. Moreover, we observed involvement of the Irg system also in *M. glareolus* during *T. gondii* infection. We are currently using our stablished organoid system to evaluate bradyzoite infections, which better recapitulate natural infections and will help to further understand the role of the Irg system during host-parasite interactions in the intestinal epithelium.
Conclusions: Experiments using primary fibroblasts and macrophages of *M. glareolus* have pointed to a control of *T. gondii* replication. We are the first to evaluate the Irg system"s relevance in intestinal cells, which are the first interaction point of the parasite with its host. We were able to show involvement of the Irg system and a strong trend towards parasite control in a rodent species other than the traditional *Mus musculus* model.

155/EKP

Differences in the ability of *C. albicans* strains 101 and SC5314 in colonising the murine gut

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Introduction: *Candida albicans* colonises various mucosal surfaces including the gut and oral cavity. In the latter, colonization efficacy depends on strain-specific characteristics and on induction of host responses: while *C. albicans* strain SC5314 is cleared from the oral mucosa of experimentally infected immunocompetent mice, strain 101 can persist for a prolonged period of time.
Method: To determine whether these strains also differ in their ability to colonise the murine gut, we used C57Bl/6 mice in specific—pathogen free (SPF) and germfree (GF) status and colonized them by single and/or mixed oral gavage. To further investigate the impact of the oral niche on gut colonisation, we infected mice sublingually. Fungal colonization was monitored by plating of faecal samples over 14 days.
Results: In single strain colonisation, the faecal burden of strain 101 was higher than that of strain SC5314 in SPF mice, whereas colonization levels were similar in GF mice. In contrast, SC5314 outcompeted 101 during co-colonisation in both GF and SPF mice. The findings were corroborated in the oro-pharyngeal (OPC) infection model. *In vitro* experiments also indicate a strain-intrinsic and general growth advantage of SC5314 in comparison to 101 under certain environmental conditions.
Conclusion: Thus, both strains are capable of colonizing the murine gastrointestinal tract, with 101 having an advantage when cohabiting with resident microbiota while SC5314 has an intra-species advantage. The result that two *C. albicans* strains that differ significantly in their ability to colonise the oral mucosa, show a more uniform behaviour in gut colonization than in the oral mucosa suggests that strain-specificity of *C. albicans*-host interactions may vary between specific anatomical sites.

156/EKP

Characterising mutational effects of the CFTR using patient-specific airway organoids

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Introduction: Cystic fibrosis (CF) is a multi-organ disease which is caused by mutations of the CFTR gene and primarily impacts fluid homeostasis by impairment of ion transport at epithelial barriers. The lung tissue of CF patients is predominantly affected, since CFTR mutational effects lead to accumulation of thickened mucus, which in turn is thought to allow pulmonary pathogens to persevere in the lung. This can lead to chronic inflammation and irreversible tissue damage. Over 2000 mutations within the CFTR gene have been reported so far, painting a complex CF disease phenotypic landscape hindering development of generalized effective therapies. However, recent advances and development of CFTR modulator drugs considerably improved CF therapy for many patients. It is still unclear how different CFTR mutations precisely impact airway epithelial cells at the cellular and molecular level and how they contribute to the severity of CF disease, in particular, in the context of infections. A comprehensive characterization and quantification of different CFTR mutational

effects and their consequences on organizational and functional aspects of the airway epithelium could elucidate this and help to adapt existing and develop novel therapies.
Methods: We are currently establishing a biobank of human airway organoids which are derived from nasal brushings of cystic fibrosis patients and will perform baseline characterizations and quantifications of the airway epithelial structure and function in airway organoid-derived monolayer (ODM) using live cell imaging approaches and image analysis. In a second step we will characterize airway epithelial structure, function as well as host tropism and immune responses after infection of ODMs with the CF relevant pathogens Mycobacterium abcessus (MABS), Pseudomonas aeruginosa and Exophiala. Finally, we will analyze the functional impact of different modulator treatments on the airway epithelium before and after infection using a forskolin-induced swelling assay.
Results: We could successfully establish CF patient-specific airway organoids and establish infection parameters for MABS showing that the pathogen can actively infect the airway epithelium in vitro. Internalization of MABS could be shown by immunofluorescence stainings of ODMs.
Discussion: Here, we show that CF patient-specific airway organoids and ODMs can be established from nasal brushings of CF patients. Both models will further be used as a valuable tool to study various bacterial and fungal infections in the context of CF, as has shown our infections with MABS. Our long-term goal is to comprehensively characterize and quantify the effects of CFTR mutations and their consequences on organizational and functional aspects of the airway epithelium.

Clinical Cases in Medical Microbiology (StAG KM)

157/KMP
BCG disease of the pleura
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Background: *Mycobacterium tuberculosis* (MTB) var. Bacillus Calmette-Guérin (BCG) is an attenuated live vaccine used worldwide to protect against disseminated tuberculosis (TB) and to treat bladder cancer. BCG disease is rare in the absence of primary or acquired immunodeficiencies that result in an impaired interferon-gamma (IFN-γ) -mediated immunity, such as Mendelian susceptibility to mycobacterial diseases (MSMD) or acquired immunodeficiency syndrome (AIDS). Here we describe the case of a 60-year-old female patient with advanced tumour disease and cultural evidence of BCG in the pleural effusion.
Case Report: A 60-year-old female smoker with chronic obstructive pulmonary disease GOLD 2 and lung cancer stage cTx cT1b N3 M0 in remission was hospitalised with septic shock due to pneumococcal pneumonia. The course was complicated with the occurrence of a lung abscess, uncomplicated parapneumonic pleural and pericardial effusions, and secondary organising pneumonia. Bronchoscopy excluded cancer recurrence. Antibiotic treatment and systemic corticosteroids (prednisone with an initial dose of 20 mg daily and slowly tapering) for four months resulted in clinical improvement and complete resolution of the lung infiltrates, pleural and pericardial effusions on CT imaging. Eight weeks after the thoracentesis, one of three aspirates of the pleural effusion grew mycobacteria of the MTB complex, which were later identified as BCG. The cultures of the bronchial secretions remained negative. The patient could only remember a BCG vaccination in her youth without being able to give further details. For the treating clinicians, the significance of BCG in the pleural effusion remained unclear. Possible laboratory contamination was excluded by whole genome sequencing (WGS) of both the clinical isolate and the laboratory strain.
Conclusions: The detection of MTB complex not infrequently leads to diagnostic ambiguities in complex clinical situations such as that described in our case study. With WGS, specialised laboratories are now able to assist in clarifying the clinical situation.

Infection Immunology (FG II)

158/IIP
Comparative performance measurement of particle agglutination assays for antibody detection against *Treponema pallidum pallidum*
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Introduction: The particle agglutination assay is an essential part of the stepwise diagnostic of syphilis according to the actual guideline. The assay confirms the presence of antibodies against *Treponema p. pallidum*. Particle assays differ in the use of gelatin particles (TPPA), bird erythrocytes (TPHA) or latex particles (TPLA) as antigen carriers. The TPPA from Fujirebio is regarded as gold standard in syphilis diagnostics. However, Fujirebio decided to stop the production in 2022. For TPHA, the Paul-Ehrlich-Institute published an advice against its use after a series of false-negative results for blood donations in 2020. The need of an alternative for the TPPA makes a comparative performance measurement of particle agglutination assays inevitable to define new guidelines for future syphilis diagnostics.
Material and Methods: Serum specimens were pre-analyzed with the screening test Alinity i Syphilis TP CMIA (Abbott). Based on in-house validation results of 0.3–0.9 were evaluated as borderline. The following particle agglutination assays were included for comparison: Sekure TPLA Assay (SEKISUI Diagnostics, TPLA), TPPA (Serodia Fujirebio Inc.), TPHA (Bio-Rad Laboratories Inc.) and TPHA (Newmarket Biomedical Ltd.). Correlation coefficient (R) was calculated.
Results: For comparison, 684 specimens were analyzed in both TPHA tests and TPPA showing 555 negative and 129 positive results in TPPA. The TPHA tests showed a correlation of R=0.87 to TPPA results. For TPLA analysis, high variation in titer levels of TPPA and titer units of TPLA were observed and a correlation of R=0.73 was calculated.
One aim of our study was to test the reliability of TPHA and TPLA regarding the detection of highly infectious specimens like from patients with primary affection. Analysis of a group with known primary affection (n=22) revealed a detection rate of 13.6 % for both TPHA assays (table 1). Detection rate of TPLA was 40.9 % and 100 % in CMIA, if borderline values were included. Another important study group consists of specimen with residual antibody findings past infection and treatment. Here, differences can be seen according to the titer level in TPPA. Specimens (n=27) with residual antibodies and titer level ≤320 were detected with a rate of 22.2 and 33.3% in TPHA tests (table 2).
Discussion: The aim of our study was to compare particle agglutination assays with the gold standard TPPA to identify alternative assays. Analysis by TPLA showed insufficient correlation and instable titer level detection. TPHA assays show almost no differences between manufacturers. Both assays fail in the detection of highly infectious specimen from patients with primary affection. Additionally, specimen with low titer levels of residual antibodies were almost not detected. Taken together, it seems that none of the tested particle agglutination assays could replace the diagnostic reliability of the no longer produced TPPA. Therefore, the current guideline has to be revised.

Fig. 1

Table 1: Detection rate of specimen with known primary affection.

n=22	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
Positive	100.0 %	100.0 %* (63.6%)**	40.9 %	13.6 %	13.6 %
False Negative	0.0 %	0.0 %* (36.4 %)**	59.1 %	86.4 %	86.4 %

*Borderline values are included. **According to the manufacturers interpretation criteria.*

Fig. 2

Table 2: Detection rate of specimen with residual antibody findings past infection and treatment.

n=27	TPPA (Fujirebio)	CMIA (Abbott)	TPLA (SEKISUI)	TPHA (Bio-Rad)	TPHA (New Bio)
Positive	100.0 %	100.0 %*(81.5 %)**	92.6 %	74.1 %	77.8 %
False Negative	0.0 %	0.0 %	8.1 %	21.6 %	18.9 %
TPPA ≥640 (n=18)	100.0 %	100.0 %**	100.0 %	100.0 %	100.0 %
TPPA ≤320 (n=9)	100.0 %	100.0 %* (44.4 %)**	77.8 %	22.2 %	33.3 %

*Borderline values are included. **According to the manufacturers interpretation criteria.*

159/IIP

Screening in the context of syphilis diagnostics – threshold ranges for automated Syphilis immunoassays

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Introduction: Automated syphilis screening tests are used in laboratory routine. Positive samples are further tested according to the syphilis stepwise diagnostic. During evaluation studies prior to the introduction of screening tests into routine use, it was noticed that some positive sera were not detected. There is no defined threshold range from the manufacturer for those routinely used screening assays. Therefore, we have tested whether the definition of a threshold range can identify samples, which can then be further investigated with other tests and reduce the number of false negative results.

Material/ Methods: In total, 2091 samples were included in this study. Samples were first analyzed with Architect Syphilis TP CMIA (Abbott) and Elecsys Syphilis TP ECLIA (Roche) and then further characterized according to the syphilis stepwise diagnostic using TPPA, IgG-FTA-ABS-Test, 19S-IgM-FTA-ABS-Test, RPR-Test and immunoblot if required. Specificity was calculated for both screening tests. Sensitivity for both screening tests was on the one hand calculated according to the manufacturer's evaluation instructions (S/Co-Value <1.0= negative, S/Co-Value ≥1= positive) and on the other hand including a threshold range of 0.3 - <1.0. Samples within the threshold range also followed the stepwise diagnostic.

Results: The measured collective included all stages of Syphilis. The treponema antibody status was positive for 173 (8.3%) samples and negative for 1,914 (91.3%) samples; 4 (0.4%) samples were classified as indeterminate. Both tests (ECLIA and CMIA) gave false positive results twice each (1912/1914), resulting in a specificity of 99.9% each. According to the manufacturer's evaluation instructions, the sensitivity for ECLIA was calculated with 98.3% (170/173) and sensitivity for CMIA with 95.4% (165/173). For both tests, sensitivity was additionally calculated with a threshold range (0.3 - <1.0) through which the sensitivity could be improved to 100% (173/173 positive samples detected). In this context, for ECLIA 17 samples and for CMIA 22 samples were diagnosed within the threshold range (0.3 - <1.0). Further testing revealed that 3 (ECLIA) and 8 (CMIA) samples were positive, hence initial results were false negative.

Discussion: The introduction of a threshold range of 0.3 - <1.0 led to a significant increase in diagnostic confidence. It is especially important to correctly diagnose samples in an early seroconversion phase, because those patients are highly infectious (e.g. for blood bank screenings). Besides, the effort of additional sample measurement is manageable with about 1% of all negative tested samples.

160/IIP

OTUD7B fosters experimental cerebral malaria by inhibiting dendritic cell death

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Cerebral Malaria (CM) is a severe neurological complication of human malaria caused by the parasite *Plasmodium falciparum.* According to the WHO, 241 million cases of malaria were reported in 2020, resulting in 627 000 deaths globally. Studies using experimental cerebral malaria (ECM), a murine disease model of cerebral malaria caused by *Plasmodium berghei* ANKA (*Pb*A), have shown that dendritic cells (DCs) play an important role in the priming of parasite specific CD8+ T cells, which mediate the disruption of blood brain barrier and cause disease pathology.

OTUD7b, is a deubiquitinating enzyme which regulates both the canonical and the non-canonical NF-κB pathways. It removes K11, K48, and K63 linked polyubiquitin chains from target proteins, thereby regulating various cellular functions. It has been shown that in CD8+ T cells OTUD7b is essential for conferring protection against intracellular bacterial pathogen, *Listeria monocytogenesis*. However, the impact of OTUD7b on the course of ECM remains unknown. Therefore, to study the dendritic cell-specific function of OTUD7b in ECM, we generated novel conditional knockout mice in which *Otud7b* is specifically deleted in dendritic cells.

Upon *Pb*A infection, CD11c-Cre Otud7bfl/fl mice were protected from ECM while the WT mice succumbed to the infection. Flow cytometry analysis showed a decrease in numbers of splenic DCs and CD8+ T cell population and concomitantly reduced accumulation of CD8+ T cells in the brain of CD11c-Cre Otud7bfl/fl mice. Furthermore, OTUD7b deficient DCs showed impaired activation of NF-κB and MAP Kinase signalling pathways and were more susceptible to apoptotic form of cell death.

Taken together our data show that deficiency of OTUD7b induces DC cell death and thereby impairs the intracerebral accumulation of disease causing CD8+ T cells, thus protecting the mice from ECM. Collectively these results identify OTUD7B as a potential therapeutic target in DC to prevent ECM.

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Seroprevalence of humoral immunity against *Staphylococcus aureus* vaccine candidate Coproporphyrinogen III oxidase (CgoX)

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Staphylococcus aureus (*S. aureus*) represents a serious infectious threat to global public health. There is a high unmet medical need for a vaccine a to prevent or clear *S. aureus* infections. We have previously characterized a S. aureus vaccine candidate, coproporphyrinogen III oxidase (CgoX), which fulfils essential housekeeping functions in heme synthesis. Immunization with recombinant CgoX provided protection against lethal *S. aureus* infections in a murine sepsis model. Similarly, a monoclonal antibody (anti-CgoX-D3), raised against a highly conserved CgoX-D3 epitope, efficiently provided protection against S. aureus infection in mice. One major obstacle in vaccine development against *S. aureus* infection is the high prevalence of pre-existing antibody titers against more than 1,000 S. aureus antigens in healthy individuals, which, however, do not not generally protect against S. aureus infection. Thus, a first aim was to determine seroprevalence of anti-CgoX and anti-CgoX-D3 IgG titers in non-infected and *S. aureus*-infected patients. We developed an enzyme

linked immunosorbent assay (ELISA) to detect anti-CgoX and anti-CgoX-D3 antibodies in plasma samples by using whole protein CgoX and CgoX-D3 epitope peptide, respectively. In preliminary experiments, the majority of 28 non-infected and 13 S. aureus-infected patients (50% and 75%, respectively) had antibodies against CgoX, however only one patient with *S. aureus* infection had measurable antibodies against the CgoX-D3 epitope. Clearly, the real challenge for any *S. aureus* vaccine candidate is to demonstrate that the immune response elicited by the S. aureus vaccine is protective. To this end we set up a biomarker assay that tests the ability of a serum or plasma sample, to protect phagocytes from *S. aureus*-induced cell death. We noticed that GMP grade anti-CgoX-D3 exerted a dose-dependent, neutralizing activity against *S. aureus*-mediated death of phagocytic host cells. By combination of primary murine phagocytes or long-term cultured THP-1 cells on the one hand, with *S. aureus* or *E. coli* transformed with either wild type CgoX or mutant CgoX lacking the D3 epitope on the other hand, we established a cytoprotection assay that distinctively measures CgoX- and CgoX D3 epitope-specific protectivity. Notably, only the very serum plasma that showed binding to the D3 epitope, tested also positive in the THP-1 protection assay. These findings suggest that protective activity corresponds to D3 epitope binding but not with high antibody titers against full length CgoX. These data also suggest that in humans, immunization with the entire CgoX protein might not be suited to significantly improve the already existing (and non-protective) immunity against *S. aureus*. In contrast, active immunization with the CgoX-D3 epitope seems to be a valuable modality to elicit a new quality of protective antibodies against *S. aureus* in humans.

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Deficiency of Sentrin-specific protease-1, -2 and -5 in THP-1 derived macrophages confers protection against *Listeria monocytogenes* infection

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Introduction: *Listeria monocytogenes* is a facultative intracellular Gram-positive bacterium which can cause severe systemic and brain infections in immunocompromised patients. Cell-mediated immune responses are important to control listeriosis and the innate and adaptive immune response are critically regulated by posttranslational modifications including SUMOylation of signaling molecules. SUMOylation is a process in which Small Ubiquitin-Like Modifier proteins (SUMO) are covalently attached to lysine residues of target proteins to regulate diverse cellular functions. The sentrin-specific proteases- (SENP) 1-7 mediate the deconjugation of SUMO moieties by cleaving the isopeptide bond between SUMO and lysine residues of the target proteins but their function in listeriosis is largely unknown. Therefore, we aim to investigate the role of sentrin-specific protease- (SENP) 1, -2 and -5 in listeria-infected human THP1-cells as a model for human macrophages.
Material and methods: SENP1-, 2- and 5-deficient THP1-cells were generated using CRISPR-Cas9 system and differentiated into macrophages using PMA. Wildtype (WT) as well as SENP1-, 2- and 5-deficient macrophages were infected with *L. monocytogenes* (MOI=1) for one hour followed by gentamycin treatment (30 µg/ml) to remove the extracellular bacteria. 1.5 and 24 hours post infection, cells were lysed and cell lysates were plated on BHI agar. Bacterial colonies were counted microscopically after incubation at 37°C for 24 h.
Results: The bacterial load was significantly reduced in SENP1-, 2- and 5-deficient THP1-macrophages after 1.5 and 24 hours of infection compared to the wild-type cells. Additional WB analysis from Listeria-infected WT and SENP5-deficient THP1-cells showed an increased activation of NF-kB in SENP5-deficient THP1-macrophages compared to WT cells as indicated by reduced levels of the NF-kB inhibitor IkBα.
Discussion: Our initial experiments show that deletion of SENP1, 2 and 5 in THP1-cells improves the pathogen control during listeria infections, thereby identifying SENP1, 2 and 5 as a potential therapeutic target in the treatment of listeriosis.

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Automated prediction of *S. aureus* MHC class I epitopes at a global scale

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Introduction: A hallmark of the adaptive immune response is the interaction of surface MHC class I bound peptide complexes and the T cell receptor of CD8+ T cells leading to T cell activation. The importance of the antigen processing and presentation pathway for the generation of viral epitopes is undisputed. In addition, there is evidence that cellular infection by bacteria such as *Staphylococcus aureus* is accompanied by presentation of MHC-bound peptide epitopes which can be recognized by specific cytotoxic T cells. To verify the involvement of a CD8+ T cell response in the elimination of bacterial pathogens, identification of specific bacterial MHC class I epitopes is necessary and still at the beginning. One problem is the high bacterial protein content which makes it difficult to search for immunogenic proteins or even specific epitopes. Therefore, we established a bioinformatic approach to screen the whole proteome of bacteria for epitope candidates and to identify highly immunogenic regions with many putative epitopes for different MHC-class I haplotypes.
Methods: We developed a computational tool to retrieve proteome datasets and to predict associated epitopes. Complete datasets were further filtered by selecting different *S. aureus* strains or by protein localization. Amino acid sequences were posted to IEDB"s Analysis Resource and predictions were performed for different MHC-I alleles. For each protein the epitope density, i.e. the number of predicted epitopes per total possible 9mer peptides, was calculated. In proteasomal processing experiments synthetic precursor peptides containing overlapping regions of epitopes binding to different MHC class I haplotypes were digested *in vitro* with purified 20S proteasomes and the resulting peptide fragments were analysed by HPLC-MS. The putative epitopes were tested for their MHC binding affinity.
Results: We were able to retrieve datasets for MHC class I-epitopes derived from different *S. aureus* strains and proteins. The selected protein candidates have predicted epitopes for all chosen HLA haplotypes which were visualized by heatmaps where regions of overlapping epitopes are highlighted. Finally, we were able to investigate proteasomal processing and MHC-binding of the identified epitopes. Currently, these peptide epitopes are analysed regarding their capacity to stimulate specific human CD8+ T lymphocytes.
Discussion: The results are based on stochastic predictions. So far, four well-annotated *S. aureus* strains which display highly conserved proteins were included in our analysis. For future experiments the number of strains will be extended to strains isolated from patients. In summary, we developed a powerful tool to predict a number of possible epitope candidates which have to be further tested for their relevance in CD8+ T cell responses and may provide the basis for novel vaccination strategies.

Microbial Pathogenicity (FG MP)

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Genomic, transcriptomic, and phenotypic investigation of pathogenic *Escherichia coli* reveals unique markers such as the L-sorbose phosphotransferase system (PTS)

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Introduction: Since the discovery of penicillin in 1928, the over- and misuse of antibiotics resulted in the emergence of multidrug-resistant (MDR) bacteria including carbapenem-resistant and extended-spectrum beta-lactamases (ESBL)-producing Enterobacterales. The facultative pathogen *Escherichia (E.) coli* is often responsible for a wide range of infections. Previously, the spread of particular bacterial subtypes, so-called international, high-risk clonal lineages that combine MDR phenotype with fitness and virulence, has been recognized. These include the *E. coli* sequence types (ST)131 and ST648 and others, which place a high burden on public health. The treatment with conventional antibiotics is increasingly difficult. Thus, this project aims to develop new treatment strategies, where identified and verified pathogenic markers might be leveraged as novel therapeutic targets and the healthy intestinal microbiome of the host is not addressed.
Material and Methods: A bioinformatics approach analyzing 22,267 pathogenic and commensal *E. coli* genomes was performed to create a patho-coregenome including unique markers of pathogenic STs. Based on these genomic outcomes, phenotypic assays were conducted to verify the results. In addition, growth kinetics and Northern blotting were performed to analyze the expression of selected markers in pathogenic and commensal STs.
Results: The patho-coregenome analysis revealed the operon for an L-sorbose phosphotransferase system (PTS) among 27 identified markers. The use of different pH indicators in solid and liquid cultures confirmed the unique utilization of L-sorbose in pathogenic STs encoding the L-sorbose PTS. Northern blotting verified the induction in ST131 and ST648 in the presence of 1% (m/v) L-sorbose during early stationary phase on transcriptomic level. When comparing the growth kinetics of all STs, it became evident that pathogenic STs showed diauxic growth and a significant increase in cellular densities in the presence of L-sorbose. These changes in growth behaviors were associated with the induction of L-sorbose utilization.
Discussion: This study identified and verified the L-sorbose PTS as a unique marker of pathogenic *E. coli* STs on phenotypic and transcriptomic levels. PTSs not only provide the ability to utilize specific sugars but are described to be involved in the regulation of pathogenic processes. Literature indicates the importance of such specific metabolic abilities to colonize certain niches. The improved colonization might then support the development of MDR strains. Identification of the L-sorbose PTS in pathogenic *E. coli* contributes to a better understanding of the success features of MDR, high-risk clonal lineages. Moreover, inhibiting such colonization could be a new promising therapeutic approach. Proteome analyses are currently conducted to further investigate the co-regulation of other pathways and the suitability of this target for novel therapeutic strategies.

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Identifying the fibronectin binding regions and genomic variation of *Bartonella henselae* adhesin A

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Introduction: *Bartonella henselae* is a facultative intracellular zoonotic pathogen and is the causative agent of cat scratch disease and other clinical entities such as endocarditis and bacillary angiomatosis. Bacterial adhesion is crucial in the early stages of pathogenic infections. Accordingly, *B. henselae* principally targets endothelial cells in which the *Bartonella* adhesin A (BadA) is essential for adherence to host cells and extracellular matrix proteins such as fibronectin (Fn). Furthermore, BadA is a trimeric autotransporter adhesin (TAA), is modularly structured including a head domain, a long and repetitive neck/stalk region, and a conserved C-terminal anchor domain, and is responsible for angiogenic reprograming of host infected cells. The identification of BadA-Fn binding regions would support the development of new "anti-adhesive" components that inhibit the initial adherence.
Methods: The *badA* genomic island of eight *B. henselae* strains was analysed using long-read PacBio SMRT sequencing. Various truncated BadA (fusion) proteins were constructed in a *B. henselae* Marseille BadA-transposon mutant (ΔBadA-T) via Gibson Assembly® technology. Surface expression of BadA proteins was assessed via confocal laser scanning microscopy using rabbit anti-BadA IgG antibodies and transmission electron microscopy. Identification of potential Fn binding site(s) was performed via ELISA and fluorescence microscopy. Immunogenicity of few truncated BadA proteins was assessed via Western blotting using human sera of 13 patients diagnosed with for example cat scratch disease.
Results: The *badA* gene (8.7 to 13.2 kb long) and flanking *badA* pseudogenes show a high intraspecies variability, occasionally including a peculiar 18 nt-long repeat sequence motif. *B. henselae* Marseille contains a *badA* gene of 11,922 bp and consist of 30 neck/stalk domains displaying a repetitive superimposed domain sequence pattern. Furthermore, six additional truncated BadA mutants were each expressed on the outer membrane of *B. henselae* Marseille ΔBadA-T. Truncated BadA constructs retain their immunodominant characteristic. The BadA neck/stalk domain composition influences Fn binding capacity with a focus on residue motifs present in domains 19 and 27.
Discussion: The variable and repetitive genomic *badA* island indicates frequent reshuffling of homologous domain regions, facilitated via active recombination mechanisms, potentially contributing to differences in virulence and host immune evasion. Presumably, BadA adheres to Fn in a cumulative effort with quick saturation, primarily via species-conserved unpaired β-sheet hairpins of particular domains repeatedly present throughout the neck/stalk region. The localisation of the main BadA-Fn binding regions provides a basis towards new "anti-adhesive" components in preventing initial adherence of *B. henselae* and other TAA-expressing pathogens in the course of infection.

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Fibronectin-mediated host cell adhesion as a general principle in infections with human pathogenic bacteria

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Introduction: Binding to fibronectin (Fn) has been reported for many Gram-positive and -negative bacteria. Fn-mediated binding to host cells represents a plausible mechanism for bacteria-host cell interaction. In this study, we examined the relevance of Fn in host cell binding of various human pathogenic bacteria (*Acinetobacter baumannii*, *Bartonella henselae*, *Borrelia burgdorferi*, *Staphylococcus aureus*) to human endothelial cells (ECs).

Material & Methods: RNA expression in ECs for extracellular matrix (ECM) protein genes was assessed via transcriptome analysis (RNAseq). Bacterial binding to Fn was evaluated using an *in-house* indirect ELISA. Infection experiments were performed using Fn-expressing or Fn-knockout ECs (LentiCRISPR). The number of bacteria adherent to host cells was quantified via qPCR. Localization of adhered bacteria to cellular Fn fibres was assessed by immunofluorescence and high-resolution confocal microscopy (CLSM).
Results: Transcriptome analysis of ECs revealed that *FN1* (fibronectin) was the most abundantly expressed ECM protein-coding gene and was listed under the ten highest expressed genes in ECs. Bacterial adhesion to Fn occurred in a dose-dependent manner for all bacteria analyzed in this study. CLSM analysis revealed a preferred binding of bacteria to Fn-fibres. Loss-of-function infection experiments demonstrated a reduction in bacterial binding to Fn-deficient cells (*A. baumannii* -25%, *B. henselae* -58%, *B. burgdorferi* -34% and *S. aureus* -79%).
Discussion: Our findings confirm the general role of Fn in the adhesion process of pathogenic bacteria to human cells and suggest that this interaction is a much more general principle in infection biology than acknowledged before. Perspectively, targeting Fn binding seems to be a conceivable strategy for inhibiting bacterial adhesion to host cells.

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Analysis of *Yersinia*-mediated cell death pathways in human macrophages

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Introduction: Infection with the enteropathogenic bacterium *Yersinia enterocolitica* mediates cell death and inflammation in macrophages. This is triggered by the effector protein YopP which is injected by a type III secretion system into host cells. YopP induces apoptosis through the deactivation of several TLR-induced signalling pathways. This involves the inhibition of TAK1 through YopP which leads to activation of RIP1 that in turn activates caspase-8. Studies in mouse macrophages show that caspase-8 can then activate the pore forming protein gasdermin D, resulting in pyroptosis and the release of interleukins. Consequently, GSDMD seems to play an important role in modulating cell death response upon *Yersinia* infection. The related protein gasdermin E, on the other hand, does not seem to have similar effects. Even though it is cleaved into its active fragment, there are no signs of GSDME influencing either cell death or the release of IL1b in mouse macrophages. While the roles of gasdermins during *Yersinia* infection in murine macrophages have thus been the focus of several studies, their relevance in human macrophages has not yet been extensively investigated.
Methods: Our research focusses on investigating the involvement of gasdermins in human macrophages infected with *Yersinia* with special emphasis on the role of YopP. This is accomplished by studying protein processing, interleukin release and cell death rates in primary human macrophages and PMA-differentiated THP-1 cells upon infection with wild type *Yersinia enterocolitica* and *Yersinia* strains lacking YopP. Furthermore, involvement of the catalytic activity of RIP1 was investigated using a RIP1 kinase inhibitor.
Results: So far, our data showed that while caspase-8 was activated during *Yersinia* infection, GSDMD seemed not to be processed and thus might not trigger pyroptosis or influence interleukin release in human macrophages. The related GSDME, on the other hand, was processed into its active form along with caspase-3, which is responsible for cleaving GSDME. Furthermore, we investigated cell death rates and interleukin release upon *Yersinia* infection. We found that absence of YopP decreased cell death and increased the release of IL1b and TNFa. Additionally, *Yersinia*-induced cell death in human macrophages seemed not to depend on the RIP1 enzymatic activity as RIP1-kinase inhibition did neither increase nor decrease cell death rates.
Discussion: The absence of active GSDMD coupled with the processing of GSDME might suggest that *Yersinia* infection leads to the activation of different cell death pathways in human and murine macrophages. Further studies are required to address whether GSDME triggers pyroptosis in human macrophages and how it may affect cell death or interleukin release.

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Staphylococcus aureus strains Newman and its variant Newman D2C differ markedly in their abilities to adhere to and to form biofilms on plastic-like artificial surfaces

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Introduction: *Staphylococcus aureus* Newman is one of the most frequently used laboratory strains in the staphylococcal research community, for which at least two variants exist: the highly mouse pathogenic and cytotoxic derivative Newman, and the variant Newman D2C, distributed by the American Type Culture Collection as ATCC25904. The latter Newman derivative harbors among others point mutations in the global response regulator genes *agrA* and *saeR*, which strongly impairs the capacity of Newman D2C to express exoproteins such as nucleases and hemolysins, and renders this derivative less pathogenic in mouse models of infection. As larger differences in the capacity of strain Newman to form biofilms under in vitro conditions were reported between various research labs, and given the impact of nuclease and *agr* on biofilm formation of *S. aureus*, we speculate that these differences might be due to the utilization of different Newman derivatives, which were inadvertently all indicated as *S. aureus* "Newman".
Methods: *S. aureus* strains Newman and Newman D2C were tested for their capacities to express exonucleases, to adhere to and to form biofilms on polystyrol (PS)-based plastic surfaces in vitro, and to affect infectivity in a murine foreign body-related *S. aureus* infection model.
Results: Spotting fluorescence-stained exponential growth phase cells of strains Newman and Newman D2C on PS-based petri dishes revealed that Newman D2C cells adhered in significantly larger quantities to this kind of plastic surface than cells of strain Newman. Similarly, Newman D2C cells produced significantly larger biomasses on PS in the static 96-well cell culture plate-based biofilm assay after 24 hours of growth in Tryptic Soy Broth at 37°C than strain Newman. When cells of strains Newman and Newman D2C were used to infect peripheral venous catheter (PVC) fragments implanted into the flanks of mice, no clear differences in the bacterial cell numbers of the vegetation formed on the PVC tubing were observed for both strains. However, mice challenged with strain Newman produced significantly larger edema sizes at the PVC implantation sites and higher bacterial loads in the tissues surrounding the PVC fragments at 10 days post infection than mice infected with the Newman D2C variant, which is in line with the in vitro observations that the former derivative secretes larger amounts of nucleases into the extracellular milieu than strain Newman D2C.
Conclusions: Our results demonstrate that strains Newman and Newman D2C differ in their capacities to adhere to PS-based plastic surfaces and to form biofilms in vitro. Researchers working with strain Newman should be aware of this fact and should report which Newman variant they used in their studies.

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Elucidation of nodes of interaction between Yersinia outer proteins (Yops) and host cells via TurboID proximity labeling

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Introduction: The main virulence system of pathogenic *Yersinia* spp. is a type III secretion system that mediates the translocation of effector proteins (Yersinia outer proteins; Yops) in infected cells. These Yops interact with host proteins and exhibit a manifold of functions in modulating host cell immune responses. Nonetheless, the molecular mechanisms of the crosstalk of Yops with host cellular proteins is often ill defined. In recent years proximity labeling (i.e. TurboID) proved to be a powerful tool for interactom studies. Here, we created four Yop-TurboID fusion constructs to identify Yop interactions and to broaden the knowledge about host cell responses in regard to infection with *Yersinia* spp.
Material/Methods: TurboID is a protein, which biotinylates proteins in close vicinity (~ 10 nm) to the protein of interest. Fusion constructs between TurboID and YopP, YopQ, YopE and YopH were created and introduced into the corresponding knockout Yersinia lineage. HELA or J774 cell lines were infected and immunoprecipitations of biotinylated proteins via streptavidin-beads were performed. These were subsequently analysed by mass spectrometry.
Results: Our results indicate robust biotinylation of host cell proteins by TurboID-tagged Yops. Accordingly, known targets of YopP were detected to be biotinylated by YopP-TurboID translocated by the Yersinia type III secretion system into infected host cells. Further data for YopP and for other Yops are currently under analysis.
Discussion: Here, the TurboID proximity labeling system was used to analyse the interactoms of four Yops, which was done, to our knowledge, for the first time under physiological in vitro infection conditions. Proximity labeling via TurboID appears to be a powerful tool for the elucidation of the interactoms of translocated bacterial effector proteins, including non-stable interaction nodes.

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Intestinal epithelial barrier dysfunction caused by the cyanotoxin microcystin visualised by super-resolution STED microscopy in Caco-2 cell monolayers

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Introduction: Microcystin from fresh water cyanobacteria *Microcystis* spp. is an emerging contaminant, causing diarrhea after ingestion of freshwater. However, the role of this toxin on intestinal barrier function and its mechanisms in provoking diarrheal symptoms is not known. Our study focused on the action of the toxin leading to diarrhea mechanisms such as epithelial barrier disruption in a small intestinal Caco-2 cell model.
Method: Human Caco-2 epithelial cell monolayers were treated with microcystin-LR (MC-LR) and then characterized for barrier function on the functional and molecular level by electrophysiological measurements of electrical resistance and Western blot or STED microscopy. Tight junction (TJ) changes and morphological changes were analyzed in conjunction to the changes in transepithelial electrical resistance (TER).
Results: Microcystin-treated Caco-2 cells revealed a reduction of TER to 65% of the initial value after 24 h. Concomitantly, permeability for fluorescein was 2.6-fold increased (P<0.001, n=8, student's t-test with Welch's correction). In Western blot analysis for barrier-forming TJ proteins, claudin-1 expression was reduced, while expression of claudin-3 and claudin-4 was unchanged. However, TJ integrity was compromised by undulations, fraying and splitting of the TJ domain of the epithelial cells. This could be visualized by immunostaining for TJ proteins and super-resolution STED microscopy. Epithelial apoptosis induction did not contribute to the barrier disruption. The perijunctional cytoskeletal actomyosin contraction was found to be responsible for the TJ de-regulation and the barrier dysfunction.
Discussion: Ingestion of the cyanotoxin microcystin leads to epithelial leakiness, contributing to a leak flux type of diarrhea as pathomechanism. Unopposed myosin light-chain phosphorylation triggers the cellular mechanism of TJ rearrangement. The diarrhea by microcystin is explained by the passage of water and solutes through an opened paracellular pathway.

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Establishing novel therapeutic compounds against *Helicobacter pylori* motility and flagella and target identification

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Background: New treatment options against the widespread cancerogenic gastric pathogen *Helicobacter pylori* are urgently needed, due to rising antibiotic resistance and side effects of combination therapy.
Results: Screening various compound libraries for inhibitors of *H. pylori* flagellar biosynthesis and motility using a luciferase reporter strain, we identified numerous virulence blockers ("antimotilins") that inhibit *H. pylori* motility or the flagellar type III secretion apparatus. We identified compounds that either inhibit both motility and the bacterial viability, or the flagellar system only, without negatively affecting bacterial growth. Novel anti-virulence compounds which suppressed flagellar biosynthesis in *H. pylori* were active on pure *H. pylori* cultures *in vitro* and partially suppressed motility directly, reduced flagellin transcript and flagellin protein amounts. Two main actives identified were termed Active1 and Active2, which had divergent effects on bacterial viability and flagellar regulation. We successfully performed a proof-of-principle treatment study in a mouse model of early chronic *H. pylori* infection, and demonstrated a significant effect on *H. pylori* colonization for one antimotilin termed Active2, even as a monotherapy in a therapeutically meaningful dose. Plasma immune parameters and diversity of the mouse intestinal microbiota were not significantly affected by Active2.
In the next step of our project, we aim to specifically identify the target(s) of selected compounds. For this purpose, we performed swimming assays, transcript quantification of important targets and comprehensive transcriptome analyses. Those assays revealed that antimotilin compound Active2 showed a pleiotropic effect with a strong bias on *flaA* regulation, motility and flagellar assembly.
Conclusions: The novel antimotilins active against motility and flagellar assembly bear promise as a viable alternative to commonly used antibiotic-based combination therapies for treating and eradicating *H. pylori* infections. Further development will benefit from identifying and characterizing molecular targets and improving the active compound properties.

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***Clostridioides difficile* binary toxin (CDT) effects on the epithelial barrier function**

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Introduction: *Clostridioides difficile* (*C. difficile*) infection (CDI) is the most common cause of antibiotic-associated colitis. Main virulence factors produced by *C. difficile* are the glycosylating toxins TcdA and TcdB, and additionally in hypervirulent *C. difficile* strains the binary ADP-ribosylating toxin CDT. The presence of this CDT toxin is associated with an increased lethality, indicating additive pathomechanisms with severe outcome. The objective of our study was to investigate the impact of CDT on the intestinal epithelial barrier and to enlighten the underlying cellular mechanisms in CDI.
Method: Human colonic HT-29/B6-GR/MR epithelial cell monolayers were treated with sublethal concentrations of CDT.

The functional integrity of the epithelial barrier was assessed measuring the transepithelial electrical resistance (TER) and the paracellular fluxes of 332 Da fluorescein and 4 kDa FITC-dextran. To analyze the impact of the induction of cell death, LDH-release was measured, and the rate of epithelial apoptotic cells was determined by TUNEL-staining. We examined changes in subcellular location of bi- and tricellular tight junction proteins (TJ), using confocal laser-scanning microscopy (LSM). Western Blot analysis of TJ proteins was performed to check for changes in protein expression.

Results: Apical treatment of HT-29/B6-GR/MR cell monolayers with a sublethal concentration of CDT reduced the TER to 58±3% (n=6) of the initial value when compared to untreated control after 24h (100±2%; n=6; $p<0.05$ as indicated by Student's t-test). The paracellular fluxes of the markers fluorescein and 4 kDa FITC-dextran were 3.3-fold and 5-fold increased, respectively. With these sublethal CDT concentrations, necrosis induction, measured by LDH release, and the rate of apoptotic cells, measured by TUNEL assay, were unchanged. However, changes in the subcellular distribution of TJ proteins were seen using LSM, and the TJ protein tricellulin was redistributed off the tricellular TJ domain in CDT treated cells.

Discussion: In conclusion, we showed a compromised epithelial barrier function by CDT in a human intestinal colonic cell model, as indicated by a decrease in TER and an increase in the paracellular permeability for small macromolecules such as fluorescein and 4 kDa FITC-dextran. Pointing to barrier dysfunction and leak flux as diarrheal mechanism. However, this cannot be attributed to the induction of apoptosis and necrosis, but rather to an opening of the paracellular leak pathway as the result of epithelial tight junction alterations.

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A new assay for measuring Type VI Secretion *in vivo* in *P. aeruginosa*

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Introduction: *P. aeruginosa* (*Pa*) is a potential human pathogen causing infections ranging from skin and soft tissue infections to pneumonia and septic shock. It can express a variety of virulence factors, one of which are its three Type VI secretion systems (T6SS). Those needle-like secretion systems deliver effector proteins into eukaryotic host cells or competing bacteria [1]. One effector protein of the T6SS of *Pa* is the cell-wall amidase AmpDh3 [2]. In addition to being an anti-bacterial effector, AmpDh3 plays a role in cell wall recycling in *Pa* and has an impact on AmpC-mediated beta-lactam resistance [3]. Although T6SS are known to play an important role in infection and pathogenesis [4], to our knowledge, direct translocation of a T6SS-effector from cell to cell has not been shown in vivo without interfering with the T6SS's regulation itself. In this project, we aimed to establish a novel assay to measure the translocation of AmpDh3 in order to gain further insight into the regulation and impact of Type VI secretion.

Method: By fusing a small subunit of a luciferase to AmpDh3 and expressing the other subunit in a competitor bacterium, we can detect the translocation of AmpDh3 in vivo upon co-incubation via a luminescence signal. We used genomic knock-out mutants to confirm the T6S-dependency.

Results: Using this assay, we observed a luminescence signal arising from translocation of AmpDh3 following co-incubation of the two strains, reaching its peak after 1.5 h and decreasing thereafter. The signal is diminished in T6S-deficient strains and increased in strains over-producing AmpDh3 compared to the wild-type.

Discussion: We present the first assay to observe the activity of a T6S-dependent effector delivery system from prey to predator in vivo without interfering with the regulation of the T6SS itself. This will allow us to investigate the regulation mechanisms of T6S under different growth conditions, in different mutant backgrounds and with different prey species. Combined with results from competition or promotor activity assays, this will allow us to understand the role of T6SS in competitiveness of *Pa*.

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***In silico* analysis of *Klebsiella pneumoniae* lipoproteins and elaborating a molecular toolbox to study host-pathogen interactions**

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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* cause urinary tract and bloodstream infections as well as pneumonia and hypervirulent are responsible for liver abscesses and meningitis. Treatment is complicated by the high antimicrobial resistance of *K. pneumoniae* strains and a vaccine for prevention is not available. Because *K. pneumoniae* infections are a major threat new anti-virulence strategies have to be evolved. Lipoproteins represent promising candidates for protein-based vaccination strategies due to their surface-exposure, conservation and potential immunogenicity. Hence, a function-structure analysis of lipoprotein is required to understand their role in host-pathogen interactions and elucidate their immunogenic potential.

Methods: The genome of *K. pneumoniae* ATCC BAA 2146 was analyzed in silico to identify genes encoding for lipoproteins using locateP and LipoP. One hundred and twenty-three genes were identified and gene specific primers were designed for PCR amplification, cloning into expression vector pTP1 and heterologous expression in *E. coli* to generate a *K. pneumoniae* protein library for heterologous expression in *E. coli*. Transposon mutants were used to analyse the morphology and physiology of defined *lipoprotein* mutants. To generate isogenic *K. pneumoniae* mutants, an insertion-deletion mutagenesis strategy was established and mutants with antibiotic resistance genes but also markerless mutant were generated.

Results: We selected 37 out of 123 predicted lipoproteins of *K. pneumoniae* ATCC BAA2146 because of their conservation in comparison to other strains, and because of their proposed molecular weight and outer membrane localization. While some are hypothetical other candidates are with unknown or only predicted functions. We expressed and purified the majority of

selected lipoproteins as His6-tagged nonlipidated proteins heterologously in *E. coli*. The mutagenesis of *K. pneumoniae* ATCC BAA2146 resulted in a nonencapsulated D*wza*-mutant and lack of capsule was verified by electron microscopy and alteration of biofilm formation.
Conclusion: In this study, we started to elaborate a molecular toolbox for *K. pneumoniae* aiming to investigate in future the role of *K. pneumoniae* lipoproteins for bacterial fitness and virulence. Importantly, we established the generation of isogenic mutants and obtained e.g., a capsule knockout strain, which can be used for in vitro cell-culture based infection experiments and proteome analysis.

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The two-Cys-type TetR repressor GbaA confers resistance under disulfide and electrophile stress in *Staphylococcus aureus*
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Staphylococcus aureus has to cope with oxidative and electrophile stress during host-pathogen interactions. The TetR-family repressor GbaA was shown to sense electrophiles, such as N-ethylmaleimide via monothiol mechanisms of the two conserved Cys55 or Cys104 residues in vitro.
Results: In this study, we further investigated the regulation and function of the GbaA repressor and its Cys residues in *S. aureus* COL. The GbaA-controlled *gbaAB-SACOL2595-97* and *SACOL2592-90* operons were shown to respond only weakly to oxidants, electrophiles or antibiotics in *S. aureus* COL, but are >400-fold derepressed in the *gbaA* mutant, indicating that the physiological inducer is unknown. Moreover, the *gbaA* mutant remained responsive to disulfide and electrophile stress, pointing to additional redox control mechanisms of both operons. Thiol-stress induction of the GbaA regulon was strongly diminished in both single Cys mutants, supporting that both Cys residues are required for redox-sensing in vivo. While GbaA and the single Cys mutants are reversible oxidized under diamide and allicin stress, these thiol-switches did not affect the DNA binding activity. The repressor activity of GbaA could be only partially inhibited with NEM in vitro. Survival assays revealed that the *gbaA* mutant confers resistance under disulfide and electrophile stress, which was mediated by the *SACOL2592-90* operon encoding for a putative glyoxalase and oxidoreductase.
Conclusions: our results support that the GbaA repressor functions in the defense against oxidative and electrophile stress in *S. aureus*. GbaA represents a 2-Cys-type redox sensor, which requires another redox-sensing regulator and an unknown thiol-reactive ligand for full derepression of the GbaA regulon genes.

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Impact of glycosphingolipids on meningococcal pathogenicity in an Air-Liquid-Interface model of the nasopharyngeal epithelial barrier
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Introduction: *Neisseria meningitidis* (*Nm*) is a major cause of bacterial meningitis and sepsis. A critical step in the pathogenesis of *Nm* is the passage through the epithelial barrier of the nasopharynx, a step that is still poorly understood. Recent published data on human brain microvascular endothelial cells have shown that *Nm* interacts strongly with GM1, a glycosphingolipid (GLS) strongly abundant on the epithelium (1). This interaction seems to be essential for the ability of *Nm* to invade the cells.
The aim of this study was to investigate the role of GLS on the ability of *N. meningitidis* to overcome the epithelial barrier in the nasopharynx. We hypothesize that interaction between *Nm* and GLS (e.g. GM1) is an important perquisite for bacteria to enter and overcome the epithelial barrier without destroying it.
Methods: Human lung epithelial cells (Calu-3) were grown on permeable cell culture inserts as liquid – liquid (LLI), or air – liquid interface (ALI) model and further characterized by immunofluorescence imaging, Transepithelial electric resistance (TEER) and permeability (NaF and Fitc-dextran) measurements. The cells were exposed to different *Nm* strains and their effect on the barrier integrity was evaluated by TEER measurement and qPCR analysis of tight junction protein expression. The ability of the bacteria to cross the barrier was determined by transmigration experiments in the presence or absence of cholera toxin b subunit (CtxB), blocking the interaction between *Nm* and a specific GLS subset. GM1 distribution on the cells and accumulation at the site of bacterial interaction with the cells were visualized by structured illumination and direct stochastic superresolution microscopy (SIM / *d*STORM).
Results: Here we demonstrate that only the Calu-3 air – liquid interface model exhibits distinct epithelial barrier properties, such as its multilayer structure and mucus production. Whereas the barrier integrity was generally not weakened during infection, the distribution of GM1 (or closely related GSL) has shifted from a uniformly distributed pattern to clustering and strong accumulation around the bacteria. The ability of *Nm* to overcome the epithelial barrier was found to be depended on the strain used. In addition, the ability of *Nm* to transmigrate through the barrier was affected by the pretreatment of the cells with CtxB, which resulted in a decrease in bacterial transmigration through the epithelial barrier.
Discussion: The results demonstrate that the ALI model offers various advantages over the classical LLI model. In addition, the ability of *Nm* to initially cross the epithelium of the nasopharynx is not based on the disruption of the barrier, but strongly relies on the interaction with host GSL.
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177/MPP
Regulation of *Staphylococcus aureus* capsular polysaccharide synthesis on the single cell level – *cap* transcript level does not correlate with promoter activity or polysaccharide synthesis
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In *Staphylococcus aureus*, the capsular polysaccharide (CP) protects against phagocytosis while also impeding adherence to host cells. Synthesis of the CP was shown to be highly heterogeneous within colonising *S. aureus* populations (1). The biosynthetic enzymes responsible for CP synthesis are encoded by the *capA-P* operon with the principal promotor (P*cap*) located upstream *capA*. We used different assays to detect promotor activity, native transcripts and CP formation on the single cell level using promotor-fluorescent protein fusion (P*cap-cfp/yfp*), *in situ* hybridization (mRNA-FISH) and CP immunofluorescence, respectively. All methods verified the strong growth phase dependency and high cell variability of *cap*/CP expression. However, *cap* transcription proceeded CP synthesis: promotor activity and mRNA FISH signals were found at mid-exponential growth phase, while CP formation could only be detected in stationary phase bacteria. Combination of the different methods allowed to analyse whether the different signals co-occur within individual cells. However, P*cap* driven *cfp/yfp* expression (fluorescence) does not correlate with the native *cap* mRNA level

(mRNA-FISH) or CP synthesis (immunofluorescence). Little correlation was also found, when *cap* and *cfp/yfp* mRNA species, both resulting from P*cap* activity, were compared using dual mRNA-FISH. Thus, mRNA structure/stability and other post-transcriptional mechanisms are likely detrimental for the timely CP synthesis in a sub-population of non-growing bacterial cells.
George SE. et al (2015) Mol Microbiol.98:1973; Keinhörster D. et al (2019) Mol Microbiol. 112:1083

178/MPP

The essential genome of *Streptococcus canis* – a comparison to group A, B and C streptococci

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Streptoccocus canis is a leading pathogen in dogs with skin infections and bacterial keratitis as the most prevalent clinical manifestations. In the years 2005 to 2008, *S.canis* infections were shown to account for 22.4% of the 394 streptococcal canine infections recorded in that timeframe [1]. Due to the lack of vaccines, treatment of *S.canis* infection has primarily been via the use of ß-lactam antibiotics. This project aims to provide a comprehensive approach in understanding molecular mechanisms for disease manifestation and increasing prevalence of *S.canis.*
In this study, Transposon Directed Insertion-site Sequencing, a mutant library approach paired with high throughput sequencing, has been conducted on an *S.canis* strain issued from a clinical case of septicemia. This approach produced a mutant library of approximately 70 000 unique mutants. This equates to a unique transposon insertion every 30 bp in the genome of *S.canis.* The essential genome of *S.canis* was therefore determined and compared to the essential genomes of human pathogens *S.pyogenes*, *S.agalactie* and the animal pathogen *S.equi*. We show that the essential genome of *S.canis* comprised 30,2 % of its total genome. This is a considerably higher proportion compared to the 12%, 13.5% and 19% respectively corresponding to *S.pyogenes*, *S.agalactiae* and *S.equi* [1]. Furthermore, we show that the dominant Kegg categories of *S.canis'* essential genome most resembles those of *S.equi*. Finally, the identification of essential Kegg categories exclusive to *S.canis, such as two-component systems* could aid in distinguishing its pathogenesis from other streptococcal species.
In conclusion, the essential genome will allow a better understanding of the virulence mechanisms of *S.canis*, which may enable the elaboration of new vaccines. This will further increase canine welfare and may give new insights into the invasive capacities of other streptococcal pathogens.
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Investigations into an overlooked slow-growing skin bacterium – *Staphylococcus saccharolyticus* - as a possible causative agent of implant-associated infections

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Introduction: Orthopedic implant-associated infections (OIAIs) are one of the most serious complications in orthopedic surgery. OIAIs are frequently caused by staphylococcal species, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*. However, also numerous other, often low-virulent bacterial species are culprits of OIAIs. An important source of OIAI-causing bacteria is the patient"s skin microbiota.
A recent study posited that a slow-growing, anaerobic coagulase-negative staphylococcal species, *Staphylococcus saccharolyticus*, constitutes a very common member of the human skin microbiota. However, only few clinical cases, where *S. saccharolyticus* was isolated and suspected to be the cause of disease, have so far been reported. It is possible that this bacterium is more frequently present in clinical samples but is overlooked or considered as contaminant.
The aim of this study was to investigate the pathogenic potential of *S. saccharolyticus* in order to test if this bacterium has the necessary traits to cause OIAIs.
Material and Methods: Clinical OIAI isolates of *S. saccharolyticus* were used in this study and an array of experiments was carried out, including growth and biofilm assays, cell culture-based infection experiments and genome and transcriptome analyses.
Results: Although *S. saccharolyticus* has previously been described as a slow-growing anaerobic species, a re-evaluation of its growth behavior revealed that some strains of *S. saccharolyticus* are able to grow aerobically under CO2-enrich conditions. Biofilm and autoaggregation formation was monitored, revealing the ability of *S. saccharolyticus* to form distinct biofilms as well as aggregates. Transcriptome analysis of biofilm-grown and planktonic cells discovered the identity of biofilm-related genes. The inflammatory potential of *S. saccharolyticus* was determined in cell culture experiments, revealing a profound induction of proinflammatory cytokines in macrophage-like cells.
Discussion: Taken together, the results of this study suggest that *S. saccharolyticus* has a substantial pathogenic potential and can be a likely cause of OIAIs and, possibly, other types of deep-seated infections.

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Mucoidy in *Staphylococcus aureus* colonising the airways of people with cystic fibrosis is caused not only by the 5bp deletion in the *ica* locus but also by mutations in *icaR*

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Background: *Staphylococcus aureus* has been identified as a major respiratory pathogen isolated from the airways of people with cystic fibrosis (pwCF). Persistent bacterial colonization/infection of the lungs allows adaption of this organism to the CF-environment. Less is known about the presence of *S. aureus* with a mucoid phenotype in CF airways. Recently, we recovered *S. aureus* isolates with mucoid phenotypes (PTs) from respiratory specimens of pwCF, that constitutively overproduced biofilms consisting of polysaccharide intercellular adhesin (PIA), which was due to a 5bp-deletion (5bp-del) in the intergenic region (IGR) of the intercellular adhesion (*ica*) locus [1]. Neither the prevalence nor any potential impact of such mucoid *S. aureus* PTs on pulmonary disease is yet known.
Methods: The prevalence of mucoid *S. aureus* in pwCF with chronic *S. aureus* colonization/infection was determined in a prospective multicenter study. Respiratory specimens were sent to the central laboratory in Münster, Germany and 10 *S. aureus* isolates were sub-cultured on Columbia Blood (CB) and Congo Red (CR) agar, which allows to distinguish mucoid from non-mucoid *S. aureus* PT [1]. Biofilm formation was tested in a static 96-well plate assay and the isolates were subjected to *spa*-sequence typing and sequencing of the *ica*-operon.
Results: Fourteen CF centers recruited 611 pwCF. Mucoid *S. aureus* strains were isolated from 41 of 435 *S. aureus* positive specimens (9.4%). So far, *spa*-typing was performed for 835 *S.*

aureus isolates of pwCF with mucoid isolates with t091 being the most prevalent *spa*-type (12%). Most mucoid isolates were high biofilm producers. Until now, the analysis of the 5bp-del was performed for isolates of 19 pwCF and was detected in mucoid *S. aureus* isolates of 9 pwCF from 7 different centers, while the 5bp-del could not be determined in mucoid isolates of 10 pwCF. So far, unrecognized mutations in *icaR*, the repressor of the *ica*-operon, were identified in mucoid isolates of 6 patients from 6 centers. Mucoid isolates of 6 patients carried different mutations in *icaR*, while mucoid isolates of 6 patients carried no mutations in the *ica* locus.

Conclusions: We were able to identify an unexpected high prevalence of mucoid *S. aureus* PTs in pwCF. No specific *S. aureus* clone was associated with mucoidy. In about 50% cases, the 5bp-del was identified indicating that this mutation is common during chronic *S. aureus* infection in pwCF. In addition, we identified new mutations in *icaR* and other so far unidentified mutations, which most likely lead to mucoidy of *S. aureus* and which will be elucidated in future studies.

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181/MPP

Impact of focused high-energy extracorporeal shockwaves on the gene expression of *in vitro* biofilm of *S. aureus*

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Introduction: Bacterial biofilm formation on medical implants is a major issue in many surgical disciplines with high therapeutical failure rates. The biofilm matrix protects the bacteria against the immune system and antibiotics. Therefore, antibiotics as a stand-alone therapy do not reduce biofilm on implants sufficiently. It is important to look for new approaches in treating implant-associated infections. Raising susceptibility of the causitive agents to antibiotics although hiding in a biofilm could be one approach. Focused high-energy extracorporeal shockwave therapy (fhESWT) is a non-invasive alternative for the treatment of e.g. arthrodesis nonunions. Moreover, in further studies on the use of fhESWT, it has been shown that this therapy also reduces the number of *S. aureus* in a biofilm *in vitro* and *in vivo*, especially in combination with antibiotics. The underlying mechanism for this effect has not yet been determined. It is known that fhESWT alters gene expression of some genes in eukaryotic cells. Therefore, in this study, we investigated the effects on gene expression in bacterial cells after application of shockwaves to *S. aureus* biofilm. In addition, we looked at the growth behavior of the bacteria and the ability to form a new biofilm after fhESWT.

Methods: The biofilms were cultivated on titanium discs in brain heart infusion + 1% glucose using *S. aureus* strain SH1000. After 48h the discs were transferred to sterile bags filled with PBS, and shockwaves (500 impulses with 23k) were applied to the discs. Immediately thereafter, the bacteria were detached from the discs and RNA was isolated. We examined the gene expression of global regulators (agr, sigB, sarA, sae) as well as genes important among others for biofilm formation (icaA, icaB, icaC, icaD, eno, cna, fnbA) by RT-qPCR. In addition, we performed growth curves using the detached bacteria and analyzed the ability of the bacteria to form a new biofilm after fhESWT by crystal violet staining. *S. aureus* biofilms that did not receive fhESWT but were otherwise treated the same as the other samples served as controls in all experiments.

Results: Our current results show no differences in gene expression between the fhESWT-treated and non-treated samples. Also, no change in growth behavior or biofilm formation ability was observed.

Discussion: We have established a method for culturing an *S. aureus* biofilm on titanium discs that is of sufficient bacterial number to analyze gene expression after shockwave therapy. There was no distinct difference in gene expression of the genes studied. No changes and thus also no enhancement of the proliferation of bacteria and the ability to form biofilms were seen either. We consider the latter results as positive with regard to the possible use of fhESWT for the treatment of biofilms. Since previous studies have shown a decrease in biofilm after the treatment, further efforts should be made to explain the effect of fhESWT on bacterial biofilm.

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Characterisation of the *sraP*-homologous gene cluster in coagulase-negative *staphylococci*

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Introduction: Coagulase-negative staphylococci (CoNS) are a heterogenous group of bacteria that colonize skin, but also cause an increasing number of serious infections, such as foreign body-associated infections and native valve endocarditis. The pathogenesis of these infections is characterized by the bacteria's capacity to bind to immobilized human platelets and to form biofilms. While underlying mechanisms in such pathogenic events have been intensively studied in *Staphylococcus aureus*, not much has been known from CoNS so far. In *S. aureus*, the serine-rich adhesin for platelets (SraP) has been identified as a pathogenicity factor in an animal model of infective endocarditis. SraP is a large surface glycoprotein and its gene is part of a gene cluster that also encodes the genes for its secretion and glycosylation.

Methods: Analysis of sequenced whole genomes of CoNS strains revealed the presence of *sraP*-homologous gene clusters. To study the importance of the *sraP*-homologous gene cluster in the CoNS pathogenicity, we determined its prevalence in clinical versus harmless skin isolates in the species *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus lugdunensis* by PCR. Furthermore, we performed a quantitative biofilm assay and a flow-cytometric platelet adherence assay to correlate biofilm formation and binding to platelets, respectively, with the presence of the *sraP*-homologous gene cluster.

Results: PCR analyses demonstrated that in *S. epidermidis* the prevalence of the *sraP*-homologous gene cluster is quite high and similar among isolates: 43/48 (90%) of clinical and 19/22 (86%) of harmless skin isolates carried the *sraP*-homologous gene cluster. In contrast, the prevalence of the *sraP*-homologous gene cluster in *S. haemolyticus* is much higher with clinical isolates: 30/35 (86%) of the clinical isolates, but only 2/8 (25%) of the skin isolates harbored the gene cluster. In *S. lugdunensis*, a portion of the gene cluster was found with only one clinical isolate (1/20).
While in *S. epidermidis*, *sraP*-positive isolates formed significantly more biofilm than *sraP*-negative isolates, such a significant difference could not be observed with *S. haemolyticus*. Analysis of the bacterial adhesion to platelets revealed that there is no significant difference between *sraP*-positive and *sraP*-negative isolates (tested only in *S. epidermidis*).

Conclusions: The prevalence of the *sraP*-homologous gene cluster is high in clinical *S. epidermidis* and *S. haemolyticus* isolates, but barely detectable in *S. lugdunensis* isolates. This might be due to less conserved sequences in *S. lugdunensis* and therefore needs to be further analysed. In *S. epidermidis*, but not in *S. haemolyticus*, the presence of the *sraP*-homologous gene cluster correlates with increased biofilm formation. Thus, the findings of this study suggest that the *sraP*-homologous gene cluster might play a role in CoNS pathogenicity, which however needs to be further investigated.

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Analysis of proteochemical properties of the *Burkholderia* virulence factor BimC

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Introduction: Human-pathogenic bacteria of the *Burkholderia pseudomallei* group are able to invade mammalian host cells and remodel part of the cellular actin by inducing actin polymerization at the bacterial cell pole termed actin tail formation. This process contributes to intracellular persistence and dissemination within the host by cell-to-cell spreading of the bacterial pathogen. Generation of actin tails depends on the bacterial virulence factor BimA which is anchored to the bacterial outer membrane in a polarized fashion and promotes actin filament nucleation. Currently, it is not known how BimA activity is functionally regulated. One key regulator could be BimC, a member of the glycosyltransferase family.
Objectives: This work focuses on the structural and functional analysis of the *Burkholderia thailandensis* BimC which is found at least by some studies to be involved in polar localization of BimA and appears to be essential for BimA-dependent actin tail formation. Biochemical properties of BimC, an unconventional heptosyltransferase, are not fully understood so far. Furthermore, gene annotations appear to be incomplete in some cases missing essential biochemical features of the protein.
Methods: Multiple sequence alignments and homology modeling of BimC using the closely related heptosyltransferase TibC of human-pathogenic *E. coli* was used to identify structural similarities between both proteins. For further experimental studies, expression, and purification of BimC was successfully established. Purified recombinant BimC protein was used for the analysis of the BimC structure using SAXS.
Results: The closely related dodecameric heptosyltransferase TibC shows strong structural similarities to BimC - especially to its putative catalytic domain - as previously reported by other groups. Upon closer inspection by multiple sequence alignments, significant differences in protein sequences can be found that could affect the structure and function of BimC, such as the missing TibC-like oligomerization site. Nevertheless, first analysis of the BimC structure in solution using SEC-SAXS suggests that BimC can indeed form decamers in an unknown manner. Additionally, mobilization of BimC across the bacterial cell membrane was characterized by fluorescent reporter protein assay indicating an important step in BimC activation.
Conclusion: The potential BimC decamer could act as highly efficient machinery for functional regulation of BimA-dependent actin polymerization at the bacterial cell pole of certain pathogenic *Burkholderia*, and therefore having a key role in the infection mechanism. Further investigation of the BimC structure is required, but also functional assays in respect to enzymatic function, subcellular localization, and possible interactions of BimC with other bacterial proteins.

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Carbon source utilisation in hybrid STEC/UPEC indicates adaptation to intestinal and extraintestinal milieu

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Introduction: Recently emerged hybrid *Escherichia coli* display high virulence and potential for outbreaks. While the number of hybrid *E. coli* increased within the last decade, their adaptation, virulence, and outbreak potential remain largely unknown. The Shiga toxin-producing and uropathogenic *E. coli* (STEC/UPEC) serogroup O2:H6 are phylogenetic intermediates between Shiga toxin-producing (STEC) and uropathogenic (UPEC) *E. coli* and can cause both diarrhea and urinary tract infections[1]. Phenotype analysis associated with virulence traits supports the intermediate position of these hybrids and indicates adaptation to both the intestinal (IM) and extraintestinal milieu (EM)[2].
Methods: To further investigate the adaptation of the STEC/UPEC hybrid to both IM and EM, carbon source utilization was tested using a Phenotype MicroArray screening growth on 95 carbon compounds. We compared an O2:H6 strain to the commensal K-12 strain MG1655, the UPEC strain 536, and the canonical STEC strains Sakai and B2F1.
Results and Discussion: Analysis of the growth data shows high similarities between the growth of the O2:H6 strain and 536 supporting a morphed evolution from a UPEC into a hybrid via acquisition of STEC virulence factors[3]. Notably, the growth on amino acids and peptides is overall higher in 536 and O2:H6 compared to the canonical STEC strains. Amino acids count as the major metabolite within the urine, which supports the adaptation of the hybrid to the EM[4]. Interestingly, high metabolic differences were observed between the STEC strains B2F1 and Sakai, with Sakai showing a more limited utilization of the screened carbon sources. When comparing the hybrid to both STEC strains the growth observed resembles B2F1 more than Sakai. This reflects the closer phylogenetic relation between the hybrid O2:H6 and B2F1 as compared to Sakai[3].
Conclusion: Overall, the hybrid O2:H6 strain most often shows carbon utilization comparable to UPEC strain 536 and otherwise an intermediate growth between 536 and the canonical STEC with a closer resemblance to B2F1. This supports its hybrid status and shows an adaptation to both intestinal and extraintestinal growth.

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Gastrointestinal Infections/ Microbial Pathogenicity (FG GI/FG MP)

185/GIMPP

Proteomic changes of *Enterococcus faecium* and *Enterococcus faecalis* during bile acid stress

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Introduction: *Enterococcus faecalis* and *Enterococcus faecium* belong to the commensal human intestinal microbiome as glucose fermenting facultative anaerobes. However, both bacteria can cause severe nosocomial infections once they leave their natural environment – the human gut.
One of the diverse functions of bile acids is their antimicrobial activity. Growth of *E. faecalis* and *E. faecium* is indeed reduced in the presence of bile acids. The steroid acids formed in the liver (primary bile acids) or as metabolic side product of intestinal microbes (secondary bile acids) can disrupt the bacterial cell membrane and thus reduce the amount of pathogens in the gut. Between 200 and 600 mg of bile acid is produced by the human liver per day. Approximately 40 % of the bile acid pool are cholic acid (CA), 40 % chenodeoxycholic acid (CDCA) and 20 % deoxycholic acid (DCA).

In this project, the proteomic stress response of *E. faecalis* and *E. faecium* towards three different bile acids was analyzed.
Material and methods: *E. faecalis* and *E. faecium* were grown in M17 broth with different concentrations of CA, CDCA and DCA. Growth curves were generated and IC50 values were determined for each bile acid. Protein extraction was performed from samples grown at sublethal bile acid concentrations of 0.05 % and 0.1 %. Quantification of the proteome was done via SWATH-MS (sequential window analysis of all theoretical mass spectra). Perseus v1.6.2.2 was used for statistical data analysis.
Results: 1410 proteins were identified for *E. faecalis* and 1400 proteins for *E. faecium* by SWATH-MS. PCA plots were created to show the clustering of the proteome for CA, CDCA and DCA at the two applied concentrations. Up- and down regulated proteins in response to bile acids were identified by volcano-plots. For all three applied bile acids, concentrations of 0.05 % resulted in a higher number of up- and downregulated proteins than 0.1 %. Venn-diagrams were used to identify proteins that were consistently up- or down regulated in all bile acid treated samples. At 0.05 % bile acids, 71 proteins were commonly upregulated in *E. faecalis* and 88 *in E. faecium*. 379 proteins were commonly downregulated in *E. faecalis* and 459 in *E. faecium*. These commonly regulated proteins were assigned to functional super categories, revealing a particular high number of upregulated proteins involved in metabolism, while proteins involved in DNA synthesis were downregulated.
Discussion: Comparative proteomic analysis after treatment of *E. faecalis* and *E. faecium* with three different bile acids allowed us to identify a subset of proteins comprising the "general bile acid stress proteome", with proteins commonly up- or downregulated in response to all tested bile acids.

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Barrier dysfunction caused by clostridial toxins in a colon organoid-derived 2D infection model

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Introduction: *Clostridioides difficile*-induced enteritis is one of the most relevant nosocomial infectious diseases. In *C. difficile* infection (CDI) the main virulence factors produced by the bacteria in the intestine are high-molecular weight AB-toxins (TcdA and TcdB) causing *C. difficile*-associated diarrhea, inflammation and pseudomembranous colitis. Hypervirulent strains produce a binary ADP-ribosylating toxin (CDT) that impairs the actin cytoskeleton. However, functional studies on pathomechanisms responsible for epithelial barrier dysregulation in intestinal organoid models are lacking. The objective of this project is to identify the epithelial barrier pathology with regard to alterations of tight junction proteins and to enlighten molecular mechanisms for diarrhea and inflammation.
Method: Confocal laser-scanning microscopy (CLSM) was performed, in order to characterize the subcellular localization of tight junction proteins, epithelial leaks and epithelial cell death phenomena. Potential protein expression changes were assessed by western blotting. Primary cell culture from human intestinal organoids was established. Statistical analyses were performed using Student"s t-test in GraphPad Prism software.
Results: Two-dimensional and polarized monolayers on PCF-filter inserts were established from patient-derived colonic organoids. All three clostridial toxins caused a reduction in transepithelial electrical resistance in colonic HT-29/B6, as well as intestinal organoid-derived monolayers. Concomitantly, we detected an increase in paracellular permeability for the two fluorescent tracers fluorescein (332 Da) and FITC-dextran (4 kDa). In CLSM, tight junction proteins were affected on a subcellular level, such as claudin-4, which seems to be in many regions redistributed from the bicellular tight junction into cytoplasmatic cellular compartments. After toxin treatment cell shape and cell sizes changed dramatically in the epithelial monolayer and F-actin structure was impaired.
Discussion: We established a new organoid-derived cell monolayer culture as infection model for the human colon epithelium, in which electrophysiological measurements are applicable. With the help of this model, we were able to identify tight junction changes leading to intestinal epithelial barrier dysfunction. This may lead to increased invasion of luminal microbial pathogens and microbiota-associated molecular patterns, resulting in inflammation and tissue damage promoting CDI. Therefore, our findings provide more details in understanding pathomechanisms of *C. difficile*. To further characterize the intestinal barrier dysfunction after toxin treatment, we will use the Ussing chamber technique with impedance spectroscopy. Immunological aspects of the leaky gut concept in chronification of the colitis will be enlightened in a co-culture model.

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Analysis of innate immune response of gastrointestinal epithelium to EPEC infection using human 3D organoids derived 2D monolayers as a model

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Introduction: The innate immune response of the host cell activated by pattern recognition receptors (PRRs) and microbe associated molecular patterns (MAMPs) initiates NF-kB response that results in inflammatory cytokine production upon microbial exposure. Profiling of human intestinal organoids grown from the different segments of the gastrointestinal tract show segment specific gene expression. This can be utilised to study gastrointestinal infection using patient derived primary cells. Enteropathogenic E. coli (EPEC) is human specific, gram-negative bacteria that causes diarrhoea in infants and children in developing countries leading to severe illness and even death. The key component of EPEC pathogenicity is type III secretion system (T3SS) that inject effector proteins into the host cell to block the NF-kB pathway and prevent inflammatory response.
Material/methods: Organoids are generated *in vitro* from resection material from human Jejunum. Here we establish 2D cultures from 3D organoid cultures. Barrier function is measured using transepithelial electric resistance (TEER). Using different media composition, we analyse the differentiation of the cells in 2D using qRT-PCR and immunofluorescence. We infect the primary monolayers with EPEC. Attachment of bacteria is determined by microscopy and FACS.
Results: Within 7-8 days, organoid-derived cells form a tight monolayer. The monolayers are stable for 10 days. Using different media composition, cultures can be directed either towards adsorptive lineages, enterocytes, or secretory cells (goblet cells marked by MUC2 and enteroendocrine cells marked by CHGA). EPEC can attach and infect Jejunum monolayer cells and form characteristic actin-rearrangements ("pedestals") on the apical side of the cells, one of the hallmarks of EPEC infection. With FACS analysis we detect about 50% of cells EPEC+ in 2D monolayers compared to 75% in HeLa cells.
Discussion: Primary human jejunum monolayers grown from 3D jejunum organoids show characteristics similar to that of physiological conditions namely, tight junction formation and expression of various differentiated cells. This provides an easily achievable tool to better understand the interaction of pathogens with the host cells, which is otherwise difficult to investigate in human setting. This therefore provides a platform to identify the importance of both EPEC and host factors for selective infection in the human gastrointestinal tract.

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Happy Birthday – local outbreak of monophasic *Salmonella* Typhimurium due to consumption of grilled suckling pig at a birthday party

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Introduction: After a private party in February 2022, 16 out of 18 guests developed gastrointestinal symptoms (in part severe gastroenteritis with bloody diarrhea) after consumption of grilled suckling pig with dumplings and sauce. Out of those 16, six had to be treated stationary at a hospital. The suckling pig had been previously prepared at a local butchery, and was then grilled at the party-site for approx. six hours by the hosts, while being doused with beer, which was collected as sauce. Two people, who did not attend the party but ate meat with sauce and dumplings, which had been delivered to them, were affected as well. Therefore, involvement of other served foods (i.e. salads) was implausible.
Material: Stool samples of eight affected guests and two employees of the local butchery were investigated, as well as seven bacterial isolates of affected patients from third party laboratories and two samples of meat and dumplings (for two patients both a stool sample and a bacterial isolate were present).
Methods: Bacterial culture examination of stool and food samples for 2 x 24hours with several selective and non-selective culture media for enteric pathogens. MALDI-TOF MS for species identification. Slide agglutination for serotype-differentiation of *Salmonella* sp. Multiplex-real-time-PCR for identification and confirmation of monophasic *Salmonella* Typhimurium. Whole genome Sequencing via NGS (Next Generation Sequencing) and data analysis using core genome (cg)MLST (Multilocus Sequence Typing).
Results: No pathogenic bacteria were detected in the two stool samples of the butchery"s employees, but seven out of eight examined stool samples and both food samples contained *Salmonella* sp. Those, as well as the third party and food bacterial isolates were identified as monophasic *Salmonella* Typhimurium (antigenic formula O4 : Hi : -). NGS-Analysis revealed that all 16 isolates form a cluster with zero allelic differences (cgMLST clonal complex-type 9996).
Discussion: As all investigated isolates of *Salmonella* are clonal, according to the molecular-genetic analysis, this outbreak is assumed to be local and caused by consumption of contaminated food. Possible vehicles are meat, dumplings and sauce, as they were the only food consumed by all affected individuals. Sensory examination of the food samples revealed that the core of the meat still was pinkish in color and therefore not thoroughly cooked. Comparisons with other in-house isolates from 2014 to 2022 as well as two simultaneously occurring, cross-country outbreaks with *Salmonella* Typhimurium in Germany showed no epidemiological linkage, supporting the assumption of an isolated local outbreak. The occurrence of *Salmonella* spp. is common in pig populations, so the contamination of raw pig meat cannot be completely excluded during slaughtering. Therefore, a correct preparation and handling of meat including a sufficient heating step is necessary to prevent such local *Salmonella*-outbreaks.

Bacteriophages today - from basic research to patient treatment (FG MV)

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Interdependency between bacteriophage resistance and antibiotic sensitivity in MDR *Klebsiella pneumoniae*

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Introduction: The continuing rise and spread of multi-drug resistant bacteria (MDR) represent a global health concern. However, this development does not mean that traditional antibiotics will eventually wind up as being entirely useless. A number of studies have shown that the concerted activity of phages and "useless" antibiotics control the bacteria stronger than the phage alone and that phage resistance emergence can be reduced. In addition, development of phage resistance can occur at the expense of re-converting bacteria to an antibiotic sensitive genotype. Here we describe a newly isolated phage belonging to Drexlerviridae (Webervirus) with lytic activity and positive interactions with antibiotics against drug resistant clinical isolates of *Klebsiella pneumoniae.*
Methods: Phage LAPAZ was isolated from a 5-year old sewage sample stored at 04 ˚C to ensure a storable and robust phage. Host range, burst size and phage adsorption profile of LAPAZ were evaluated, as well as phage morphology. Phage stability was determined under different temperatures and pH values. Phage interactions were assessed with ciprofloxacin, meropenem, gentamicin, ampicillin, and ceftazidime. Whole genome sequencing was performed for the phage as well as for the parental and emerging resistant bacterial host.
Results: Phage LAPAZ lysis 14% of tested strains. Its genome consists of 51,689 bp and encodes for 84 ORFs, of which 40% could be assigned to proteins of known functions. The latent period is 30 min with an average burst size of 27 PFU/cell. Upon exposure up to 50˚C the phage titer only decreased by 10%. Phage infectivity is maintained over a pH range from 4 to 10 (with pH 6 being optimal). Unlike exposure to the phage alone, a complete bacterial eradication was achieved when combining LAPAZ with sub-inhibitory concentrations of meropenem. Phage resistance emergence could be markedly delayed with the co-presence of ciprofloxacin. Conversely, emerging phage resistance came along with a 16 times higher sensitivity to ciprofloxacin. Whole genome analysis revealed only one mutational aberration in the phage resistant strain affecting a membrane transport protein belonging to the Major-Facilitator-Superfamily (MFS).
Discussion: We speculate that this frameshift mutation compromises ciprofloxacin efflux efficiency in the bacterial host and that the non-mutated protein might be involved in phage receptor binding. Phage LAPAZ is comparably stable and might thus suit one important requirement for phage therapy along with its positive "cooperation" with antibiotics.

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Molecular cross-talk between Sa3int phages and their *Staphylococcus aureus* host

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Introduction: 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. 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, leading to the loss of 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).

Methods and Results: We constructed and integrated Sa3int phages into different phage-cured *S. aureus* strains and found significant differences in phage transfer and induction 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 and performed differential RNAseq to determine transcriptional units and gene expression differences between low and high transfer strains. By analysis of a set of mutants and promoterfusion constructs, some bacterial and phage genes were identified which are likely to play a role in the regulation of the strain dependent phage life cycle.

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Restoration of antibiotic susceptibility in *Klebsiella pneumoniae* using capsule type specific non-lytic bacteriophages

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Introduction: The alarming rise of multi-antibiotic resistant bacteria is probably the greatest Health challenge of our time from a one-health perspective, with multiantibiotics- resistant organisms detectable in hospitals, livestock and in the environment. In terms of medical treatment challenges the focus is on ESKAPEE organisms, which cause the highest case fatality.

The use of bacteriophages (phages) is now considered again as an alternative treatment option in Western Europe and America. In comparison to Eastern European countries with well-established phage therapy guidelines, there are still concerns in the West regarding the use of phages as standardized treatment.

Combined treatment with phages follwed by antibiotics is the most promising approach and is based on the hypothesis that some phages can restore antibiotic susceptibility in MRGN bacteria for example by capsule depolymerisation.

Material and Methods: This project aims to to test this hypothesis using wzi capsule typed non-lytic phages on 4MRGN *Klebsiella pneumoniae* (Kp) to restore antibiotic susceptibility. The capsule of Kp consists of capsule-polysaccharides (CPS), which represent the first receptor for the phage. The second receptor is located on the cell surface and is strain dependent. Consequently, a phage specific for a certain capsule type (CT) does not necessarily bind to the second receptor to lyse the Kp, but the loss of CPS due to phage depolymerase activity may make MRGN Kp susceptible to antibiotics, that depend on efficient crossing of the CPS barrier.

The target Kp was infected with different (lytic and non-lytic) phages combined with different antibiotics in various concentrations and the the optical density was measured over 16 hours to show the growth kinetics.

Results: The results show the different growth kinetics under antibiotic treatment comparing different phage infection settings. The non-lytic capsule type specific phage decreases the growth of the targeted Kp in combination with antibiotics, that were previosuly tested as ineffective.

Discussion: Preliminary results are promising. In our gram negative model organism Kp, phages are highly specific and a lytic phage will have to be isolated, characterized and prepared separately for the therapy of over 80 different capsule types and further restriction factors found in clinical isolates, which is time consuming.

Improving therapy by restoring antibiotic susceptibility, even if a lytic activity cannot be achieved, is a important milestone. In future studies we will investigate whether the same results can be achieved using phage depolymerases expressed in isolation. If the loss of capsule makes the Kp more susceptible to immune effectors this would be an added therapeutic benefit.

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Isolation and characterisation of novel *Bordetella avium* bacteriophages

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Question: Bordetellosis, caused by *Bordetella avium*, is a common respiratory disease in poultry of all ages. The infection progresses with a high morbidity and low mortality. Diseased animals show reduced body weight gain due to their poor general condition, causing high economic losses in commercial poultry farms. The use of antibiotics to prevent bordetellosis may result in the selection of resistant *B. avium* isolates. A future alternative approach for the treatment of bordetellosis could be the use of bacteriophages. However, little is known about *B. avium* bacteriophages and none have been isolated from the environment of poultry farms so far. Therefore, the objective of this study was to isolate and characterize *B. avium* bacteriophages**.**

Methods: Drinking water and fecal samples from hobby and commercial poultry farms were used for the screening of phages capable of lysing *B. avium* in plaque assays. Phages were purified by repeated plating and were concentrated by plate lysate and polyethylene glycol / sodium chlorid precipitation. Isolated phages were characterized by whole-genome sequence analysis, electron microscopy and host range determination. To reveal the binding sites of bacteriophages on the surface of bacterial cells, treatment of bacteria with either sodium periodate or proteinase K followed by phage infection was performed.

Results: In the course of the present study, seven different phages were isolated. Electron microscopy analysis revealed that all phages belong to the *Myoviridae* family (order *Caudovirales*). Sequence analysis revealed a size range of phage genomes between 39-43 kbp. The genomes showed considerable similarities in genes coding for structural proteins (genes responsible for DNA packaging, head assembly, tail assembly) and differences in genes responsible for lifecycle regulation (genes responsible for cell lysis, regulation, integrase, replication and other functions). Despite genetic evidence for lysogeny, the seven phages showed strong bacteriolytic activity. In total, all available 51 *B. avium* isolates could be lysed by the isolated phages. However, phage vB_BaM-IFTN3 had the smallest host range with only 14 lysed isolates, while the other phages were able to lyse 40-50 isolates each. Treatment of bacteria with sodium periodate inhibited phage absorption, indicating that all seven phages bind to lipopolysaccharide (LPS) structures of *B. avium*.

Conclusion: The isolation and characterization of these phages showed the in vitro effectiveness of phages against *B. avium* field isolates. However, it needs to be clarified whether they undergo a lysogenic cycle in order to assess the risk for therapeutic use.

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Isolation of *Staphylococcus aureus* bacteriophages for possible therapeutic application

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Introduction: The increasing difficulty in treating antibiotic-resistant bacteria has given new importance to phage therapy after a long period of negligence. Although *Staphylococcus* (S.) *aureus* is part of the physiological skin and mucosal flora, it can cause serious soft tissue infections, pneumonia, and blood stream infections in healthy and immunocompromised individuals.
Material / Methods: S. aureus bacteriophages were isolated from sewage water and analyzed regarding their host range and stability. Transmission electron microscopy was used for determination of bacteriophage morphology. For host range analysis, 60 clinical, MRSA, and PVL-producing *S. aureus* isolates were used.
Results: We isolated five different bacteriophages from sewage water samples. Three phages showed a broad host range (> 90%) even eliminating MRSA or PVL-positive strains. Isolation of bacteriophages and the subsequent production of high titer lysates were met with less challenges than expected. The bacteriophage solutions can be stored at 4°C over a period of several months with a minimal but gradual decrease in titer.
Conclusions: These findings demonstrate that candidate therapeutic bacteriophages can be isolated and analyzed relatively easily and rapidly. The results achieved, can be seen as first steps towards clinical trials in the future.

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Bacteriophage Φ6 (phi 6) is a suitable surrogate for SARS-CoV-2 for evaluation of an ozone based automated room disinfection system

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Background: The presence of coronaviruses on surfaces in the patient environment is a potential source of indirect transmission. Manual cleaning and disinfection measures do not always achieve sufficient removal of surface contamination. This increases the importance of automated solutions in the context of final disinfection of rooms in the hospital setting. Ozone is a highly effective disinfectant which, combined with high humidity, is an effective agent against respiratory viruses. As a consequence of biosafety concerns and high demands for working with SARS-CoV-2, surrogate viruses were used in this study. The effectiveness of a fully automatic room decontamination system based on ozone was tested against the enveloped bacteriophage Φ6 (phi 6) from the family of the *Cystoviridae* and bovine coronavirus (bCoV) L9, as surrogate viruses for the pandemic coronavirus SARS-CoV-2.
Methods: For this purpose, various surfaces (ceramic tile, stainless steel surface and furniture board) were soiled with the surrogate viruses and placed at two different levels in a gas-tight test room. After using the STERISAFE™ Pro automatic decontamination device according to the manufacturer's instructions, the surrogate viruses were recovered from the surfaces and examined by quantitative cultures (plaque forming units for phages and TCID50 for bCoV L9). Then, reduction factors were calculated.
Findings: The ozone-based room decontamination device achieved virucidal efficacy (reduction factor >4 $\log_{10}$) against both surrogate organisms regardless of the different surfaces and positions confirming a high activity under the used conditions. Due to a higher concentration of inoculated phages compared to bCoV L9 on ceramic tiles and stainless steel the calculated reduction factor was slightly higher for Φ6. Interestingly, on furniture board for Φ6 less phages could be recovered from the untreated control surface compared to ceramic tiles and stainless steel. Therefore, on this surface the calculated reduction factor was lower for phages compared to bCoV L9.
Conclusion: Ozone is highly active against both SARS-CoV-2 surrogate organisms. As different behavior of microorganisms can be observed on various inanimate surfaces normative testing on only single types of materials might not be sufficient for a full understanding of the efficacy of disinfection processes. Due to its culture conditions, the use of phage Φ6 enables save and accelerated processing in the testing of disinfection systems and might be used also for contamination of surfaces in field tests of disinfection processes.

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Identification of a novel bacteriophage receptor in *Staphylococcus epidermidis*

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Introduction: Bacteriophages are the most abundant biological entities on earth and considered important for bacterial population dynamics and horizontal gene transfer. Phages infecting the genus *Staphylococcus* are most thoroughly investigated for their prominent member *Staphylococcus aureus.* For attachment and infection, bacteriophages need to bind a specific surface structure via their receptor binding proteins (RBPs). In case of *S. aureus*, these receptors are glycosyl moieties of the cell wall bound glycopolymer wall teichoic acid (WTA), but for coagulase-negative *Staphylococci* (CoNS), these phage receptors remain largely unknown.
Methods: Since CoNS usually share a similar WTA backbone consisting of poly-glycerol-phosphate (GroP), we used the well-known coagulase-negative species *Staphylococcus epidermidis* for identification of the bacteriophage receptor binding site. We therefore created a transposon mutant library of *S. epidermidis* strain 1457 and challenged it with siphovirus ΦE72. Survivors were screened for a resistance phenotype, which was subsequently verified via clean knockouts, resulting in a partial resistance of *S. epidermidis* in the presence of ΦE72.
Results: The identified genes encode enzymes involved in the synthesis and transfer of sugar precursors, which are probably involved in wall teichoic acid synthesis or glycosylation. This resistance mechanism is mediated via a decreased adsorption capacity of the phage to the cell envelope and is universal for other *S. epidermidis* phages. We therefore assume that the identified enzymes are responsible for the synthesis of a phage receptor, most likely connected to the glycosylation of GroP-WTA present in most CoNS.
Discussion: Up to now, research on CoNS has been neglected, especially regarding phage receptor biology and horizontal transfer via bacteriophages. Since CoNS might function as hubs for antibiotic resistance genes, understanding phage mediated horizontal gene transfer is essential to prevent spreading of antibiotic resistance between different bacterial species.

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Construction of a T7 phage-based CRISPR-Cas delivery system targeting carbapenem resistant bacteria

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Introduction: In the light of the antimicrobial resistance crisis it gets important to develop new strategies to target antibiotic resistant bacteria. While conventional antimicrobial therapies also harm the healthy microbiome, we aim to design a phage-based device for selectively interfering with carbapenem resistant *Enterobacteriaceae*. The goal of this approach is a gene-directed

re-sensitization of antibiotic resistant microorganisms. While other studies have demonstrated the proof-of-concept of CRISPR-Cas9-based inactivation of bacterial genes, adequate delivery systems have not yet been fully explored.
Methods: The phage T7 was selected to transduce a CRISPR-Cas9 system targeting carbapenem resistant genes into designated and clinically relevant hosts. T7 was propagated using *E.coli* DSM-613 as a host. Phage lysates were harvested by standard methods comprising centrifugation and filtration. The plasmid pSB1C3 was used as a backbone for the incorporation of the T7 packaging signal, CRISPR-Cas9 targeting bla_{OXA-48} and GFP by HiFi DNA Assembly (*New England BioLabs, NEB*). The fragments were produced by PCR using the Q5 polymerase from *NEB*. Chemically competent *E. coli* BL21 and DSM-613 were prepared using 0.1 M $MgCl_2$-$CaCl_2$ and competent cells were then transformed with plasmids by the heat-shock method. Finally, bla_{OXA-48} was inserted into the pET101 plasmid and transformed into the laboratory *E. coli* BL21 strain. This strain serves as an initial model system to ensure that no further overlapping resistance genes might compromise the re-sensitization efforts. The resistance profile was evaluated by disc diffusion test and minimum inhibitory concentration (MIC).
Results: The final plasmid "pSB1C3-T7 packaging signal-CRISPR-Cas9-GFP" was successfully constructed and transformed into the susceptible *E. coli* hosts BL21 and DSM-613. By infection with T7 wild-type phage and subsequent collection of the lysates, T7 particles packaged with CRISPR-Cas9 were obtained. The packaging efficiency is currently assessed by phage plaque assay and will be further evaluated by digital droplet PCR. Sensitivity levels of *E. coli* BL21 expressing OXA-48 were decreased but further optimization is necessary.
Discussion: As a next step, transduction and re-sensitization of carbapenem resistant *Enterobacteriaceae* will be analyzed. Furthermore, an extension of the host range by generating hybrid phages with exchanged tail fibers will be pursued [1]. Finally, this system will be applied for the targeted manipulation of individual bacterial species within complex microbial communities.
Reference:

1. Yosef, I., et al., *Extending the Host Range of Bacteriophage Particles for DNA Transduction.* Mol Cell, 2017. **66**(5): p. 721-728 e3.

Molecular Infection Epidemiology (FG MS)

197/MSP
Higher levels of neutralising anti-Panton-Valentine-leukocidin-antibodies in Africans compared to Germans

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Background: While the prevalence of Panton-Valentine leukocidin (PVL) among *Staphylococcus aureus* is low in Europe (<5%), markedly higher rates are reported from sub-Saharan Africa (ca. 50%). Carriage of the pore-forming toxin is strongly associated with severe skin and soft tissue infection (SSTI), such as necrotizing fasciitis and pyomyositis. A key event in the pathogenesis of SSTI could be the cytolysis of granulocytes by PVL. The objective of the multicenter study was to measure anti-PVL-antibodies in Africans and Germans and to assess the neutralization capacity towards the cytotoxic effect of PVL on granulocytes.
Methods: Only sera from healthy/asymtomatic Caucasians (n=22) and Africans (n=22) from Nigeria and Gabon were included. Antibody titers were measured (in arbitrary units [AU]) using ELISA. Human neutrophils were isolated from venous blood employing dextran-sedimentation and density gradient centrifugation. The neutralizing effect of antibodies in individual or pooled serum was tested on granulocytes from one African and German donor at different serum concentrations. Cell damage of granulocytes exposed to 5 nM recombinant PVL was analyzed by flow cytometry.
Results: Levels of anti-PVL-antibodies are significantly higher in Africans compared to Germans (1.9 vs 0.7 AU, $p<0.0001$). The sera neutralized the cytotoxic effect of PVL on African and German granulocytes in a dose dependent manner. Neutralization of PVL on granulocytes from both the German and the African donor was stronger by pooled sera from Africans (half-maximal inhibitory concentration (IC_{50}) = 0.27% and 0.47%, respectively) compared to Germans (IC_{50} = 3.51% and 3.59% respectively). A correlation between anti-PVL-antibody titers in donor sera and protective effect on granulocytes was observed.
Conclusion: Compared to Germans, Africans have higher serum levels of neutralizing antibodies (NAbs) against PVL. The protective effect seems to be solely titer-dependent. It remains unclear whether or at what level these NAbs can be used to predict protection against PVL-related diseases.

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Identifying pathogen-specific targets based on resistance plasmid-host interaction and adaptation mechanisms on genomic, transcriptomic and proteomic levels

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Introduction: High-risk pandemic clonal lineages of *Escherichia (E.) coli* and *Klebsiella (K.) pneumoniae* are found worldwide and in different habitats in the One Health context. While antimicrobial resistances (AMR) increasingly emerge, the development of new antibiotics declines. Multidrug-resistant (MDR) bacterial pathogens such as carbapenemase- and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae are classified as "critical" by the WHO underlining the urgent need for new and alternative therapeutic strategies. AMR spread is mostly driven by mobile genetic elements (plasmids) and their transmission among bacteria. We hypothesize that the bacterial host undergoes certain adaptation processes associated with the plasmid itself and/or the chromosome upon resistance plasmid acquisition. These processes involve genes and/or intergenic regions, the transcriptome and/or proteome. Here, we present an approach focusing on plasmid-host interaction and adaptation processes to identify potential new targets in bacterial pathogens, and their applicability.
Material and Methods: For both *E. coli* and *K. pneumoniae*, sequence type-specific ESBL-plasmids were selected and transferred into ESBL-plasmid-free acceptor strains by electroporation to generate ESBL-carrying transformants. We then performed plasmid profile analysis (PPA) and randomly amplified polymorphic DNA PCR (RAPD) to confirm correct plasmid acquisition and exclude contamination. A preliminary microevolution experiment (ME) in standard medium (LB) with antibiotic (cefotaxime) supplement for selection pressure was performed for 12 hours in a 96-well plate format and repeated four times, approximately resulting in 100 bacterial generations. Prospectively, we will perform further MEs with additional media and set-ups, as well as subsequent phenotypic screening assays to rapidly identify putative interaction and adaptation processes. These will then be investigated in-depth on genomic, transcriptomic, and proteomic levels.
Results: So far, we have obtained 16 ESBL-transformants, which were verified by RAPD and PPA. The initial microevolution experiment revealed that the evolved representatives (generation 100) showed a delayed lag phase compared to their corresponding parent (generation 0) without any additional changes in growth.
Discussion: These preliminary results suggest that possible plasmid-host interactions and/or adaptations happen fast. However, it remains to be further investigated, whether and how bacterial changes (such as a prolonged lag phase) are due to media

adaptation. This is one of the reasons why we will perform MEs and phenotypic analyses in different settings. Phenotypic differences between parental and evolved generations will serve as a preliminary indicator for performing subsequent detailed analyses on genomic, transcriptomic, and proteomic levels to identify targets, which are potentially associated with plasmid-host interaction and adaptation.

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WGS-based comparison of clinical *Klebsiella pneumoniae sensu lato* complex isolates with and without resistance to third generation cephalosporins

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Background: Several species of the *Klebsiella pneumoniae sensu lato* complex (*K. pneumoniae, K. variicola, K. quasipneumoniae*) are important nosocomial pathogens with high dynamics in development of resistance especially to third generation cephalosporins and carbapenems. Production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases is the main cause of these resistances that emerged in different clonal lineages of *Klebsiella* in hospitalised patients worldwide. This study aimed to analyse the nationwide phylogeny of *K. pneumoniae sensu lato* complex isolates with and without resistance to third generation cephalosporins.
Methods: *Klebsiella pneumoniae sensu lato* complex isolates were collected prospectively at 23 diagnostic laboratories in two time periods (2016 and 2019) of the resistance surveillance study performed by the Paul Ehrlich Society for Infection Therapy. For whole genome sequencing (Illumina) we randomly included 303 isolates without third generation cephalosporin resistance (147 isolates in 2016 and 156 isolates in 2019) and 52 third generation cephalosporin-resistant isolates collected in 2016. The selected 355 isolates were from blood (n=113) and other materials (urine, respiratory tract, wounds). Genome sequences were assembled using unicycler and further analysed using core genome multilocus sequence typing (cgMLST). Resistance genes were identified using ResFinder.
Results: MLST/cgMLST enabled assignment of 152 different sequence types (STs) to the 355 isolates and identification of *K. pneumoniae* as the dominant species (n=327) followed by *K. quasipneumoniae* (n=19) and *K. variicola* (n=9). The most frequent STs in the 52 third generation cephalosporin-resistant isolates were ST15 (15,4%; n=8), ST307 (9,6%; n=5) and ST219 (7,7%; n=4). For the 303 isolates without third generation cephalosporin resistance ST45 and ST37 were detected both in 2016 (3,4%; n=5 and 2,7%; n=4) and in 2019 (5,2%; n=8 and 5,8%; n=9). Furthermore, ST23, ST25 and ST35 were detected in 2016 (2,7%; n=4 each); and ST14, ST17 und ST20 were detected in 2019 (3,9%; n=6 each). ESBL gene *bla*CTX-M-15 was determined in 38 of 52 third generation cephalosporin resistant isolates (73%), the remaining 14 isolates produced other ESBLs, e.g. SHV-12, CTX-M-1, CTX-M-14 and CTX-M-32.
Conclusions: *Klebsiella* isolates with and without resistance to third generation cephalosporins belong to various clonal lineages, while the frequency of certain clonal lineages differs strongly between the two groups. The detected and worldwide spread epidemic clonal lineages ST15, ST307 and ST219 were associated exclusively with ESBL production and isolates of the known hypervirulent lineage ST23 were susceptible to third generation cephalosporins. Presence of resistance genes other than ESBL genes, virulence genes and plasmid types requires further analyses.

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Unraveling a cluster of nosocomial infections of *Bacillus cereus* in a neonatal intensive care unit using core genome multilocus sequence typing

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Question: The *Bacillus cereus* group are environmental bacteria, which can be responsible for serious nosocomial infections or outbreaks in immunocompromised patients. The ubiquitous presence of these bacteria demand high-resolution typing method to delineate cluster-related from unrelated isolates. Here we analyzed a suspicious accumulation of *B. cereus* detections in a neonatal intensive care unit (NICU), including a blood-stream infection (BSI), with *B. cereus* as the causative pathogen, using an ad-hoc whole genome sequencing (WGS)-based core genome multilocus sequence typing (cgMLST) scheme.
Methods: Between 06/2020 and 10/2021 a total of 340 patients were admitted to a NICU, where all neonates are screened weekly for anal or nasopharyngeal colonization by different pathogens. Due to the increased number of *B. cereus* detections during the regular surveillance, skin, wounds, and the environment on the NICU were additionally sampled. In total, 28 isolates from patient and environmental samples that had been identified as member of the *B. cereus* group, were included in the analysis. By using publicly available WGS datasets, we defined an *ad-hoc* cgMLST scheme for cluster analysis.
Results: We used *Bacillus cereus* strain M3 (NZ_CP016316.1) as the seed genome and 6 additional genomes to define an *ad-hoc* cgMLST scheme comprising 2,759 target genes. All 28 isolates underwent WGS and subsequent MLST and cgMLST typing using the novel cgMLST scheme. Several different STs were present with ST127 as the most common ST with thirteen isolates. Based on the cgMLST scheme, ten of the thirteen ST127 isolates were closely related and formed a cluster with a maximum distance of 5 alleles. The remaining ST127 isolates were more distantly related exhibiting a minimal allelic distance of 31. Three pairs of strains formed separate clusters. The remaining isolates showed no relatedness.
Conclusions: Utilizing the cgMLST scheme, it was possible to accurately find clusters of closely related isolates while excluding coincidentally detected *B. cereus* isolates. Some isolates showed no genetic relatedness, underlining the ubiquitous nature of this microbe, while other isolates appeared to be closely related indicating preceding transmissions. In summary, this study showed that *B. cereus* can be typed using an *ad-hoc* created cgMLST scheme to resolve clusters with a sufficient discriminative power even among closely related isolates.

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The geographical distribution of human cutaneous and visceral leishmania species identified by molecular methods in iran – a systematic review with meta-analysis

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Introduction: Leishmaniasis is one of the most common vector-borne parasitic diseases in Iran. *Leishmania* species identification is necessary for epidemiological aspects, precise prognosis, control and treatment of the disease.
Material / method: We systematically searched all the studies, reports, and documentation related to species identification and geographical distribution of causative agents of cutaneous (CL),

mucosal (ML), and visceral leishmaniasis (VL) using DNA-based molecular diagnostic techniques in Iran. International databases including PubMed, ScienceDirect, Embase, Google Scholar, Scopus, and Web of Science were systemically searched for English articles and Iran's databases including SID, IranMedex and Magiran were searched for Persian reports and articles. Searches were performed from 1999 to 2019 (20 years). The current review was conducted using the keywords: cutaneous leishmaniasis, visceral leishmaniasis, *Leishmania* species, Human, Molecular, PCR, and Iran. The study quality was evaluated using the NOS checklist. This meta-analysis procedure was accomplished using STATA, version 2.7.9.

Results: Of the 3,426 records identified in the initial search, 154 articles met inclusion criteria and qualified for the systematic review and meta-analysis. In subgroup analysis, the pooled frequency of causative agents of CL isolates was 67.3% (95% CI: 59.51–74.67%) for *L. major* and 32.1% (95% CI: 24.72–39.87%) for *L. tropica*. In addition, the pooled frequency of causative agents of VL isolates was 97.1% (95% CI: 94.6–98.8%) for *L. infantum* and 2.9% (95% CI: 1.12–5.37%) for *L. tropica*.

Discussion: The findings of this study showed that the main causative agents of CL and VL in Iran are *L. major* and *L. infantum*, respectively. Moreover, kinetoplast DNA (kDNA) and internal transcriber spacer (ITS) were the most used markers for identifying *Leishmania* species. The current study provides valuable data to encourage and direct researchers as well as public health managers in the comprehensive leishmaniasis control and prevention planning in Iran.

Pathogen genome-based Prediction of Antibiotic Resistance (FG MS)

202/MSP

Impact of growth-speed selection without the involvement of antibiotics in quinolone-resistant mutants of *Escherichia coli* with declined growth speed

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Question: Multiple-drug resistant (MDR) enterobacteria, especially 3- and 4-MRGN are a major challenge to healthcare systems worldwide. Beside ß-lactams, fluoroquinolones (fq) like ciprofloxacin (CIP) remain an effective last-resort antibiotic against life threatening gram-negative infections. Fluorquinolones show a rapid bactericidal activity due to an irreversible block of bacterial DNA gyrase and topoisomerase IV bound to dsDNA breaks. Clinically relevant resistance to these synthetic drugs includes target mutations in genes gyrA and parC and increased MDR-efflux by upregulation of the ArcA/BTolC efflux system. While combinations of these mutations are detectable both, in patient isolates as well as in mutants selected in vitro in a few selection steps. However, the latter shows strongly reduced fitness (growth rate) and diminished expression of virulence factors (type-1 fimbriae), whereas patient isolates retain wild type-like behaviour. To identify the genetic basis for the adaption of fluoroquinolone resistant mutants to a restored fitness/virulence, high-level fqR in-vitro mutants were grown under fluoroquinolone-free conditions for up to 300 generations. Clones isolated after up to 300 generations were analyzed for growth rate, fq susceptibility and occurrence of additional mutations.

Methods: A high-level fqr in-vitro mutant *E. coli* MIVb derived from a fq susceptible wildtype strain (WT) (Heisig, Tschorny, 1998) was inoculated for 25 days in quinolone-free LB growth medium (generation time = 120 min). Samples were harvested after 25, 100, 200 and 300 generations, and plated on LB Agar plates, containing CIP (0.5xMIC) and antibiotic-free LB Agar

Growth rate was determined as background-corrected absorption (BCA), using a OCelloScope (BioSense Solutions) with UniExplorer software

Antibiotic susceptibility was determined as minimal inhibitory concentration (MIC) in MH broth according to standard procedure

Genomic DNA was isolated (Monarch Genomic DNA Purification Kit) and sequenced at GeneWIZ

Results: After 25 generations growth in antibiotic-free medium, highly fqR-mutant MIVb of *E. coli* WT showed a reduction in CIP MIC and a more than restored growth rate compared to its parent. Results from whole genome sequencing reveal an *acrA* mutation in these strains.

Conclusions: The results of a genetic and functional comparison of high-level fqR *E. coli* strains selected in-vitro and isolated from patients reveal significant differences in growth rate and extend of fqR phenotype. WGS data indicate a role of altered activity of the AcrAB-TolC MDR efflux pump in the process of adaption to a compensated fitness of fqR mutants of *E. coli*.

Fig. 1

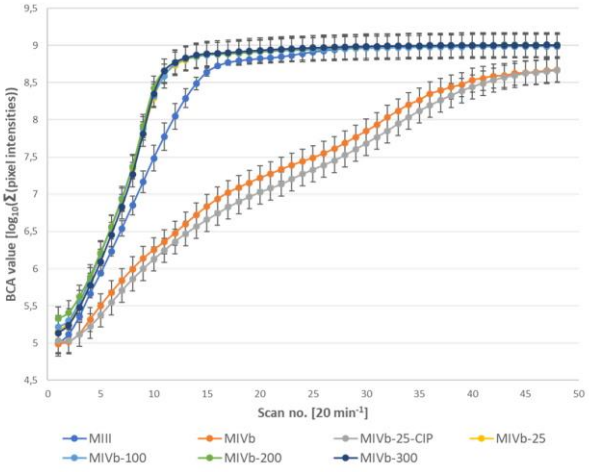

Figure 1: Bacterial growth, indicated as background-corrected absorption of growth speed-selected successor strains of *Escherichia coli* MIVb.

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WGS-Analysis of non-betalactamase-mediated carbapenem resistance in the nonfermenter *A. baumannii*

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Question: Carbapenems have emerged to retain a high efficacy against otherwise resistant bacteria. Due to the serious situation of increasing carbapenem-resistant species, physicians and researchers are interested to elucidate the nature and contributing factors of this resistance. About five percent of the carbapenem resistant *A. baumannii* isolates, that were sent to the National reference laboratory for Gram-negative bacteria, do not own a carbapenemase, despite of the strain-specific OXA-51-like carbapenemase. Here we report other genes, which may convey carbapenem resistance in these isolates.

Methods: WGS was performed on a set of carbapenem-resistant NRC isolates of *A. baumannii* without carbapenemase production. They were sequenced by Illumina and Nanopore technology to create a hybrid assembly for better quality. Genes that are associated with carbapenem resistance such as porins, efflux pumps and PBPs were selected and further compared to the genome of the carbapenem-sensitive reference strain ATCC17978.

Results: The sequencing results of the NRC isolates confirm the absence of any carbapenemase gene despite the strain-specific *bla*OXA-51. Furthermore, overexpression of *bla*OXA-51 by IS*Aba1* could be excluded. In comparison to the sensitive reference strain, we have observed several point mutations located in regulator genes of efflux pumps; such as *adeR* and *adeS*, in the efflux pump genes themselves; such as *adeH*, in most of the PBP genes and in some porin genes such as gene *omp33-36*. Many of these point mutations, like A136V in AdeR, were conserved among the NRC isolates. Another interesting observation was a different porin pattern of the NRC isolates compared to the ATCC 17978 strain. Most of them exhibited *oprD* and some *oprD* like and *ompW* like family proteins, that could be found in ATCC 17978 and are not yet annotated for *A. baumanni*.

Conclusion: A variety of factors such as increased expression of efflux pump genes, structural changes of efflux pumps, PBPs or porins due to point mutations and the loss of porins could possibly contribute to carbapenem resistance in non-carbapenemase producing *A. baumannii* isolates. Further investigation and mutagenesis of the respective candidate genes is needed to confirm the impact of the described findings on carbapenem resistance.

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Enhanced expression of sigma E factor increases carbapenem resistance in *Klebsiella pneumoniae* with *bla*$_{OXA-48}$

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Background: In Germany, *Klebsiella pneumoniae* with *bla*$_{OXA-48}$ 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*$_{OXA-48}$.
Materials/methods: Twenty clinical *K. pneumoniae* isolates with *bla*$_{OXA-48}$ 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. Found mutations were edited in the primary clinical isolates using CRISPR/Cas 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 literature. But not all mutants revealed defects in OMP genes. Two spontaneous mutants had an insertion element in their sigma E-operon in the anti-sigma E factor gene *rseA*. Inhibited expression of *rseA*, *rseB* and *rseC* in the spontaneous and genetically constructed mutants leads to an enhanced expression of *rpoE* alongside with a downregulation of porin genes after 4 h and 12 h of incubation. Diminished production of OMPs could also be seen in SDS-PAGE for the spontaneous and genetically constructed mutants. These mutants showed significantly elevated MICs of carbapenems and cephalosporins, but also lower growth rates. Clinical *K. pneumoniae* isolates with high MICs of carbapenems will be screened for similar mutations to check if this resistance mechanism also occurs in clinical context.
Conclusions: A few mutations can have a huge impact on MICs of carbapenems in *K. pneumoniae* with *bla*$_{OXA-48}$. The resistance levels can be biased not only by mutations in the porin genes themselves, but also via altered genes regulating porin gene expression. This is an important point to remember for future approaches to predict resistance levels from WGS data.

205/MSP

Metagenomics analyses reveal a wide distribution of antimicrobial resistance genes from active sludge and wastewater samples

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Introduction: Antimicrobial resistance (AMR) has been recognized as a major threat to public health. Wastewater treatment plants (WWTPs) serve as a reservoir of resistomes and a media for AMR transport and proliferation. However, the consequences of AMR gene abundance and diversity in WWTPs on human, animal, and ecosystem health are still little considered.
Aim: In this study, we aimed to assess the presence of antimicrobial resistance (AMR) genes in publicly available active sludge and wastewater metagenomes to comprehensively analyze AMR gene compositions and abundance.
Method: We retrieved 165 public metagenomes from various active sludge and wastewater treatment plants covering 14 countries. Subsequently, metagenome-assembled genomes (MAGs) were recovered using MetaWRAP. We defined as MAGs the bins recovered by MetaWRAP with a quality score above 50 (quality score = % completeness – (5 x % contamination)). Completeness and contamination are calculated by measuring the presence and absence of 122 known single-copy genes. MAG taxonomy was assigned using GTDB-tk and AMR genes were predicted using DeepARG. We set a 35% similarity and E-value of 10-10 threshold to avoid the risk of false-positive prediction of reads as ARG-like. We defined resistome as the collection of AMR genes in a specific MAG.
Result: We recovered 5917 MAGs of high and medium quality. MAGs with a quality score above 50, completeness exceeding 90% and contamination below 5% were considered high-quality. MAGs with a quality score greater than 50, completeness between 50-89% and contamination below 10% were regarded as medium quality. These MAGs covered a total of 73 different phyla; 70 phyla were found with AMR genes. We found resistomes in 5291 (89.51%) MAGs with 13224 AMR genes. These AMR genes were dereplicated into 343 unique AMR genes and distributed in 27 AMR classes. The most dominant AMR genes were multidrug (4188), with 105 unique AMR genes, glycopeptides (2765) with 24 unique, bacitracin (997) with two unique, tetracyclines (654), with 24 unique and beta-lactam (430) with unique 40. A total of 679 AMR genes predicted by DeepARG were unclassified and may target novel classes of antibiotics. Further, our data revealed multidrug (31.67%) and glycopeptides (20.90%) as the most prevalent AMR classes within our data. Our data also showed *Proteobacteria*, *Bacteroides* and *Chloroflexota* phylum are the major pool of resistomes.
Discussion/Conclusion: Our study provided a comprehensive overview of AMR genes in different active sludge and wastewater treatment plants, highlighting the association of AMR genes with MAGs. It also links the resistome interconnectivity within the microbiome. This research's outcome may help design new management strategies to diminish AMR transmission and evolution in constructed ecosystems.

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Role of biocides in selection of methicillin-resistant, *mec*-negative *Staphylococcus aureus*

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Question: *Staphylococcus aureus* MRLM (methicillin resistant lacking *mec*) strains are easily misclassified as methicillin susceptible based on the exclusive detection of *mec* genes or PBP2a. Hence, these isolates pose a threat to public health and represent a diagnostic and therapeutic challenge. While we have recently shown that mutations in the phosphodiesterase GdpP are a common cause of the MRLM phenotype, it is still largely unknown how this phenotype is selected in the clinical setting. Since many MRLM isolates originate from nasal screenings and occur independently of prior antibiotic therapy, we investigated the potential role of biocides in selection of these isolates isolates.
Methods: By broth microdilution, MICs were determined for the following biocides: Benzalkonium chloride, Cetylpyridinium chloride, Didecyldimethylammonium chloride, Octenidine, Chlorhexidine, Sodium hypochlorite, Polyhexanide, Potassium peroxomonosulphate and Triclosan. MICs were compared within MRLM-MSSA strain pairs. The pairs were either clinical strains from one patient each or pairs of *gdp*P mutants and isogenic strains complemented with a functional *gdp*P. In addition, in vitro

selection experiments were carried out with various antiseptic compounds and strains generated under selection were tested for their sensitivity to biocides and antibiotics in order to investigate possible cross-adaptations.
Results: Initially, no MIC differences were found between MRLM and MSSA strains. It was possible to adapt MSSA strains to grow in gradually increasing concentrations of several biocides tested. However, no increase in biocide and antibiotic MICs has yet been detected in the adapted strains. To gain further insights into the adaptive effects, we are currently sequencing the genomes of the adapted strains.
Conclusions: Under the conditions used here, we have not yet succeeded to show a correlation between adaptation to biocides and the development of *mec*-independent methicillin resistance. Nevertheless, the possibility of a correlation should not be completely ruled out. Therefore, we are currently varying our selection experiments with regard to the biocides used as well as the selection process. Investigations into possible effects of biocides on antibiotic resistance are of particular clinical importance, since the use of biocides in medicine and food production, but also in private households, appears rather unsupervised and an influence on the ability to treat bacterial infectious diseases cannot be ruled out.

207/MSP
***Escherichia coli* with decreased susceptibility towards colistin from infected pigs, cattle and poultry collected within the national GE*RM*-Vet resistance monitoring**
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Introduction: Antibiotic resistance is a major threat in human as well as in veterinary medicine. In human medicine, colistin is used to treat infections with multidrug-resistant Gram-negatives as a last-resort drug. Since the description of mobile colistin resistance (*mcr*) genes it is known that not only chromosomal, but also plasmid-mediated resistance occurs suggesting an enhanced resistance transmission. *E. coli* with decreased susceptibility towards colistin also arise as pathogenic agents in veterinary clinical cases. Resistance profiles, molecular resistance markers and molecular relationships of these *E. coli* from infected pigs, cattle and poultry from the German national resistance monitoring program "GE*RM*-Vet" were gathered within this study.
Material and methods: 241 *E. coli* isolated in Germany from diseased pigs, cattle and poultry with a MIC of >2µg/ml for colistin were analyzed. The isolates were collected at the Federal Office of Consumer Protection and Food Safety (BVL) in 2004-07 and 2017-20. Antimicrobial susceptibility testing of 24 antimicrobial agents relevant in veterinary treatment was conducted according to current CLSI-Vet guidelines (with interpretative criteria for 6 substances/drug classes). Illumina MiSeq-whole genome sequencing was performed after library preparation (Illumina *DNA prep kit*) followed by *de novo* assembly with the AQUAMIS pipeline and BakCharak-analyses.
Results: First results revealed colistin resistance was found to be mediated by plasmid-encoded *mcr* genes in about 50% of *E. coli* isolates. Data from this study suggest an increase of plasmid-borne resistance determinants over time. The dominant subtype of *mcr* was *mcr-1*. It was detected in *E. coli* from pigs, cattle and poultry and mainly occurred in recent isolates. The *mcr-4* type was detected in pig isolates, in early as well as in recent *E. coli* while the *mcr-5* subtype was only seen in *E. coli* in the 2004-07 collection. In certain isolates, plasmid-mediated quinolone resistance genes (PMQR) were also detected next to mobile colistin resistance genes. About two thirds of *E. coli* tested exhibited a multidrug-resistance phenotype (resistant towards at least 3 drug classes), among them certain isolates were resistant to four different drug classes. About one sixth of recent isolates showed next to decreased colistin susceptibility also decreased ciprofloxacin susceptibility.
Discussion: These results indicated an increase of mobile colistin resistance genes in veterinary pathogenic *E. coli* with decreased colistin susceptibility over time and identified multidrug-resistant isolates. For the surveillance of colistin and further resistances antimicrobial susceptibility testing combined with whole genome sequencing provides a valuable tool in veterinary bacteriology to identify emerging trends especially with regard to the One Health concept, considering the resistance situation in animals, humans, livestock and the environment.

Epidemiology and Antimicrobial Resistance of Zoonotic Pathogens (FG MS/FG ZO)

208/MSZOP
Bacterial resistance evolution towards disinfectants and antimicrobial surfaces and development of a standardised test
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Question: Disinfectants and antimicrobial surfaces (AMCs) are important tools to prevent the spread of pathogens and antimicrobial resistant bacteria. However, concerns have been raised about the possibility for the evolution and selection of resistance against disinfectants and AMCs. In turn, resistance against disinfectants and AMCs can be associated to antibiotic resistance due to cross-resistance - a single mechanism conferring resistance to a disinfectant and an antibiotic- and co-resistance - two distinct mechanisms physically linked on e.g. a plasmid. The risk for resistance and cross-resistance during use of biocides (including disinfectants and AMCs) must be evaluated during authorization according to the EU biocidal product regulation. However, to date there is a lack of standardized methods that support risk assessment during the authorization process.
Methods: We used adaptive laboratory evolution (ALE) experiments which are based on repeated exposure of bacteria to disinfectants or AMCs. The experiments are followed by phenotypic (antimicrobial susceptibility testing) and genotypic (whole genome sequencing) characterization of the evolved strains. The basic idea of these experiments is to expose bacteria to lethal conditions and select for mutants with increased survival. This approach is fundamentally different to other ALE experiments, which commonly select for increased growth at subinhibitory concentrations. Selection for increased survival represents a selective pressure that more realistically reflects selection under in-use conditions of disinfectants and AMCs.
Results: First, we studied adaptation of *E. coli* during repeated disinfection with benzalkonium chloride in a suspension assay. The experiments showed a 2000-fold increase in survival within 5 exposure cycles. The adaptive changes are linked to highly parallel mutations in genes related to lipid A biosynthesis, less negative cell surface charge, reduced growth rate and increased competitive ability in the presence of certain antibiotics (1). We use the same approach to develop standardizable ALE experiments based upon accepted standards that are used to determine the efficacy of disinfectants (EN 13697) and antimicrobial surfaces (ISO 22196). The results highlight pronounced adaptation of different test strains towards surface disinfection (benzalkonium chloride and isopropanol) and AMCs (copper).
Conclusion: Bacteria can adapt with increased survival towards lethal stress imposed by disinfectants and AMCs. The adaptive ability of bacteria to disinfectants and AMCs can be determined in a standardized manner.
(1) Nordholt, N., Kanaris, O., Schmidt, S., and Schreiber, F.. Persistence against benzalkonium chloride promotes rapid evolution of tolerance under periodic disinfection. 2021. Nature Communications 12:6792

209/MSZOP

Effects of livestock-associated environmental conditions on the transcription of genes involved in SCC*mec* mobility in MRSA ST398

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Introduction: Previous research on methicillin susceptible *Staphylococcus aureus* (MSSA) belonging to livestock-associated (LA-) sequence type (ST) 398, isolated from pigs and their local surroundings, indicated that differences between these MSSA and their methicillin resistant predecessors (MRSA) are often limited to the absence of the staphylococcal cassette chromosome *mec* (SCC*mec*) and few single nucleotide polymorphisms [1]. Since SOS-induced activation of the large serine recombinases (*ccr*), involved in site-specific mobilization of SCC*mec*, has been reported [2], we investigated the effects of typical LA environmental conditions on the expression of genes known for their association with the element"s mobility.

Material/Methods: In-depth genomic and transcriptomic analyses were performed using the LA-MRSA ST398 strain IMT38951 (isolated from a pig) and its methicillin susceptible descendant IMT38951_42. We further evaluated the transcriptomic responses of *ccr* genes and other factors involved in the mobilization of SCC*mec* elements in MRSA ST398 across pig-farm associated environmental conditions, i.e. contact with host proteins (10% porcine serum) and an increased ammonia concentration (0.3%).

Results: Full genomic reconstruction of the SCC*mec* integration site downstream of *rlm*H (previously: *orf*X) revealed a mosaic-structured region that includes a putative replicative SCC*mec*Vc. The latter element is absent from the MSSA chromosome through homologous recombination that likely occurred between short repetitive sequences. Further analysis indicated the presence of a complete replication machinery [3] in the deleted SCC*mec*Vc element. Exposure of MRSA ST98 to 0.3% ammonia (elevating the pH of the culture to 9) revealed limited activation of genes belonging to the SOS-response after 10 and 60 min of exposure. However, neither exposure to ammonia, porcine serum or a combination of both induced any significant differential expression of the two *ccr*C variants present in that region nor other mobility-associated genes encoding transposases or replication initiation proteins.

Discussion: The *ccr*-driven excision of SCC*mec* from its chromosomal integration site is known to affect the amount of 15 bp direct repeats (DR) across the particular region. Since the number of DR remained unchanged, other mechanisms likely caused the loss of SCC*mec* in IMT38951_42. Typical environmental LA conditions also failed to significantly induce *ccr* transcription, suggesting that some LA-MRSA might be able to dismiss a fully replicative SCC*mec* element, while activation of the *ccr* genes by cleavage of LexA from its promoter region [3] seems to depend on provocation of more sufficient SOS-responses. Further research on these mechanisms that are likely involved in facilitating the spread of SCC*mec* is warranted.

1. Chlebowicz et al. (2010). .Antimicrob Agents Chemother 54, 783-791.
2. Liu et al., (2017). Nucleic Acids Res. 20;45(7):3944-3952.
3. Bebel et al. (2020). eLife.55478

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ST131-*fimH*22 *Escherichia coli* of poultry reveal a close relationship to human clinical isolates from Germany

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Background: *Escherichia coli* sequence type 131 (ST131) has emerged globally as the most important extraintestinal pathogenic (ExPEC) lineage causing predominantly bloodstream and urinary tract infections in humans. Multidrug-resistant ST131 typically possess fluoroquinolone and extended-spectrum beta-lactamase (ESBL) genes, which significantly affects treatment success. The pathogenic potential is further enabled by the presence of an extensive variety of virulence factors. However, such isolates not only occur in humans, but are also frequently isolated from livestock animals and animal-based food products. In 2014 and 2020, four ST131-*fimH*22 *E. coli* were isolated from turkey or chicken feces and from chicken meat as part of the national zoonosis monitoring in Germany. To investigate a possible transmission of ST131-*fimH*22 *E. coli* along the food production chain, we conducted whole-genome sequencing (WGS)-based analysis of four livestock and food isolates in comparison with 26 additional publicly available (Enterobase) data sets of ST131-*fimH*22 *E. coli* obtained from humans, livestock animals, foodstuff and the environment from Germany.

Methods: A total of 30 ST131 *E. coli* from Germany possessing the fimbrial adhesin allele *fimH*22 were investigated. In light of the One Health perspective, genome comparisons and WGS-based *in silico* characterizations were conducted using the web-based tools of the Center for Genomic Epidemiology. Furthermore, isolates were assessed for their pathogenic potential.

Results: All 30 ST131-*fimH*22 *E. coli* belonged to the phylogroup B2 and serotype O25:H4. SNP-based phylogenetic analysis using a human isolate as reference revealed a homogeneous distribution and clustering of isolates was independent of their source or year of isolation. Genomic differences of *E. coli* within and cross-sectors ranged from 1 to 59 SNPs. 90% of the population carried at least one ESBL-/AmpC-gene with *bla*CMY-2 (60%) as the most abundant. In particular, isolates, which acquired the most resistance genes, were recovered from poultry feces or meat. Notably, ST131-*fimH*22 *E. coli* show high similarity in their virulence gene content of which six isolates of different sources, including human, poultry, meat, and the environment, exhibited an identical virulence gene profile. In contrast, certain virulence-associated genes were only present in human isolates such as *afaACD* (host colonization), *cnf1* (toxin), *nfaE* (non-fimbrial adhesion), *papA* (colonization of the urinary tract) and *senB* (plasmid encoded enterotoxin).

Conclusion: Our study reveals a close relationship between ST131-*fimH*22 *E. coli* from different sources in Germany highlighting a possible transmission route along the food production chain with poultry as possible source. A study suggested recently that ST131-*fimH*22 *E. coli* might be foodborne uropathogens, which we supported by our data. Carriage of certain virulence factors might be decisive for infection development in the human host.

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Evaluating disinfectant efficacy against *Salmonella* biofilms

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Introduction: Bacteria can form matrix-embedded aggregates called biofilms, in which individual cells are protected from adverse environmental conditions. When compared with planktonic cells, they show an increased tolerance towards disinfectants, resulting in severe problems in livestock husbandry and the food processing industry. Currently, disinfectant efficacy is evaluated against planktonic cells, thus, posing the risk of inadequate recommendations regarding their effectiveness against biofilms. Here, we demonstrate the applicability of a highly reproducible method for testing the efficacy of disinfectants on biofilms for testing *Salmonella* biofilms.

Methods: *Salmonella* Typhimurium biofilms (reference strains ATCC 14028 & ATCC 13311) were grown on porous glass beads according to a previously described "Bead Assay for Biofilms"

protocol. After two days in LB without NaCl at 20°C, biofilms were exposed to different concentrations of peracetic acid (PAA) for 10 min, or glutaraldehyde (GA) for 30 min. Disinfectant efficacy was evaluated by assessing the number of recoverable viable colony forming units (CFU). Successful disinfection was defined as a ≥5 $\log_{10}$ reduction of CFU compared to an untreated control. Assessment of disinfectant efficacy against planktonic *Salmonella* was performed using the EN 1656 standard using the same strains and conditions.

Results: Enumerating *Salmonella* in untreated biofilms revealed a cell count of 7.4 $\log_{10}$ CFU/ml (±0.3), which is sufficient for disinfectant efficacy testing, and also demonstrated the high comparability of individually cultured biofilms. For PAA, a ≥5 $\log_{10}$ reduction in the CFU of *Salmonella* biofilms was achieved with a PAA concentration of 0.1% (w/v), whereas 0.002% PAA was sufficient for disinfection of planktonic cells, thus demonstrating the increased tolerance of *Salmonella* organized in biofilms. Similarly, higher concentrations of GA were needed for disinfection of biofilm-embedded bacteria (0.1%-0.5% w/v), as compared to the concentration required for the inactivation of bacteria in suspension (0.03%). In addition, comparable CFU recovery rates and disinfectant testing results were obtained when the experiments were performed by two different technicians, emphasizing the intra-laboratory repeatability and robustness of the method.

Discussion: We demonstrate here that concentrations of PAA and GA, which proved to be sufficient for inactivation of cell suspensions, failed to eradicate *Salmonella* biofilms. This suggests that routinely applied disinfection programs may not be sufficient to effectively eradicate biofilms containing *Salmonella*. The Bead Assay for Biofilms proved to be a reproducible and repeatable method to evaluate disinfectant efficacy against *Salmonella* biofilms. Further studies are needed to assess the inter-laboratory reproducibility of the method and to evaluate its potential as candidate for the standardization of disinfectant efficacy testing for bacterial biofilms.

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Antimicrobial photodynamic therapy with different photosensitizers – *Escherichia coli* biosensors reveal a substance-specific primary site of attack

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Introduction: Due to the antibiotic crisis, novel antimicrobial therapies are urgently needed. In contrast to classical antibiosis, the antimicrobial properties of chemical compounds in antimicrobial photodynamic therapy (aPDT) first need to be activated. This reduces the probability for resistance development and aPDT is therefore believed to be a more sustainable form of antimicrobial therapy. aPDT only requires a light-sensitive dye called photosensitizer (PS) that generates highly cytotoxic reactive oxygen species upon irradiation with visible light. Even though many different PSs have been synthesized until today, there is still a lack of experimental data on the precise mechanism of action and the primary targets of the PS in the bacterial cell, which is still limiting the use of this form of therapy. We used here a set of *Escherichia coli* (*E. coli*) K-12 MG1655-based biosensor strains in order to elucidate mechanism of action and primary targets of different PS.

Methods: Lambda red mediated recombineering was used to construct a set of complex stress gene promoter- fluorescent reporter gene reporter modules that allow for the real-time detection of different types of stress in *E. coli* K-12 MG1655. In addition, recombineering was used to construct sets of regulator mutants for each sensor strain. A microplate plate reader was used to detect fluorescence signal and optical density in the presence/absence of the test substance in real time on the population level. Fluorescence microscopy and flow cytometry were used to assess differences in stress gene promoter activities within the bacterial populations.

Results: Our results indicated that the PS methylene blue caused mainly oxidative stress in the cytosol while the PS 2(3), 9(10),16(17), 23(24)-tetrakis-[3-(N-phenyl) pyridyloxy]-phthalocyaninato dihydroxy-silicon(IV) tetrabromide caused mainly envelope stress to *E. coli* at physiological concentrations. The stress response was specific for both PS, as a stress response signal from the respective promoter was absent in sensor strains lacking specific transcriptional regulators for oxidative and envelope stress.

Conclusion: Our results indicate that even though the basic mechanisms of action of different PSs may be identical, the primary site of action of the drugs likely depends on their overall chemical properties. Moreover, we show here that *E. coli* can be directly used not only as a sensor for the identification of the type and extent of stress, but also for the localization of the primary targets of the stressor.

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***Mammaliicoccus sciuri* – a common resistance gene reservoir?!**

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Question: We previously performed studies on the antimicrobial resistance (AMR) profiles of coagulase-negative staphylococci (CoNS) from dust and manure samples in conventional pig farms in Germany. Unexpectedly, we found high AMR rates among CoNS in pig farm environments, which were mainly driven by *Mammaliicoccus sciuri* isolates that were overabundant in the sample. Interestingly, we also detected decreased susceptibilities towards last resort antibiotics such as linezolid and daptomycin in some of the *M. sciuri* isolates. Here, we set out to determine the AMR gene content of the *M. sciuri* strain collection by whole genome sequencing in comparison with *M. sciuri* strains from other geographic origins. Moreover, we elucidate the high-level daptomycin resistance (DAP-R) mechanism in one of the *M. sciuri* isolates.

Methods: Our livestock-associated *M. sciuri* strain collection (Bacterial isolates from industrialised pig farms (dust samples) in Germany (n=120) and from grazing goats in Nigeria (n=35) were analysed regarding genetic relatedness and pathogenicity starting with antibiotic resistance. These data were compared to the published *S./M. sciuri* genomes deposited at NCBI database (n=62). Furthermore, transcription profiling upon DAP exposure was analysed to identify candidate DAP-R genes. Genes of interest were subcloned and heterologous expressed to prove correlation to DAP-R. Furthermore, whole-genome sequencing was performed.

Results: 87.6 % of German and Nigerian isolates were phenotypically multidrug resistant (MDR). *mecA1* and *salA* are present in all isolates and genes mediating resistance to lincosamide/streptogramin, methicillin and tetracyclines are the most common AMR genes in the collection. In general, isolates from Nigeria were less resistant than isolates from Germany.
Transcription profiling upon daptomycin exposure identified an ABC transporter homologue (named *drcAB*) as candidate DAP-R gene in *M. sciuri* TS92. *drcAB* genes are highly upregulated upon daptomycin exposure and subcloning and heterologous expression of *drcAB* causes high-level DAP-R in *S. aureus*. Whole-genome sequencing revealed that the *drc* cluster is located on a novel staphylococcal cassette chromosome (SCC) element, thus it is potentially mobile.

Conclusion: Although the exact functions of the membrane-associated Drc proteins are not fully understood yet, the data strongly suggest that *drc*AB is capable to mediate high-level DAP resistance in staphylococci. Investigations are currently under way to explore the genetic origin of the gene cluster and its potential to spread via horizontal gene transfer. The data highlight *M. sciuri* (or

coagulase negative staphylococci in general) as potent reservoir of AMR genes. Thus, *M. sciuri* poses a potential thread by acquiring and transfer of AMR genes among other staphylococcal species like *S. aureus*.

Exit Strategies of Intracellular Pathogens (FG EK/FG MP)

214/EKMPP

Candida albicans immune evasion strategies during interaction with macrophages

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Candida albicans is a commensal fungus that is part of the healthy human microbiome. However, the fungus also represents a predominant cause of nosocomial fungal infections by causing life-threatening systemic candidiasis in immunocompromised patients. During infection, *C. albicans* is taken up by macrophages. Internalized *C. albicans* cells are enclosed in the phagosome which harbors unfavorable conditions like restriction of nutrients or presence of antimicrobial substances. Nevertheless, *C. albicans* is able to survive, form hyphae and escape from the macrophage. We aim to unravel the strategies that *C. albicans* uses for intracellular proliferation and exit from macrophages.
Most studies on *C. albicans* – macrophage interactions were performed with murine phagocytic cells, in which fungal escape is already visible 6 h post infection. We focus on an *in vitro* model of primary human blood monocyte-derived macrophages, which show different infection kinetics with escape events 8-10 hours post infection.
Previous studies have assigned *C. albicans* escape and host cell damage mainly to mechanical forces and glucose consumption of growing *C. albicans* hyphae as well as NLRP3 inflammasome-dependent inflammatory cell death (pyroptosis). We have identified the fungal peptide toxin candidalysin (encoded by the gene *ECE1*) as a new player in fungal escape from the macrophages by causing damage to host membranes. Although candidalysin activates the NLRP3 inflammasome, it is dispensable for pyroptosis. Our future work will analyze whether candidalysin is involved in induction of necroptosis or other types of programmed cell death.
While it is already well understood how *C. albicans* adapts its metabolism to the challenging nutrient conditions in the phagosome, it is not fully understood which nutrients are central for fungal proliferation and escape. Our data indicate that *C. albicans* has access to cytoplasmic or external nutrients. The length of intracellular hyphae was reduced when macrophage infection was done in nutrient-scarce HBSS (Hanks′ Balanced Salt Solution) as compared to more nutrient-rich standard cell culture medium. Also, fungal cells pre-starved for biotin formed shorter filaments. Based on these data, we will systematically examine how the macrophage infection environment as well as the fungal nutritional conditions before infection might impact *C. albicans* escape and also macrophage polarization. Finally, the transcriptional profiling of macrophage-internalized *C. albicans* wild type *versus ece1*Δ/Δ deletion mutant cells will help to reveal potential effects of candidalysin-mediated membrane damage on nutrient acquisition.
Taken together, our experiments will reveal the mechanisms behind filamentation of *C. albicans* inside macrophages and ultimately escape from these immune cells.

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Intracellular *Staphylococcus aureus* induced cell death and cytokine release in human macrophages

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Professional phagocytes play a key role in the host defense against bacterial pathogens by recognizing, engulfing and eradicating invading bacteria. *S. aureus* has evolved strategies to avoid the host immune response, either by hiding inside of the phagocytic cells or escaping from within cells by the induction of a so far unknown type of cell death. We show that two major regulators of *S. aureus* namely AgrABCD and SaePQRS act in concert to facilitate escape of the bacteria after initial uptake by human macrophages. Sae-regulated two-component pore-forming toxins LeukocidinAB (LukAB) and/or Panton-Valentin Leukocidin (PVL) are required for the escape of *S. aureus* from within human macrophages after phagocytosis. Non-toxic strains were able to persist in macrophages without causing cell-death.
LukAB when added exogenously potently trigger the activation of the NLRP3 inflammasome, which promote IL-1ß secretion. The NLRP3 Inflammasome activation leads to the death of primary human monocytes, which is called Pyroptosis. Conversely, the role of LukAB, when expressed intracellularly by *S. aureus* and its effects on the NLRP3 inflammasome and the cell-death pathway Pyroptosis are not well understood.
Checking the three main cell-death pathways Apoptosis, Pyroptosis and Necroptosis we could show that the pore-forming toxin LukAB induced from within macrophages is enough to induce cell-death, independent of pyroptosis and apoptosis. The intracellular activation of the NLRP3 inflammasome was also LukAB independent. However, necroptosis seems to be involved. Thus, intracellular toxin dependent cell-death and cytokine release are due to different mechanisms. This leads to the question which role plays lukAB intracellular and how does it act in Macrophages?

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Characterisation of cellular exit strategies of intracellular *Yersinia pseudotuberculosis*

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Yersinia pseudotuberculosis is a Gram-negative pathogen that causes zoonotic infections. After oral uptake *Y. pseudotuberculosis* passes through the gastrointestinal tract until it reaches the terminal part of the ileum. To get access to the lymphatic tissue beneath the epithelium, bacteria specifically adhere with the outer membrane protein invasin to the β1 integrins on the apical part of the M-cells leading to integrin clustering and finally internalization of the bacteria by the so-called zipper mechanism. Intracellular bacteria are enclosed in vacuoles (*Yersinia* containing vacuoles, YCV) with autophagy-related characteristics and fusion of YCV with lysosomes is actively blocked by *Y. pseudotuberculosis*, creating a niche for replication. Upon an unknown mechanism, *Yersiniae* can egress from infected cells and subsequently, reach the underlying Peyer's patches. The gentamicin protection assay represents a convincing method to assess and quantify adherence and internalization capacities of bacteria. We recently adjusted the protocol to also enumerate bacteria released from infected cells after prolonged incubation by high throughput flow cytometry. Using this system, we identified pharmaceutically approved compounds that prevent bacterial egress from infected Hela cells. Interestingly, blocking autophagy by chloroquine not only impaired the intracellular replication of *Y. pseudotuberculosis* but also decreased the release of bacteria to the extracellular environment. These results suggest that *Y. pseudotuberculosis* may manipulate the intersection between autophagy and secretory pathways. This observation was additionally confirmed by immunofluorescence colocalization studies of intracellular *Yersinia* with autophagy markers. To transfer our research into a model of gastrointestinal relevance, we employed the 2D Caco-2 trans-well filter system. This culturing method results in polarized cells, partially re-differentiated into M-cells, with a basolateral and apical site and

formation of tight junctions, leading to an increase in trans electrical epithelial resistance (TEER). We monitored the change of TEER over time upon *Yersinia* infection and quantified the bacterial release on the apical and basolateral site. *Y. pseudotuberculosis* could be recovered from the lower trans-well chamber, suggesting that bacterial egress was promoted at the basolateral site of infected Caco-2 cells. As no breakdown of TEER was observed during this process, we concluded that the *Yersinia* exit strategy preserves the integrity of the host cell monolayer and might use a non-lytic exocytic mechanism. Preliminary electron microscopy images support this assumption. However, the molecular mechanisms that allow pathogenic *Yersinia* to subvert autophagy and promote egress have not been identified.

217/EKMPP

The protease activity of *Pb*SERA4 is required for efficient *Plasmodium* exit from the liver

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Before *Plasmodium* parasites infect red blood cells and cause malaria, the parasites first undergo an essential round of replication in the liver. The number of hepatocytes that are infected after *Plasmodium* transmission by mosquito is limited. Therefore, parasites must exit the liver and enter the blood stream efficiently following liver-stage replication to establish infection of the blood. To egress from hepatocytes, the parasites escape the intracellular parasitophorous vacuole and are subsequently released from the infected cell in merosomes, which are host-plasma-membrane-derived vesicles containing infectious parasites. We recently showed that a *Plasmodium* serine repeat antigen, *Pb*SERA4, is important during exit of *Plasmodium berghei* from the liver. Parasites lacking *Pb*SERA4 are less efficient in forming merosomes compared to the wild-type strain, and consequently these *Pb*SERA4-deficient parasites are slow to establish infection in the blood following liver-stage development. *Pb*SERA4 is a putative cysteine protease; however, its proteolytic function has not yet been confirmed experimentally. To determine if *Pb*SERA4 protease activity is crucial for its function in liver-stage exit, we generated parasites with a specific point mutation in the putative active site. When animals are infected with these *Pb*SERA4-C517A parasites, in which the active-site cysteine was replaced with alanine, there is a delay before the onset of the blood-stage infection similar to that seen with the *Pbsera4*- knockout parasites. These results suggest that *Pb*SERA4 is a functional protease and that this proteolytic activity is required for its role at the culmination of the liver infection stage.

218/EKMPP

Using population dynamics and transposon-directed insertion site sequencing (TraDIS) to identify bacterial factors essential for the egress from the neonate epithelial cell

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Introduction: Non-typhoidal Salmonella (NTS), e.g. S. Typhimurium are a global health problem in human and veterinary medicine. In industrialized countries, immunocompetent adults usually suffer from self-limiting gastroenteritis after oral uptake. However, low hygiene conditions and age (children younger than 5 years), are considered as risk factors for systemic distribution which often lead to severe and life-threatening infections. This is why in our study we decided to work with a pre- established neonatal mouse model [1]. To overcome the epithelial barrier, NTS use a variety of effector proteins, encoded by Salmonella Pathogenicity Islands (SPIs). E.g. SPI2 and its effector molecules play an important role for the exit of the Salmonella containing vacuole (SCV). Even though in the previous study we were able to find S. Typhimurium in different tissues in our neonatal mice, the subsequent evasion mechanisms and other steps in pathogenesis still are not fully understood. In my study, our aim is to identify important genes for egress mechanisms.
Material and method: We will use Wild-type isogenic tagged strains (WITS) approach to label strains and figure out if there are certain genes which are important to exit the SCV and if we can find out bottle necks during the exit of the SCV. With the help of Transposon Directed Insertion Sequencing (TraDIS) we want to sequence the Tn5 mutant library, we created. The inoculum (input pool) will be administered orally to newborn mice and will be compared to populations isolated from different tissues (output pool). The output pool supposed to have those mutants that were able to egress the SCVs and spread into different organs. Other mutants where the transposon blocked the function of an important egress gene will be missing in the output pool.
Results: We were able to establish the WITS approach for different Salmonella strains in our lab. And right now we try to use the TraDIS method to sequence our Tn5 mutant library.
Discussion: The results of the study will help us to better understand the process of egress and systemic spread and potentially assist in development of new treatment and prevention strategies for NTS infections in the newborn.
[1]. Zhang et al (2014), Age-Dependent Enterocyte Invasion and Microcolony Formation by Salmonella, PLOS pathogens

Referenzzentren & Konsiliarlaboratorien (StAG RK)

219/RKP

An optimised Elek test for the identification of toxigenic corynebacteria

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Question. The classical Elek test is the gold standard for detection of the diphtheria toxin in *Corynebacterium diphtheriae* and *C. ulcerans*. However, due to its sophisticated methodological requirements the classical Elek test is preformed mainly by specialized reference laboratories. Therefore, a more robust test method with imporved sensitivity and specificity for detecting free diphtheria toxin is urgently needed. The goal of the study was to optimize the Elek test so that it could detect toxigenic corynebacteria, including those with minimal toxin production.
Methods. 30 Diphtheria toxin gene positive *C. ulcerans* strains of human and animal origin, isolated in Germany in 2011-2022 were studied. Melted Elek agar (WHO Diphtheria Manual, 2021) was supplemented with 20% bovine serum and poured into a 5.5 cm Petri dish. A 6 mm paper disk impregnated with a diagnostic diphtheria antitoxin (Microgen, Russia) was placed on the agar and 6 rounded bacterial culture plaques were inoculated around the disc. The result of the reaction was regarded as positive if a precipitation line was formed between the bacterial plaque and the disk with antitoxin after 24 hours of Elek test incubation at 37°C.
Results. All 30 isolates studied were toxin positive in the optimized Elek test. Immunoprecipitation with clear bands occurred more rapidly when a volume of the medium 5 ml, concentration of the antitoxin 2.5 IU per disc and a heavy inoculum were used. The optimal distance between the bacterial plaque and the antitoxin disc was found to be 6 mm. Diameter of the plaques was also 6 mm. We noticed that the Elek precipitation lines were better expressed if the plaque with the test culture was placed in between the plaques of the toxigenic control strain.
Conclusions. This method was based on the Elek test modification which has proven itself well in assessing the toxin-expressing capacity of *C. diphtheriae* isolates in times of diphtheria epidemics in Russia in the 1990s. The most important factor for the sensitivity of the method was the distance of the inoculum from the antitoxin

disk. The specificity of the test has been increased with the use of a purified by specific sorption diagnostic antitoxin, which does not produce false positive precipitation lines. These modifications allow to clearly identify the toxigenic *C. ulcerans* that are recognized as an emerging zoonotic pathogen causing human diphtheria in the industrialized countries.

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Azithromycin resistance newly detected in invasive *Neisseria meningitidis* strains isolated in Germany 2021

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Question: Azithromycin has been shown to eradicate meningococcal colonization, is easy to applicate and has a good safety profile. Thus, it is recommended as one of few antimicrobial substances for the post-exposure prophylaxis of close contacts to an invasive meningococcal disease (IMD) case. In 200 invasive Neisseria mengingitidis isolates from Germany from the years 2006 to 2018, no azithromycin resistant isolate was detected (Krone et al., J Antimicrob Chemother 2020;74(4):984-987). During the COVID-19 pandemic, despite recommendations against the use, azithromycin sales increased in many countries worldwide (Belleudi et al., Front Pharmacol (2022);13:825479; Del Fiol, et. Al., Front Pharmacol (2022):13:844818).
Methods: Minimal inhibitory concentrations (MIC) of all cultured invasive meningococcal isolates sent to the German National Reference Laboratory for Meningococci and Haemophilus influenzae were determined and interpreted by broth microdiluation according to the standards of the Clinical and Laboratory Standards Institute. Isolates are judged susceptible against azithromycin if the MIC was 2 mg/L or below.
Results: 47 IMD isolates were tested with one isolate not be able to grow in the used media. 40 of the 46 isolates (87.0 %) tested susceptible while 6 isolates tested resistant (13.0 %) with a MIC between 4 and 16 mg/L.
Conclusions: In 2021, for the first time, azithromycin resistant *N. meningitidis* isolates were detected In Germany. This fact puts the use of azithromycin as post exposure prophylaxis of close IMD cases into perspective. Further data on potential resistance mutations in the isolates and data from the years 2019 and 2020 will be presented on the conference.

221/RKP

***Corynebacterium silvaticum* – a new zoonotic agent in butchers?**

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Objectives: *Corynebacterium silvaticum*, a unique group of nontoxigenic diphtheriatoxin gene bearing NTTB corynebacteria (formerly called "*Corynebacterium ulcerans* wild life cluster"), was recently described as a novel zoonotic *Corynebacterium species* causing caseous lymphadenitis in wild boar and roe deer. In contrast to the emerging zoonotic pathogen *Corynebacterium ulcerans* it has not been found in humans before. We report on the first case of a human infection in a butcher presenting axillary lymphadenitis and abscess formation presumably originating from a previously hunted and slaughtered infected wild boar.
Materials & Methods: Strain identification was performed by biochemical differentiation and MALDI-TOF analysis (MALDI Biotyper; Bruker Daltonics, Germany). Next generation sequencing (NGS) analysis was carried out with MiSeq whole genome sequencing and data analysis by cgMLST. Average nucleotide identity (ANI) value comparison was performed to analyse species boundary. Susceptibility testing was performed according to CLSI and EUCAST guidelines. Toxigenicity was verified by real-time PCR and the modified Elek-test.
Results: A 37 years old male patient had a three weeks history of a predominantly indolent tumorous lesion in the region of the lateral thorax and right axilla. Sonography and MRT results indicated an abscess formation, lymphadenopathia and adiponecrosis. After surgical intervention a 7 x 6 x 3cm tissue sample was histologically analysed and an intraoperative bacterial swab was investigated. After 48 hours the bacterial swab grew a pure culture of a *Corynebacterium* strain, identified as *C. ulcerans* using MALDITOF MS, but showing atypical colony morphology and sensitivity against clindamycin. Further analysis in the German Consultant Laboratory for Diphtheria including DT-PCR, modified Elek-test, NGS and ANI the strain was identified as NTTB *C. silvaticum* strain – so far the first strain isolated from a human being. The patient was successfully treated with surgical intervention and cefuroxime for 14 days without further complications aside of a self limitating postoperative lymph fistula. No secondary human cases occurred. The patient reported on a recent hunting and slaughter event of a wild boar presenting suspicious mesenterial lymph nodes.
Discussion: Our case report indicates that *C. silvaticum* may be a new zoonotic source of human infections in hunters and butchers with the most probable route of infection via direct contact to infectious tissue and possible microtrauma lesions during the slaugther process. The diagnosis of potentially toxigenic *Corynebacterium species C. diphtheriae, C. ulcerans, C. pseudotuberculosis* and *C. silvaticum* is essential for tracing infectious sources and initiate adequate therapy and precaution measures.

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Antimicrobial activity of Ceftolozane-Tazobactam, Ceftazidime-Avibactam and Cefiderocol against multidrug-resistant *P. aeruginosa*

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Background: *P. aeruginosa* possesses has a high intrinsic resistance and the outstanding capacity to develop resistance to nearly all available antibiotics. Multidrug-resistant (MDR) *Pseudomonas aeruginosa* increasingly cause healthcare-associated infections, while treatment options are limited. In the last few years, ceftolozane-tazobactam (CTB), ceftazidime-avibactam (CZA) and cefiderocol (CFDC), three new cephalosporins with promising activity against MDR gram-negative organisms, became available.
Methods: In this study, we determined the antimicrobial activity of CTB, CZA, CFDC against 223 MDR *P. aeruginosa* clinical isolates recovered at the University Hospital Frankfurt by using MIC test stripes. For a subset of isolates, we compared the performance of MIC test stripes with microdilution (CZA, CTB) or agar dilution (CFDC). Detection of genes encoding metallo-β-lactamases VIM and IMP, the extended spectrum ß-lactamase (ESBL)/carbapenemase GES and the virulence-associated traits ExoS and ExoU was performed by PCR.
Results: For MDR *P. aeruginosa* isolates, the MIC50 / MIC90 of CTB, CZA and CFDC were 8/>256 mg/L, 16/>256 mg/L, and 0.25/1 mg/L, respectively. CFDC showed the highest susceptibility rate (97.3%) followed by CZA (48.4%) and CTB (46.6%). In 73

(32.7%) isolates carbapenemase gene *bla*VIM and in 5 (2.2%) isolates *bla*GES was detected (with a positive association of *exoU* and *bla*VIM), while *bla*IMP was not detected. Co-resistance for CTB and CZA was found in 44.8% of isolates while 9.4% or 6.7% of MDR *P. aeruginosa* were susceptible to either CTB or CZA only.
Discussion: This study underlines that CTB, CZA and CFDC are important options for the treatment of infections due to MDR *P. aeruginosa* with CFDC currently being the most active available anti-pseudomonal β-lactam agent. In case of MDR *P. aeruginosa*, CTB, CZA and CFDC should be tested. However, to prevent further resistance selection, prescribing practice of these agents should be carefully, best within an existing antibiotic stewardship program.

223/RKP
Mandatory notification of panton-valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* (MRSA) in Saxony, Germany – analysis of cases from the city of Leipzig in 2019
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Background: In Germany the legal basis for the control of infectious diseases is provided by the Protection against Infection Act. In addition, there are laws and ordinances in the individual federal states extending these reporting obligations. Only in Saxony the detection of a PVL-positive MRSA has to be notified by name from the laboratory to the local health authority (LHA). The LHA reports the case to the state health authority and takes concrete infection control measures after assessing each individual case. We analysed MRSA isolates from the respective cases in 2019, which were send to the National Reference Centre (NRC) for strain characterization and typing.
Material & Methods: Strains were collected and identified as PVL-positive MRSA in local microbiological laboratories and sent to NRC for Staphylococci and Enterococci. Only the first isolate per patient was included. Antibiotic resistance testing was done by broth microdilution. Molecular characterization was performed using *spa*- and SCC*mec*-typing, MLST and the PCR-detection of marker genes associated with distinct MRSA lineages. Demographic and clinical data of the individual cases were assessed and the LHA performed epidemiological investigations.
Results: Thirty-nine (index) persons, diagnosed with a PVL-positive MRSA, were initially reported to the Leipzig LHA in 2019. Most of the patients suffered from skin and soft tissue infections (SSTI). For 21 of them household contacts were screened for MRSA. A total of 63 contacts were screened, 18 of which were colonized with a PVL-positive MRSA. The median age of altogether 58 individuals was 23.5 years. In over half of the cases the home country was not Germany and/ or a history of travel or migration was reported.
Molecular characterization of the 58 isolates revealed the presence of various epidemic community-associated MRSA lineages. Lineages like "USA300", including the North American Epidemic (ST8-MRSA-IVa) or the South American Epidemic Clone (ST8-MRSA-IVc), and the "Bengal Bay Clone" (ST772-MRSA-V) were more prevalent than the "Taiwan Clone" (CC59-MRSA-V), "Southwest Pacific Clone" (ST30-MRSA-IV) or "African Clone" (ST88-MRSA-IV). In eight out of nine households the contact persons were colonized with the same clone as the respective index, therefore an epidemiological linking is very likely.
Conclusions: PVL-positive MRSA primarily cause SSTI and are often spread in households. They are frequently acquired in endemic areas, e.g. by international travelers or patients with migration background. The obligation to report PVL-positive MRSA enables us to collect data on the origin and spread of these clones within (community)-settings. But the epidemiological investigation and tracking of patients is complex and time-consuming, as this has to be realized mainly by the responsible LHA.

224/RKP
Whole genome-based surveillance of German clinical *Campylobacter jejuni isolates* identifies growing time-stable clusters
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Background: Campylobacteriosis continues to cause the highest number of notifiable disease cases among bacterial food-borne infections in Germany and Europe. However, the majority does not have an obvious epidemiological link and is therefore considered sporadic. The main sources of infection are chicken and other types of meat, raw milk, and animal contact. The infection is typically self-limiting and restricted to the gastrointestinal tract. In rare cases (1-5%), extra-intestinal manifestations like blood stream infections or reactive-arthritis can be observed. *C. jejuni* is also leading cause of the rare Guillain-Barré-Syndrome, which is an acute inflammatory polyneuropathy. Here we present results of our nationwide whole genome-based Campylobacter surveillance program, which has been established in 2020, but also included retrospective samples. For the collection of *Campylobacter* isolates, numerous primary laboratories contributed nationwide.
Materials & Methods: *Campylobacter* spp. From clinical cases were cultivated under microaerophilic conditions on CCDA and species determination was carried out by PCR. Phenotypic AMR testing was performed using broth micro dilution assay according to EUCAST. Whole genome sequencing was performed with Illumina NextSeq, yielding paired end sequence reads.
Results: In 2021 we analyzed 1713 isolates by means of whole genome sequencing. This amounts to an increase of approximately 30% analyzed isolates compared to the year 2020. Our samples represent ~ 3% of the notified 57421 Campylobacteriosis cases of 2021 in Germany. 1364 isolates (79.6%) belonged to the species *C. jejuni*, followed by 241 isolates *C. coli* (14.1%), and 2 isolates (0.1%) *C. fetus*. Including 224 isolates from a sentinel program before 2020, our whole-genome-sequence database consisted of 2239 *C. jejuni* sequences spanning the years 2012-2021. We detected 251 *C. jejuni* cgMLST clusters with a maximum allele distance of 4. The majority of clusters consisted of 3 isolates. However, 12 clusters consisted of at least 20 isolates (e.g. CT53, CT117 and CT1542) and the largest cluster consisted of 86 isolates (CT2151). All of the larger clusters included isolates from two consecutive years, some even three years and were distributed across several states.
Discussion: A nationwide whole genome-based surveillance program has been successfully established for Campylobacteriosis. Several larger *C. jejuni* clusters of >20 isolates have been detected, which are geographically diverse and span across 2-3 years. Whether these clusters originate from the same source is currently being evaluated in an integrated approach together with epidemiologists and consumer protection experts.

225/RKP
Antimicrobial efficacy of inhaled antibiotics against *P. aeruginosa* in Cystic Fibrosis
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Background: Cystic fibrosis (CF) is characterized by chronic suppurative infection of the airways leading to an ongoing tissue inflammation and a continuous loss of lung function. *Pseudomonas aeruginosa* is one of the major pathogens in chronic CF lung infection and associated with a high morbidity and mortality in this population. Inhaled antibiotics, such as colistin, tobramycin,

aztreonam and levofloxacin, belong to standard suppressive antimicrobial therapy for chronic *P. aeruginosa* lung infection, generating high local antibiotic concentrations in the airways, with low systemic drug concentrations and negligible systemic side effects while. Microbiological criteria to assess effectiveness of inhalative agents are so far not available.

Methods: In this study, we investigated the microbiological efficacy of colistin, tobramycin, aztreonam and levofloxacin against *P. aeruginosa* CF isolates with variable minimal inhibitory concentrations (MIC values up to ≥ 1024 mg/L) by using artificial sputum medium (ASM) to simulate CF lung environment. By *in vitro* killing assays, we tested the susceptibility of *P. aeruginosa* after exposition with three different high-level antibiotic concentrations achievable after inhalation therapy.

Results: In general, we observed a dose-dependent bacterial killing, while levofloxacin was the most potent bactericidal agent. With increasing MIC values the bactericidal effect decreases, except for levofloxacin that showed complete killing up to MIC values of 512mg/L. For the other agents, significant bacterial killing (about 80% after 3h) was observed: for colistin, tobramycin and aztreonam up to MIC values of 8mg/L, 32-64 mg/L and 64-256 mg/L, respectively. Above these values, killing efficiency was highly variable and correlates only poorly with in vitro MIC.

Discussion: Mentioned MIC values may represent preliminary clinical breakpoints for the efficacy of anti-pseudomonal suppressive inhalation therapy in CF lung infection. This in vitro study may help to guide the selection of the most active inhalative agent for CF lung infection based on routine in vitro MIC values. Lung adapted *P. aeruginosa* CF isolates with resistance against colistin, tobramycin, aztreonam and levofloxacin may exhibit highly variable killing rates in ASM.

226/RKP

Ciprofloxacin resistance in invasive *Haemophilus influenzae* infections in Germany 2019 and 2020

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Introduction: Antibiotic resistance in *Haemophilus influenzae* (H.i.) rises globally. Fluoroquinolones are still potent antimicrobial agents, even though their use has been restricted due to important side effects. There are currently no data for ciprofloxacin resistance in invasive H.i. infections in Germany. Therefore, the aim of this study was to analyze the epidemiology of ciprofloxacin resistance in invasive H.i. cases in Germany in 2019 and the following pandemic year 2020.

Material/Methods: All invasive H.i. strains collected from 2019 and 2020 in Germany at the NRZMHi were included in this study. Antibiotic susceptibility testing was performed by gradient agar diffusion (GAD). All strains were tested for ampicillin, cefotaxime, and ciprofloxacin susceptibility. Susceptibility testing results were interpreted according EUCAST guidelines. Ciprofloxacin resistance was confirmed by microdilution and genetic analysis of QRDRs (GyrA, GyrB, ParC, ParE).

Results: A total of 1104 isolates were examined in the study. With 379 cases in 2020, submissions in the pandemic year were reduced by 48%. The median age of the patients was 72,5 years, gender ratio (m/f) was 1,1. Regarding GAD results, a prevalence of 0,72 % (8/1104) of ciprofloxacin-resistance was observed. Microdilution confirmed 6 cases, resulting in a prevalence of 0,54 %. Correlation between both methods can be considered significant (Pearson"s correlation coefficient, r=0,934, p<0,001).

All ciprofloxacin resistant strains showed at least one mutation in either GyrA or ParC. No mutations in QRDRs were found in any sensitive strains tested for reference.

Discussion: Ciprofloxacin remains an effective agent to treat H.i. infections with a resistance prevalence < 1% in Germany. The mutations found in our study have been published previously and are possible explanations for the increased minimal inhibitory concentrations. Analysis of further mutations in QRDRs is necessary to fully understand its effect on minimal inhibitory concentration rates. Our strains of 2020 comprise only a subset of isolates found in the pandemic, since the first COVID19-cases in Germany only appeared as of March 2020. Due to the ongoing pandemic, continued surveillance is desirable to assess the impact of COVID-19 on invasive H.i. in Germany and their antibiotic resistance.

227/RKP

Epidemiology of invasive *Haemophilus influenzae* infections 2021 in Germany

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Introduction: The present pandemic has also influenced infections other than COVID-19. Here, we present epidemiologic data of invasive *H. influenzae* infections in Germany in 2021.

Materials and Methods: Besides notification according IfSG, diagnostic laboratories send invasive Hi isolates to the NRZMHi. Submissions were analysed for the calendar year 2021. Coverage was assessed by comparing the NRZMHi cases with the number of notified cases (SurvStat@RKI). Resistance testing was done using gradient agar diffusion.

Results: The NRZMHi received 366 submissions, from which 290 invasive *H. influenzae* isolates could be confirmed. In one case, *H. influenzae* was detected by PCR from cerebrospinal fluid only. In five cases, *H. parainfluenzae* from blood cultures were found. Since 366 invasive cases have been registered via statutory notification, a coverage of 79.5% can be assumed for the laboratory surveillance of the NRZMHi.

Serotype distribution was similar to previous years with non-typeable *H. influenzae* (NTHi) representing the majority (n=176, 61%). The most frequent serotype found among invasive capsulated isolates was serotype f (Hif, n=62, 21%), followed by serotype e (Hie, n=24, 8%). Hib, which used to be the most common serotype before the introduction of Hib vaccination in Germany, was found in 16 cases (6%). Hia was almost as frequent with 13 invasive cases (5%).

Invasive infections affected mostly elderly patients with highest case numbers in the age group of >64 years (58%). In 44 cases children <5 years were affected (15%).

The NRZMHi has tested ampicillin susceptibility of 289 viable invasive *H. influenzae*. Thereby, 39 (13%) were ampicillin resistant (MIC > 1 µg/ml). Among these resistant isolates, 23 (8% of all tested isolates) showed β-lactamase production. Resistance to cefotaxime was found in five isolates (2%).

Discussion: One year into the COVID-19 pandemic, registered case numbers of invasive *H. influenzae*-infections have increased since 2020, when only 487 invasive H. influenzae-infections were registered in Germany. However, the case numbers in 2021 remained low compared to prepandemic years. At the same time, estimated coverage of the laboratory surveillance compared to statutory notification data did not differ to previous years. The age group most affected was typically elderly patients. However, since the number of children affected was not altered, whereas over-all incidence was reduced, this lead to a higher proportion of invasive *H. influenzae*-infections in children compared to prepandemic years. The changes in incidence did not affect serotype composition and antibiotic susceptibility. Invasive Hia cases were remarkably high, which is in line with observations of rising incidences of this serotype in other countries. However, the very small numbers make interpretation of this phenomenon difficult, and invasive Hia infections in Germany require further observation.

228/RKP

Invasive group-B streptococcal infections in Germany

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Introduction: The German National Reference Center for Streptococci (GNRCS) has monitored invasive infections associated with *Streptococcus agalactiae* (Group-B streptococcus, GBS) in Germany since 1990. Here, we report on the progression of invasive group-B streptococcal disease (IBD) between 2010 and 2021 with special attention to serotype distribution and antimicrobial resistance.
Material/method: Species identification was based on haemolysis-assessment, Lancefield-typing, catalase-, pyrrolidonyl-arylamidase- and leucine-aminopeptidase-test. Serotype assignment was performed using multiplex-PCR techniques and serum-agglutination. Determination of minimum inhibitory concentration for eleven antibiotics was executed following CLSI guidelines.
Results: In the study period, 1240 cases of IBD were reported. More than 90% of the isolates were cultivated from blood with serotypes III (34%), Ia (21%) and V (17%) dominating. In the age group 51-90 years, the serotype distribution was considerably diverse, whereas in newborns a domination of serotype III was observed, mainly in late onset disease (LOD). In early onset disease (EOD) the serotype distribution was more various, comparable to GBS isolates from asymptomatic anal-vaginal-swabs. IBD isolates were mostly susceptible to penicillins, cefotaxime, vancomycin and chloramphenicol, but non-susceptibility to tetracycline remained stable at ≈80%. Erythromycin non-susceptibility increased from ≈1% in 2010 to ≈33% in 2021 and levofloxacin-resistance increased from ≈1% to ≈7%. First penicillin/amoxicillin resistant isolates were observed for serotypes Ia (2 in 2018) and Ib (1 in 2019), which are increasing, especially in EOD and the elderly.
Discussion: IBD in Germany mainly affects older people and newborns, with serotype III dominating LOD. The observed gain in antimicrobial resistance, especially the appearance of penicillin/amoxicillin non-susceptible isolates in the expansionary serotypes Ia and Ib is alarming.

229/RKP
Epidemiology of invasive meningococcal disease 2021 in Germany
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Introduction: The implementation of protection measures against COVID-19, e.g. social distancing and the use of face masks, at the end of March 2020 influenced other infectious diseases including invasive meningococcal disease (IMD) as well as *Haemophilus influenzae* and *Streptococcus pneumoniae* infections. Not only in Germany, but worldwide, the number of cases declined significantly (Brueggemann et al., Lancet Digit Health 2021)**.** The measures have a continuing impact on IMD in Germany, i.e. case numbers are still on a very low level. Here, we present data of IMD cases submitted to the reference laboratory for meningococci and *Haemophilus influenzae* (NRZMHi) in 2021.
Materials and Methods: IMD isolates and clinical samples of suspected IMD cases were send on a voluntary basis by diagnostic laboratories to the NRZHMi. Routinely, isolates were analysed by serogrouping, whole genome sequencing and antimicrobial susceptibility testing (gradient agar diffusion), whereas clinical samples were analysed by serogenogrouping and finetyping.
Results: Meningococci were confirmed in normally sterile sites from 58 patients. Since 71 IMD cases had been notified to the RKI, a coverage of 81% can be assumed for the laboratory surveillance at the NRZMHi.
As in all previous years, serogroup B was the most prevalent serogroup with almost 70% in 2021. The proportions of serogroups C and W were almost comparable to those of previous years, whereas only 3.4% of the cases were caused by serogroup Y in comparison to 18.9% in 2019 and 20.0% in 2020.
Antimicrobial susceptibility for penicillin, cefotaxime, ciprofloxacin and rifampicin of 47 viable isolates was tested, of which four isolates were penicillin resistant and one isolate was resistant to penicillin, cefotaxime and ciprofloxacin.
Whole genome sequencing revealed a highly diverse population with no hints for local clusters.
Discussion: In the second year of the COVID-19 pandemic the numbers of IMD cases are still on a low level. The most remarkable change was the very low number of serogroup Y cases. It remains speculative whether IMD case numbers will return to pre-pandemic numbers.

230/RKP
Epidemiological surveillance and resistance data – the first two years of the National Candidemia Surveillance Network
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Introduction: Invasive *Candida* infections pose a health threat to the community and are of public concern. In recent years, a decent change of species spectra and resistance patterns is observed, which may impact future treatment strategies. We established the first German sentinel network in 2020 to closely monitor these dynamics and to enable surveillance of new pathogens like *C. auris*.
Objectives: The sentinel network will monitor species distribution and the development of antifungal drug resistance for invasive yeast infections in Germany.
Materials & Methods: Ten German microbiological labs participate in this network collecting relevant *Candida* species derived from blood cultures implicating invasive bloodstream infection. Epidemiological and resistance data are assessed by the NRZMyk. Data were classified by Date and place of specimen, species ID, method of species ID, antimicrobial susceptibility testing (AST) results of a diverse range of antifungals and method of AST.
Results: During the first two years data acquisition 966 strains and 21 different species could be preserved (514 in 2020 and 452 in 2021). All Species-IDs were confirmed by MALDI-TOF. At least 5000 MIC-values were analysed. In both years ~6% of the isolates show secondary resistance against ≥1 antifungal substance (according to either EUCAST or CLSI). MDR resistant strains were found. Interestingly, not all phenotypic echinocandin resistant isolates harbour target gene mutations and lose reduced susceptibility upon retesting.
Epidemiological data is currently assessed by each participating partner focussing on incidence and prevalence of candidemia.
Conclusion: Data acquisition through this national surveillance network and a database with open access to all participating partners enables systematic research projects, epidemiological studies and active surveillance concerning the prevalence of fungal bloodstream infection and emergence of antifungal drug resistance.

231/RKP

Mycobacterium chelonae in bioprosthetic valve endocarditis

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Introduction: *Mycobacterium chelonae* has been reported as rare cause for infectious endocarditis and has been in particular associated with bioprosthetic heart valves. We report on three patients with molecular detection of *M. chelonae*, originating from bioprosthetic heart valves or conduit from one manufacturer. This led to further investigations of unused, originally packaged prosthetic heart valves.

Materials & methods: We routinely use FISHseq, the combination of Fluorescence in situ Hybridization (FISH) with broadrange 16S rRNA-gene PCR and sequencing to visualize and localize bacteria in native as well as prosthetic heart valves in endocarditis patients.

In cases that yielded *M. chelonae* complex, results were confirmed by Kinyoun- and Auramin-stain and in one case next generation sequencing. The mycobacteria were further differentiated as *M. chelonae* in an independent laboratory.

Results: From 2020 to 2022, we identified three patients with *M. abscessus/chelonae* complex in tissue from explanted prosthetic heart valves, respectively a valved conduit. The prosthetic materials from the three patients were sent in for analysis because of suspected endocarditis and yielded exclusively *M. chelonae*. Morphologically intact, FISH-positive mycobacteria were visualized within the tissue. The bioprostheses were from the same manufacturer and had been implanted for 4 years, 3.5 months, and 12 days, respectively. This led to the analysis of two unused prosthetic heart valves of the same company, which yielded the identical finding. Until now, *M. chelonae* could not be cultured from any of the materials.

Discussion: We found *M. chelonae* on previously implanted and on new bioprostheses from the manufacturer BioIntegral. Whether the contamination is restricted to certain products or batches of this manufacturer is under investigation. Further studies investigating the clinical relevance of these findings are underway.

Conclusion:

- In patients implanted with BioIntegral biomaterial and suspected infection or even only dysfunction of the valve, *M. chelonae* should be considered and appropriate diagnostic testing should be performed.
- Such cases should be notified to public health and regulatory authorities.
- The processes and safety testing of bioprostheses should be reconsidered.

232/RKP

Low temperature vacuum drying-a future storage option?

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Background: Nowadays it is becoming more and more important to think about sustainability and our ecological footprint. Concurrently, national reference centers (NRC) in Germany are committed to store submitted patient samples for at least to ten years. However, this requires an enormous amount of space for conventional used freezers (-80°C). Beside this, electricity costs and CO2 consumption can also not be neglected. Furthermore, if it comes to a breakdown of freezers alternatives storage options are limited. Therefore, we want to employ low temperature vacuum drying (LTVD) in our routine processes as a future storage option.

Material & Methods: During LTVD samples will be dried under very gentle conditions and energy costs are decreased up to 40 % in comparison to freeze drying. Furthermore, only one large device (vacuum oven (VO) 29cool from Memmert) is required for this method which is another advantage. In the case of probiotic samples in the food area, the LVTD method was examined successfully by Prof. Foerst (chair for system process engineering-TU Munich). The temperature range of the VO belongs from 5-80 °C. At 5°C working temperature the sample itself possesses a temperature under the freezing point.

Results: We examined survival rates of bacterial strains after storage at -18°C and different time points. Additionally, bacterial strains were frozen supplemented with various adjuvants like sorbitol, trehalose and skimmed milk powder to increase the yield of survival. All strains (gram +/gram -) showed significant higher survival rates after three months of storage when an adjuvant was added in comparison to strains which were frozen without adjuvants. Supplementation with trehalose yielded in highest survival rates.

Discussion: However, purchase of trehalose is very expensive in terms of the number of samples that are submitted to the NRC. The combination of sorbitol and skimmed milk powder offers a cheaper alternative without any major differences concerning survival rates. The best option would be skimmed milk powder alone but therefore more experimental investigation is needed. All in all, in future perspective low-temperature vacuum drying could offer an alternative method in terms of sample storage within our national reference centers and other institutions with strain collections.

Breaking News in der Lebensmittelmikrobiologie (FG LM/ FG ZO)

234/LMZOP

Studies on the microflora during spontaneous sauerkraut fermentations

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Introduction: Traditional sauerkraut fermentation is the result of the spontaneous growth and activity of the microflora of white cabbage. Various fermentation conditions influence the fermentation process. In this study, the microflora of spontaneous sauerkraut fermentations in the brine of two fermentation barrels was examined. Sampling during the whole fermentation time gave an overview of the diversity and succession of the microflora.

Methods: Samples of the natural brine of two barrels were taken from the beginning to the end of the fermentations. The pH values were measured. The samples were analyzed on the colony counts of lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonadaceae* and fungi. Diluted samples were spread on MRS agar with natamycin, VRBD agar, CFC agar, and malt extract agar with chloramphenicol, respectively. The aerobic incubation of CFC agar and malt extract agar with chloramphenicol and the anaerobic incubation of MRS agar with natamycin and VRBD agar was performed at 30 °C for three days. Optically distinguishable colonies were isolated randomly. Isolated strains of bacteria were classified by Gram staining and by catalase test or oxidase test. Yeasts and molds were morphologically confirmed.

Isolates of lactic acid bacteria were identified by comparative sequence analyses of the genes of the 16S rRNAs. After enrichment of the strains in MRS bouillon, extraction of the DNA, amplification of the genes by PCR and sequencing was done. The sequences were analyzed using the Basic Local Alignment Tool (BLAST) within the database of sequences of 16S RNAs of the National Center of Biotechnology Information (NCBI).

Results: *Enterobacteriaceae* and *Pseudomonadaceae* were detected in the first phase of fermentations. 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 brine. During the whole fermentation process, yeasts and molds could be detected.
Out of heterofermentative lactic acid bacteria *Lentilactobacillus diolivorans*, *Levilactobacillus brevis*, *Levilactobacillus parabrevis*, and *Leuconostoc mesenteroides*, as *Leuconostoc mesenteroides* ssp. *jonggajibkimchii* were identified. Out of homofermentative species of lactic acid bacteria *Lactiplantibacillus paraplantarum*, *Lactiplantibacillus plantarum*, *Lactiplantibacillus xiangfangensis*, *Latilactobacillus curvatus*, and *Pediococcus parvulus* were identified.
Conclusions: All species found have already been described before in relation to the fermentation of vegetables. Diversity and succession of lactic acid bacteria isolated out of spontaneous sauerkraut fermentations were different in both barrels.

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Insights into the bactericidal effect of the disinfectant chlorine dioxide to hygienic relevant indicator microorganisms

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Introduction: Chlorine dioxide is valued as a potent bactericidal agent that produces less harmful side products than other chlorine-based disinfectants. Since the damaging effect of ClO2 is still incompletely known, this study aims to elucidate its mode of action by analyzing the short-term effect of ClO2 on various microbial pathogens based on their prevalence causing water and foodborne infections and hence, their role as indicators of environmental and food hygiene.
Material & Methods: The experimental set-up of this study involves gram-negative pathogens such as *Legionella pneumophila*, *Serratia fonticola*, *Pseudomonas aeruginosa* and *Escherichia coli* as well as the gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*. The spectrum of methods to analyze the mechanism and outcome of short-term disinfection involves confrontation assays with different concentrations of ClO2 for varying incubation times to bacteria. To determine the bacterial survival after ClO2 treatment, bacteria underwent cultivation on agar plates to obtain colony-forming units (cfu). The flow cytometry technique (FCM) was applied to assess the bacterial membrane integrity following the live/dead ratio, as well as to monitor enzymatic esterase activity after ClO2 exposure. Damage to DNA by ClO2 was investigated on a molecular approach using a RT-PCR assay.
Results: The confrontation assays resulted in a dependency of ClO2 efficiency and applied bacterial densities, regardless on incubation time. In detail, cfu reduction plateaued mostly for bacterial solutions with 105-107 cfu/ml treated with 1 mg/l ClO2, but 4.6 mg/l ClO2 caused either disinfection (reduction of bacterial load by 3-5 log10 levels) or resulted in the total eradication of the pathogens. High ClO2 concentrations caused an increase of membrane permeability leading to a transition of bacterial cells from an intact to a dead state. Interestingly, a manifold but temporarily increase of bacterial esterase activity after exposure to 0.4 and 1 mg/ l ClO2 for 30 min, respectively, was plotted for waterborne bacteria. Finally, exposure of intact bacterial cells to high-dosed ClO2 (5 mg/ l) did not reveal a significant damage of bacterial DNA by ClO2 as assessed by RT-PCR.
Discussion: ClO2 is a potent and fast reacting disinfectant or eradication agent for gram-positive and negative bacteria in context of water and food contaminations. The results of a ClO2 treatment show clear dependencies in efficiency with regard to bacterial density and applied concentration, while the contact time was of less importance. Regarding the mechanism of action, ClO2 primarily targets disintegration of bacterial membranes, which is associated to a first enhancement of enzymatic activity that declines throughout the ClO2 treatment. ClO2 causes a rather delayed or partial hydrolysis of bacterial DNA.

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Microbiological quality and safety of raw milk from vending stations in Germany

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Question: Growing demands by an increasing number of consumers regarding minimally processed, fresh and natural products have modified the supply of food within the last years. This could be observed across all product groups, and has also affected dairy products like raw milk. Consumers" motivations for drinking raw milk are usually related to health benefits, the desire for more natural products, taste, and support of local farmers. On the other hand, there is a well-recognised link between raw milk consumption and food-borne infections, in particular in relation to *Campylobacter* spp. and Shiga toxin-producing *Escherichia coli* (STEC). In Germany, most food-borne outbreaks with high evidence were associated with raw milk consumption in 2018. As in the previous year, *Campylobacter* and raw milk were the most frequently reported pathogen and causative food combination. To collect data about the microbiological status of raw milk sold direct via vending systems in Germany, the quality and safety of raw milk was investigated with regard to hygiene parameters and the presence of selected foodborne pathogens. Furthermore, aspects of the technology of the vending machines, the operating procedure and cleaning routine at the farm were recorded and analysed. From these results, correlations between individual parameters (such as machine type and product temperature) and the microbiological status are derived.
Methods: Up to this date, one hundred and sixty-five samples raw milk samples from milk vending machines from nine different federal states of Germany have been examined.
Results: The total viable count (TVC) was higher than 5 log cfu mL-1 in 32.8% of the milk samples. Counts of *Pseudomonas* spp., *Enterobacteriaceae*, yeasts and moulds were detected at median values of 3.6 ± 1.5 log cfu mL-1, 2.4 ± 1.6 log cfu mL-1, 2.9 ± 0.8 log cfu mL-1 and 1.2 ± 0.7 log cfu mL-1, respectively. Overall, only 41% of the raw milk samples were of satisfactory quality from a microbiological point of view. *Campylobacter jejuni*, *Arcobacter butzleri*, shiga toxin-producing *E. coli*, *Yersinia enterocolytica* and *Listeria monocytogenes* could be shown to occur in different raw milk samples after enrichment.
Conclusion: The study shows clearly that raw milk from vending systems on farms can be problematic from both a safety and a quality point of view and implies a hygiene deficit stemming either from conditions on the farm or from cleaning and sanitation of the milk vending system. Beyond that, when considering spoilage microorganisms such as *Enterobacteriaceae*, *E. coli*, *Pseudomonas*, yeasts and moulds, only 41% of the samples were microbiologically acceptable, regarding to the recommendations for sale as milk as grade I. In summary, our data and on-site observations/investigations stressed the importance of ensuring correct and efficient cleaning and disinfection procedures of vending systems and of boiling the raw milk before consumption.

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***Pseudomonas* spp. in raw milk – determination of entry routes, characterisation of biofilm forming und peptidase activity**

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Introduction: Biofilms can be formed on the surfaces of dairy processing equipment and are a potential source of product contamination. Especially members of the genus *Pseudomonas* are reported as the most important milk spoilage microorganisms. The

residual activity of their extracellular enzymes after UHT treatment may lead to technological problems (off-flavors, physico-chemical instability) during the shelf life of milk and dairy products. The aim of the study was to determine entry routes of pseudomonads at farm level and to analyze the biofilm formation ability of raw milk-relevant *Pseudomonas* spp. and their spoilage potential by determining peptidase activity.
Methods: Potential entry routes of *Pseudomonas* spp. on producer farms were investigated during 8 farm inspections in northern Germany. *Pseudomonas* and total bacterial counts were determined at all sampling points and compared between farm and sampling point. The biofilm mass produced was quantified by crystal violet assay of *Pseudomonas* spp. on stainless steel and rubber surfaces, and bacterial counts were determined in mono-biofilms during a 6-day incubation at 6 °C in milk medium. Fluorescamine assay was used to determining peptidase activity of biofilm-associated cells compared to that of planktonic cells. Additionally, biofilm morphology was analyzed by using scanning electron microscopy.
Results: The determined *Pseudomonas* numbers of airborne measurements in the area of the milking parlor and the water samples showed low numbers, making an entry via these routes unlikely. *Pseudomonas* counts ≥ 4 log cfu/cm2 were detected at various locations including spigot and teat cup holders. Biofilm formation was found to be strain-specific and dependent on surface material, as well as on milk medium concentration. Scanning electron micrographs showed a strain-specific biofilm formation of *Pseudomonas* spp. on stainless steel. Raw milk-associated multi-species biofilms on stainless steel and rubber surfaces showed temperature-specific and surface-dependent growth by scanning electron microscopy. The peptidase activity of *Pseudomonas* spp. was also found to be species- and isolate-dependent using the fluorescamine assay of *Pseudomonas* biofilms. Furthermore, the formation of peptidases was detected in *Pseudomonas* mono-biofilms at a higher level when compared to planktonic growth.
Conclusion: High *Pseudomonas* counts on seals made of rubber, e.g. the spigot and the teat cup collector, indicate favored growth of biofilms at these sites. The results indicate that biofilms represent another entry route of pseudomonads into raw milk and may contribute to milk spoilage through increased peptidase production in this growth form. Manual cleaning of critical points in milk production is recommended, as well as regular maintenance of parts with milk contact to prevent premature aging.

238/LMZOP
Is fresh produce a vehicle for the spread of antibiotic resistance genes?
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Introduction: Fresh produce is often consumed raw and can therefore be a potential vehicle for dissemination of bacteria to consumers via the farm-to-fork-route. The goal of this work was to identify fresh produce as a potential vehicle for antibiotic-resistant bacteria and antibiotic resistance genes.
Materials and methods: Enterobacteria from fresh produce were phenotypically and genotypically tested for antibiotic resistance. The genomes were then sequenced to identify the resistance genes and their genomic location in the bacteria, i.e. whether present on the chromosome or on a plasmid. Complete chromosome and plasmid DNA sequences were generated after hybrid assembly of short read (MiSeq) and long read (MinION) sequencing data. Enterobacterial strains containing plasmid sequences with a plasmid transfer system and antibiotic resistance genes were selected for conjugation experiments at different nutrient availability and temperature conditions. Furthermore, exogenous plasmid isolation was performed by putting the recipient strain directly onto the fresh product or by adding the recipient strain to an enriched sample.
Results: Conjugation was detected at 37°C, 21°C and 10°C; however, conjugation was only possible at 10°C after a selective enrichment (LB-broth + antibiotics for transconjugant selection) step at 37°C. After the multiplex PCR that identified transconjugants failed to detect the newly acquired plasmid (primer target: plasmid replication protein), sequencing revealed that recombination occurred between the plasmids of the donor strain. The *tetA* resistance gene that was responsible for the acquired tetracycline resistance of the recipient was found on different extrachromosomal DNA sequences in the transconjugants. There might be more combinations of plasmids than we detected, because we only selected for the ones with the tetracycline resistance. Transconjugants were detected after exogenous plasmid isolation that was done directly on the fresh produce. Further studies have to confirm these results and the plasmids have to further be tested for antibiotic resistance profile and replication type.

239/LMZOP
Fast bacterial identification method for screening of faecal samples
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Introduction: Most bacterial identification methods require extensive culturing, strain purification and lengthy DNA extraction protocols. This can lead to unnecessary expenses and time lags on isolating irrelevant colonies when targeting specific bacteria to be isolated from complex microbiological ecosystems. Our aim was to develop a fast and robust method for identification of human faecal bacteria, particularly lactobacilli, bifidobacteria and *Bacteroides*.
Methods: Human faecal samples were cultured anaerobically on de Man Rogosa and Sharpe (MRS), lithium propionate MRS (LP-MRS) or *Bacteroides*-specific *Bacteroides fragilis* bile-esculin (BBE) agar plates. 180 single colonies were picked and inoculated in the same broth media using Hungate tubes or in brain heart infusion-supplemented (BHIS) medium for those previously cultured in BBE agar. The bacteria were collected for sonication-based DNA extraction, followed by PCR amplification of the complete 16S rRNA gene and Oxford Nanopore Technologies (ONT) MinION high throughput sequencing with a Flongle adapter. Sequence analysis was performed using NanoCLUST and RStudio was used for bioinformatics analysis.
Results: The most frequently isolated bacteria from both MRS and LP-MRS were bifidobacteria, lactobacilli and *Enterococcus*. *Bacteroides* and *Parabacteroides* were the most commonly isolated genera from BBE agar. For 110 of the 125 colonies further investigated, 100% of the sequenced reads were attributed to a single species, suggesting that these were pure colonies, while the remaining 15 colonies presented up to three different bacterial species.
Discussion: The MRS and the LP-MRS media did not have a significant effect in exclusively selecting for lactobacilli and bifidobacteria, respectively. Furthermore, their colonies were rarely distinguishable during the culturing process and many other genera were able to grow in these media. The proposed bacterial identification method is thus advantageous when there is interest for particular genera for which there are no selective media, because it avoids time-consuming purification of unrelated colonies and it relies on the complete 16S rRNA gene for higher identification accuracy. This method can then be used for screening for pure cultures or specific microorganisms.

Microbe-Host Interactions (FG PW)

241/PWP
The tropism of SARS-CoV-2 and implications for a systemic disease
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Introduction: The coronavirus disease 2019 (COVID-19) indicates infections with the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) as a systemic disease. Therefore, to understand the pathogenesis of SARS-CoV-2, we analyzed the viral load and the innate immune response *in vitro* and *in vivo.*

Material/Methods: We investigated the viral distribution within the human body and the correlation with the tissue damage in deceased COVID-19 patients. In addition, the organ tropism was analyzed in the human alveolus-on-a-chip system, composed of epithelial/endothelial cells and macrophages, to investigate the viral load and the immune response.

Results: Our results demonstrated high viral loads in most of the lungs of deceased COVID-19 patients measured by real-time PCR. We were even able to verify viral particles in the lung tissue by using transmission electron microscopy and many of the lungs were severely damaged by the infection. Additionally, viral RNA was detected throughout various extrapulmonal tissues and organs, e.g. in the gastrointestinal system and cardiovascular system to a much lower extent than in the lung. Despite the presence of viral RNA histological analysis did not reveal severe tissue damage. In addition, our *in vitro* data in the human alveolus-on-a-chip system show that SARS-CoV-2 efficiently infected epithelial cells whereas the adjacent endothelial layer was neither infected nor did it show productive virus replication. Nevertheless, the endothelial-epithelial barrier function was activated by a strong inflammatory response and severely damaged, which could contribute to the viremia observed in many patients.

Discussion: Our study demonstrates the dissemination of viral RNA throughout the body, which supports the hypothesis that there is a maladaptive host response that fails to clear the viral particles resulting in viremia and multiorgan dysfunction. Based on our i*n vitro* data, we suggest that although the efficient replication of SARS-CoV-2 is dependent on the epithelium, also the neighboring endothelial cells are affected by the epithelial cytokines. This systemic response results in the damage of the epithelial/endothelial barrier function and a strong viral dissemination.

242/PWP

Deficiency of deubiquitinating enzyme OTUB1 leads to impaired pathogen control in macrophages during Salmonella infection

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Salmonella Typhimurium (STm) is a gram-negative bacterium, which can cause non-typhoidal gastrointestinal tract disease upon ingestion of contaminated food or water. *S.* Typhimurium first invades intestinal epithelial cells and then infects baso-laterally located macrophages. Thus, macrophages play an important role to contain the pathogen; however, they also serve as a preferable niche for intracellular bacterial growth resulting in systemic spread. Invasion and replication within the macrophages triggers activation of pro-inflammatory signaling pathways, which are responsible for the generation of anti-bacterial responses. These pathways are tightly regulated by posttranslational modifications including ubiqutination/deubiquitination. Ubiquitination is a process where small regulatory proteins known as ubiquitins are attached to the signaling molecule which either regulates its stability or interaction with other proteins. Ubiquitination is reversed by a process known as, deubiquitination where deubiquitinating enzymes remove ubiquitin chains from the substrate. OTUB1 is one such versatile deubiquitinating enzyme (DUB), which preferentially cleaves K48 ubiquitin chains. In addition, OTUB1 can bind to the ubiquitin conjugating enzyme E2 and prevent the ubiquitination of the substrate. However, specific role of OTUB1 in macrophages is not well studied. We generated conditional OTUB1 knock out mice (LysM-Cre OTUB1fl/fl) with deletion of OTUB1 in macrophages. Subsequent in vivo infection with SL1344 wild-type STm showed that LysM-Cre OTUB1fl/fl mice had higher pathogen load and inflammation in spleen, liver and small intestine compared to the OTUB1fl/fl mice. We confirmed the OTUB1 dependent control of STm in vitro in infected bone derived macrophages (BMDMs). The impaired control of *Salmonella* in-OTUB1-deficient BMDM was paralleled by an impaired activation of key protective pro inflammatory NF-κB and MAPK signaling and a reduced production of anti bacterial reactive oxygen species. Thus, OTUB1 plays role in the regulation of pro-inflammatory immune signaling and thereby contributes towards the control of intracellular *Salmonella*.

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Insights into genomic Anaplasma phagocytophilum variant analyses to unravel microbe-host associations of this emerging pathogen

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Introduction: Anaplasma phagocytophilum is a tick-transmitted intracellular alpha-proteobacterium. This zoonotic pathogen can cause granulocytic anaplasmosis in humans, horses, dogs and cats and tick-borne fever in ruminants. The main vector in Central Europe for this emerging pathogen is the hard tick Ixodes ricinus. For A. phagocytophilum the associations between vertebrate reservoirs and the vector are described as wide-ranging with a high variability of different genetic variants in different host species. It was previously shown that also the pathogenicity differs depending on the combination of the pathogen variant and the infected host species. This study aims to genotype samples of four partial genes (16S rRNA, groEL, msp4, msp2) of A. phagocytophilum from different mammalian host animals and present new methodological approaches for interaction analyses.

Material/Methods: Samples were screened by PCR and sequences of the four gene loci were generated for different positive isolates from variable host species by Sanger-sequencing. Additionally, sequences of these loci which were available in public databases were also included. This led to a total of 1280 sequences for further investigation Minimum spanning nets and correlation nets were applied to display relationships among different variants and show the complex variant combination options in different hosts.

Results: Out of these 1280 sequences, 89 msp2, 692 16S rRNA, 234 msp4 and 265 groEL were analysed. In total we found 40 different msp2 variants in 14 host species, 51 different 16S rRNA variants in 20 hosts species, 72 different msp4 variants in 18 host species and 67 different groEL variants in 20 host species. The correlation nets showed that the genetic variability of the loci and the detected variants highly depend on the host species. The minimum spanning nets showed separation of variants originating from wildlife animals only and a set of variants shared by wild and domestic animals. The most striking detail we observed and confirmed was, that cattle seem to play a central role in the complex transmission cycles, maybe bridging the vector-wildlife cycle to infections of humans and domestic animals.

Discussion: Here a comprising study with 1280 sequences of four gene loci included is presented. A large amount of gene variants occurring only in specific hosts could be found. Additionally, different hosts showed unique but also shared variant combinations. The use of newly combined results of network phylogeny and correlation nets brightens the picture of host-

pathogen interactions and may be a starting point to predict future spread and infection risks of A. phagocytophilum in Europe.

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Zinc metalloprotease ProA as a versatile virulence factor in human lung tissue, cellular infections and immune evasion of *Legionella pneumophila*

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Introduction: The environmental bacterium *Legionella pneumophila* parasitizes protist species of its aquatic habitat but also represents the infectious agent of Legionnaires' disease, a severe and life-threatening pneumonia. Inhalation and replication of the pathogen inside human alveolar macrophages lead to severe tissue damage. In this context, our recent publications illustrated and specified crucial importance of the zinc metalloprotease ProA as an extracellular virulence factor of *L. pneumophila*.
Material/method: By applying our unique infection model of human lung tissue explants, we were able to characterize the versatile role of ProA on different organizational levels during infection. Vital specimens were obtained from surgery patients after lobectomy and inoculated with *L. pneumophila* mutant strains or the purified protease. Subsequent experiments comprised histopathology and transmigration studies, cell line reporter and infections, protein degradation assays and structural analyses of ProA.
Results: We showed that the tissue-destructive protease ProA improves bacterial replication and dissemination in human pulmonary explants. Further histological analyses enabled to quantify a characteristic thickening of alveolar septa due to ProA-mediated degradation and derangement of collagen IV. Moreover, the interplay of ProA with different host factors or even bacterial FlaA promotes immune evasion. Hence, increased susceptibility of a *proA*-deficient mutant to human serum suggested importance of the protease to control the humoral response. In a HEK-Blue hTLR5 model system, it became also evident that flagellin-mediated stimulation of the TLR5-NF-κB signaling pathway is reduced by degradation of FlaA monomers via ProA. Interaction of the enzyme with its versatile spectrum of substrates was evaluated by successful crystallization and structure determination of ProA at 1.48 Å. Despite high similarities to homologous virulence factors, substrate recognition of these proteases seems to depend primarily on specific amino acids in defined binding motifs and less on general structural similarities.
Conclusion: For the first time, the virulence factor ProA was analyzed extensively in a physiological human background. Overall, it was shown to exhibit a key role for the pathogenesis of Legionnaires' disease by destroying pulmonary tissue, inhibiting host defense mechanisms and promoting proliferation and distribution of *L. pneumophila* in the human lung.

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Toxins of different bacteria impair platelets in different ways

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Introduction: *Streptococcus pneumoniae, Streptococcus agalactiae* (Group B streptococci, GBS*)* and *Staphylococcus aureus* are causative agents of severe invasive diseases. These three pathogens express toxins as major virulence factors: pneumolysin (Ply, *S. pneumoniae*), alpha hemolysin (Hla, *S. aureus*) and ornithine rhamnolipid pigment (pigment, GBS). Thrombocytopenia and disseminated intravascular coagulation are frequently observed during bacteremia. Therefore, an impairment of platelets by bacteria or bacterial factors can by assumed. In the presented study we analyzed how the bacterial toxins Ply, Hla and pigment interfere with platelet function and platelet viability. Further, we aimed to neutralize these toxins with specific antibodies as well as pharmaceutical immunoglobulin preparations.
Methods: Washed platelets were isolated from healthy volunteers. Platelet activation and function were assessed by measuring CD62P expression, localization studies of CD62P, platelet aggregation, release of intracellular Ca2+ and platelet viability. Thrombus formation was measured in whole blood. All experiments were performed in the presence or absence of pharmacological immunoglobulin preparations (IVIG)
Results: Pore formation in platelet membranes by Ply leads to immediate lysis of platelets and loss of platelet function. In comparison, Hla pore formation induced initially platelet activation as shown by extracellular CD62P staining, platelet aggregation and calcium release. Nevertheless, activation was rapidly followed by platelet lysis, leading to impaired thrombus formation and thrombus stability in whole blood. In contast to Ply, Hla could not beneutralized by IVIG. Similar to Hla, GBS pigment first activated platelets but destroyed them over time via necrosis.
Discussion: In the presented study we show that the pneumococcal Ply, GBS pigment as well as staphylococcal Hla impaired platelet viability and platelet function. The molecular mechanisms leading to platelet damage as well as the ability of neutralization differed between the toxins.
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Deciphering the role of proteins involved in capsule and teichoic acid anchoring and influence on the innate immune response

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Introduction: Teichoic acids (TAs) are important components of the cell wall of the pathobiont *S. pneumoniae* and are either bound to the peptidoglycan (PGN) as wall teichoic acids (WTA) or anchored in the cell membrane as lipoteichoic acids (LTA). The linkage to either PGN or a glycolipid is the only difference between pneumococcal WTA and LTA, as both share the same biosynthetic pathway and repeating units. While the teichoic acid ligase TacL has been identified as the enzyme responsible for anchoring of the LTA, the LytR-Cps2A-Psr (LCP) protein family is assumed to be involved in the anchoring of WTA and capsule to PGN.
Material/methods: Aim of this study is to elucidate the role of the individual LCP proteins in the anchoring of WTA and capsule to PGN and to characterise the altered bacterial cell physiology of specific *LCP*/*tacL* deletion mutants.
Isogenic single mutants of the *lcp* genes and *tacL* were generated in *S. pneumoniae* D39 as well as various double mutants. For phenotypic characterisation, pneumococcal growth in complex and chemically defined medium and autolysis behaviour were analyzed. Further, the susceptibility of the individual mutants to various cell wall antibiotics was tested. As LCP proteins are

suspected to be involved in WTA and capsular polysaccharide (CPS) linkage, the TA and capsular content was investigated by flow cytometry and electron microscopy.
Our previous data suggested that the loss of TacL activity induces alterations in the membrane integrity (Heß et al., 2017), the abundance of various lipoproteins was compared between the mutant D39Δ*tacL*, its isogenic wild-type and the complemented mutant. Flow cytometry and Western blotting were used to quantify the amount of various lipoproteins. Furthermore, the cytokine release from dendritic cells after bacterial infection was determined.
Results: Taken together, our results suggest that LCP proteins have a direct influence on capsule anchorage and are able to compensate partially or completely for the loss of one LCP protein by another. In addition, the loss of individual LCP proteins leads to an altered cell morphology and physiology. Further, we showed that the loss of TacL leads to significant changes in the abundance of surface-exposed and membrane-anchored lipoproteins, and their immunostimulatory properties.
Discussion: This study highlights the importance of LCP proteins in anchoring WTA and CPS to the PGN. This is an important step towards understanding the mode of action of individual LCP proteins and their influence on the WTA content as well as their influence on bacterial physiology. We also show the influence of TAs on bacterial physiology and their significance in the interaction with immune cells.

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Lugdunin – impact on the nasal microbiota and insights into the regulation of its biosynthesis and transport

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The vast majority of systemic bacterial infections is caused by facultative, often antibiotic-resistant pathogens colonizing human body surfaces. Pathogen eradication from the microbiomes of risk patients could help to reduce the numbers of opportunistic infections.
Nasal carriage of *Staphylococcus aureus* predisposes to invasive infection. Nasal *Staphylococcus lugdunensis* strains produce lugdunin, a novel thiazolidine-containing cyclic peptide antibiotic prohibiting colonisation by *S. aureus*, and a rare example of a non-ribosomally synthesized bioactive compound from human-associated bacteria. In two independent studies human nasal colonisation by *S. lugdunensis* was associated with a significantly reduced *S. aureus* carriage rate suggesting that lugdunin or lugdunin-producing commensals could be valuable for preventing staphylococcal infections. Long-term analysis of the nasal microbiomes of *S. lugdunensis* carriers revealed the preferred co-occurrence of *S. lugdunensis* with the genera *Corynebacterium* and *Dolosigranulum* and the tendency towards reduced microbiome diversity.
In recent studies, we have shown that not only lugdunin but also its synthetic enantiomer has an identical antibiotic activity. Furthermore, two independent ABC-transporters are responsible for the secretion of and self-resistance against lugdunin in *S. lugdunensis*. Preliminary experiments showed that lugdunin activates expression of the transporters as well as the biosynthesis genes. Therefore, we intended to elucidate the regulation of the *lug*-genes in more detail. In this study, a heterologous expression system of putative promotor regions and suspected regulators of the lugdunin transporter and biosynthetic gene cluster was constructed. Whereas LugR is encoded upstream of the biosynthetic genes *lugABCD*, LugJ is encoded upstream of the ABC transporters *lugIEFGH*. The experiments clearly confirmed the auto-inducing activity of lugdunin on its own biosynthesis and transport, whereas the synthetic enantiomer shows no inducing activity.

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The influence of postnatal infectious challenge on enteric tissue maturation

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Introduction: Gastrointestinal infections caused by *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) remain a major health problem for neonates and young infants worldwide. A better understanding of the mechanisms responsible for disease development and progression during the postnatal period is crucial to improve therapeutic options. Here, using our murine neonatal *Salmonella* infection model [1], we characterised the influence of *S.* Typhimurium infection on intestinal epithelial cell proliferation, differentiation, and function in newborn mice.
Material/ Methods: We analysed the gene (RT-PCR and RNA-Seq) and protein (immunofluorescence staining) expression and metabolic profiles of *S.* Typhimurium-infected and age-matched healthy animals as well as various gene-deficient newborn mice at four days post infection, as well as of *ex vivo* stimulated 3D stem cell organoid cultures.
Results: Immunofluorescence and gene expression analyses revealed alterations of the proliferative and metabolic activities as well as of the antimicrobial peptide repertoire of epithelial cells in infected animals. Several target genes exhibited expression levels comparable to those usually observed in more mature animals. Those alterations were not observed in infected newborn mice lacking MyD88 signalling in their hematopoietic cell compartment. Stimulation of intestinal organoids with different cytokines recapitulated the phenotype in part.
Discussion: Together, our results point towards an accelerated maturation of the innate immune and metabolic functions of the newborn small intestinal epithelium upon infection with *S.* Typhimurium *via* an immune cell-mediated mechanism. Our work is expected to gain further insight into the short- and long-term consequences of neonatal enteric infections on intestinal homeostasis and disease susceptibility. Additionally, our results might identify therapeutic target structures to improve the clinical outcome of neonates from infection.
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The redox-sensing protein Rex as transcriptional regulator is involved in metabolism and oxidative stress response in *Streptococcus pneumoniae*

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Introduction: Pneumococci colonize asymptomatically the upper nasopharyngeal cavity, but upon an external trigger, pneumococci disseminate and cause serious infections. During colonization pneumococci have to adapt to different environmental conditions. Expression of different genes are under control of the redox-sensing regulator Rex, which consists of the N-terminal helix-winged-helix domain and a C-terminal NADH binding Rossman fold domain (Sickmier et al. 2005). Rex is regulating its own expression as well as a number of glycolytic enzymes and fermentative enzymes. Enzymes involved in oxidative stress response are also regulated by Rex. For further studies *rex* was

mutated in strain D39. Mouse coinfection experiment demonstrated that Rex is involved in maintaining pneumococcal fitness and robustness under *in vivo* conditions.
Methods: *in vivo* infection experiments were performed by infecting intranasally CD-1 mice with bioluminescent *S. pneumoniae* D39*lux* and its isogenic *rex*-mutant. Rex was heterologous expressed in *E. coli* and purified by affinity chromatography. EMSA were performed with different promoter fragments of genes encoding glycolytic, fermentative or genes for NAD synthesis or oxidative stress response. Northern hybridizations were conducted to analyse the different expression of certain genes under anaerobic or microaerophilic conditions.
Results: These studies demonstrated that pneumococcal Rex is acting as repressor for glycolytic and fermentative encoding genes. By EMSA analyses we observed binding of Rex to promoters of glycolytic encoding genes like *gap*N, *enolase*, *pgk* as well as to different *adh* encoding genes. In addition genes involved in NAD synthesis are also under Rex promoter control as well as genes necessary under oxidative stress conditions like *spx*B or *sod*A. In a mouse competition model we demonstrate that wild-type pneumococci outcompeted the *rex*-mutant.
Conclusion: The importance of Rex on the regulation of different genes involved in glycolysis, fermentation, NAD synthesis and in oxidative stress response was observed.
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Determinants of the interaction of *Aspergillus fumigatus* and Eosinophils in the context of allergic bronchopulmonary aspergillosis

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Allergic bronchopulmonary aspergillosis (ABPA) is a severe and incurable disease that affects approximately five million people worldwide with pre-existing conditions such as asthma or cystic fibrosis. This hypersensitivity pneumonia is predominantly caused by the omnipresent environmental mould *Aspergillus fumigatus* and is characterized by an overshooting inflammatory type 2 immune response, accompanied by a massive influx of eosinophils and their secreted cytokines into the lung. To gain in-depth insights into the transcriptional regulation of *A. fumigatus* after eosinophil confrontation and into the signaling events triggered thereby in eosinophils, we used an *in vitro* co-culture system with murine bone marrow-derived eosinophils being confronted with *A. fumigatus*. By screening a gene deletion strain library, we identified six previously uncharacterized transcription factors of *A. fumigatus* that influence eosinophil activation as well as fungal susceptibility to eosinophil-mediated killing. In this setting, the absence of a particular transcription factor leads to reduced eosinophil activation and simultaneously reduced killing. Interestingly, these readouts may also be affected in opposite ways. Furthermore by using small molecule inhibitors, we identified important signaling modules of eosinophils in the course of *A. fumigatus* confrontation. As a result, the activation of eosinophils is influenced in a stronger manner than their antifungal activities. The identification of crucial *A. fumigatus* transcription factors and signaling cascades of eosinophils during their interaction provides new fundamental insights into the host-pathogen interaction in the context of ABPA to yield therapeutic perspectives.

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Immunglobulin M-degrading enzyme of *Streptococcus suis* (Ide*Ssuis*) – modulation of B-cell function

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Introduction: *Streptococcus suis* (*S.suis*) is one of the most important bacterial pathogens in pigs and also an emerging zoonotic pathogen with human cases mostly in Asia. In pigs, it causes mainly meningitis, arthritis, septicaemia and sudden death (Segura et al. 2016). Disease mostly occurs in piglets after weaning, at a time of development of adaptive immunity (Rieckmann et al. 2018). *S. suis* is also a very successful colonizer of mucosal surfaces in pigs of varies ages (Vötsch et al. 2018).
The cysteine-protease Ide*Ssuis* is host and isotype specific as it only cleaves porcine IgM between constant domain C2 and C3 (Seele et al. 2013). Cleavage of soluble IgM by IdeSsuis has been shown to be a novel complement evasion mechanism as it reduces labelling of bacteria with C3b and subsequent opsonophagocytosis (Seele et al. 2015; Rungelrath et al. 2018). This study was initiated by the finding that recombinant Ide*Ssuis* also cleaves membrane-bound IgM on the surface of porcine B cells.
Objective: We investigate the working hypothesis that *S. suis* modulates B-cell function through cleavage of the IgM-type B-cell receptor (BCR) by Ide*Ssuis*.
Material and Methods: Cleavage of BCR by recombinant Ide*Ssuis* and respective variants as well as BCR recovery was analyzed by flow cytometry of porcine B cells. Furthermore, loss-of-function experiments with supernatants of different *S. suis* strains including specific mutants were conducted.
The proportion of B-cells positive for tyrosine-phosphorylated phospholipase C-γ2 (PLC-γ2) was analyzed as a parameter of early B-cell activation after treatment with different variants of Ide*Ssuis* and *in vitro* stimuli.
Results: Ide*Ssuis* but not its inactive variant cleaved the IgM-type BCR. After cleavage, recovery of BCR took at least 20 hours.
Stimulation with cross-linking IgM F(ab)2-specific antibodies induced tyrosine phosphorylation of PLC-γ2 in IgM+ B-cells, but not in IgG+ B-cells. After cleavage of the IgM-type BCR by Ide*Ssuis*, there was no longer an increase in PLC-γ2+ cells in the IgM+ B-cell population.
After stimulation with the tyrosine phosphatase inhibitor pervanadate both IgM+ and IgG+ B-cells showed an increase in the number of PLC-γ2+ cells as well as in PLC-γ2+ levels, independent of an intact or cleaved BCR.
Discussion: We present *in vitro* data indicating that cleavage of the IgM-BCR by Ide*Ssuis* diminishes early B-cell activation. This finding suggests modulation of antigen-dependent B-cell responses by *S. suis* through Ide*Ssuis* expression. We plan to investigate this hypothesis *in vivo*.

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Morphological changes of T-lymphocytes exposed to bacterial determinants vary depending on species and strain

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Introduction: Sepsis is a leading cause of morbidity and mortality worldwide, affecting millions of people every year. Delays in appropriate treatment initiation significantly decrease survival rates, underlining the importance of early diagnosis and pathogen identification. Due to the very heterogeneous nature of this syndrome, identification of patients at high risk for subsequent organ failure remains a challenge. Currently, white blood cell morphology is under investigation as a new biomarker for the detection of septic patients. In this proof-of-concept study, we analyzed changes of T-cell morphology in response to different bacteria and their determinants by digital holographic microscopy (DHM). Thereby we aim to investigate the potential of this technique to detect specific morphological changes depending on the pathogen, which may offer a new approach for early sepsis diagnosis.

Methods: Primary T-cells were exposed to different gram-negative and gram-positive bacterial strains either using membrane vesicles (MVs), sterile culture supernatant or living bacteria. DHM was applied to capture holograms every 3 minutes for up to 24 h to record changes of cell morphology over time. Quantitative phase images were numerically reconstructed and image segmentation was applied to analyze morphological parameters, i.e. area, circularity and mean phase contrast, on a single cell level.
Results: Exposure to living bacteria as well as MVs and sterile culture supernatants led to changes in cell morphology, e.g. cell area, circularity and mean phase contrast. Interestingly, the cellular response varied between different species and strains of the causative bacteria and we observed a concentration dependent effect. Morphological changes varied in not only the response time and intensity but also the overall pattern.
Conclusion: These findings clearly show that the response of T-cells to living bacteria and bacterial determinants varies depending on the causative agent. For timely and proper initiation of antibiotic treatment, discriminating gram-negative from gram-positive infection would already be of advantage. Our results suggest that examination of lymphocyte morphology by DHM might provide a promising tool for the early detection of bacterial infections and potentially distinguishing between different causative species.

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Characterisation of *B. thailandensis* E264 mutant strains in cellular infection assays

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Introduction: The genus *Burkholderia* contains several human-pathogenic bacteria species. Among them, *Burkholderia pseudomallei*, *B. mallei*, and *B. thailandensis* are closely related. *B. pseudomallei* is endemic in Southeast Asia and in Northern Australia and causes the tropical human disease melioidosis. *B. thailandensis* is a genetically closely related species but with much lower virulence and is therefore well-suited as model organism. The intracellular lifestyle of these *Burkholderia* species relies on different virulence factors, which enable active escape from the phagosome, actin-based intracellular motility, and fusion of host cell membranes into multi-nucleated giant cells. The bacterial protein BimA is essential for actin tail formation but seems to require activation by a yet unknown mechanism. At present, it is not fully understood how actin-dependent motility and cell-to-cell spread is regulated on the molecular level. Potentially involved are *Burkholderia* proteins BimC, BimD, and BipC. Here, we present results of cellular infection assays using different *B. thailandensis* gene knockout strains to further elucidate critical bacterial factors determining *Burkholderia* virulence.
Methods: *B. thailandensis* E264 fluorescent reporter strains were generated by using the mini-Tn7 transposon system for site-specific genome tagging. Gene knockouts in *B. thailandensis* were constructed by allelic exchange using lambda-red recombineering and artificially generated homology fragments. The latter contained a tetracycline resistance cassette flanked by Flp recombinase target (FRT) sites for flippase-mediated marker excision. The characterization of the intracellular lifestyle of *B. thailandensis* sfGFP reporter and gene knockout strains was carried out by infection assays using primary human macrophages as host cell model. Infected macrophages were fixed, stained, and analyzed via confocal fluorescence microscopy.
Results: Generation of *Burkholderia* knock-out strains was efficient using the lambda red recombinase system. Importantly, quick removal of the Flippase encoding vector and lambda rec vector by counter-selection against a temperature sensitive plasmid *ori* resulted in satisfying yields of the desired gene knockout strains. In the selected host cell model, *B. thailandensis* phagosome escape and actin tail formation could be clearly observed. Interestingly, actin tails polymerized at the bacterial cell pole showed a rather remarkable phenotypic diversity. Gene deletion mutants were analyzed in the same cellular infection model.
Conclusions: The results obtained so far indicate an essential contribution of the *B. thailandensis* virulence factors BimA and BipC to the actin-dependent intracellular life cycle of the pathogen. If these proteins interact in cooperative manner to enable *Burkholderia* intracellular motility requires further investigation.

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Comparison of the diversity of anaerobic-cultured gut bacterial communities on different culture media using 16S rDNA sequencing

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Introduction: Antimicrobial resistance constitutes one of the major human health hazards worldwide. Failure of identification of pathogenic bacteria and their resistance patterns leads to dependence on empirical treatment regimens, which contributes to the emergence of new resistant strains. Microbiological culturing is the first step in this process, where anaerobic strains are among the most demanding bacterial communities to culture. Anaerobes need special growth media and several days for optimal growth, which results in many different growth media being used for diagnostic purposes. To understand how the choice of growth media would impact the diversity of cultured anaerobic communities, we compared the standard anaerobic culture media in Freiburg University microbiology laboratory, the "yeast extract cysteine blood agar" (HCB) plate with the newly modified culture media "modified peptone-yeast extract-glucose"(MPYG), enriched with certain vitamins for optimal culturing, to see whether differences of bacterial growth between the plates can be found.
Methods: Stool samples from 8 human subjects with various medical conditions were cultured each on a standard HCB and a supplemented MPYG cultural media plate. After Anaerobic cultivation for 2-5 days, swabs were taken horizontally from each plate and diluted in PBS buffer for further DNA extraction steps. The isolated bacterial DNA was amplified using primers targeting the V1-2 region of the bacterial 16S rDNA gene for Illumina NGS analysis. The generated sequencing data were analyzed using the DADA2 workflow in the R Program.
Results: The Analysis of the sequencing data showed tight clustering of sequencing samples belonging to the same patient. Various differences could be observed between the two culture media groups in bacterial richness and evenness. While the HCB media seemed to support the growth of a more diverse microbial community, for some specific taxa - like various genera of the family Lactobacillaceae - the MPYG media was more favorable. However, no statistical significance in alpha- and beta-diversity were observed between the groups.
Conclusions: Both media are very suitable for anaerobic culturing, as they support the growth of a broad variety of clinically relevant bacteria on phylum, genus and species level. HCB plates supported the growth of more diverse anaerobic microbial communities, proofing it to be a fitting culture medium for standard microbiological diagnostics. Nevertheless, because the MPYG plates improved the growth rates of certain taxa, they could be more suitable for targeting specific pathological conditions. This work emphasizes the impact of growth media choice on culture of anaerobic bacteria and the importance of adjusting culture conditions depending on the target species.

Fig. 1

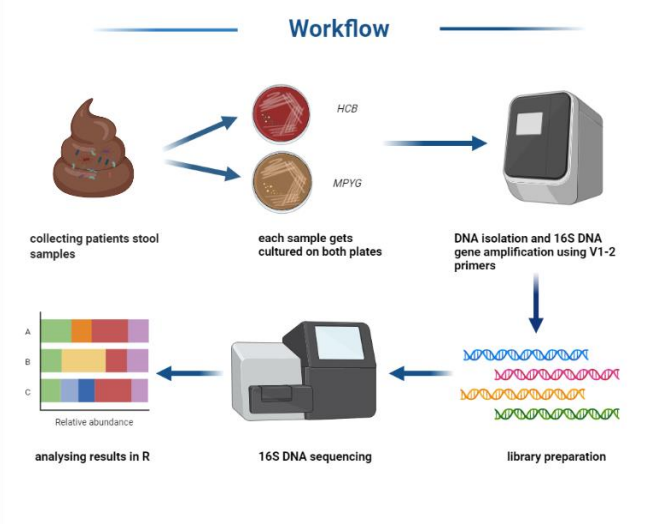
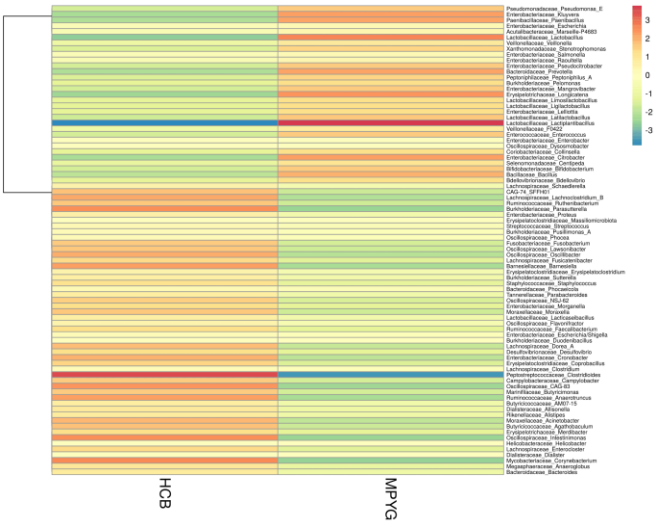


Fig. 2



Genomics and Phylogeny of host-associated Microbes (FG PW)

255/PWP

Phylogenetic analysis and complete genome sequence of *F. tularensis* subsp. *holarctica* strain A-1341 isolated from a patient suffering from tularemia in Brandenburg, Germany

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Introduction: *Francisella tularensis* is an intracellular pathogen causing tularemia in a variety of hosts including humans, rodents and rabbits. The disease tularemia, also known as rabbit fever, is characterized by various clinical symptoms depending on the route of infection and ranging from skin lesions to severe forms of pneumonia. *F. tularensis* ssp. *holarctica* (*Fth*) is the most clinically relevant subspecies in European countries including Germany. Using whole genome sequencing methods (canonical Single Nucleotide Polymorphism (canSNP) typing and whole genome SNP typing), it has been shown that European *Fth* strains belong to a monophyletic population. However, European *Fth* strains differ genetically constituting two main phylogenetic clades: B.6 (Biovar I) and B.12 (Biovar II) possessing erythromycin sensitivity (B.6) and erythromycin resistance (B.12), respectively. Recent reports indicate also putative differences in pathogenicity. Moreover, main clade B.12 can be divided into two subclades: B.12/71 and B.12/72.

Objectives: We aimed to generate and publish a reference genome sequence for subclade B.12/71. Also, B.12/71-specific characteristics shall be identified in comparison to B.12/72 and B.6.

Methods: The whole genome sequence of the German human *Fth* isolate A-1341 was sequenced using Illumina and Nanopore technology; assembled and annotated using the reference genome *Fth* A-271 (B.12/72, accession no. CP048229.1) and Geneious. Nine *Fth* strains (three B.6, three B.12/71, three B.12/72) were used for comparative proteomic analysis and growth experiments to detect clade-specific differences.

Results: In this study, we confirmed the phylogenetical separation of strains belonging to clade B.6, B.12/71 and B.12/72. Also, we present the whole genome sequence of the reference strain (A-1341) of B.12/71 revealing B.12/71-specific differences (including deletions and canSNPs in genes involved in replication, metabolism and outer membrane stability). Strains belonging to B.6, B.12/71 or B.12/72 showed a clade-specific growth pattern in liquid media. The phylogenetic clade discrimination was confirmed by proteomic analysis revealing distinct protein abundance pattern for each clade and subclade, respectively.

Conclusion: In this study, we present the whole genome sequence of a B.12/71 reference strain. Also, we firstly described phenotypical differences of strains belonging to B.6 and B.12. However, further research is necessary to identify a putative relationship between different phylogenetic *Fth* clades and their distribution, phenotype and pathogenicity.

256/PWP

Loss-of-function mutation in mutL is present among genetically diverse *Staphylococcus aureus* during chronic airway infection in cystic fibrosis

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Introduction: The hereditary disease cystic fibrosis (CF) is associated with recurrent and chronic bacterial airway infections due to the mutated cystic fibrosis transmembrane conductance (CFTR) channel. Caused by various CFTR-mutations, mucociliary clearance is impaired leading to long-term colonization and infection with typical CF-related pathogens such as *Staphylococcus aureus.* In this study, we investigate the evolution and diversity of *S. aureus* in the lung of a CF patient while long-term colonization.

Methods: Over a time-period of twenty-one years, from 1995 until 2016, fifty *S. aureus* isolates from respiratory samples of one individual CF patient were collected at routine visits. Additionally, forty isolates from one single sputum were cultured in 2015. All ninety isolates were subjected to whole genome sequencing (WGS). Based on the genes of the core genome multilocus sequence typing (cgMLST) scheme maximum-likelihood phylogeny (MLP) and Bayesian evolutionary analysis sampling trees (BEAST) analysis was performed. Small nucleotide variants of the cgMLST genes and mutations of *mutL/mutS* mismatch repair genes were assessed.

Results: Over the course of this time-period, colonization with eight different *spa*-types (t021, t012, t056, t338, t550, t331, t364, t2351) was observed. Parallel colonization with isolates of different *spa*-types was found from 1998 until 2010. Thereafter, *spa*-type t021, which first appeared in 2002, was the only *spa*-type present since 2011. The MLP tree exhibited close genetic relatedness of *spa*-type t021, t012 and t338 isolates, whereas the other isolates were genetically more diverse. Interestingly, a distinct group of isolates in 2014/2015 showed a close genetic relatedness to two isolates from 2007/2008 (minimum allelic distance = 2, maximum allelic distance = 4), whereas the other isolates since 2010 were more genetically different from this group. Those isolates exhibited a broader diversity with a substantially larger number of genes that showed SNVs (median allelic distance = 25). All these isolates displayed a loss-of-function mutation of *mutL* caused by a frameshift-mutation.

Conclusions: We speculate that that *mutL* loss-of-function mutation could be a potential cause for diversity in the evolution of

S. aureus in the CF lung. Whether a *mutL* loss-of-function mutation leads to improved adaptation of *S. aureus* to CF airways remains open and will be investigated in further studies.

Metagenomics & Microbial Bioinformatics (FG PW)

258/PWP

Fate of pathogenicity and antibiotic resistance genes during thermophilic composting of human excreta

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Agriculture is challenged by weather changes and extremes caused by global climate change. Sustainable agricultural practices are therefore necessary for climate change adaptation and mitigation. Recycling human excreta via thermophilic composting could be one means for enhancing the supply of organic fertilizer. However, the potential spread of pathogens and antibiotic resistance genes (ARGs) is a major concern. In this context, we have assessed the effect of thermophilic composting of human excreta from dry toilets together with straw and green cuttings on the microbial community, the pathogenicity and on ARGs.

Compost samples from the start and the end of the composting process were compared by metagenomic sequencing of total DNA extracts. In addition, we have assessed the fate of ARGs as influenced by thermophilic composting by TaqMan qPCR.

Bacteria comprised around 70 % relative read abundance of the compost community in the start samples with highest relative abundances of the phyla *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes*. These phyla decreased in relative abundance during composting in favor of *Chloroflexi*, *Planctomycetes* and Eukaryota and Archaea. Most potential human pathogens exhibited declines in relative abundance during composting. Strong decreases to 17-35 % of initial relative abundance were found for *Enterococcus* spp., *Listeria monocytogenes*, *Pseudomonas* spp., *Staphylococcus* spp., and *Stenotrophomonas maltophilia*. Genes assigned to bacterial pathogenicity declined, as well. Significant decreases were found for type IV secretion system genes, as well as for those contributing to bacterial motility, biofilm-formation, and toxin production. ARGs of most antibiotic classes exhibited significant decreases from start to end of composting. This was confirmed by TaqMan qPCR for genes conferring resistance to sulfonamides, tetracyclines, aminoglycosides, and erythromycin.

The microbial community was re-shaped during five months of thermophilic composting. Decreases in the relative abundances of pathogens and pathogenicity factor genes indicate that the composting process sanitized the material. Besides, ARGs declined during the treatment, suggesting thermophilic composting as a promising method to treat human excreta prior to application as a fertilizer in agriculture.

Microbiome/Microbiota (FG PW)

259/PWP

Screening for cervical cancer in Bejaia, Algeria – involvement of human papillomavirus (HPV) and human herpes virus (HHV)

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Background: Cervical cancer (CC) is the fourth most common malignant neoplasia in women worldwide. Persistent human papillomavirus (HPV) infection is the main factor, but not sufficient for the development of this disease(1). In addition, the role of the human herpes virus (HHV) also appears to be a subject of debate. Since the main route of transmission of HPV and HHV is sexual, it is fair to assume that co-infection of HPV and HHV is a decisive factor in the development of cervical cancer(2).

Material & Methods: Cervical cancer screening campaigns were carried out in different regions of Bejaia province, between 2019 and 2021. Cervical smear samples were collected and analyzed to verify the presence of infectious agents and cell abnormalities and lesions.

Results: During this period, 1774 women were screened. 27 women were diagnosed with a cell abnormality (ASC-US, ASC-H, LSIL, HSIL, AGC). HHV was identified in 16 women. However, HPV was identified only in 10 women.

Conclusion: Statistics and data on the presence and even the involvement of HPV and HHV are lacking in Algeria, that is why it is necessary to remedy to the situation.

Key words: Cervical cancer; women; human papillomavirus (HPV); human herpes virus (HHV), Bejaia

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260/PWP

Viral genome sequences detected after metagenomic analysis of seafood samples

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Introduction: Analysis of seafood metagenomes may provide relevant information on its microbiota accross the different existing taxons and also reveal new taxonomic groups. One of the advantages of metagenomic studies is that they may provide new data on viruses without the needs for specific enrichment or concentration and in a non-specific way. The aim of the present study was to analyse the virus-specific sequences from metagenomic data obtained from seafoods.

Methods: A metagenomic study was carried out on seafood samples purchased at local supermarkets. Each set of samples combined DNA extracted from five different samples bought at different sampling points. The following sets of samples were analysed: oysters (3 sets), rainbow trout (3 sets), raw shrimps (3 sets) and boiled shrimps (2 sets). Sequences assigned to viral genomes were retrieved and their relative abundances compared.

Results: Phylum *Nucleocytoviricota* was the main group detected in one oysters sample and in two rainbow trout samples, but it was not detected in shrimps. In oysters, this phylum was represented mainly by Fam. *Mimiviridae* and Unclassified *Nucleocytoviricota*. In rainbow trout, it was represented mainly by

Fam. *Iridoviridae* (including the Erythrocytic necrosis virus) followed by Unclassified *Pimascovirales* and Unclassified *Megaviricetes*. Phylum *Adintoviridae* (Fam. *Adintoviridae*) was only detected in one rainbow trout sample, with low relative abundance. Phylum *Hofneiviricota* (Fam. *Inoviridae*) was detected in oysters and in shrimp, with low relative abundances in most cases. Phylum *Uroviricota* was the most abundant group in all oysters and shrimps and also in one rainbow trout sample. This phylum included mainly representatives of Order *Caudovirales* (Fams. *Ackermannviridae*, *Autographiviridae*, *Myoviridae*, *Podoviridae*, *Siphoviridae* and Unclassified *Caudovirales*). The main representatives were Unclassified *Myoviridae* and Unclassified *Podoviridae*. Sequences for bacteriophages specific to relevant bacterial groups (*Pseudoalteromonas*, *Psychrobacter*, *Vibrio* or *Brochothrix*) had very low relative abundances. The remaining sequences were assigned to Unclassified *Caudoviricetes*, Unclassified *Uroviricota* or Unclassified Viruses (amounting up to 27.3% in one case).

Discussion: The results of the present study revealed differences in the viral sequences retrieved according to the seafood source. The retrieved sequences corresponded to both prokaryotic and eukaryotic viruses. Some of the eukaryotic viral sequences detected (e.g., Erythrocytic necrosis virus in rainbow trout samples) would suggest diseased fish.

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261/PWP

Siderophore-based interactions between *S. aureus* and the nasal microbiota

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Introduction: The opportunistic bacterial pathogen *Staphylococcus aureus* can be found as a commensal in the nasal microbiota of 20-30% of the human population. Why some individuals can be colonized by *S. aureus* and some cannot is unclear. As *S. aureus* carriage is an important source of hospital-acquired infections, addressing this question is of particular relevance. Epidemiological studies of the nasal microbiome have revealed positive and negative correlations between *S. aureus* and other nasal commensals, suggesting cooperative and antagonistic interactions among species. Among these interactions, competition for iron is suggested to shape the composition of nasal bacterial communities. Indeed, iron plays a fundamental role in various metabolic processes in bacteria, and its availability is restricted in nasal secretions due to high amounts of the host-secreted iron-chelating molecule lactoferrin. Iron acquisition in bacteria relies on the secretion of iron-binding siderophores. *S. aureus* produces two distinct siderophores, staphyloferrin A and staphyloferrin B, and is also able to use xenosiderophores that are produced by other bacterial species. We tested whether siderophore-based interactions may be responsible for the presence/absence of *S. aureus* within the nasal microbiome.

Materials/Methods: We screened the ability of nasal bacterial isolates from several genera to produce siderophores using the chrome azurol S overlay assay. Also, we examined the ability of nasal isolates to consume staphyloferrin A and B, as well as the ability of *S. aureus* to use xenosiderophores in *in vitro* co-culture experiments. Additionally, we used a cotton rat nasal colonization model to investigate whether siderophore acquisition is relevant during *in vivo* colonization.

Results: *In vitro*, three siderophore-based interactions were identified. Several commensals consumed staphyloferrins without producing siderophores themselves. Others consumed staphyloferrins while simultaneously producing siderophores that could not be utilized by *S. aureus*. Finally, some bacterial species consumed staphyloferrins while producing siderophores that also supported *S. aureus* proliferation. All the interactions that resulted in siderophore stealing by commensals reduced *S. aureus* proliferation. Furthermore, we observed reduced levels of nasal colonization in cotton rats of an *S. aureus* strain, carrying impaired siderophore uptake systems (knock-out of the ATPase *fhuC*), compared to the wildtype. This led to the finding that siderophore uptake is important during *in vivo* nasal colonization.

Discussion: Our data indicate that siderophore production and acquisition is an important trait during nasal colonization and is involved in a wide range of bacterial interactions. We also show that several commensals hinder *S. aureus* proliferation by decreasing iron-availability, suggesting that these commensals might be used as nasal probiotics to reduce *S. aureus* colonization in humans.

262/PWP

Fungal metagenomics of a pressurised smoothie drink

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Introduction: Smoothies are widely consumed as healthy drinks composed mainly of fruit and vegetable mixtures. Smoothies are often stabilized by high hydrostatic pressure (HHP) processing, because HHP has little or no impact on the food organoleptic and nutritional properties. Yet, it is important to know the impact of HHP treatments on microbial populations and how these influence the product stabilization and shelf life. The aim of the present study was to determine the impact of different HHP treatments on fungal populations of a smoothie through metagenomic data analysis.

Methods: A smoothie drink was prepared from red pepper, carrots and orange juice. The smoothie was pressurized at 450 or 600 MPa at temperatures of 22ºC or 50ºC and refrigerated stored for 20 days. A control not pressurized was also included. After DNA extraction and shotgun metagenomic analysis, ITS sequences were retrieved and compared.

Results: About 99.7% of the ITS sequences retrieved from control samples at time 0 corresponded to Phylum *Mucoromycota*. This phylum was represented mainly by Fam. *Glomeraceae* with Gen. *Rhizophagus* (96.92%). Changes in fungal populations detected during refrigerated storage of the control samples included an increase in the relative abundance of *Ascomycota* (specially at days 10 and 20 of storage). *Ascomycota* were represented mainly by Fams. *Candida* (*Saccharomycetales* incertae sedis), *Pichiaceae*, unclassified *Saccharomycetales* and unclassified *Ascomycota*. The genera *Pichia* and *Candida* reached relative abundance values of 14.2 and 4.8%, respectively. In the pressurized samples, no changes were observed during storage and Fam. *Glomeracea* remained at values between 90 and 98.7%. *Rhizophagus* was also the main genus detected in all the pressurized samples (96.1 to 96.9% relative abundances).

Discussion: The results of the present sudy suggested that fermentative yeasts accounted for microbial growth during refrigerated sturage of the smoothie and possibly were also involved in spoilage. By contrast, pressutization of samples would avoid the proliferation of fermentative yeasts and contribute to product stability. Carrouts would be expected to be the main source of contamination with the (more pressure resistant) mycorrhizal fungus *Rhizophagus.*

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263/PWP

Genetic and phenotypic identification of the metabolic features of nasal commensals in *Staphylococcus aureus*-positive and –negative bacterial communities

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Question: The asymptomatic nasal carriage of *Staphylococcus aureus* increases the risk of infection, urging the identification of the factors that enable *S. aureus* to colonize the nasal cavities. In

this context, the role of the resident microbiota in creating a favourable or hostile nutritional milieu to *S. aureus* nasal colonization has long been neglected. This project aims to identify the main nutritional interactions between nasal commensals and their impact on *S. aureus* physiology with the long term goal to develop nutritional exclusion strategies. We present here the first step of the project, consisting in the identification of the metabolic potential of nasal bacterial communities.

Methods: Bacterial species were isolated from the anterior nostrils of 11 volunteers to constitute a collection of 291 isolates. Assays on solid and liquid medium were performed to assess their ability to metabolize various molecules that are present in the nasal environment, *eg*. proteins (heme, haemoglobin, mucins), lipids (triolein, trilinolein, stearic acid, cholesterol), exogenous DNA and putrescine. In parallel, the genomes of the bacterial isolates as well as the nasal metagenome of each volunteer were analysed using three sequencing approaches (16S, Illumina and Nanopore). The compositions of the nasal communities that were identified through sequencing and culture-based methods were compared, and the presence of relevant metabolic pathways was inferred from the analysis of nasal bacterial genomes and metagenomes.

Results: Depending on the volunteer, 16% to 45% of the species that were identified through 16S sequencing could be cultivated and isolated. 16S analysis and enumeration on plates highlighted (i) consistent community structures between the two approaches, (ii) a fraction of cultivable species comprising 43 to 98% of the total nasal communities and (iii) the presence of 3 *S. aureus* nasal carriers among the 11 volunteers. The first results of the metabolic screening show that DNAse and lipolysis activities are well conserved among *S. aureus* isolates. In contrast, the tested metabolic potential of *S. epidermidis* isolates is highly diverse between and within volunteers, and that of *Corynebacterium* spp very low.

Discussion: The data suggest that DNAse and lipolysis activities are important for *S. aureus* nasal colonization, while other staphylococci appear to be metabolically more versatile. Illumina and Nanopore sequencing analysis will reveal the degree of conservation of these pathways among nasal strains. Pairwise co-culture experiments will unravel hub nasal commensals and metabolic activities as well as the transcriptional and phenotypic responses of nasal *S. aureus*. The identification of the nasal species and nutritional mechanisms that restrict *S. aureus* proliferation will pave the way for the development of strategies to prevent *S. aureus* nasal colonization.

265/PWP

Eliminating microbial surface contamination in the SIRIUS habitat during simulated space missions using innovative antimicrobial coatings

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Isolation conditions, microgravity and radiation during long-term space missions affect the human immune system and can alter microbial community compositions within the spacecraft. Increased microbial virulence and antibiotic resistance put astronauts at increased risk of infectious disease. In addition to health concerns, microbial surface contamination can corrode technical equipment. Effective antimicrobials are needed to eliminate microbial contamination within crewed space crafts.

We assessed two innovative antimicrobial surface coatings, AGXX® and GOX, for their long-term antibacterial effect during the 4-months SIRIUS-19 simulated space mission. AGXX®, a contact catalyst, efficiently eliminates pathogenic bacteria, including multiresistant organisms, filamentous fungi, yeasts, and viruses by release of ROS. GOX consists of chemically modified graphene oxide with a positive surface charge which immobilizes bacteria via electrostatic interaction. Samples coated with AGXX® and GOX were exposed inside the SIRIUS habitat and assessed after 1, 2 and 4 months of isolation. Survival and growth of airborne heterotrophic, aerobic bacteria on the surfaces were assessed by molecular analysis and cultivation.

Bacterial growth was inhibited in the presence of AGXX® regardless of the carrier material. Human-associated, biofilm-forming *Staphylococcus* spp. dominated the bacterial community, most were resistant against erythromycin, kanamycin, and ampicillin. A bactericidal effect was exhibited only by AGXX®-coated cellulose fleece. GOX-cellulose fleece effectively immobilized bacteria. Sequence analysis of 16S rRNA gene amplicons revealed that the isolated *Staphylococcus* spp. did not dominate the overall bacterial community, accounting for only 0.1–0.4% of all sequences.

As part of the SIRIUS-21 study, we are evaluating new antimicrobial agents in addition to AGXX® and GOX: QAC and QAC-C8 are quaternary amine compounds. Like GOX, they bind bacterial cells by means of electrostatic force, without the expensive graphene component. Two cationic antifungal peptides, AFP from *Aspergillus giganteus* and AnAFP from *Penicillium chrysogenum*, are also studied for their efficacy against surface colonization by filamentous fungi. Changes in the bacterial and fungal community composition will be assessed at 6 and 8 months by comparing molecular fingerprints. The goals of the study are: (i) recording the microbial diversity (prokaryotes and eukaryotes: bacteria, yeasts, filamentous fungi) on the novel antimicrobial surfaces, (ii) comparing the prokaryote microbial diversity obtained in SIRIUS-19 (0-4 months) with that of SIRIUS-21 (0-8 months) and (iii) assessing the effect of the antimicrobials on the germination capacity of surface-contaminating *Bacillus subtilis* endospores. The results will provide new information on the microbial diversity within the SIRIUS habitat and the potential of the antimicrobial surface coatings for use in future, long term space missions.

Postersession 02
06. Sep. 2022 • 17:30–19:00

Zoonoses (FG ZO)

266/ZOP
Establishment of microfluidic parameters for characterisation of streptococcal attachment to human primary endothelial cells

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Introduction: *Streptococcus equi ssp. zooepidemicus* (SEZ) is a zoonotic pathogen that infects mainly horses and is additionally associated with endocarditis, meningitis, and respiratory diseases in humans. SEZ can adhere to human cells, but underlying pathomechanisms are not yet clarified. Assuming that systemic infections require multifactorial interactions with the cell surface, we aim to identify the bacterial adherence factors, which mediate bacterial cell attachment during an endocarditis infection.
Material/method: Primary human cardiac endothelial cells (HCMEC) serve as model cells to analyse SEZ adherence to the vasculature inducing heart infection. We performed cell culture infection analyses and microscopically quantified SEZ-attachment to HCMEC at various incubation times and bacterial multiplicity of infections (moi). In order to mimic the altered shear force conditions during heart valve infection, we established a microfluidic pump system that enables the culture of cells under a defined medium flow. In this system, a pneumatic pump presses medium over a confluent grown HCMEC layer, which has been seeded onto specialized micro slides allowing microscopic visualization.
Results: Immuno fluorescence staining confirmed SEZ adherence to HCMEC in a standardized cell culture infection and at different shear forces. In addition, a surface expression profile of selected endothelial receptors of primary HCMEC in static cell culture and after 48 h of flow cultivation was determined by flow cytometry.
Discussion: At later project stages, a Transposon library will be used to identify new bacterial adherence factors comparing the sequences of the bacterial in and output pools via Transposon directed insertion sequencing (TraDIS). The cell culture infection under optimized microfluidic conditions will provide essential information to understand the pathomechanism of a zoonotic SEZ endocarditis and will pave way for the development of new diagnostic and therapeutic strategies.

268/ZOP
Gene amplification based heteroresistance in a multi-resistant clinical *Enterobacter cloacae* complex strain

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Introduction: Heteroresistance (HR) describes the ability of a subpopulation within a population of mainly susceptible bacteria to grow in the presence of inhibitory antibiotic concentrations. HR causes treatment-failure and is often underdiagnosed in standard diagnostics. HR manifests itself as isolated colonies (CFU) appearing in the inhibition zone of disk diffusion assays (figure 1). Here, we provide a mechanistic explanation for HR in a clinical *Enterobacter cloacae* complex (ECC) strain (IMT49658).
Materials & Methods: For phenotypic characterisation we conducted population analysis profiles (PAPs) on Mueller-Hinton II agar around the breakpoint concentration (16 µg/ml) of ceftazidime (CAZ) and continuous subcultivation on non-selective media without antibiotic. To analyse expression and to quantify genomic content of β-lactamase genes we performed qRT-PCR. Whole genome sequencing (WGS) was used to compare the genome of resistant and susceptible population entities. Furthermore, we exposed IMT 49658 to long-term heat stress. Effects on growth in resistant and susceptible population entities were investigated using competition assays and ScanLag, a tool for the quantification of single cell lag times.
Results: PAPs confirmed HR with growth up to 16-fold the breakpoint concentration due to a transient resistant subpopulation, which was reversible after subcultivation on non-selective media. WGS identified a gene-amplification region comprising the AmpC β-lactamase *bla*$_{DHA-1}$ gene in the resistant subpopulation. qRT-PCR showed that the amplification decreased when the resistant subpopulation reverted to susceptibility. Heat stress led to gene loss of *bla*$_{DHA-1}$ and the disappearance of HR in disk diffusion assays, confirming the role of this gene for HR. In competition assays, the gene-amplification was associated with a loss of biological fitness. ScanLag revealed a heterogeneous lag time profile for resistant subpopulations growing on 32 µg/ml CAZ. Here, CFUs appearing early maintained higher copy numbers of *bla*$_{DHA-1}$ than late occurring CFUs. However, on LB plates without CAZ selection, both resistant and susceptible CFUs showed homogeneous lag times.
Discussion: WGS revealed gene amplification of the β-lactamase *bla*$_{DHA-1}$ as a cause for HR in IMT 49658, which is in accordance with recent findings [1]. Resistant subpopulations with gene amplifications are transient, and as a result difficult to detect in infections, endangering antibiotic treatment success in human and veterinary medicine. Understanding the processes leading to gain and loss of gene amplification in heteroresistant phenotypes require urgent studies. It likely involves multifactorial processes involving stress-triggers, stochasticity, microbial priming, or metabolic states of a population.

1. Nicoloff, H., et al., *The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification.* Nat Microbiol, 2019. **4**(3): p. 504-514.

Fig. 1

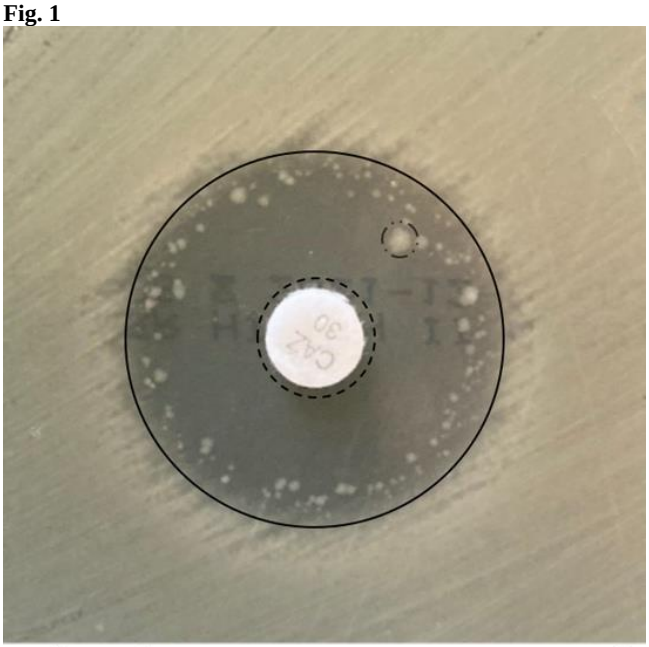

figure 1: Heteroresistance in an agar-disk diffusion assay

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Comparative viral metagenomics of *M. fuliginosus* bats inhabiting Wavul Galge cave, Sri Lanka

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Question: The mammalian order Chiroptera comprises numerous taxonomically diverse species of bats that are distributed worldwide. They are described as the natural reservoir hosts of numerous viruses from a diverse range of viral families (i.e. Filoviruses, Coronaviruses etc.). By spillover and zoonotic transmission, some viruses cause diseases of different severity in humans. Although an increasing number of bat-associated human pathogenic viruses were discovered in the past, the full picture of the bat viromes are not explored yet.
Methods: With this pilot study we analyzed the virome composition of different sample types from *Miniopterus fuliginosus* bats, inhabiting the Wavul Galge cave (Sri Lanka). Oral swabs, feces and urine swabs were taken depending on availability. Samples were analyzed using metagenomic NGS and the data were evaluated with regard to the detected viruses. Virome compositions were compared for the different sample types and phylogenetic analyses were calculated for viruses of particular interest.
Results: In feces and urine samples, we detected two different Coronaviruses related to bat alphacoronavirus isolate batCoV/MinFul/2018/SriLanka (OL956935) and alphacoronavirus HKU8 isolate BtMf-AlphaCoV/GD2012 (KJ473797), both strains have also been identified in Miniopterus bats. In urine swabs, a paramyxovirus related to Jingmen Miniopterus schreibersii paramyxovirus 1 (MZ328288) was identified. Furthermore, sequences related to Picornaviridae, Retroviridae, Iflaviridae, unclassified Riboviria, Astroviridae were identified in feces samples, as well as further Astroviridae in urine swab samples. No further viruses were detected in oral swab samples.
Conclusions: The comparative virome analysis in this study revealed significant differences between the sample sources. The detection of two different Coronaviruses in the samples indicate a possible co-infection and the potential general persistence of these viruses in *M. fuliginosus* bats. Based on the phylogenetic analyses, the identified viruses are rather related to bat-associated viruses. There were no signs of resemblance to zoonotic and human pathogenic viruses. In-depth sequence analyses will be necessary to allow for more precise statements. In future studies, the feasible and non-invasive collection of urine, feces and orals swabs will be applied. These sample sources also represent different typical viral shedding routes. Furthermore, the seasonal variation of the virome will be analyzed to identify possible seasonal shedding patterns for particular viruses.

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Changing tick fauna of Berlin and Brandenburg – a result of climate change and possible consequences for tick-borne diseases

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Introduction: Tick-borne diseases are the most common vector-borne diseases in Germany. They represent a major threat to public health. The infection risk for tick-borne diseases depends among other things on the prevalence of pathogen in ticks, tick occurrence and tick activity. The latter two are strongly influenced by Climate change.
Especially in the years since 2018, climate change-typical characteristics of the weather became apparent. We investigated the resulting consequences for the tick fauna in Berlin and Brandenburg.
Methods: We have been conducting standardized surveys of tick occurrence and activity at 16 sites in Berlin, Brandenburg and the Lower Rhine region since 2008 and compared them with other sites in Germany.
Results: Significant changes in the occurrence and activity of ticks were observed since 2008. While the castor bean tick probably disappeared in some formerly heavily infested areas due to drought, the ornate cow tick spread to areas where it had not previously been detected.
Ticks not previously observed in Germany like the relict tick appeared and almost disappeared again. The Situation of ticks of the genus *Hyalomma* described in 2018 with high numbers of individuals remains unclear.
The changes differ depending on the investigated area. Findings that were typical for Berlin and Brandenburg e. g. the disappearance of the castor bean tick were not observed in other areas of Germany like the Lower Rhine region. Here the number of ticks increased from 2008 to now.
First results of pathogen screening will be presented.
Discussion: Occurrence and activity of ticks showed clear deviations from the traditionally described pattern. The most important consequences that follow from this are the extension of the tick season. The most common tick in Germany, the castor bean tick, starts activity in some investigated areas already in January and the tick season occasionally ends in December. Additionally, the emerging tick species in Germany, ornate cow tick was found questing next to snow. Both findings result in a potential tick bite risk comprising the whole year. New tick species like the relict tick or the genus *Hyalomma* are described to carry pathogens not endemic in Germany. Until now it is unclear whether potential pathogens could be transmitted by these species in Germany.

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Identification and characterisation of an immuno-active factor belonging to the arginine deiminase pathway of *Enterococcus* spp. involved in immunomodulatory effects of probiotic *Enterococcus faecium* SF68

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Introduction: The bacterial probiotic strain *Enterococcus faecium* strain SF68 (NCIMB 10415) has been shown to alleviate symptoms of human and animal intestinal inflammation. In a previous study, we observed significant lower expression of immune-associated genes of intestinal tissues and associated lymphoid organs in post-weaning piglets supplemented with this strain prior to animal feeding trial (1).
Material & Methods: To identify and characterize accountable factor(s) involved in observed immunomodulatory effects on host cells, both commensal and pathogenic *Enterococcus* isolates were evaluated using porcine and human intestinal epithelial cells harboring an NF-κB reporter to determine effects on host cell NF-κB activation. We selected NF-κB as one of the main inflammatory transcription factors involved in both innate and adaptive immune responses particular in intestinal epithelial cells (2). Moreover, cytotoxic effects and responses to TLR- and NOD protein-ligands were determined.
Results: Cell-free whole cell lysates of *E. faecium* exhibited reversible inhibitory effects on NF-κB activation of epithelial cells. Such effects were not observed using lysates of *E. avium*, *E. gallinarum*, or *E. casseliflavus*. An ammonium sulfate fraction of *E. faecium* lysates with inhibitory effects prominently included arginine deiminase (AD), identified using MALDI-TOF. The *E. faecium arcA* gene cloned into *E. avium* conferred the same NF-κB-inhibitory effects on porcine and human intestinal epithelial cell lines as the *E. faecium* SF68 strain.
Discussion: Our observations indicate that the arginine deiminase of the AD pathway in probiotic strain *E. faecium* SF68 is most

likely responsible for the NF-κB inhibitory effects *in vitro*, and possibly involved in the *in vivo* immunomodulatory effects of the probiotic *E. faecium* SF68 strain observed in prior animal trials.

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Renal cell lines as *in vitro* model environment for hantaviral infection may show different effects than primary cells

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Acute kidney injury (AKI) is frequently observed in infectious diseases. Eurasian hantaviruses are causing a zoonosis transmitted via rodents. Human infection is characterized by infection of glomerular and tubular cells leading to AKI. Because infected rodents only show asymptomatic infection, no suitable small animal model has been established so far. The analysis of underlying pathomechanisms therefore relies on in vitro infection studies and requires adequate cell culture model systems to identify target cells and functional consequences. Renal cells are highly specialized and differentiated cell types and immortalization procedure often changes their characteristics. Therefore, we tested the suitability of different renal cell lines compared to primary cells as in vitro infection model for infection-induced AKI.

We used a podocyte cell line (CIHP), a glomerular mesangial cell line (CIHGM-1) and two tubular cell lines (HK-2 and TH-1) and their corresponding primary cell types derived from two donors. We compared entry receptor expression by flow cytometry and permissivity for infection with Eurasian hantaviruses between cell lines and primary cells. In addition, we analyzed functional effects (viability, motility, adhesion) in infected primary cells and in infected cell lines.

All primary cells and cell lines express the entry receptor for hantaviral infection. Primary podocytes, mesangial cells as well as their cell line counterparts CIHP and CIHGM-1 are permissive and release infectious hantaviral particles. The same is valid for primary tubular cells and cells of the tubular cell line HK-2. In contrast, cells of the tubular cell line TH-1 only exhibit a poor initial, but abortive infection. Viability of cells is neither affected in infected primary nor in immortalized cells. As observed for infection of primary podocytes and tubular epithelial cells, infected HK-2 cells exhibit an impaired migration and adhesion capacity. CIHGM-1 showed a reduction of motility while primary mesangial cells did not. No effects on adhesion were present in both, primary mesangial cells and cell line.

Our study indicates that the choice of a cell line as in vitro infection model for renal cells may influences the observed alterations in physiology. Using cell lines requires the verification in primary cells of different donors. Both, permissivity and functional effects may differ between cell culture systems. However, the detailed comparison of cell lines with dissimilarities in permissivity and effects may also allow the identification of restriction factors and mechanisms. In conclusion, the interpretation of results from cell line infection studies should be performed carefully.

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Phenotypic characterisation of *Coxiella burnetii* isolates from different host species using tissue cell culture infection models

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Background: *Coxiella* (*C.*) *burnetii*, the etiological agent of the worldwide distributed zoonotic disease Q fever, causes an acute flu-like illness or may manifest as a chronic disease in humans. In ruminants an infection is often asymptomatic or abortion, weak offspring and fertility disorders may occur. Differentiation of *C. burnetii i*solates has a long history and genomic groups or genotypes are correlated with disease outcome and/or host. Besides, host factors have been proposed to influence disease manifestation and outcome.

Method: The aim of the project is to show isolate-specific differences in cell culture models resembling entry side (monocyte-derived macrophagesTHP-1) and replication niches (bovine trophoblasts F3, udder parenchyma cells PS). *C. burnetii* Nine Mile phase I and six field isolates of different host origin (human, sheep, goat, cattle) and disease outcome (acute, normal birth, abortion and milk shedding) are characterized phenotypically by replication and invasion rate as well as targeted gene expression.

Results: THP- 1 cells were permissive for Nine Mile I and isolates from sheep and goat with replications rates around 2 after 7 days but with invasion rates < 1% after 4h for the field isolates. Replication in F3 was higher for goat isolates compared to the other isolates. Invasion rates were low with < 3% for all isolates except for the cattle isolate with > 15%. PS were permissive for all isolates with replication rates of approximately 2 after 7 days except for the cattle isolate. Invasion rates of approximately 5% were detected after 4h for most isolates with a maximum of > 10% after 4h. There was no correlation between replication and invasion.

Conclusions: Based on replication and invasion assays, we can detect phenotypic differences of the here tested isolates. Targeted gene expression analyses are ongoing. Isolates, which show the most significant differences, will be selected for proteome analysis. This might support the identification of potential factors and adaption mechanisms that could be relevant for the isolate-specific virulence.

Mikrobiologische Diagnostik (StAG DV/FG DKM)

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Holistic system for the rapid establishment of an antibiogram for nosocomial infections

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Introduction: Bacterial infections become more and more difficult to treat as antimicrobial resistance (AMR) rises. In 2019, over 1 million worldwide deaths were attributed to bacterial AMR. Some of these bacteria are able to cause blood-stream infections and sepsis. Sepsis is a life-threatening infection that affects the whole body, with septic shock being a progression of sepsis with multiorgan failure and a greater risk of mortality. Time is crucial in sepsis diagnosis as the mortality odds increase every hour without antibiotic treatment. However, pathogen isolation and identification with subsequent antimicrobial susceptibility testing usually takes between 1-5 days. Thus, this collaborative study aims to develop a

holistic system for the rapid establishment of an antibiogram for nosocomial infections. The system will be based on a microfluidic chip on which analyses will be performed.

Material and methods: We acquired a total of 300 clinical isolates of species of interest (SOI), which were *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecium*, *Acinetobacter* spp., *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. From these, 10 isolates of each species were randomly chosen for subsequent analyses including growth kinetics, media adaptation and determination of minimum inhibitory concentrations (MIC). The generated data will be used as references to control assay compatibility with the microfluidic chip. Experiments for the different SOI are currently performed with and without antibiotics in combination with a fluorescent nucleic acid dye (FNAD), which will be used for the automated detection of growth for the antibiogram on the chip. Media adaptation is necessary for enhancing bacterial growth and thus shortening the incubation time for the device.

Results: Growth kinetics were tested in different media. Growth improvement for all species was obtained for MH2 medium supplemented with 5 g/L peptone. Especially the enterococci showed increased growth compared to MH2 medium without the supplement. Medium adaptation was necessary to obtain a universal medium in which most SOI grow and MIC tests can be performed. The latter were done with and without FNAD revealing decreased breakpoints with added FNAD. As FNAD slightly inhibits growth, we applied rather low concentrations (0.5 µM). The SOI seem unable to adapt to antibiotics and FNAD simultaneously. We thus added FNAD after approx. 40 % if the total incubation time, which resulted in similar MIC breakpoints.

Discussion. Bacterial concentrations in blood samples are low and thus, the holistic system needs to detect bacterial growth in low quantities. We plan to use the fluorescence signal of cells with bound FNAD for bacterial growth detection. The holistic system is supposed to generate results in a fraction of the time previously needed for sepsis diagnosis. Basic tests have been completed and the holistic system/chip-associated experiments will start soon.

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Can ejaculate-washing with sodium chloride increase the sensitivity of culture?

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Introduction: Bacteria in the ejaculate can be associated with male infertility. Therefore, microbiological diagnostics become crucial. Antibacterial substances (e.g. proteases, Zn-containing proteins) in ejaculate impair cultural growth of bacteria. Washing the ejaculate to remove antibacterial substances could increase the sensitivity of bacterial detection by culture. This has previously been shown for *Neisseria gonorrhoeae*. The aim of this study was to compare the detection and amount of colony forming units in ejaculate samples with and without pre-culture washing.

Materials and Methods: Ejaculates, which are obtained for routine clinical workup to assess male infertility were included in this study. One aliquot (≈3 ml) of each patient was allocated to the control arm (no washing) and to the test arm (pre-culture washing). Test samples were diluted with 2 ml sterile 0.45% saline, vortexed and centrifuged (5 min, 5000 U/min). 2 ml of the supernatant was removed. 10 µl of the pellet was used for culture on Chocolate agar (incubation at 35 ± 2°C in CO_2 for a maximum of 24 h), MacConkey agar (incubation 35 ± 2°C for a maximum of 24 h, aerobic conditions), Schaedler agar (incubation at 35 ± 2°C for a maximum of 48 h, anaerobic conditions), each. Control samples were plated on the same media without washing. Species identification was performed by MALDI-TOF.

The proportion of detected species between the two arms was compared using the Chi^2-test. The number of colony forming units (total and species-specific) was compared between the test and the control arm using Mann-Whitney-U-Test. P-values <0.05 were considered statistically significant.

Results: A total of 186 samples were included. The dataset was stratified into five groups (gram-negative rods [GNR], anaerobes [AN], *Enterococcus* sp. [EC], coagulase-negative staphylococci [CNS], viridans streptococci [VS]). Compared to the control arm, the test arm revealed significant lower proportions for CNS (59.1% vs. 44.6%, p<0.005) and VS (53.8% vs. 41.9%, p<0.05). Additionally, lower proportions of GNR (16.1% vs. 15.1%, p=0.886), AN (19.9% vs. 17.2%, p<0.5) and EK (25.3% vs 23.1%, p=0.63) were observed. The medians of CFU were lower in test samples compared to the control samples (6.5 $x10^3$ vs. 2.5 $x10^3$, p<0.0005) in total and in each analysed group as well.

Discussion: Pre-culture washing of ejaculate results in a decrease in total bacteria count and culture positive samples. Unlike *N. gonorrhoeae*, ejaculate washing does not increase the sensitivity in the detection of bacteria in ejaculate samples.

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Comparative evaluation of the performance, workflow and associated costs of a multiplex PCR panel versus conventional methodologies in diagnosis of infectious gastroenteritis

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Introduction: Infectious gastroenteritis is a common reason for consulting a physician in low, middle and high-income countries. Although most cases of gastrointestinal illness are self-limiting, identification of the etiologic pathogen by stool specimen analysis usually is required for clinical and epidemiological reasons.

Due to the broad range of causative pathogens, the examination of a stool specimen is labor-intensive and usually requires different methodologies such as cultivation, antigen tests or PCR. Over the last few years, multiplex PCR tests became available. For instance, the BioFire GI panel detects 22 pathogens rapidly in about one hour. This study was aimed at evaluating the performance, workflow (hands-on-time, time-to-result), as well as associated costs of stool specimen management using the BioFire GI panel versus conventional laboratory methods.

Materials and methods: Stool specimens were evaluated prospectively in the microbiology routine testing from July 2019 to December 2020. Pathogen detection rate, hands-on time, time to result and material and personnel costs were determined for the BioFire GI panel and conventional methods.

Results: The detection rate of enteropathogens was higher with 45.6% using the multiplex PCR panel compared to 15.0% using conventional methods. The BioFire GI panel presented results in a median time of 2.2 hours compared to cultural testing with 77.5 hours and antigen testing with 22.1 hours. Based on list prices, the BioFire GI panel was approx. ten times more expensive compared to conventional methods whereas hands-on-time where significant lower using the BioFire GI panel.

Conclusion: Multiplex PCR panels such as the BioFire GI panel are valuable tools for laboratory identification of infectious agents causing diarrhea. The higher costs of such a multiplex PCR panel might be outweighed by the better performance, ease of handling, rapid results and consequently optimized patient management including isolation measures and prompt targeted anti-infective therapy.

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Nanomotion technology with the help of machine learning allows for highly accurate classification of antibiotic susceptibility of various pathogens

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Rapid and accurate determination of antimicrobial susceptibility is paramount to improving patient treatment. It helps reduce the usage of broad-spectrum antibiotics and, therefore, contributes to curbing the spread of antimicrobial resistance (AMR). Nanomotion-based Antibiotic Susceptibility Testing (AST) could help achieve these goals. Based on technology prior developed for atomic force microscopy, it records vibrations of living bacteria attached to microcantilevers. Greater variances of the microcantilever deflections refer to higher metabolic activity. As cellular processes of resistant bacteria are differently affected by a given antibiotic than those of susceptible bacteria the nanomotion signal will convey this difference. The signal is however very rich and complex and machine learning is required to develop classification models that can reliably discriminate between susceptible and resistant isolates distributed over a wide range of MICs.
During the DGHM conference, we will elucidate the development of a highly accurate nanomotion-based AST involving a cloud-based analysis and classification model for *Enterobacteriaceae* exposed to one concentration of cephalosporins and fluoroquinolones based on four-hour recordings. For anonymized patient samples and spiked *E. coli* and *K. pneumoniae* blood cultures we achieved performances over 97% compared to standard hospital diagnostics. Similar performances were achieved for other Gram-negative species often found in bloodstream infections as *P. mirabilis*, *E. cloacae* and *C. freundii*.
The technology is per se growth-independent and species agnostic making it especially attractive for bacteria in physiological states of non-growth or bacteria exhibiting in general slow replication rates which are therefore difficult or impossible to address with current diagnostic methods. In several collaborations, we are developing the AST for *M. tuberculosis*, elementary bodies of *C. trachomatis* and *H. pylori*. Its underlying principle is universal to all living cells and opens the door for various research applications.

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Screening for hypervirulent *Klebsiella pneumoniae* isolates in routine microbiological diagnostics

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Introduction: Hypervirulent *Klebsiella pneumoniae* strains (hvKp), in contrast to classical *K. pneumoniae* strains, can cause invasive community-acquired infections in healthy patients of all ages. In this study, *K. pneumoniae* isolates from routine microbiological diagnostics were tested via string-test and PCR for virulence genes to screen for hvKp.
Materials/methods: For the study period of six months, all *K. pneumoniae* isolates from various clinical specimens were tested by string-testing. 100 isolates with a positive result (≥ 0.5 mm) were cultivated on different conditions (aerobe, microaerophile). For all conditions, the string-lengths were measured for string-length comparison. A multiplex PCR for common genes (*magA*, *iutA*, *rmpA2* and *rmpA*) associated with hypermucoviscosity and hypervirulence was used for all isolates. A *K. pneumoniae* isolate with a positive string-test and detection of the aforementioned genes is defined as "putative hvKP". Furthermore, determination of antimicrobial resistance was assessed by disk diffusion method according to EUCAST.
Results: Most frequently string-test psoitive *K. pneumoniae* were isolated from urine (58%), followed by respiratory samples (18%), various specimens (13%) and blood culture (11%). No isolate showed resistance to carbapenems. In total, seven ESBL-producing strains were detected. The prevalence of strains identified as putative hvKp (string-test+ and PCR+) was 9%. Statistical analyses revealed that hvKp strains showed significantly ($p < 0.05$) longer strings under anaerobic growth, compared to other conditions. These isolates were susceptible for all tested antibiotics.
Discussion: In conclusion, nearly 10% of string-positive *K. pneumoniae* isolates from routine microbiological diagnostics were putative hypervirulent *K. pneumoniae*. For optimized patient care and epidemiological surveillance, the establishment of a test panel for the detection of hvKp strains in routine diagnostics can be an option.

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Direct identification of bacteria from positive blood culture bottles by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry – a systematic review

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Question: Bloodstream infections (BSI) are a leading cause of death worldwide. Therefore, numerous methods have been established to identify causing pathogens directly from positive blood cultures bottles. Among these methods is the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF) and in the past years different MALDI-TOF-systems, protein extraction methods and blood culture systems have become available so that various MALDI-TOF-protocols have been developed.
Here, we perform a systematic review aiming to evaluate direct identification protocols of bacterial pathogens from patients with BSI using MALDI-TOF with the following review questions:

- What is the correct identification ratio (CIR) of MALDI-TOF based rapid identification of bacteria from positive blood cultures 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 blood cultures compared the SOC for (i) different mass spectrometer and blood culture systems and for (ii) different bacterial (sub)groups and species?
- Are there significant differences in the cost effectiveness for different mass spectrometer and blood culture system combinations?

Methods: The search strategy includes an electronic search of various databases using the keywords "bacteremia", "bloodstream infection", "sepsis", "blood culture", "mass spectrometry", "matrix assisted laser desorption ionization time-of-flight", "MALDI TOF", "direct identification", "rapid identification", "rapid diagnostic test" and "RDT".
All studies comparing MALDI-TOF based identification of bacteria directly from positive blood cultures with the identification involving prior subculture of positive blood culture bottles are included.
Data regarding study characteristics, experimental setup, economic performance and numbers of correctly identified bacteria in various sub-groups compared to the reference method are extracted by two reviewers and used for downstream meta-analysis.
The meta-analysis will be performed using the R software. Separate meta-analyses will be performed for single bacterial groups and species. Heterogeneity among studies within each subgroup will be evaluated using appropriate measurements.
Results: Currently, 95 studies out of 1300 studies screened have been included for further analyses. Study details have been deposited at the PROSPERO International prospective register of systematic reviews (PROSPERO 2022 CRD42022293236).
Discussion: The findings of this meta-analysis will be discussed to evaluate whether mass-spectrometry based bacterial identification directly from positive blood bottles is eligible to be used as a routine method for bloodstream infections and whether there are significant differences in the identification results for different (combinations of) blood culture and/or MALDI-TOF systems and/or among different bacterial (sub)groups.

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Improvement of syndromic screening of respiratory and enteric pathogens with rapid PCR cycling

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Introduction: Genetic Signatures specialises in the development of syndromic multiplex PCR test kits, under the *EasyScreen*TM brand, for detection of over 100 clinically relevant pathogens. These kits are underpinned by a unique 3baseTM technology and intended for use in medium- to high-throughput pathology laboratories. There is a need to further increase laboratory testing to provide faster time-to-result to support better patient outcomes. To answer this need, we sought to reduce PCR cycling times for six key *EasyScreen*TMrespiratory and gastrointestinal detection kits.

Materials and methods: To enable faster PCR cycling, we investigated a range of PCR mastermixes, reverse transcriptases, and PCR cycling conditions for *EasyScreen*TM Respiratory (RP007, RP012, RP017) and Enteric Detection Kits (EV002, EB001, EP005). See Table for pathogen targets. Primer and probe concentrations were re-optimised, and assays were re-designed where needed. One standard PCR cycling program was selected (per PCR platform) and assays were re-optimised to align to this program. Samples for analytical sensitivity and specificity testing were quantified reference bacterial, viral, protozoan and fungal targets purchased from various sources (eg ATCC, Vircell). Clinical samples were from our in-house collection, including previously characterised positive samples.

Results: Following reagent and assay optimisation, the PCR cycling times were reduced to approximately 62min (BioRad CFX-96); 67min (BioRad CFX-384), or 78min (QuantStudio5-384), significantly reducing the time to result. This was accomplished by a combination of switching to antibody hot-start polymerases, re-optimised primer and probe concentrations, and assay designs. All previous analytical sensitivity and analytical specificity claims were either met or exceeded, and positivity when clinical samples were tested was equivalent to the slower cycling time versions.

Discussion: The PCR cycling times have been substantially reduced, to approximately 1hour, for a range of *EasyScreen*™ Respiratory and Enteric Pathogen Detection kits, enabling faster time-to-result. Additional*EasyScreen*™ Detection kits are being optimised to suit the faster cycling times.

The regulatory status of *EasyScreen*™ Detection Kits varies in each region. Contact Genetic Signatures or your local distributor for more information.

Fig. 1

Detection Kit	Target 1	Target 2	Target 3
Respiratory RP007			
Panel A	Influenza A	Rhinovirus	
Panel B	Influenza B	RSV A/B	hMPV
Panel C	PIV1/3	Enterovirus	PIV4
Panel D	*B. pertussis/ parapertussis*	*M. pneumoniae*	Adenovirus
Respiratory RP017			
Panel E	HKU1	NL63/229E	OC43
Panel F	*B. pertussis*	*Pneumocystis jirovecii*	*B. parapertussis*
Panel G	PIV4		
Respiratory RP012			
RP012	CoV-2 M-gene	CoV-2 N-gene	
Enteric EV002			
Panel A	Norovirus GII	Astrovirus	Rotavirus A
Panel B	Sapovirus	Norovirus GI	Enterovirus
Panel C	Adenovirus	Bocavirus	AdV 40/41
Enteric EB001			
Panel A	*Campylobacter* spp	*Salmonella* spp	*Shigella*/EIEC
Panel B	*C. difficile*	*Yersinia*	*Listeria*
Enteric EP005			
Panel A	*D. fragilis*	*C. cayetanensis*	*Cryptosporidium* spp
Panel B	*B. hominis*	*E. histolytica*	*Giardia* spp

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MALDI-TOF mass spectrometry for differential identification of adult *Schistosoma* worms – diagnostic accuracy and effects of storage media on the spectra profiles

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Introduction: In the diagnostic parasitology laboratory, pathogen identification is mainly based on microscopy. However, exact species identification cannot always be achieved. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is the standard diagnostic technique for the identification of bacteria, mycobacteria, and fungi. Recently, it was shown that MALDI-TOF can also be used for helminth identification if specifically developed databases are used. In this study, we evaluated the capacity of MALDI-TOF MS to identify adult *Schistosoma* species, and we analyzed the influence of different sample storage media.

Material/method: 62 adult *Schistosoma* spp. (including females, males and paired adults) stemming from experimentally infected mice were subjected to this study. 4 worms (2 x *Schistosoma mansoni*, *2 x Schistosoma japonicum*) were used to generate reference spectra for identification, which were added into a previously developed in-house database for MALDI-TOF mass spectrometry-based identification of various helminth species. The remaining 58 adult worms were subjected to MALDI-TOF to evaluate the database"s diagnostic accuracy regarding helminths. The analysis was carried out with a Microflex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany), and the Maldi Biotyper Compass Explorer© software was used for species identification. The acquired MALDI spectra were tested with the commercially available database for bacteria and fungi as well as a previously developed in-house database for helminths. Log-score values (LSVs) above 1.7 were considered as reliable identification, and a score ≥2.00 represent species confirmation. Additionally, the influence of two storage media (RNA*later*®, and 70% (v/v) ethanol) was evaluated using classification algorithms installed in the online Clover MS Data Analysis® software (https://platform.clovermsdataanalysis.com/, Clover BioSoft, Granada, Spain).

Results: When we employed our in-house database utilized for helminths, MALDI-TOF mass spectrometry identified all 58 *Schistosoma* samples successfully with LSVs ≥ 1.7, corresponding to a reliable identification at the genus level. Accurate identification at the species level (LSVs ≥2.0) was achieved for 81% (47/58) (Table 1). A principal component analysis (PCA) indicated an influence of the storage media, as samples stored in RNA*later*® clustered together in a different pattern than samples stored in 70% (v/v) ethanol (Figure 1).

Discussion and conclusion: Our results confirm that MALDI-TOF mass spectrometry can be successfully used for helminth identification and differentiation, including schistosomiasis. Both ethanol and RNAlater are adequate storage solutions for later MALDI-TOF-based testing. Further research and database validation is needed to confirm the technique"s potential as a diagnostic tool for parasitic pathogens.

Fig. 1

Table 1. External validation: blind-test identification of *Schistosoma* specimens stored in different media (70% (v/v) ethanol, and RNAlater) using our in-house database implemented in MBT compass explorer software (Bruker Daltonics, Bremen, Germany).

			External Validation							
			Identification results							
			LSV≥1.7				LSV≥2.0			
Species	Preservation Media	Number of samples	Paired adults (male/fem.)	Female	Male	Total	Paired adults (male/fem.)	Female	Male	Total
S. mansoni	Ethanol	18	6/6 (100%)	6/6 (100%)	6/6 (100%)	18/18 (100)	6/6 (100%)	5/6 (83.33%)	0/6 (100%)	11/18 (61.11%)
	RNAlater	20	10/10 (100%)	5/5 (100%)	5/5 (100%)	20/20 (100%)	10/10 (100%)	4/5 (80%)	5/5 (100%)	19/20 (95%)
S. japonicum	Ethanol	10	4/4 (100%)	3/3 (100%)	3/3 (100%)	10/10 (100%)	4/4 (100%)	0/3 (0%)	3/3 (100%)	7/10 (70%)
	RNAlater	10	4/4 (100%)	3/3 (100%)	3/3 (100%)	10/10 (100%)	4/4 (100%)	3/3 (100%)	3/3 (100%)	10/10 (100%)
Total		58	26/26 (100%)	17/17 (100%)	17/17 (100%)	58/58 (100%)	24/24 (100%)	12/17 (70.59%)	11/17 (64.71%)	47/58 (81.03%)

Fig. 2

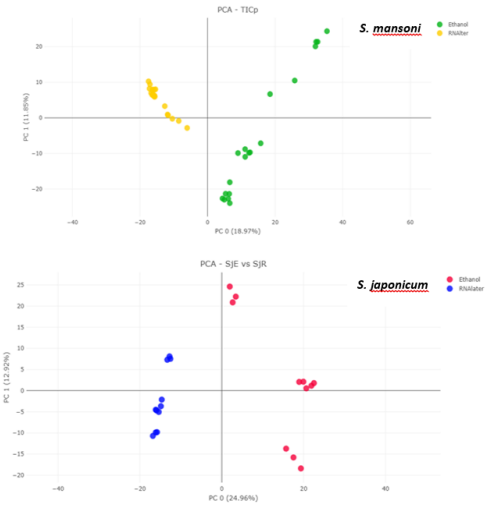

Figure 1. Influence of storage media: classification of adult *Schistosoma* species using a two-dimensional view of a principal component analysis (PCA) based on a peak matrix generated with a threshold of 1% and total ion current (TIC) normalization.

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Application of FT-IR spectroscopy for the automated classification of *Legionella* spp. isolates from waters

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Background: *Legionella* spp. is the causative agent of legionellosis. This opportunistic pathogen has been identified as an increasing public health concern since 1976, being responsible for the death of thousands of people worldwide. Despite at least 20 species of the genus *Legionella* have been associated with human infections, *Legionella pneumophila* (Lp) is the causative agent of more than 90% of the most severe forms (Legionnaires" disease and Pontiac fever). *Lp* presents a variety of 16 serogroups (SG2-16), with serogroup 1 (Lp SG1) being the most clinically relevant (accounting for 80–90% of Legionnaires" disease cases), while the other serogroups are less common and only occasionally cause legionellosis.

Agglutination and immunochromatographic tests are the reference methods for the identification of *Legionella* serogroups. Furthermore, PCR tests can be employed, as long as they comply with ISO 12869 (2012).

In this study, we evaluated FT-IR spectroscopy for the classification of *Legionella* isolates, applying an automated classifier built using artificial intelligence.

Materials and methods: Thermal and drinking water samples were submitted to membrane filtration and diluted on selective agar medium following the ISO 11731:2017. The samples were cultured in buffered charcoal yeast extract (BCYE) agar (48±2 h at 37 °C). *Legionella* confirmation was carried out by Vircell Virapid Legionella culture immunochromatographic test or *Legionella* agglutination Latex test. In addition, 10 *L.* non-*pneumophila* (Lnp) reference strains were employed for the next analytical step. *Legionella* cultures were analyzed by the FTIRS-based IR Biotyper system (IRBT - Bruker Daltonics, Germany). The dataset included n=35 Lp SG1, n=28 Lp SG2-15 and n=23 Lnp. The classifier was built using artificial neural networks (ANNs), trained and validated to discriminate Lp SG1 and Lp SG2-15. It delivers a result in terms of class and reliability score (<1 highly reliable, 1 ≤ score ≤ 2 moderately reliable, >2 non-reliable), based on the spectral distance of the tested samples to the spectra used to train the ANNs.

Results: Overall, 62/63 (98.4%) Lp strains were correctly classified by IRBT classifier, with high (71.4%) or moderate (26.9%) reliability. One Lp SG1 isolate was classified correctly, but with non-reliable score. Among Lnp isolates, 21/23 (91.3%) were classified with non-reliable score (correctly, as Lnp isolates were not included in the training set), while 2/23 (8.7%) were misclassified as Lp SG2-15.

Discussion. In this study, FT-IR spectroscopy showed to be an innovative, reliable and user-friendly approach for discrimination of *L. pneumophila* SG1 and SG2-15 isolates. The automated classifier showed high accuracy, with only one isolate classified with a non-reliable score but still correctly. Further development would be necessary to expand the discriminatory power of the system, in order to enable also the differentiation between Lp and Lnp isolates.

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Comparison of the automated plate assement system (APAS) to manual plate reading of surveillance screening cultures – a diagnostic study

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Comparison of the automated plate assement system (APAS) to manual plate reading of surveillance screening cultures.

Question/Background: Screening samples are one of the most frequent samples sent for culturing in clinical microbiology labs. Therefore, reading and processing of these plates account for a relevant part of daily work. The objective of this study was to evaluate the performance of the automated plate assement system (APAS) in comparison to manual read by experienced technicians.

Methods: Screening samples for methicillin resistant *S. aureus* (MRSA) and vancomycin resistant enterococci (VRE) from primary, secondary and tertiary care centers were randomly selected and prospectively evaluated. Samples sent for MRSA were plated either manually or by an automated streaking system on brilliance ™ MRSA and CNA media biplates (Oxoid, Thermo Fisher). Plates were incubated 36ºC for a minimum of 18 hours. Samples sent for VRE were plated on brilliance ™ VRE (Oxoid, Thermo Fisher) incubated 36ºC for up to 48 hours. The APAS independance (Clever Culture Systems) is a standalone module with an AI software solution for reading and sorting incubated agar media plates. The interpretation followed manufacturers recommendations. Technicians read plates prior APAS. Results were evaluated independently and compared afterwards.

Results: Overall 818 samples were included into this study (MRSA n=714; VRE n=104). A total of 18 samples (n=2.2%) sent for MRSA and 8 samples (7.7%) sent for VRE showed true positive growth. The APAS achieved a concordant result in 694 samples (84.8%) for MRSA and 102 samples (98.1%) for VRE, respectively. For both types of samples no false negatives were described (Table 1.). For MRSA 24 samples were false positive (2.9%). Whereas for VRE 5 samples were false psoitive (4.8%).

Conclusion: The APAS correctly read and identified the majority of plates, producing satisfactory results. Implementation of the APAS Independence potentially decreases the workload for technicians.

Caption

Table 1. Diagnostic performance of the automated plate assement system (APAS) with screening samples compared to manual reading and processing.

	VRE		**MRSA**	
	(n= 104)		(n=714)	
Statistic	**Value**	**95% CI**	**Value**	**95% CI**
Sensitivity	100.0%	63.1% to 100.0%	100.0%	81.5% to 100.0%
Specificity	95.0%	88.6% to 98.3%	96.6%	94.9% to 97.8%

Positive Likelihood Ratio	19.8	8.4 to 46.5	29.0	19.6 to 43.0
Negative Likelihood Ratio	0.0		0.0	
Positive Predictive Value (*)	61.5%	40.5% to 79.0%	42.9%	33.6% to 52.6%
Negative Predictive Value (*)	100.0%		100.0%	

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Comparison and improvement phenotypic assays for the detection of common and rare carbapenemases

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Introduction: The rapid and reliable detection of Carbapenemase-producing Enterobacterales (CPE) is of utmost importance for the initiation of adequate treatment and infection control measures. Most carbapenemase tests target only the main four enzymes and less common variants are often not detected. Therefore, assays based on hydrolysis (activity tests) are recommended in the German guideline and by CLSI in addition to differentiation tests such as PCR or immunochromatographic assays. The activity tests include colorimetric tests (e.g., Carba-NP) and different variants of the carbapenem inactivation method (CIM).

The goal of this study was to compare the activity tests Carba-NP, NitroSpeed-Carba NP, sCIM and the modified zCIM test.

Materials and Methods: The challenge collection included 168 clinical isolates, 129 CPE vs 44 non-CPE (FIG 2), which were all characterized by whole genome sequencing (WGS). All isolates were grown on Columbia blood agar. The colorimetric *Carba-NP* test was carried out as previously described, using Triton X-100 for bacterial lysis. A colour change from red to yellow or a significant colour change in comparison to the control tube was regarded as positive (FIG 1). The *sCIM* test was carried out as previously described using imipenem disks, with a cut-off of ≤20mm for carbapenemase-positive isolates. In addition, a second sCIM test was performed, using faropenem disks and a cut-off of ≤14mm. *NitroSpeed-Carba NP* was performed as previously described. The *modified zCIM (mzCIM)* is a variation of zinc-supplemented CIM (zCIM) test, but contains a higher concentration of zinc sulfate (1.5 mM ZnSO4), an increased bacterial inoculum and the bacterial suspension was incubated for 4h instead of 2h.

Results: Sensitivity and specificity were 98.4%/100% for mzCIM, 99.2%/97.7% for sCIM imipenem, 89.5%/100% for sCIM-faropenem, 83.9%/84.1% for NitroSpeed-Carba NP and 87.1%/100% for Carba-NP. Sensitivity for KPC, VIM, GIM and IMP was 100% for all assays, while it was lower for GES, IMI, OXA-23 or OXA-58, FIG 2.

Discussion: While all tests demonstrate good performance for the common carbapenemases KPC, NDM, VIM and OXA-48, sensitivity significantly decreases for low hydrolyzing enzymes, e.g. OXA-23, OXA-58 or GES. Both the sCIM imipenem test and the modified zCIM showed the overall best performance; for mzCIM a further increase in sensitivity and specificity was recorded in comparison to zCIM in previous studies. Colorimetric tests have the advantage of more rapid results compared to CIM-based tests, but were more difficult and subjective to read. Carba-NP demonstrated moderate sensitivity on class D carbapenemases, while NitroSpeed-Carba NP performed poorly against class A, B and D carbapenemases and yielded most false-positive results.

Fig. 1 Set up for the different activity tests: a) sCIM; b) CARBA NP; c) Nitro Speed Carba NP; d) mzCIM

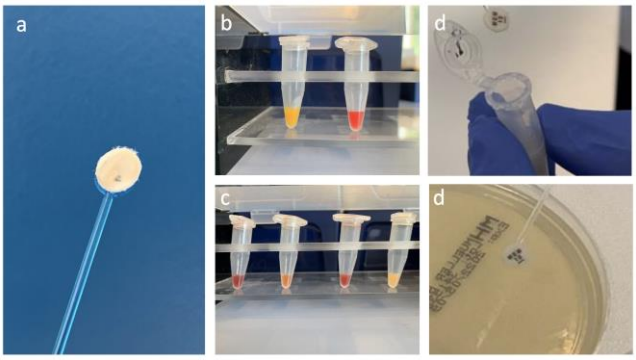

Fig. 2 Performance of the different assays for the detection of carbapenemases

Performance of the four phenotypic assays for detection of carbapenemase production

	mzCIM		sCIM Imipenem		sCIM Faropenem		NitroSpeed-Carba NP		Carba NP	
	Sens %	Spec %	Sens %	Spec %	Sens %	Spec %	Sens %	Spec %	Sens %	Spec %
All carbapenemases (n=124)	98.38	100	99.19	97.73	89.52	100	83.87	84.1	87.1	100
Ambler Class A (n = 24)	100		100		100		95.45		100	
KPC (n = 10)	100		100		100		100		100	
GES (n = 3)	100		100		100		66.6		100	
IMI (n = 9)	100		100		100		88.89		100	
Ambler Class B (n = 52)	100		100		100		96.15		100	
NDM (n = 16)	100		100		100		81.25		100	
VIM (n = 22)	100		100		100		100		100	
IMP (n = 11)	100		100		100		100		100	
GIM (n = 3)	100		100		100		100		100	
Ambler Class D (n = 43)	97.67		97.67		69.77		65.11		65.11	
OXA-23 (n = 9)	100		88.8		55.5		22.2		11.1	
OXA-48 (n = 8)	100		100		100		87.5		100	
OXA-58 (n = 11)	91		100		36.4		45.5		36.4	
OXA-162 (n = 5)	100		100		80		100		100	
OXA-181 (n = 4)	100		100		75		100		100	
OXA-232 (n = 1)	100		100		100		100		100	
OXA-244 (n = 2)	100		100		100		100		100	
OXA-245 (n = 2)	100		100		100		100		100	
OXA-370 (n = 1)	100		100		100		100		100	
Double/tripple Carbapenemases (n = 5)	80		100		100		80		80	

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Comparison of two VRE screening agars for the detection of vancomycin-resistant *enterococci* in rectal swab specimens

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Background: The number of vancomycin-resistant enterococci (VRE) infections in Germany have permanently increased. Screening for VRE in rectal swab specimens, isolation and intensified hygiene management are important tools in preventing VRE infections in high risk populations [1]. Because of the emergence and spread of VRE strains reliable and early detection of VRE-colonization is important for infection control. We compared the performance of Brilliance VRE agar with that of in-house VRE screening agar to screen rectal swab specimens for VRE.

Methods: Rectal swab specimens were obtained from patients in January 2021. The swab specimens were inoculated onto VRE screening agar (BBL Enterococcosel agar with 6 mg/l of vancomycin; in-house production) and Brilliance VRE agar (Oxoid; Thermo Fisher Scientific). Plates were incubated at (35 ± 1) °C and were read at 24 h and 48 h. Presumptive VRE colonies (blue and violet on Brilliance VRE agar and brown black on in-house medium) were identified using MALDI-TOF MS. Antimicrobial susceptibility testing were performed using agar microdilution method and Vitek2 (bioMérieux). Identification and vancomycin susceptibility results were used to calculate the specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of each medium.

Results: Summarizing obtained data from 622 rectal swabs the Brilliance VRE agar detected 64,8 % of VRE after 24 h incubation and 35,2 % of VRE after 48 h incubation. In comparison the in-house VRE screening agar detected 13,0 % of VRE after 24 h and 87,0 % after 48 h incubation. The specificity was 95,94 % (95 % CI: 93,96-97,41 %) for Brilliance VRE and 90,65 % (95 % CI: 87,95-92,92 %) for in-house VRE screening agar, when

considering only species-specific colored colonies of the isolates on both media. The sensitivity was 100,0 % (95 % CI: 93,62-100,0 %) for Brilliance VRE agar and 96,43 % (95 % CI: 87,69-99,56 %) for in-house VRE screening agar. Vancomycin-susceptible *E. faecium* and *E. faecalis* grew on Brilliance VRE agar, whereas *Lactobacillus* spp. and *Pediococcus* spp. were recovered on in-house VRE agar. The PPV and NPV were 70,89 % (95 % CI: 62,0-78,42 %) and 100,0 %, respectively, for Brilliance VRE agar, and 50,47 % (95 % CI: 43,97-56,95 %) and 99,61% (95 % CI: 98,50-99,90 %), respectively, for in-house VRE screening agar. At the incubation time of 48 h, both agars showed increased sensitivity and reduced specificity.

Conclusions: Screening for VRE on Brilliance VRE agar has a higher sensitivity and specificity, is up to 24 h faster than the detection on in-house VRE agar and therefore facilitates earlier start of the hygiene measures, thereby improving patient care and reduction in costs in the hospital.

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Localisation of cefotaxime resistance genes in multidrug resistant (MDR) Isolates of *E. coli* ST131-O25b

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Introduction: The increasing number of infections due to 3- and 4MRGN Enterobacteria such as *E. coli* ST131-O25b comes with resistance to highly potent cephalosporins and fluoroquinolones besides several others [1, 3]. The genetic basis of such MDR phenotypes is a DNA region encoding several antibiotic resistance genes such as b-lactamases (preferably CTX-type) and aminoglycoside-modifying enzymes [2]. Depending on the genetic localization of this DNA region, either on the chromosome or on a plasmid, prevention of epidemiological spread of resistance requires different measures [3]. Therefore, this study aims at investigating if *E. coli* ST131-O25b transfers its high-level cefotaxime resistance.

Material and Methods: A clinical MDR *E. coli* isolate was characterized by multi-locus sequence typing (MLST) as *E. coli* ST131-O25b. To investigate if the different resistance genes are located on plasmids which also might be transferable, conjugation experiments were performed using the *E. coli* ST131 isolate as a donor strain and *E. coli* SG22215 [4] as a susceptible recipient strain. Overnight cultures of both bacterial strains were co-cultivated for six hours at 37°C. Transconjugants were selected for resistance on agar plates containing appropriate concentrations of antibiotics for selection. The transconjugants were verified by their strain-specific genetic markers (*clpP::cat*, *lac*$^-$). Plasmids were isolated via alkaline lysis and characterized by size (gel-electrophoresis). The presence of resistance-mediating genes was detected by specific PCRs and confirmed by determining the MICs of different antibiotics. We compared donor, transconjugant and recipient strain to identify transferred resistance markers.

Results: The *E. coli* ST131-O25b isolate is showing resistance to ciprofloxacin (fluoroquinolone), ampicillin and cefotaxime (b-lactams) as well as gentamicin (aminoglycoside). The transconjugants of *E. coli* SG22215 acquired resistance to ampicillin and gentamicin as well as to cefotaxime, while not to ciprofloxacin. Isolation of extrachromosomal DNA revealed the presence of ≥50 kbp large plasmids in both, the donor and the transconjugant cells. By subsequent PCR analysis CTX-M- and TEM-type *bla*-genes were detected independently in transconjugants what implicates their localization on different plasmids of *E. coli* ST131.

Discussion: The transferability of large plasmids encoding resistance genes for 1st to 3rd generation b-lactams as well as aminoglycosides by conjugation indicates a major future risk for the spread of plasmid encoded multiple drug resistance from the global high-risk clone *E. coli* ST131-O25b to other Enterobacteria, especially *E. coli*.

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Klinische Mikrobiologie (StAG DV/FG DKM)

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Impact of the COVID-19 pandemic on the management of *S. aureus* bloodstream infection in a tertiary care hospital

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Introduction: *Staphylococcus aureus* bacteraemia (SAB) is associated with a high mortality rate. The clinical outcome of SAB patients highly depends on early diagnosis, adequate antibiotic therapy and source control. In the context of the COVID-19 pandemic, the health care system faces additional organizational challenges and the question arises whether structured screening and triaging for COVID-19 and shifting resources influence the management of SAB.

Methods: Patients (n=115) with SAB were enrolled in a retrospective comparative study with historical controls (March 2019–February 2021). The quality of SAB therapy was assessed with a point score, which included correct choice of antibiotic (narrow ß-lactam for Methicillin-sensible *S. aureus* or Vancomycin for Methicillin-resistent *S. aureus*), adequate dosage of antibiotic, sufficient duration of therapy (min. 14 days for uncomplicated SAB and 28 days for complicated SAB), early start of therapy after receipt of findings (<24 h), focus search, and taking control blood cultures 3–4 days after starting adequate antibiotic therapy. The prognostic outcome of the SAB treatment was assessed by persistent bacteremia at day 3/4. The quality of treatment before and after the onset of the COVID-19 pandemic were compared.

Results: No significant differences of the total score points were found between the pre-COVID and COVID cohort (Median = 5 vs. 5, p = 0.76). All quality indicators, except the correct duration of antibiotic therapy, showed no significant differences in both cohorts. Furthermore, there were no significant differences in the outcome between the both cohorts.

Conclusions: The treatment quality of SAB therapy were comparable before and during the COVID-19 pandemic.

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Cross-linked agarose-gelatin macro-beads as a promising substrate to study bacterial biofilms

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One of the most significant challenges in treating bacterial infections is still posed by the resistance and tolerance of biofilms against the host immune response and antibiotic treatments. Biofilms underlie chronic infections. To study biofilm-forming bacteria, new methods are needed to tackle the specific characteristics of these survival strategists. Since biofilms are often surface-associated communities, beads for cultivation are used to study biofilms experimentally. To establish a more natural surface for biofilms, we present a new type of beads based on cross-linked mixture of agarose and gelatin that is sustainable in production and leads to more robust and meaningful results than artificial material. This protocol describes the production of the beads and their use in classical quantification methods such as CFU/mL counting and Crystal Violet staining, Light and Electron Microscopy, and time/kill assays with antimicrobials for different bacterial species.

We have also studied the biofilm physiological response to different substrates by quantitative proteomics. The Agarose-Gelatin beads showed immense potential in all methods by being easy to handle and giving consistent results. Moreover, the efficiency in handling replication and the independent substrate and liquid phase manipulation can be promising.

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Antimicrobial activity and mechanism of action of *Oroxylum indicum* (L.) Benth. ex Kurz extract against aminoglycoside and lincosamide resistant clinical strain *Pasteurella multocida* causing severe respiratory infection in pigs

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Introduction: *Pasteurella multocida* is one of the most important zoonotic and nosocomial bacteria causing the severe respiratory, soft tissue infection, septicemia, and death in humans and animals. The fruit extract of tropical medicinal plant, *Oroxylum indicum* (L.) Benth. ex Kurz established for many active flavonoids including baicalein, baicalin, and chrysin, previously reported having antimicrobial activity against other important zoonotic bacteria; *Streptococcus suis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *staphylococcus aureus, and Staphylococcus intermedius* was firstly investigated against a clinical strain *P. multocida* for antimicrobial activity and mechanism of action.

Material/ methods: A clinical strain of *P. multocida* was identified from a clinical sample of a pig with severe respiratory infection and septicemia via matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). The bacterial species was confirmed by 23s rRNA-based PCR assay. The antimicrobial susceptibility profile of the bacteria was determined by standard disc diffusion assay. The *O. indicum* extract in DMSO (OPAE) was set for antimicrobial activity at concentration 0.9375-30 mg/ml. The inhibitory concentration fifty (IC50) and the minimum inhibitory concentration (MIC) were obtained using broth microdilution assay and bacterial enumeration. The data were statistically analyzed from three independent experiments in duplication and Calcusyn program. The scanning electron microscopy of the bacteria treated with 15 mg/ml OPAE at 37oC, 15h was examined for bacterial integrity and morphological status.

Results: The antibiogram of *P. multocida* strain to 16 standard antibiotics practically used in clinical veterinary medicine was characterized. The profile showed streptomycin, gentamicin, and clindamycin resistance. The inhibitory concentration fifty (IC50) of OPAE to *P. multocida* was shown at 14.98±0.35 mg/ml and the minimum inhibitory concentration (MIC) was exhibited at 29.24±0.05 mg/ml. The scanning electron microscopy of the bacteria incubated with 15 mg/ml OPAE revealed the evident bacterial morphological changes, cell wall disruption, content leakage, abnormal group of cell aggregations and cell lysis (Figure 1- 2).

Discussion: The proposed mechanisms of *O.indicum* extract to multiple drug resistant clinical strain *P. multocida* are bacterial key membrane protein dysfunction and protein synthesis interference induced cell integrity loss and cell lysis. This may relate to the aminoglycoside and lincosamide resistant manners of the bacteria. The result suggests potential use of rich flavonoid containing *O. indicum* extracts as alternative, complementary treatment to the important zoonotic bacteria especially clinical strains with drug resistant profiles. This focuses one of the keys important to veterinary medicine and human medicine for future sustainability of antimicrobial drug use and infectious disease management and control.

Fig. 1

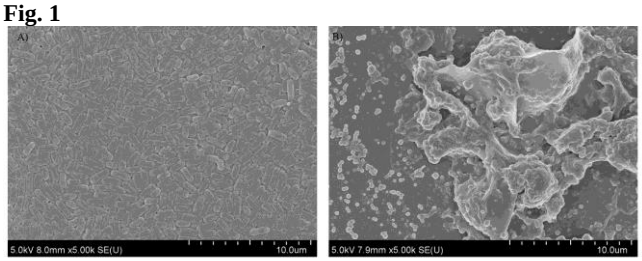

Figure 1: Scanning electron microscopy (SEM) showed evident effect of *O.indicum* extract to clinical drug resistant *P. multocida*; marked Morphological changes, mass irregular cell aggregation and lysis. A) *P. multocida*, B) *P.multocida*+OPAE 15 mg/ml.

Fig. 2

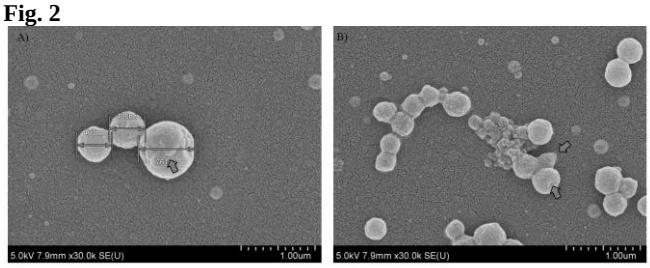

Figure 2: Scanning electron microscopy (SEM) showed evident effect of *O.indicum* extract to clinical drug resistant *P. multocida*; cell wall disruption, content leakage, abnormal cell surface integrity and lysis. A) and B) P.multocida +OPAE 15 mg/ml at 30K magnification.

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Susceptibility testing of antibiotic combinations for the treatment of chronic pulmonary infections with gram-negative pathogens from cystic fibrosis patients

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Introduction: The lungs of Cystic Fibrosis (CF) patients display a microbiologically favorable environment for different pathogens. This lead often to chronic pulmonary infections with the result of lung function decline. The treatment of these infections often proves to be difficult due to many intrinsic resistances of the main pathogens and not least the newly developed resistances during therapy. One approach to circumvent the resistances is the use of antibiotic combinations in order to break this vicious circle. Our project originated from the lack of systematic testing to predict efficacy and synergistic effects of antibiotic combinations. In our study, we first aimed to find synergistic effects of antibiotic combinations and second to establish a routinely applicable method for antibiotic susceptibility testing of CF related pathogens.

Methods: For our experiments we used clinical isolates from three different species: *Pseudomonas aeruginosa*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* complex, which are associated with increased morbidity and mortality in CF. In order to test their antibiotic susceptibility, we used broth microdilution in 96 well plates according the EUCAST guidelines. To objectify possible synergies of the combinations of two or three antibiotics, we determined the Fractional Inhibitory Concentration Index (FICI).

Results: By using antibiotic combinations, it was possible in our experiments to reduce the minimum inhibitory concentration (MIC) of certain antibiotics compared to a single antibiotic. However, the synergistic effects of the tested combinations were highly strain specific. The highest efficacy displayed Tobramycin + Ceftazidim, Tobramycin + Colistin or Tobramycin + Trimethoprim-sulfamethoxazol and the used triple combinations.

Discussion: So far, no general statement for an entire bacterial species can be made. Due to the differences in the expression of synergistic effects, we suggest combination testing to be performed on a strain-specific basis. Establishing combination testing in routine diagnostics will improve the possibilities for more reliable prediction of the effectiveness of antibiotics to treat difficult pathogens and eradicate them from the lungs of CF patients. In the future, the aim is to correlate our laboratory results with clinical

data from CF patients before and after therapy with specific antibiotic combinations.

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Let's mate – horizontal gene transfer among *Enterococci*

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Introduction: Conjugative transfer, a type of horizontal gene transfer, plays a leading role in spreading antibiotic resistance genes and development of multi-resistant bacteria. Especially, conjugative plasmids mediate transfer even among unrelated bacteria. These plasmids encode all the proteins required to transfer themselves and several antibiotic resistance genes as well. The enterococcal broad-host range inc18 plasmid pIP501 encodes a membrane-spanning molecular nanomachine, so-called Type IV secretion system (T4SS), which mediates pIP501 transfer from a donor to a recipient cell. The pIP501-encoded T4SS consists of 15 transfer (*tra*) genes (*traA* - *traO*) which are organized in a single operon.
The plasmid was originally isolated from *Streptococcus agalactia*e and serves as a model to study conjugative transfer in Gram-positive (Gram+) bacteria and to elucidate protein key players of Gram+ T4SSs.
Methods: Markerless *tra* gene knockouts of all 15 *tra* genes were generated in pIP501 to study the role of Tra proteins in pIP501 transfer by using *in vivo* biparental mating assays. Deletion mutants were complemented with wildtype *tra* genes to exclude polar effects on downstream *tra* genes. Additionally, different mutated Tra proteins and single protein domains were used as pIP501Δ*traA*, pIP501Δ*traF*, pIP501Δ*traN* and pIP501Δ*traO* complementation variants to assess the influence on pIP501 transfer rate or to obtain *in vivo* data about domain functionality.
Results: The study demonstrated the essentiality of 13 out of 15 transfer proteins. Complementation of all gene knockouts by wildtype *tra* genes fully restored conjugative transfer rate. Interestingly, the deletion of *traO* resulted in a non-significant reduction of the transfer rate compared to the wild type. The pIP501Δ*traO* complementation with truncated *traO* variants led to no significant changes in transfer rate. The markerless pIP501Δ*traN* gene knockout resulted in significantly enhanced conjugative transfer. TraN variants which will result in important changes in transfer rate will be further characterized biophysically and biochemically. Partial complementation of the *traF* knockout with single *traF* domains revealed no transfer of *E. faecalis* pIP501Δ*traF*. The same occurred by complementation of the *traA* knockout by *traA* domains. Hence, neither single TraA nor TraF domains resulted in pIP501 transmissibility.
Conclusions: Bacterial conjugation has been thoroughly studied in Gram-negative bacteria but detailed information about their Gram+ counterparts is still lacking. Furthermore, limited therapeutical treatment options harden the treatment success of patients with drug-resistant infections. Proteins involved in conjugative transfer could be selected as a target for resistance transfer inhibition. Therefore, their structural features and putative role in Gram+ T4SSs must be elucidated.

COVID-19-Infektionsdiagnostik (StAG DV/FG DKM)

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21 months of universal SARS-CoV-2 PCR admission screening in a tertiary care hospital in Germany to detect asymptomatic patients and accompanying persons

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Question: Preventing nosocomial transmissions in health care facilities has become increasingly important since the onset of the SARS-CoV-2 pandemic. Asymptomatic patients with no previous contact to a SARS-CoV-2-positive person fail to be detected through the common measure of mere screening by interviewing for symptoms and contact history. As outbreaks provoke serious consequences and may even lead to the closure of the concerned departments, measures to reliably detect SARS-CoV-2 positive patients on admission must be taken.
Methods: In this single center retrospective cohort study screening data of all hospitalized patients and accompanying persons was included from April 1st 2020 until December 31st 2021. The first oropharyngeal swab of a newly admitted patient or accompanying person taken was included in the analysis and tested for SARS-CoV-2 RNA using RT-PCR. Data on COVID-19 specific symptoms, contact history to COVID-19 cases and additional relevant data for this study were obtained from patient files. Important features and key aspects of the asymptomatic cohort were assessed. The newly-detected positivity rate was displayed on a monthly basis in comparison to the local incidences of the hospital"s catchment area. Dynamics in the hospital"s incidence rate due to different influences such as vaccination, distancing measures and different predominating virus variants were evaluated. The overall testing costs and the costs that would potentially arise from nosocomial outbreaks were juxtaposed.
Results: 705 out of the total of 111,271 screened persons (0.6%) were tested positive for SARS-CoV-2. 113 of 236 newly detected cases were only identified as SARS-CoV-2-positive through the admission PCR screening as they presented as asymptomatic cases with no previous exposure to SARS-CoV-2. 92 of these cases remained asymptomatic while 18 subsequently developed symptoms. Test specificity was high (99.997%) with only 3 false positive tests. The average number needed to test to detect an unanticipated infection was 830.
Conclusions: The universal admission PCR testing detected the high share of otherwise unidentified SARS-CoV-2-positive persons and is therefore an important infection control tool in times of the pandemic. False positive tests do not pose a relevant problem in universal PCR screening as long as testing is performed by an experienced laboratory.

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Clinical accuracy of SARS-CoV-2 rapid antigen testing in children and adolescents in comparison to RT-qPCR, November 2020 to April 2022

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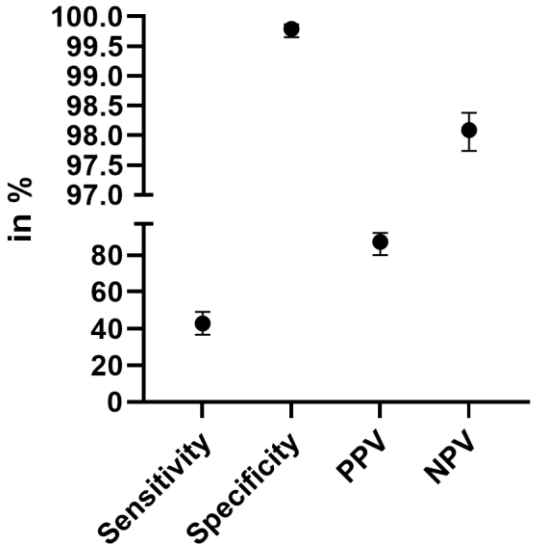
Introduction: Early detection of SARS-CoV-2 positive patients is the key to combat viral spreading – in adults as well as in the often-neglected cohort of children and adolescents. Rapid antigen detection tests (RDT) are an easily accessible, feasible, inexpensive, and point-of-care method of SARS-COV-2 diagnostics. Despite this, large-scale data of clinical RDT performance in pediatric patients especially regarding the influence of SARS-CoV-2 virus variants of concern (VOC) are rare.
Methods: This single-center and prospective analysis compares oropharyngeally sampled RDT (using NADAL®, Panbio™ and MEDsan® RDT) with standardized viral load derived from RT-qPCR as reference diagnostics in children and adolescents. From November 2020 to April 2022, RDT performance was analyzed in 7,274 cases aged younger than 18 years in a clinical point-of-care setting.
Results: Including 239 RT-qPCR SARS-CoV-2 positive cases the RDT sensitivity compared the reference standard in this paediatric cohort was 42.68 (95% CI 36.57%–49.02%) with as specificity of 99.79% (95% CI 99.66%–99.88%). RDT diagnostic accuracy depended unambiguously on specimen containing viral load implementing a restriction of RDT sensitivity for descending viral load. In case of Omicron VOC, representing 74.74% (179/239) of all RT-qPCR positive cases, RDT sensitivity was 41.67% (95% CI 34.71%–48.97%).
Figure 1: Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) in 7,274 RDT with parallel, standardized RT-qPCR
Discussion: In a pediatric population, RDT have proven to reliably detect patients with a high SARS-CoV-2 viral load. RDT can thus complement a RT-qPCR based test strategy where fast results and frequent tests are important.
Fig. 1



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Observational study of superinfections in a cohort of COVID-19 patients during inpatient treatment at a university hospital
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COVID-19 is a challenge for health care systems worldwide. A minority of COVID-19-patients develop an acute-respiratory-distress syndrome and require intensive care treatment. The majority of patients with a severe course of disease also receive empirical antibiotic treatment. Several studies from other countries have reported a rather low incidence of bacterial superinfections in COVID-19. However, questions remain regarding risk factors, predictive value and appropriate anti-infective therapy.
We analyzed bacterial superinfections in patients with COVID-19 during inpatient treatment at the university hospital Freiburg, Germany, from February 2020 to June 2021. We used multi-state statistical modeling to correlate the occurrence of superinfections with treatment in intensive care units and disease outcome. We focused on the analysis of blood cultures and respiratory materials and evaluated both pathogens and the quality of the microbiological sampling. The study collective included 722 patients. Blood cultures were sampled from approximately 87% of patients. The detection of *Enterobacterales* (mainly *E. coli* and *Klebsiella spp.*) was particularly frequent. *S. aureus* was found in 22.7% of blood stream infections, concurrent with *S. aureus* endocarditis, intravenous catheter infections or pneumonia. Respiratory materials were obtained from almost 40% of the patients. *Enterobacterales* dominated the pathogen spectrum, presumably as a result of the acquisition of a nosocomial flora during inpatient treatment for COVID-19 disease. Nevertheless, *S. aureus* was detected in nearly one third of the respiratory superinfections.
In the preliminary analysis, superinfections increased the risk of death and reduced the likelihood of discharge. These data will be useful to assess the risk for the development of superinfections in individual COVID-19 patients and will provide an estimate of the effect of superinfections on the course of the disease. In addition, the detailed understanding of microbiological superinfections is essential for evidence-based recommendations of anti-infective therapy in COVID-19 patients.

296/DVDKMP
Public health microbiology response to COVID-19 – the experience in Luxembourg
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The COVID-19 pandemic has created a new order of public health priorities and highlighted the importance of an international pandemic preparedness plan. The Department of Microbiology at the Laboratoire national de santé (LNS), with its national reference laboratory for acute respiratory infection, worked closely with the health directorate of the Ministry of Health as a part of the national efforts to respond to the COVID-19 pandemic.
In January 2020 the department implemented a rapid response plan when the first testing protocol for the detection of SARAS-CoV-2 became available. This was followed by verification of 13 RT-PCR commercial kits to identify the standard of care diagnostics tool. In parallel, sequencing protocols and an analysis pipeline within the Luxembourg Genomic and Molecular Microbiology Unit (LUX-GEMM) was established together with all partner laboratories. These efforts were identified as the first in Europe achieving the ECDC target in inclusion of representative sample, sequencing a major fraction of SARS-CoV-2 strains from COVID-19 patients, followed by rapid genome submission to open access platforms (GISAID).
Together with health authorities LNS coordinated public health initiatives including the serological component of the Large Scale Testing Program, which was internationally recognized in facilitating deconfinement. It helped to identify a maximum number of infected people, especially asymptomatic patients. Later on it monitored the population"s immunity and its link to vaccination uptake. LNS introduced mobile teams that provided sampling activities to follow the evolution of the pandemic in schools and monitored the infection rate in long term care facility. The later was cited as one of the national interventions that decreased the fatality rate due to COVID-19 compared to that reported in several countries and

In 2021, together with its partners, Microbiology was awarded the HERA incubator grant allowing it to be transformed to a public health microbiology hub in Europe and joined the WHO Biohub initiative. LNS was the first country to contribute to the sample repository. As a major public health player LNS became a European Society of Clinical Microbiology and Infectious Diseases (ESCMID) collaborative Centre (ECC) in 2022.
Research continues to be a priority and the team contributed to several national (CONVINCE study) or international consortia (e.g. ENDVOC and or ORCHESTRA). Our expertise was offered as a part of strategic planning for the pandemic preparedness research strategy in Europe, as we join BeReady consortium supported by the European commission.
Currently we are reaching out to European countries to build a digital platform for reporting cross border incidents including the genomic data, an initiative that require advocacy to address the legal framework in different European countries.

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Development and validation of a new antigen-based microarray platform for screening and detection of human IgG antibodies against SARS-CoV-2

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Introduction: Strategies to contain the current SARS-CoV-2 pandemic relies, beside vaccinations, also on molecular and serological testing. Due to vaccinations and past infections, there is an increasing need for simple and rapid serological tests for the detection of specific human IgG antibodies against antigens of the SARS-CoV-2 virus after vaccination and/or disease. For any assay development, screening for the optimal antigen is essential to get the best possible test in terms of diagnostic specificity and sensitivity. Protein microarrays allow one to screen reactivities against panels of individual antigens simultaneously, under identical reaction conditions and in a very small sample volume.
Materials/Methods: The protein microarray-based assay was developed containing a multitude of relevant commercially available SARS-CoV-2 antigen preparations which were spotted in different concentrations. A group of typical vaccine antigens of other pathogens, i.e. from *Clostridium tetani*, *Corynebacterium diphtheria* and the Measles virus, were also included. For the verification of the assay, 125 previously ELISA-tested sera were analyzed with the protein microarrays. A receiver operating characteristic (ROC) analysis was performed using ELISA results as reference and threshold values were calculated for each antigen. The optimized assay was validated by analyzing the IgG status of 131 sera from previously tested individuals and 35 sera from individuals after COVID-19 vaccinations.
Results: A diagnostic sensitivity of 1 was determined for all antigen preparations and concentrations, with one single exception. A diagnostic specificity, as well as an area under the curve (AUC) of 1 was obtained for 16 of 21 antigen preparations. For the remaining five, the diagnostic specificity ranged from 0.942 to 0.981 and AUCs from 0.974 to 0.999. Microarray evaluation of the antibody profiles of COVID-19 convalescent and post vaccination sera showed that the IgG response differed between these groups, and that the choice of the test antigen is crucial for the assay performance. Furthermore, the results showed that the immune response is highly individualized, depended on several factors (e.g., age or sex), and was not directly related to the severity of disease.
Discussion: The assay offers an ideal method for the performance evaluation of many different proteins in serological assays to determine the optimal antigen or antigen combination for diagnostic point of care test development such as Lateral-Flow tests, as well as for the development of vaccines. The assay can be expanded by adding additional or modified antigens and could also be used to detect IgM in order to monitor the serological response to acute infections. Multi-parameter serology with protein microarrays provides an optimal tool for the development of fast and economic point-of-care tests and yield more information than conventional serological methods.

Diagnostics and Clinics of Zoonotic Infections (FG DKM/FG ZO)

298/DKMZOP

Iron deprivation by oral desferoxamin application alleviates campylobacteriosis in a preclinical murine model of infection

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Introduction: Human food-borne *Campylobacter jejuni* infections are increasing worldwide and pose health and socioeconomic burdens. Given that antibiotic therapy is not recommended and antibiotic resistance of *C. jejuni*is on the rise, novel alternative treatment options for *C. jejuni* infections would be appreciable. The essential element iron triggers both, *C. jejuni* virulence as well as apoptosis in host tissues including cell destruction by generation of toxic oxygen species.
Methods: In our preclinical intervention trial, we tested potential disease-alleviating effects upon oral application of the iron-chelating compound desferoxamin in an acute murine campylobacteriosis model. Therefore, microbiota-depleted IL-10-/- mice were infected with *C. jejuni* strain 81-176 by gavage and received synthetic DF (100 mg per kg body weight per day) via the drinking water starting from day 2 until day 6 post-infection. The placebo control mice received autoclaved tap water.
Results: Whereas on day 6 post-infection gastrointestinal pathogen loads did not differ between desferoxamin and placebo treated mice, the clinical outcome was improved in the former versus the latter. Furthermore, when compared to placebo application desferoxamin treatment resulted in less distinct pathogen-induced colonic epithelial cell apoptosis, in dampened pro-inflammatory immune responses in the intestinal tract, and in alleviated systemic MCP-1 secretion.
Conclusion: Our results highlight the here applied microbiota-depleted IL-10-/- mice as reliable experimental campylobacteriosis model for the investigation of iron metabolism in *C. jejuni* infection *in vivo* and provide first evidence for disease-alleviating effects of iron-deprivation by oral desferoxamin application during acute campylobacteriosis.

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Development of a method for standardised antimicrobial susceptibility testing of *Avibacterium gallinarum* by broth microdilution and detection of antimicrobial resistance genes

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Avibacterium gallinarum (formerly *Pasteurella gallinarum*) is an opportunistic pathogen in poultry, but has also been associated with endocarditis or neonatal meningitis in humans. As there is currently no approved method for testing the antimicrobial susceptibility of this pathogen, this study aimed at developing a method suitable for diagnostic laboratories for standardized broth microdilution of *A. gallinarum*.

A total of 43 *A. gallinarum* isolates was included in the study. Species confirmation was done using a species-specific PCR assay and biochemical reactions. The epidemiological relatedness of isolates was investigated by using macrorestriction analyses. Preliminary growth experiments were conducted with six culture media, and based on the results, four media were selected to compile growth curves with four *A. gallinarum* isolates. Independent testing of minimal inhibitory concentration (MIC) determinations were then performed with five isolates to evaluate the reproducibility of MICs. For this, three microtiter plate layouts containing 24 antimicrobial agents were used. Subsequently, all isolates of the *A. gallinarum* strain collection were tested and PCR analyses were performed to detect antimicrobial resistance genes in isolates with elevated MIC values.

The species of the 43 isolates was confirmed as *A. gallinarum*. Most isolates could be distinguished by their fragment patterns, but there were three groups of clustering isolates, although they were from different bird species and years. Based on the preliminary growth experiments, four media were selected to compile growth curves: cation-adjusted Mueller-Hinton broth (CAMHB), CAMHB+1% chicken serum, CAMHB+0.0025% NADH and CAMHB+1% chicken serum+0.0025% NADH (CAMHB+CS+NADH). In all culture media, growth of approx. 107-108 cfu/ml was achieved after 20 h of incubation at 35 ± 2 °C. Although unsupplemented CAMHB was initially chosen for MIC determinations, independent testing failed to achieve sufficient homogeneity. Therefore, CAMHB+CS+NADH was selected and showed good homogeneity of MICs after 20 and 24 h. This was reflected in essential MIC agreements of 96 to 100%, exceeding the requirement of ≥90% specified in CLSI guideline M23. Testing of all isolates revealed that easily readable MICs could be obtained for all isolates. Some *A. gallinarum* isolates showed elevated MICs of enrofloxacin (≥0.5 µg/ml; n=35), nalidixic acid (≥64 µg/ml; n=35), penicillin (≥32 µg/ml; n=2), tetracycline (≥8 µg/ml; n=19) and/or trimethoprim/sulfamethoxazole (32/608 µg/ml; n=1). The following antimicrobial resistance genes were detected by PCR analyses: *bla*TEM (n=2), *dfrA14* (n=1), *sul2* (n=1), *tet*(B) (n=15), and *tet*(H) (n=5).

The study demonstrated that CAMHB+CS+NADH is suitable for standardized broth microdilution testing of *A. gallinarum* with a recommended incubation time of 20 - 24 h. At least one resistance gene was detected in 18 of 43 *A. gallinarum* isolates.

300/DKMZOP

Discrimination of growth patterns of *E. coli* including Shiga toxin-producing *E. coli* by micro-Raman Spectroscopy

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) can cause severe human diseases that are often linked to contaminated food. The amount of STEC in contaminated foodstuff is mostly very low. Moreover, STEC are accompanied by a multitude of other bacteria incl. commensal *E. coli*. Thus, laborious and time-consuming detection and isolation procedures for STEC are necessary. During these procedures, the bacteria undergo several adaptation processes that might influence the correct identification of STEC. Optical analysis procedures, such as micro-Raman spectroscopy, might represent a cheap and fast way for detection and identification of STEC. This method is able to determine a variety of chemical compounds (RNA, DNA, proteins, carbohydrates etc.) in a non-invasive and label-free manner. Furthermore, combination of Raman with laser trapping enables monitoring of a single bacteria cell in the culture media and thereby the possibility to describe the physiological changes of cells during bacterial growth.

Here, we aimed to describe the variation of *E. coli* incl. STEC during growth by applying micro-Raman spectroscopy after establishing a measurement protocol.

Material and Methods: After establishment of a measurement protocol, STEC and non-STEC strains were cultured on tryptic soy agar or in broth and sampled during lag-, log- and stationary growth phase. Finally, the cells were prepared in 4% (v/v) paraformaldehyde for 5 minutes followed by washing in phosphate buffered saline (PBS). 100 individual bacterial cells per sample were analyzed in a BioRam System (10 sec exposure with an 80 mW 785nm laser). The Raman spectral assignment for each bacterial cell was followed by principal component analysis (PCA), linear discriminant analysis (LDA) and hierarchical clustering of the spectra (HCA).

Results: Each Raman spectrum of the 100 individual cells per sample was analyzed and clustered. In total, all individual 100 cells per sample clustered together by LDA. Even, *E. coli* C600 IP could be distinguished from its Shiga toxin containing derivatives *E. coli* C600 (H19) and *E. coli* C600 (W34) by LDA in all growth phases. The different bacterial strains clearly varied in their Raman spectra under each growth (lag, log, stationary phase) condition. Here, the peaks assigned with DNA, proteins and RNA differed the most. Based on these spectral information, single cells from agar-grown colonies can be classified to the respective lag-, log or stationary growth phase.

Discussion: micro-Raman spectrosocpy is a suitable and fast tool to describe the growth kinetics of different *E. coli* and STEC. Even closely related strains could be distinguished by the applied method. However, the results imply that the growth condition of each individual cell influences the Raman spectrum and therefore the outcome. Hence, further investigation is needed to unravel the potential of Raman spectrometry in STEC detection and characterization.

301/DKMZOP

Comparison of nine assays for the detection of anti-*Echinococcus*-antibodies

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Background: Hydatidosis is a zoonosis caused by *Echinococcus* tapeworms, particularly *Echinococcus granulosus* (EG) and *Echinococcus multilocularis* (EM), and is associated with significant morbidity and mortality. Diagnosis is challenging and relies on different tools including serology, which represents a resource-efficient and patient-friendly technique.

Methods: In this study we tested sera of 50 cases of *Echinococcus* infections according to the WHO-IWGE definitions. Twenty-four infections were caused by EG and twenty by EM. Fifty sera matched for age and sex were included as controls. Each serum was tested using nine different CE labeled assays for the detection of anti-*Echinococcus*-antibodies including five ELISAs, two blots, one lateral flow assay, and one CLIA.

Results: Sensitivities ranged from 50% to 88% and specificities from 80% to 100%. Sensitivity was species-dependent. For quantitative tests, minor cut off modifications resulted in major improvements of test performance. The blots demonstrated notable differences in their ability to discriminate between EG and EM infection.

Conclusion: Serology is a valuable tool for the diagnosis of hydatidosis. Laboratories and attending physicians must be aware of the limitations of the applied assays.

Infection Control & SARS-CoV-2 (StAG HY/FG PR)

302/HYPRP

Estimation of BNT162b2 vaccine and booster effectiveness among health care workers in Germany against symptomatic and asymptomatic SARS-CoV-2 infections

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Question: Covid-19 vaccines have been shown to be a cornerstone in the combat of the ongoing pandemic. High efficacy against symptomatic disease, hospitalisation and death have been demonstrated in a plethora of publications (Polack et al. 2020, Rotshild et al. 2021). However, waning immunity and emerging immune escape variants such as omicron led to reports of decreased vaccine effectiveness over time with many countries worldwide witnessing a resurgence of COVID-19 cases. Data on real world vaccine effectiveness including asymptomatic infections is sparse.

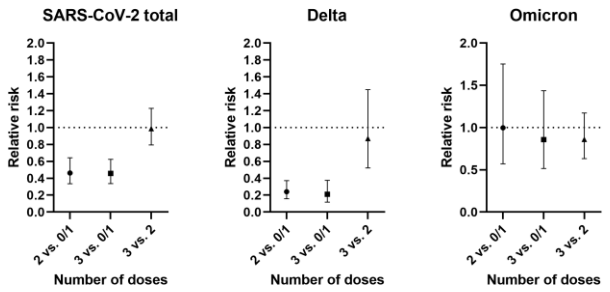
Methods: We used a retrospective observational design to estimate vaccine effectiveness among a cohort of health care workers and non-medical hospital staff in a tertiary care hospital in Germany from 1 July 2021 to 31 January 2022. We utilised data of the hospital-wide vaccination campaign and surveillance records of staff infections.

Results: Among 8493 hospital staff members, 8054 (94.8%) received two doses of BNT162b2 vaccine (or one in case of previous infection). 6985 (86.7% of the fully vaccinated) received a booster dose of an mRNA vaccine since November 2021, either BNT162b2 or mRNA-1273. We recorded 374 SARS-CoV-2 infections between July 2021 and January 2022. 126 occurred in fully vaccinated persons who had not received a booster dose, 203 in persons having received a booster dose, and 45 in not fully vaccinated individuals. Overall vaccine effectiveness (VE = 1 – relative risk) against any SARS-CoV-2 infection of fully BNT162b2-vaccinated vs. not fully vaccinated persons was 54.07% (95%CI: 38.00 - 65.75%). Effectiveness was higher against the Delta variant (76.51%; 95%CI: 64.18 - 84.52%) and not significantly different from 0 (VE: 11.6%; 95%CI: -47.5% - +46.6%) for the Omicron variant. Furthermore, we detected no significant differences comparing effectiveness of booster vaccination vs. full vaccination (VE: 1.3%; 95%CI: -22.7% - +20.5%).

Figure 1: Relative risk for both asymptomatic and symptomatic SARS-CoV-2 infection against all SARS-CoV-2 cases (n=374), Delta variant cases (n=115) and Omicron cases (n=244)

Conclusions: Vaccine effectiveness in a highly immunized and highly exposed cohort against Delta variant was high and comparable to previously reported data. However, no significant risk reduction could be observed against the Omicron variant. While having been shown to reduce numbers of symptomatic SARS-CoV-2 infections in previous studies, no additional effect of the booster vaccination on asymptomatic and symptomatic infections could be detected. Although SARS-CoV-2 vaccinations still show a strong benefit for the vaccinated individual against hospitalization or death, variant adapted vaccines seem to be necessary to obtain sterile immunity against the Omicron and potential future variants as well.

Fig. 1



303/HYPRP

Patient interrogation at hospital admission simplifies and shortens the decision for SARS-CoV-2 isolation measures

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Introduction: Patients in emergency wards need quick examination and treatment. Especially during the COVID-19 pandemic, fast identification of infectious patients that are not aware of their infection is challenging. Particularly when capacities to isolate patients are limited, RT-PCR results are necessary to reliably rule out infectiousness. If it is not possible to isolate every patient until RT-PCR results are available, a decision whether patients need to be isolated needs to be made beforehand. Therefore, we established a simple questionnaire that is answered by patients on admission. Indications for symptoms or contact to SARS-CoV-2 positive people lead to rapid and preliminary isolation.

Methods: A questionnaire was implemented in the medical information system to document possible symptoms, contact to SARS-CoV-2 positive people, stays abroad and the state of vaccination or recovery from SARS-CoV-2. When patients affirmed contact to SARS-CoV-2 positive individuals, a risk factor symbol is automatically displayed in the electronic patient record inside the medical information system.

Results: In our hospital, we experienced benefits in the clinical workflow with the questionnaire. Isolation measures could be quickly embraced when patients stated close contacts to infected people but were not yet RT-PCR confirmed for SARS-CoV-2 infection. Contact persons automatically received a risk factor symbol in the electronic patient record. Additionally, patients with recent SARS-CoV-2 infection could be easily identified. Moreover, the acquisition of the vaccination state including vaccine was advantageous to fulfill legal notification requirements to local health authorities. During periods of high incidence rates, returning travelers were legally required to undergo special isolation measures. These requirements could be rapidly implemented with the questionnaire.

Discussion: The questionnaire provided a feasible opportunity to decide upon the necessity of isolation before RT-PCR results are available. Thereby, both staff and other patients could be protected from further COVID-19 transmission. Patients that stated contact with SARS-CoV-2 positive individuals directly received a visible risk factor symbol in the electronic patient record. This is of high importance as staff is directly informed when patients have to change the wards. Additionally, ambulant medicated patients sometimes revisit the hospital and the decision for isolation can be made very easily. Similar to the symbol for SARS-CoV-2 contact persons, a symbol for SARS-CoV-2 positive patients exists. Thus, a differentiation of the two patient groups and the measures that have to be implemented is simple. Although this questionnaire gives some hints for possible SARS-CoV-2 infections, an element of risk remains. Asymptomatic infections cannot be prematurely recognized. On the other hand, symptoms like fever or respiratory distress can also be attributed to other causes or infections.

304/HYPRP

Identification of epidemiological clusters of SARS-CoV-2 by using multi-locus sequence typing

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Introduction: In periods with high incidence rates, the occurrence of SARS-CoV-2 clusters is increased (Eisenmann *et al.*, 2022). However, it remains difficult to attribute infected individuals to one or more independent transmission events during outbreak management. Especially in a hospital setting, a variety of close contact situations occurs. Staff is taking care of patients, meetings are hold, patients share sickrooms and staff outside of the patient care is present. We used multi-locus sequence typing (MLST) to identify whether SARS-CoV-2 infected people involved in putative one cluster belong to one epidemiological transmission event.

Materials and Methods: Oropharyngeal swab samples were purified and three loci (Nsp1-2, spike protein gene, N gene) of the SARS-CoV-2 RNA were amplified using RT-PCR. Subsequently, samples were sequenced by an external company. Sequences were processed in Ridom SeqSphere. Nucleotide differences were identified and loci got different allele numbers. The combination of allele numbers lead to a certain sequence type number. Thus, a local database of various sequences was compiled.

Results: Cluster events were detected at wards or departments outside of patient care. In some cases, staff as well as patients were infected and seemed to be epidemiologically connected. However, especially in periods with high incidence rates, several epidemiological transmission events could be identified within one suspected cluster. In one case, a ward meeting was suspected as event of transmission. MLST of the pretended connected cases exhibited three different sequence types, proving that SARS-CoV-2 positive individuals could be attributed to three independent events. However, COVID-19 clusters with one transmission event shown by the same sequence type in MLST were detected as well. Unexpectedly, we could identify a COVID-19 transmission of two infected chaperons which were not obviously in close contact.

Discussion: During the SARS-CoV-2 pandemic, predominance of virus variants repeatedly changed. Within this change, it is easier to differentiate independent transmission events by virus typing. In contrast, when there is only one predominant SARS-CoV-2 variant, rate of new infections can only be differentiated by detailed sequence analysis. MLST turned out to be a helpful tool. Here, some clusters were identified as being related to one epidemiological transmission event whereas others were confirmed as mutual contagion. MLST is advantageous to other tools as it is simple and fast. Measures to control outbreaks can be quickly initiated. However, this analysis is only retrospective, meaning that it cannot be used for urgent outbreak management.

Reference

Eisenmann M, Rauschenberger V, Knies K, Schwarzmann G, Vogel U, Krone M. Influence of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) vaccinations on cluster events among patients and staff in a tertiary-care hospital in Germany. 2022. ASHE.

305/HYPRP

Influencing factors of Anti-SARS-CoV-2-Spike IgG antibody titres in healthcare workers – a cross-section study

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Question: Against the background of the current COVID-19 infection dynamics with the rapid spread of SARS-CoV-2 variants of concern (VOC), above all the Omicron VOC, the immunity of healthcare workers (HCWs) against SARS-CoV-2 continues to be of high importance. Vaccination plays a central role in reducing the severity and potentially the spread of the disease. In healthcare, this is important to prevent disease-related staff shortages. However, there is a lack of data on factors influencing the humoral immune response. The aim of our study was to determine factors influencing the level of Anti-SARS-CoV-2-Spike IgG after SARS-CoV-2 infection or vaccination in healthcare workers.

Methods: 1,750 study participants were recruited who met the following inclusion criteria: age ≥ 18 years, PCR-confirmed SARS-CoV-2 infection and/or at least one dose of COVID-19 vaccination, working in health care. Anti-SARS-CoV-2-Spike IgG titres were determined by SERION ELISA *agile* SARS-CoV-2 IgG.

Results: Mean Anti-SARS-CoV-2-Spike IgG levels increased significantly with the number of COVID-19 vaccinations (92.2 BAU/ml for single dose, 140.9 BAU/ml for two doses and 1,144.3 BAU/ml after threefold vaccination). Hybrid COVID-19 immunized respondents (after infection and vaccination) had significantly higher antibody titres compared with participants after infection only (525.4 BAU/ml vs. 105.7 BAU/ml, Figure 1).

Figure 1: Distribution of Anti-SARS-CoV-2-Spike IgG levels depending on immunization scheme

Anti-SARS-CoV-2-Spike IgG titres declined significantly with time after administration of the second vaccine dose. Smoking and high age were associated with lower titres (Figure 2).

Figure 2: Anti-SARS-CoV-2-Spike IgG levels depending on individual physical properties

Conclusions: Both recovered and vaccinated HCWs presented a predominantly good humoral immune response with decreasing antibody levels over the temporal course. Smoking and higher age limited the humoral SARS-CoV-2 immunity. This reduced immune response is an important aspect as people with these risk factors are recognized as people with an increased risk for a severe course of disease.

Fig. 1

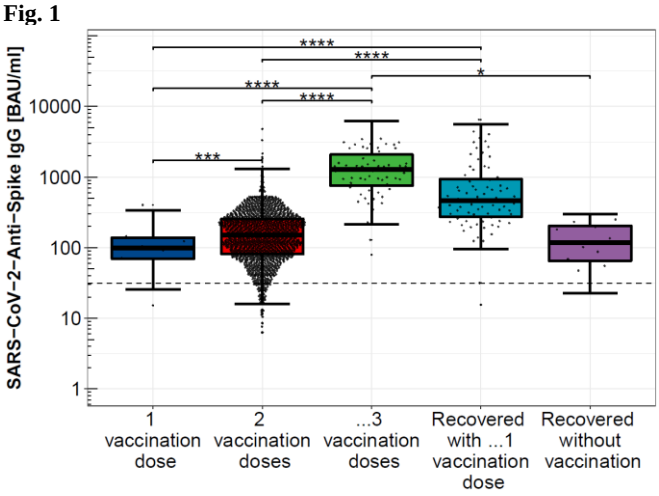
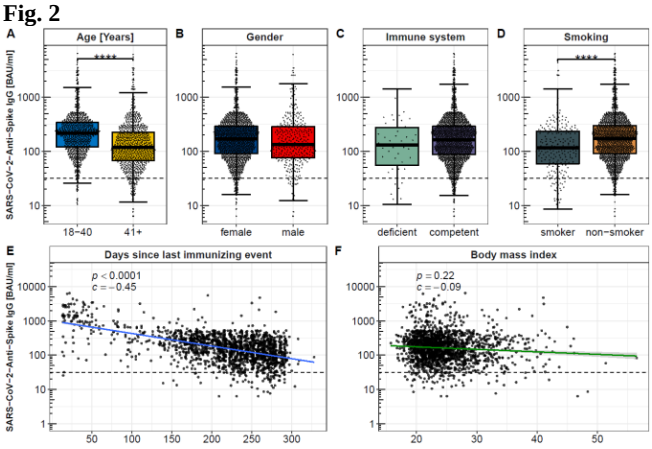


Fig. 2



306/HYPRP

Immunogenicity and safety of coadministration of COVID-19 and influenza vaccination among healthcare workers

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Question: While a third dose of COVID-19 vaccination established as one of the preventive key methods for strengthening the immune response against SARS-CoV-2, seasonal influenza vaccination has been an important infection prevention measure for years, especially among highly exposed healthcare workers (HCWs).

Methods: The study presented examines the differences in Anti-SARS-CoV-2-Spike IgG antibody formation as well as side effects after a third COVID-19 vaccination with and without coadministration of a seasonal tetravalent influenza vaccine, analysed separately according to COVID-19 vaccine. 1,231 HCWs were recruited having received a mRNA-based booster COVID-19 vaccination (mRNA-1273 or BNT162b2mRNA) at least 14 days prior to participation after basic immunisation with twice BNT162b2mRNA. Anti-SARS-CoV-2-Spike IgG titres were determined by SERION ELISA *agile* SARS-CoV-2 IgG.

Results: Median SARS-CoV-2-Anti-Spike IgG was significantly higher in persons without coadministered influenza vaccination (2,187 BAU/ml, IQR: 1,314 – 3,713 BAU/ml) compared to persons with a coadministered influenza vaccination (1,631 BAU/ml, IQR: 1,083 – 2,736 BAU/ml, Mann-Whitney test p<0.0001).

Figure 1: Anti-SARS-CoV-2-Spike IgG without and with co-administered influenza vaccination.

All side effects queried were reported less frequently in the whole coadministration group without statistical significance (Figure 2).

Figure 2: Frequency of reported side effects

Conclusions: Coadministration of the seasonal tetravalent influenza vaccine significantly limited the post-vaccination increase of Anti-SARS-CoV-2-Spike IgG levels, with a more restricted elevation in case of BNT162b2mRNA booster vaccination compared to the mRNA-1273 vaccine. Coadministration of COVID-19 and influenza vaccine does not increase side effects. Although the significance of this difference for protection against SARS-CoV-2 infection and severe COVID-19 disease course is unclear, this fact has to be considered in seasonal vaccination recommendations. Increases in mRNA dose may compensate for the lower immunogenicity in coadministration.

Fig. 1

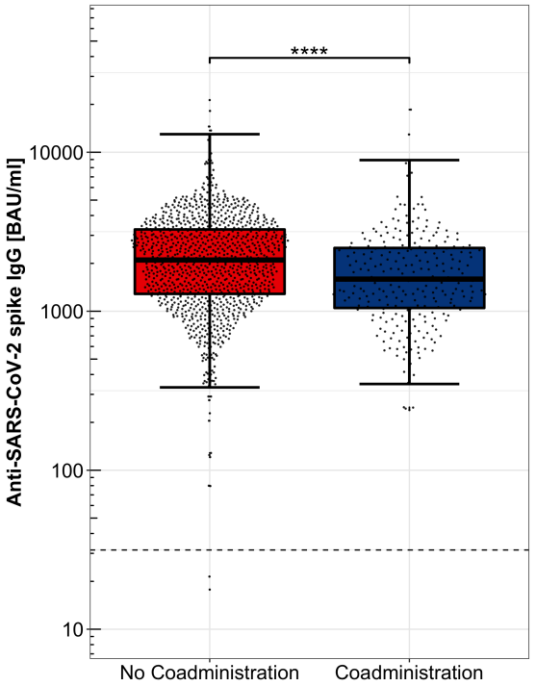
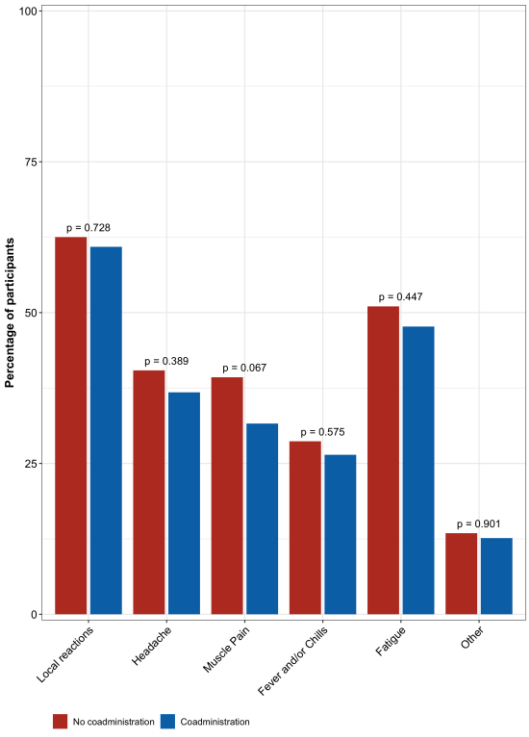


Fig. 2



307/HYPRP

Did the interventions in the cluster-randomised controlled WACH-trial contribute to pandemic preparedness? An explorative analysis of pre- and perioperative SSI-preventive compliance before and after the onset of COVID-19

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Question: The cluster-randomized controlled WACH trial ("Wound Infections and Antibiotics Use in Surgery", German Clinical Trials Register-ID: DRKS00015502) aimed at improving compliance with clinical interventions to prevent surgical site infections (SSI) by adapting the psychological tailoring approach of the PSYGIENE hand hygiene trial (DRKS00010960) from interventions addressing health care workers directly involved in patient care to infection prevention and control (IPC) teams, and other stakeholders, in nonuniversity hospitals. Specifically, the interventions included, besides written reports, 2-day workshops with feedback, supervision, simulation and teambuilding. Like other studies in the past two years, the WACH-trial unexpectedly "collided" with the COVID-19-pandemic, specifically in that the post-intervention follow-up compliance assessments lasted from June 2019 to August 2020. Thus, key pre-/post-comparisons were re-calculated for follow-up data assessed before vs. after the onset of the pandemic to eludicate whether results were affected by this event.

Methods: After briefing by the WACH-project, in-house staff in each of 6 participating hospitals observed compliance with, among others, 10 pre- or peri-operative measures in N=905 surgical procedures (general/visceral or orthopedic/trauma surgery) before and after the intervention ("tailoring"-arm) or "usual practice" period. A bundle indicator was constructed to identify procedures for which compliance with at least eight of these 10 measures had been observed. Data analysis (cluster-level) were performed using OpenEpi 3.01, chi² and Breslow–Day tests.

Results: Overall, bundle compliance in the "tailoring"-arm increased from 33% to 72% (p<0.001), whereas it decreased in the "usual practice"-arm (57% to 47%, p=0.033). The follow-up bundle compliance rates for procedures which had taken place before vs. after the onset of the pandemic ("tailoring"-arm: 152 of 250 procedures, "usual practice"-arm: 39 of 202 procedures) were 63% vs. 77% in the "tailoring"-arm (p=0.019 for the difference) and 49% vs. 36% in the "usual practice"-arm (p=0.14). In general, pre-/post-differences were more pronounced in general/visceral surgery than in orthopedic/trauma surgery.

Conclusions: The study arm-specific trends in this bundle compliance indicator - i.e., increase after tailored interventions and decrease after usual practice - continued and were even partially amplified after the onset of the COVID-19 pandemic. While obviously representing an unplanned and thus explorative analysis, results may reflect an unintended but positive side effect of the tailored interventions on the pandemic preparedness of the teams in the particpating hospitals in terms of sustaining SSI-preventive compliance. Whether this effect is especially related to interventions encompassing supervision, simulations, and teambuilding, will have to be scrutinized in future studies on pandemic preparedness in hospital settings.

308/HYPRP

T-cellular SARS-CoV-2 immunity among convalescent and vaccinated healthcare workers in relation to Anti-SARS-CoV-2-Spike IgG

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Question: During the more than two years lasting COVID-19 pandemic health care workers (HCWs) have proven as key workers dealing with the pandemic derived unprecedented burden on public health. Next to humoral, antibody-mediated SARS-CoV-2 immunity, the probably long-lasting T-cellular immune response to SARS-CoV-2 is being investigated in relation to SARS-CoV-2 infections as well as COVID-19 especially among HCWs.

Methods: Healthcare workers (HCWs) with at least two doses of EMA-approved COVID-19 vaccines were included in the study, with vaccination derived immunization only, infection derived immunization only and hybrid immunization as a combination of vaccine and SARS-CoV-2 infection derived immunization. Anti-SARS-CoV-2-Spike IgG titres were determined by SERION ELISA *agile* SARS-CoV-2 IgG. T-cellular reactivity against SARS-CoV-2 spike S1 protein as well as the nucleocapsid protein was obtained with T-SPOT®.COVID.

Results: Among 440 HCWs included in the study, 314 reported a vaccination derived SARS-CoV-2 immunity, 2 an infection derived immunity, and 124 a hybrid immunity. 84.32% (371/440) showed a T-cellular reactivity towards SARS-CoV-2 spike S1 protein (84.76% (267/314) in case of being vaccinated only; 83.06% (103/124) in case of being hybrid immunized). 21.82% (96/440) of the study participants showed a positive T-cellular reaction towards SARS-CoV-2 nucleocapsid protein (6.69% (21/314) in case of being only COVID-19 vaccinated without an anamnestic infection; 60.48% (75/124) in case of being hybrid immunized).

An extended T-cellular reactivity was obtained in comparison of hybrid SARS-CoV-2 immunized with only COVID-19 vaccinated respondents towards SARS-CoV-2 spike S1 protein (median 34 (IQR: 12.5-86.5) vs. 26 (IQR: 13-54) spots, p=0.012) and nucleocapsid protein (median 11 (IQR: 5-29) vs. 1 (IQR: 0-2) spots, p<0.001, *Figure 1*). T-cellular reactivity declined significantly with time after last COVID-19 immunizing event. Anti-SARS-CoV-2-Spike IgG levels were positively correlated with T-cellular reactivity (p=0.002125, *Figure 2*).

Figure 1: Number of Spots against S1- and N-antigen in hybrid SARS-CoV-2 immunized and only COVID-19 vaccine immunized (n = 438).

Figure 2: Correlation of Anti-SARS-CoV-2-Spike IgG levels in BAU/ml with T-cellular activity towards SARS-CoV-2 spike S1 protein measured as spot count of T-SPOT.COVID® test (n = 438).

Conclusions: Both vaccinated and hybrid immunized HCWs present a predominantly reliable T-cellular reactivity towards Spike S1 protein. Reactivity of SARS-CoV-2 sensitized T-cells declined temporarily and was positively correlated with the humoral immune reaction. Detected T-cellular reactivity among anamnestically only vaccinated and never SARS-CoV-2 infected HCWs might be explained due to restricted T-SPOT.COVID® test sensitivity especially after an extended interval from the last SARS-CoV-2 immunizing event or due to unknown SARS-CoV-2 convalescence.

Fig. 1

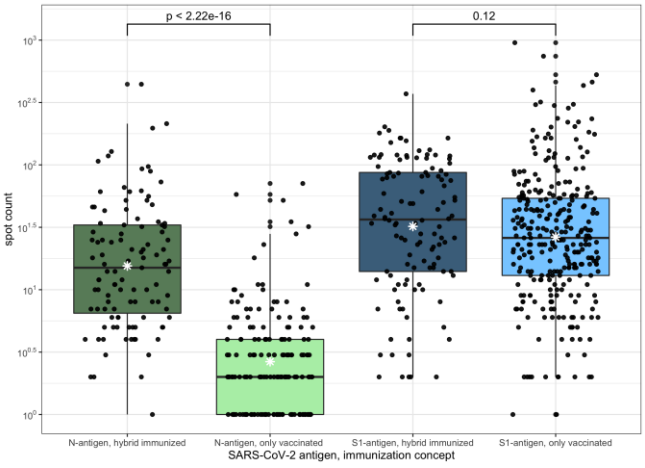
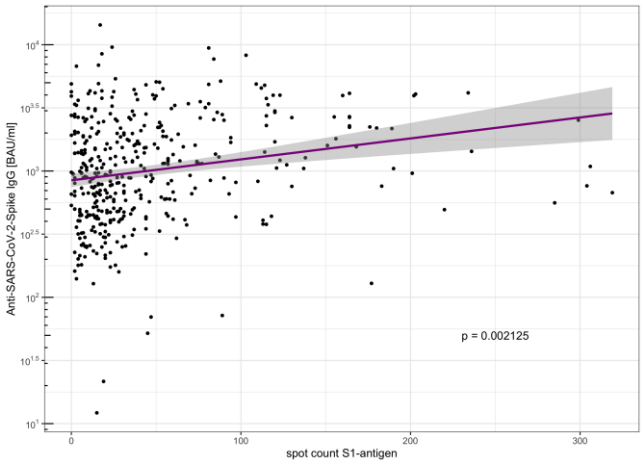


Fig. 2



309/HYPRP

Changes in management of COVID-19 reporting in a municipal public health department

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Introduction: The earliest cases of the COVID-19 pandemic were reported in Germany in January 2020. The first PCR-confirmed case in Stuttgart, the capital city of Baden-Württemberg, was reported to the municipal public health department on the 4th of March 2020; additional cases soon followed, necessitating a rapid and fundamental restructuring of the reporting management, both organisationally and in terms of digitalisation. Herein we describe the development of reporting management (esp. continuous adaptation of workflows and an in-house database) in context with case numbers over time.

Material & methods: The database was programmed using HCL Domino (formerly Lotus Notes / IBM Domino) as a framework, a system locally established in numerous municipal departments for widely varying purposes. All lab-reported data are recorded; additional data – acquired through contact with the patient or entered by the patient themself in a browser-based survey tool – are stored for purposes of individual case management and statistical analyses. Importantly, the database structure is formed around organisational changes in reporting management ("Clusters"), which allow for higher specialization and faster recruitment of new and untrained staff, to more easily adjust personnel count to increasing caseloads.

Results: As of early May 2022, more than 170.000 case files were registered, including cases of re-infection. Reporting management was split into "Clusters" with a limited number of tasks per case file. Online and interdepartmental interfaces enhanced relevant tasks. As the internal reporting management and the external requirements thereof developed and changed over the course of the pandemic, "Clusters" were added, rendered redundant and removed or had their focus changed – mirroring the dynamics of the pandemic and the tasks that public health departments were faced with. The ability for automated and specified export of statistical data is of particular relevance, as up-to-date analyses are essential for administrative decisions, politics, press, and for informing the citizenry.

Discussion: Over the course of five waves of the COVID-19 pandemic, each reaching higher case numbers than the last, the essential nature of full digitalization, new workflows and specific, highly responsive programming and IT support has become ever more evident. Lessons learned during the COVID-19 pandemic should be applied to the management of other infectious diseases, outbreaks, and potentially other topics of public health. Standard programs for case management and reporting to state and federal departments (LGA, RKI) currently in use - such as Octoware and SurvNet - have several limitations, including a lack of interdepartmental operability. Experience and knowledge gained from the establishment and use of the municipal database could assist in improving these essential software solutions as well.

Fig. 1: Weekly reported COVID-19 cases

Fig. 2: "Cluster" structure

Fig. 1

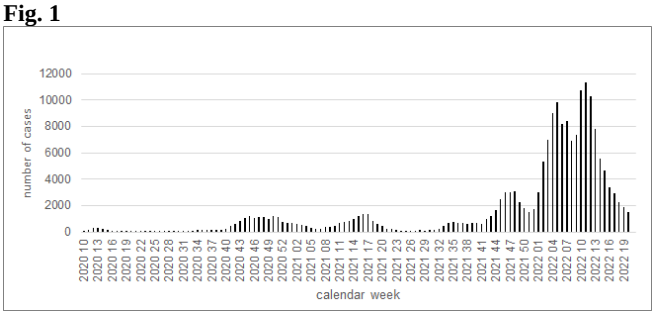
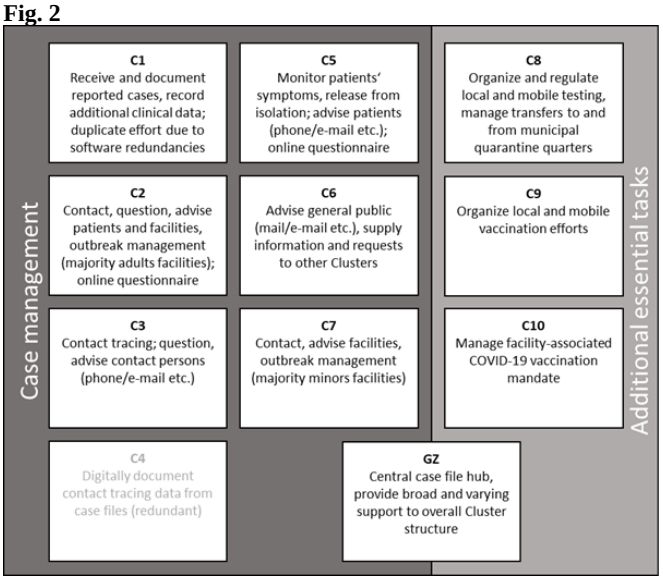


Fig. 2



310/HYPRP

Calculation of the anticipated shelf life in sterile supply by web-based analysis of long-term exposure data

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Question: Medical packaging material for terminally sterilized items is normally porous or has porous components to ensure sterilant access (steam, ethylene oxide). Airflow into the packages is caused by weather-dependent air pressure changes (Boyle-Mariotte"s law) and temperature-dependent volume changes (Gay-Lussac"s law). A data-based control of environmental airflow into the packaging and the airborne microbial challenge has been developed to estimate the maximum anticipated shelf life at the sterility assurance level (SAL) of $\leq 10^{-6}$ in a web-based analysis.

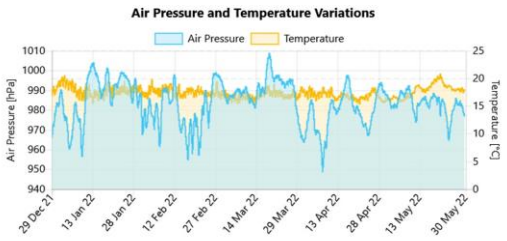
Methods: A real time data logger (PCE-THB 40, PCE Americas Inc.) was used to record the atmospheric air pressure values and the temperature variations in the storage area between 29 December 2021 and 29 May 2022. The logger saved the data on the SD memory card. After ending the monitoring period, the saving data file was uploaded from the SD card to the input mask of the website. The following inputs were entered in the dialog box: the packaging volume, the filtration efficiency of the porous component which was taken from the manufacturer"s instructions for use or from reference sources, and the airborne microbial concentration (colony-forming, CFU/m³). After uploading the file, the program plotted the measured temperature and air pressure

values and calculated the cumulative airflow into the packaging on a graph. The airborne microbial challenge (N_0) was calculated on the basis on the airborne microbial concentration (10 CFU/m³ was inputted) and the cumulative airflow into the packaging. The compatibility of the filtration efficiency with the airborne microbial challenge to maintain the sterility at the SAL was calculated according this formula: $N_0 \times (100 - \text{filtration capacity in \%}): 100 \leq 10^{-6}$. The anticipated maximum shelf life was calculated and shown as an expiration date based on the linear regression analysis. The web-based calculation of the shelf life was performed for double-wrapped paper/film pouches (filtration efficiency 99.97%) with a volume of 150 cm³, high-density polyethylene-wrapped (HDPE-) pouches (filtration efficiency 99.998%, 150 cm^3) and double-wrapped baskets with cellulose-based medical-grade paper (filtration efficiency 99.966%, 2.600 cm³).

Results: Figure 1 shows the graph of the viewing screen of the web-based analysis of the temperature und air pressure time courses between 29 December 2021 and 29 May 2022. Following air volumes that entered into the packaging were calculated: baskets 3970 cm³, pouches 229 cm³. The calculated anticipated maximum shelf life was 28 July 2022 (double wrapped paper/film pouches) and 10 October 2023 (HDPE pouches). The estimated expiration date for the baskets was the 8 January 2022, at that time the cumulative airflow was 332 cm³.

Conclusions: This web-based application offers health care professionals a data-based calculation of the maximum shelf life of terminally sterilized products.

Fig. 1



311/HYPRP

Monitoring of COVID-19 and vaccination in long-term care facilities in Germany between October 2021 and February 2022

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Introduction: Residents of long-term-care facilities (LTCF) are at high risk for a severe or fatal course of SARS-CoV-2 infection. In Germany, residents and staff of LTCF were therefore among the first to receive Coronavirus disease 2019 (COVID-19) vaccinations starting in December 2020. After initially declining COVID-19 case and death rates in the first months of 2021, SARS-CoV-2 outbreaks in LTCF were increasingly observed since August 2021. Due to a lack of data on coverage and progress of COVID-19-vaccinations and concomitantly occurring COVID-19-cases, a monthly survey has been established in these settings.

Methods: LTCF in Germany were contacted to participate in the monthly survey between October 2021 and February 2022. Facilities were asked to provide aggregated data on the total number of residents and staff and the numbers differentiated according to vaccination status (unvaccinated, fully vaccinated and boostered). In addition, data on the occurrence of COVID-19-cases and their vaccination status have been collected. Subsequently, we calculated proportions of residents and staff according to the vaccination status. Logistic regression was used to calculate the odds ratios (OR) of acquiring COVID-19 for vaccinated versus unvaccinated residents and staff and for each month of the study period, respectively.

Results: The number of participating LTCF per month ranged between 1,003-1,665 and included data of 77,779-120,892 residents and 75,829-123,427 staff. The proportion of fully vaccinated individuals increased from 87.8% (95%-confidence interval: 87.3-88.3%) to 93.7% (93.3-94.0%) in residents and from 81.1% (80.6-81.7%) to 91.7% (91.3-92.1%) in staff. Regarding booster vaccinations, the proportions increased from 48.7% (48.0-49.4%) to 76.5% (75.9-77.1%) and from 23.4% (22.8-24.3%) to 59.8% (59.1-60.5%), respectively. Among both residents and staff, vaccinated individuals had a lower risk of acquiring COVID-19 (OR for residents: 0.19 [0.11-0.34] in December to 0.51 [0.31-0.88] in January; OR for staff: 0.14 [0.10-0.21] in November to 0.54 [0.39-0.78] in January).

Discussion: Monitoring of the vaccination status revealed rising proportions of vaccinated residents and staff in LTCF during the study period, which is consistent with official booster vaccination recommendations and the introduction of the mandatory vaccination of staff, which was passed in December 2021. The remaining gaps in vaccination coverage and the observed decline in vaccination protection against SARS-CoV-2-infection highlight the need for continuing efforts to enhance vaccination coverage, especially with regard to booster vaccinations.

312/HYPRP

Investigation of the sources behind the high COVID-19 incidence rates in Saxony, August to December 2021 – insights into the potential future preventive strategies

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Introduction: In summer 2021, the 7-day incidence rates of COVID-19 were below 10 during most time in Saxony, much lower compared to the average incidence rates in Germany. However, from October on, the incidence rate in Saxony increased dramatically. At the beginning of November, it already reached above 500, which was two times higher than the average value in Germany at that time. What happened in Saxony in autumn that led to the high incidence in winter? We investigated the sources behind the high COVID-19 incidence in Saxony in last autumn and winter.

Methods: The sources of the high incidence in Saxony were investigated through several genomic epidemiology approaches. Through international collaboration with Czech and Polish partners, we performed genomic epidemiology analysis on a weekly base with samples from Saxony, and from two neighbor regions in Poland and the Czech Republic, respectively. Phylogeny analyses were used to track transmission chains. Phylodynamic approaches integrating evolutionary, demographic and epidemiological concepts have been applied to track virus genetic changes and identify emerging variants.

Results: From October to December 2021, four SARS-CoV-2 Delta sub-lineages were most frequently detected in Saxony: AY.122, AY.43, AY.36 and AY.4, indicating these variants caused most infections during that period. Through genomic epidemiology analyses, several clusters derived from community transmissions

have been identified in Saxony, including cluster AY.122, AY.43, AY.36 and a few others, most of which started from August 2021 during the travel season. Among these clusters, AY.122 cluster kept on from August 2021 till beginning of 2022. From the neighbor region in the Czech Republic, one AY.122 community transmission cluster was also detected, which similarly started from August 2021. Both AY.122 clusters in the two places display a similar transmission pattern in the dynamic changes of geographic distribution and age distribution. Moreover, we discovered that most local AY.36 samples belong to a specific locally occurred variant derived from virus evolution of previously imported AY.36, which had grown rapidly in Saxony since October and formed a big cluster with special transmission patterns.

Discussion: The results revealed that community transmission clusters developed since August play an important role in driving the surge of the incidence rates in late autumn 2021 in Saxony. Directly imported variants (e.g. AY.122) and new variants derived from further virus evolution of imported variants (e.g. the special AY.36) both caused incidence increases, but in different transmission patterns. This indicates, to prevent the possible sharp increase of COVID-19 incidence in late autumn, proper mitigation or preventive manners might should have been taken since early August, and genomic epidemiology analyses should be run regularly to monitor virus evolution and to detect emerging variants as early as possible.

313/HYPRP

A new pre-amplification method to detect very low DNA copy numbers of (multi-)resistant pathogens in complex samples

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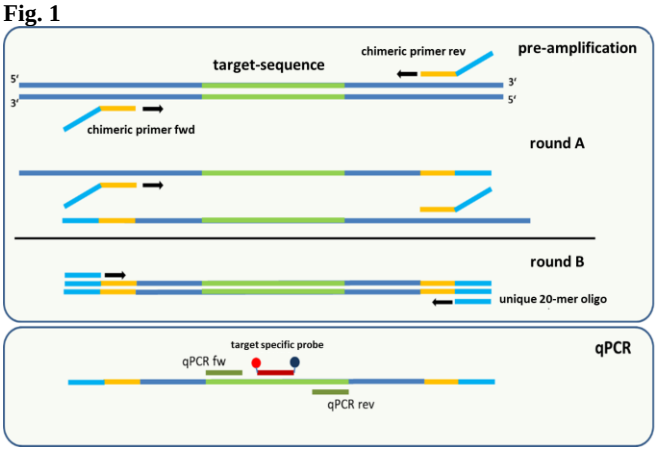
Introduction: Detecting low copy-numbers of DNA in diagnostically relevant complex samples, such as blood, is challenging. In clinical diagnostics, molecular assays gain an increasingly important role. The development of new specific and sensitive assays for the detection of pathogens is often hindered by the need to analyse complex clinical samples directly without previous cultivation. In addition to inhibitory and sample matrix effects, target molecules are usually present in very low concentrations. Up to now, most molecular tests for bloodstream infections start from a positive blood culture, where the causative pathogen and its genome is already enriched above the limit of detection for molecular tests. This requires several hours to days, which is delaying all subsequent analytical steps. Molecular assays, that directly detect pathogens in complex matrices are limited in specificity, sensitivity and ability for multiplexing analyses of different targets in one and the same assay.

Methods: Our new developed multiplex pre-amplification method could close this gap. Briefly, 21 different target DNAs of gram-negative and gram-positive bacteria with different resistance genes were used in a final concentration of 101 to 102 copy-numbers per pre-amplification reaction. Additionally, 1 µg of human DNA was included to simulate human blood background. In the first step, two chimeric primers per marker consisting of a target specific sequence (9- to 13-mer) and a unique 20-mer oligo were used to pre-amplify and tag the target-DNAs by a strand-displacement polymerase. In the second step, the tagged target DNAs were amplified to a detectable level using the unique 20-mer oligo with a standard Taq-polymerase. Verification was done by qPCR.

Results: The following targets were successfully pre-amplified and detected by qPCR: *basC* (*A. baumannii*), *khe* (*K. pneumoniae*), *gad* (*E. coli*), *cfa* (*C. freundii*), *ecfX* (*P. aeruginosa*), *gapA* (*S. aureus*), *AAC6* (*E. faecium*), *ddl* (*E. faecalis*), *lytA2* (*S. pneumoniae*), *atlE* (*S. epidermidis*), *bla*VIM, *bla*OXA-48, *bla*OXA-58, *bla*OXA-181, *bla*OXA-23, *bla*NDM, *bla*KPC, *bla*CTX-M-15, *bla*CTX-M-9, *mecA*, *vanA* and *vanB*. Initial qPCR cross experiments showed that all targets were specifically detected with no false positive signals. We could also show that for all targets a ΔCT between 15 and 18 for all initial used concentrations was achieved. Despite the high concentration of human DNA, we were able to increase the initial 101 copy-numbers per target to a minimum concentration of 104 copy-numbers.

Discussion: We could show that all 21 targets could be specifically raised to a detectable level of 104 copy-numbers in a multiplexed approach. In a next step we are planning spiking experiments with real blood samples. The purification of the bacterial DNA will be performed using existing lysis (RBC buffer) and filter systems (leukosorb).

Fig. 1



314/HYPRP

A comprehensive 16-month surveillance study of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants in Mecklenburg-Western Pomerania, Germany

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Introduction: In December of 2019, SARS-CoV-2 was identified as the causative agent of unusual viral pneumonia in the city of Wuhan, China. Similar to the global development, multiple dominating lineages have passed through Germany. Amplicon-based third-generation sequencing methods have been used to gain a better understanding of the viral origin, transmission and evolution as well as to substantiate the public health response to COVID-19. The aim of this study was to generate robust sequencing data for the public health authorities of the German federal state of Mecklenburg-Western Pomerania (M-V).

Material/Methods: In close collaboration with 13 laboratories in M-V, more than 3500 samples collected from 11/2020 to 03/2022 were applied to amplicon-based Illumina and Nanopore sequencing methods, which was adapted several times to emerging SARS-CoV-2 variants. The sequences were classified according to the international standards of Pangolin and Nextstrain lineage classification. The obtained data were analyzed focusing on viral variant determination, mutation detection, and time-resolved phylogeny.

Results: In total, 3518 virus sequences from all counties of M-V were collected and analyzed over a period of 16 months. This enabled elucidation of clusters, outbreak chains and distribution of variants, providing insights into the spread and evolution of SARS-CoV-2 in M-V. Overall, phylogenetic analyzes allow the sequences to be classified into 117 different lineages belonging to 16 clades. Alpha (20I), Delta (21A/21I/21J) and Omicron (21K/21L/21M) clades were dominant from 03/21 to 06/21, 08/21 to 01/22 and 02/22 to 03/22 respectively. Further, regional outbreaks caused by the Gamma variant or outbreaks with the Delta variant among ship crews whose ships were anchored in international harbors of M-V were discovered. In March 2022, we identified the first XM recombinant variant in M-V.

Conclusions: The closely coordinated cooperation of the laboratories and local authorities as well as the access to metadata provided by the study center guaranteed a detailed overview of the occurrence of the different virus variants in a spatio-temporal manner on a federal state level in the northeast of Germany. Our approach ensured a short turnaround time, a large coverage depth and permitted an early identification of SARS-CoV-2 variants of potential clinical relevance or concern. Thus, the utilization and in depth-analysis of locally assignable sequencing data has successfully demonstrated its value for local, regional and state-based infection control measurements. Capacities and infrastructures created during the pandemic should be maintained and expanded to increase the level of preparedness, alert and response to future infectious disease challenges.

315/HYPRP

Glove use in hospital patient care – too complex to be safe?

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Hand hygiene is a crucial factor in the prevention of nosocomial infections. During the development of an automated sensor-based system measuring and reporting hand hygiene compliance (HHC), numerous direct HHC observations were carried out for validation purposes. These observations revealed gloves were not only used for single indications of hand disinfection indications instead of disinfections but also for consecutive ones, resulting in contamination. Therefore, the aim of this study is to add a comprehensive glove-use observation system onto the well-known structure of hand hygiene compliance observation.
Methods: All 5 moments for hand hygiene compliance were observed directly according to the WHO standard. Additionally, glove use was documented, and classed in the categories: fresh glove, contaminated glove, disinfected glove and unknown. Observations were performed in one neurosurgical, one intermediate-care and an intensive care unit during 6 weeks (398 indications observed).
Results: 68, 99 and 51 indications were observed after contact to infectious material, after patient contact, after contact to the environment, respectively, for which hand disinfection compliance were 32%, 62% and 80%. Within these three "after" moments, glove use without consecutive hand disinfection was most prevalent with 64% after contact to infectious material. 82 and 98 indications were observed before patient contact or aseptic procedure, respectively. Hand disinfection compliance before patient contact was 49%, before aseptic procedure only 19%. Before patient contact, contaminated gloves were used in 9%, and in 40% for aseptic procedures.
Conclusion: In three different wards the use of gloves was common. "Before" indications demonstrated a use of gloves before patient contact, which is not always indicated. After this "before" contact the then contaminated glove was often used for an aseptic procedure. Possibly, the widespread glove use was favoured by the pandemic, during which PPE was promoted for SARS-CoV-2 patients. Surprisingly, for the "after" indications with the higher risk of self-contamination, we observed smaller percentage of hand disinfection after taking off the glove.

316/HYPRP

Task force nursing homes, homes for disabled people and ambulant care for the elderly

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Introduction: Residents of long-term nursing homes are among the risk groups for a severe course of Covid-19. Therefore, outbreak management and prevention presented a significant challenge.
How could a public health department support such facilities and protect the people most vulnerable to the disease?
Method: In order to be able to ensure close monitoring and support for the care facilities, a dedicated team was established in cooperation with the fire brigade. The aim of this team was and is to support the facilities in the implementation of infection control measures to limit outbreaks and to prepare employees for work in isolation units through targeted hygiene training.
To reduce overhead for our facilities, we coordinated all efforts undertaken by all city departments i.e. fire brigade for PCR swabs and welfare office for alternative accommodations.
To this day, the team contacts the facilities immediately after receiving positive PCR test results in order to identify contact persons, organize outbreak-related tests for residents of the facilities and explain the most important measures for immediate implementation. Since then, all cases, chains of infection and courses of events have been documented for the individual facilities. To realize imminent inspections upon knowledge of a Covid outbreak or a case, the team developed standardized forms and checklists for the various types of facilities and keeps them updated with changes in code and recommendations by the RKI.
Findings: During the entire pandemic we were able to gain insights into the procedures and processes implemented by the facilities, identify possible error sources and make recommendations applicable to the specific nursing homes.
By consolidating our efforts, we were able to reduce workload within our facilities. This let them focus on the important part: containing the outbreak.
Discussion: Since the conditions and circumstances in the supervised facilities are very different, general recommendations would not have been helpful. It was only through the close support of the facilities and the many insights on site that we are able to recommend measures that were as appropriate and feasible as possible and targeted for individual cases. It is also crucial that we have always tried to work together with the institutions in a spirit of trust, so that the aspect of the "monitoring authority" has receded into the background.

317/HYPRP

Side-by-side evaluation of three commercial ELISAs for the quantification of SARS-CoV-2 IgG antibodies

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In December 2020, WHO presented the first international standard (WHO IS) for anti-SARSCoV-2 immunoglobulin. This standard is intended to serve as a reference reagent against which serological tests can be calibrated, thus creating better comparability of results between different tests, laboratories, etc. Here, we have examined three different commercial ELISA kits for the quantification of SARS-CoV-2 IgG antibodies, namely the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) (Euroimmun, Lübeck, Germany), the SERION ELISA agile (Institut Virion Serion, Würzburg, Germany), and the COVID-19 quantitative IgG ELISA (DeMediTec Diagnostics, Kiel, Germany). According to the manufacturers, all are calibrated against the WHO IS and can provide results in either international units (IU) (DeMediTec) or arbitrary antibody units (BAU) per milliliter (Euroimmun, Virion Serion), which are numerically identical, according to the WHO. A total of 50 serum samples from vaccinated individuals were tested side by side and according to the manufacturer"s instructions. We compared the test results of all three assays with each other to assess comparability and with a quantitative in-house virus neutralization test (micro-NT). In summary, our data are consistent with other studies published on this topic that tested similar assays from different manufacturers. Overall, the agreement between quantitative ELISAs is variable and cannot be used interchangeably despite calibration against a standard. Therefore, interpretation of results must still be individualized and tailored to each case. More importantly, our results highlight that quantitative ELISAs in their current form cannot replace neutralization tests.

Prevention of Nosocomial Infections (StAG HY/FG PR)

318/HYPRP

Disinfection of vehicles for passenger transport by a dry-fog technology

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Introduction: At the moment, the disinfection of vehicles in passenger transport and rescue services is carried out by means of wipe disinfection. Although there is already work on alternative disinfection methods, fundamental studies are needed to show the feasibility and effectiveness of implementation in vehicles. [1, 2] The dry-fog technology could represent a new method for a cheap an fast acting disinfecting measure. In this work, the disinfecting potential a dry fogging technology (Apollon Biotech GmbH, Germany) was investigated for the possibility of disinfection of vehicles for passenger transport.
Methods: A public bus was used as a model for a passenger transport vehicle (Ferienfahrschule Hense GbR, Germany). In order to test the effectiveness of the method, the reduction of the microbiological load in the air was investigated in a first test. For this purpose, active airborne germ measurements were done. At the same time, the existing microbiological contamination was determined at various points in the bus by means of contact plate and swab tests. The measurements were repeated after dry-fog disinfection and the values were compared. In addition, contaminated test samples were spread (*Staphylococcus aureus* ATCC 6583; 5x104 CFU) in order to be able to calculate a standardized germ reduction.
Results: The values consistently showed a germ-reducing effect of the measure. The microbiological contamination on several testing points in the bus was reduced by 1.6 log levels in average. In addition, there was a 68% reduction in bacteria in the air after disinfection. An evaluation of the standardized test plates showed a reduction of between log 0.8 and log 3.7, depending on the test location and the distance to the disinfection device.
Conclusion: The tests confirmed the basic applicability of the process for vehicles. It should be noted, however, that the localization of the test areas in relation to the fogging device had a significant influence on the effectiveness in some cases. Further tests are necessary to determine whether the disinfection performance of the process is also sufficient for use in vehicles in the medical sector. In addition, it must be examined whether the measure could be potentially harmful to medical and technical equipment. This has already been addressed in other experiments by our research group.
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Disinfection of objects with cold plasma technology

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Introduction: There is a great demand for fast, effective and efficient disinfection processing equipment, especially among users of reusable products which are to be used in a sterile manner. Especially in the dental field, cold plasma has recently become an issue. [1, 2] In this work, a portable cold plasma disinfection device from the company logfive was tested for its effectiveness and its possible use in the reprocessing of products will be evaluated.
Methods: To simulate the disinfection of contaminated objects, support frames were first 3D printed and fitted with standardized test samples. These test samples were contaminated with different concentrations of test bacteria (Staphylococcus aureus ATCC 6583; 1x10² - 1x108 CFU/ml) and then disinfected in the cold plasma device. The residual bacterial count was determined by contact plate tests and compared with the bacterial count on untreated control plates. The difference in bacterial counts was calculated as log fold bacterial reduction.
Results: In initial tests, the basic effectiveness of the method was demonstrated using different bacterial concentrations. Here, the germ reduction was between log 2.9 and 3.6. After the expected efficiency could not be achieved, the procedure was changed again and adapted. Further improvements in the process and the repetition of the disinfection cycles increased the effectiveness of the device to log 4.7.
Conclusion: The principle effectiveness of the technology for germ reduction was confirmed by our tests. However, in order to achieve a stable germ reduction rate in the range of 5 log levels, further improvements and adjustments have to be made. Therefore, the next step will be to increase the plasma concentration in the device and thus shorten the disinfection time. If the changes achieve the desired success, the cold plasma technology will represent an alternative option to the current disinfection processes for corresponding products.
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Fatal hospital acquired legionella infection due to contaminated toilet flush water

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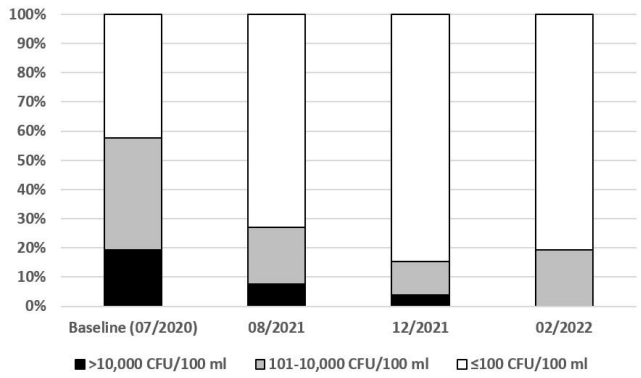
Introduction: While legionella infections caused by inhalation of pathogen-containing aerosols from showers and sinks have been increasingly described, there is only one report of toilet water as potential source of infection.
In 2020, a 45-years-old patient with known HIV infection and relapsed Hodgkin lymphoma developed a fatal hospital-acquired legionella infection two days after high-dose chemotherapy with peripheral autologous hematopoietic stem cell transplantation in our hematology unit.
Methods: Culture-based water analysis regarding contamination with *Legionella* spp. was carried out according to the *German Ordinance on the quality of water intended for human consumption*, to recommendations of the German Federal Environment Agency and to *DIN EN ISO 11731: 2019-03*. Isolates were sequenced on a MiSeq system (Illumina); sequence assembly and data analysis was performed with SeqSphere+ (Ridom) using the cgMLST database of SeqSphere+ (State 2020).
Results: Legionella contamination was found in the building"s cool water system and here in particular in the flushing cisterns (mean legionella concentration: hot water: 1 CFU/100 ml; cool water: sink/shower: 115 CFU/100 ml; toilet flush water: 7084 CFU/100 ml). In the patient"s room, legionella were found in toilet flush water (15,200 CFU/100 ml) but not in hot and cold water of shower or sink. Next-generation sequencing, followed by data

analysis and calculation of a minimum spanning tree showed identical cgMLST types for the isolates from the patient and from the flushing cistern. According to the clinic staff, the patient had repeatedly vomited into the toilet while operating the toilet flush. We hypothesized toilet flush water as origin for the fatal *L. pneumophila* infection – especially since all showers and sinks of the ward were equipped with point of use filters.
As intervention, all patients were instructed about hygiene measures in the wet room (f.e. closing the toilet lid before flushing, usage of vomit bags). Toilet cisterns were disinfected to remove possible biofilms. Despite disinfection initially reduced contamination, legionella concentration reached a potentially hazardous magnitude of more than 10,000 CFU/ml within weeks. To prevent recolonization a chlorine dioxide (ClO2) disinfection system was installed. These measures reduced legionella contamination in the toilet cisterns (see figure).
Conclusions: To the best of our knowledge, this is the second report of toilet flush water as likely origin of a legionella infection. The implementation of a ClO2 system can reduce the degree of contamination. Other prevention points are routine sampling of cold water for legionella in high-risk areas and individual education of high-risk patients regarding wet cell hygiene.
Figure: Legionella contamination of toilet cisterns in the hematological wards at baseline and after the implementation of a ClO2 system 06/2021.

Fig 1.



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Do we know what we do? Overestimation of one's own hand hygiene compliance – results from the WACH-study

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Question: Overconfidence is one of the most consistent and powerful psychological biases. Surprisingly, it has only recently been studied as a barrier to hand hygiene compliance. So far, it has been found that medical students and health care workers rate themselves superior compared to others (overplacement), and that one's education in hand hygiene and feedback is overestimated. The present study scrutinizes overestimation of one's own compliance, i.e. self-assessing one's behaviour to be more compliant than it is. Previous overconfidence research shows that such overestimation tends to be higher for difficult tasks, suggesting that the magnitude of overestimation also depends on how it is assessed. Asking health care workers to self-report compliance using a single item is common but difficult since responses must be based on some aggregation across the "5 Moments of Hand Hygiene" (WHO-5). Thus, we tested the hypothesis that self-reported overall compliance based on one item is higher than that based on WHO-5-items. Additionally, we compared both overall and WHO-5-specific self-reported compliance rates with direct observations.
Methods: In the WACH study (German Clinical Trials Register [DRKS] ID: DRKS00015502), a questionnaire survey was conducted among physicians and nurses in nine surgical clinics (general/visceral surgery or orthopedics/trauma surgery) of six nonuniversity hospitals. Self-reported compliance was assessed both by a single item and WHO-5-items using percentage scales, and compared among each other and to direct observations. Relative frequencies of the WHO-5 indications used to calculate the WHO-5-based self-reported overall compliance rate were estimated from a systematized review (1st author). In analysis, t-tests, chi²-tests, and multiple linear regressions were used.
Results: Ninety-three physicians (response rate: 28.4%) and 225 nurses (30.4%) participated. Significant compliance differences between physicians and nurses were found for direct observations (overall and for WHO-5 except "after patient contact") and were in favor of nurses, while no such differences were found for self-reports (except "after body fluid exposure" in favor of physicians). Across the WHO-5, overestimation showed inverse correlations with observed compliance (physicians: r = -0.88, p=0.049; nurses: r = -0.81, p=0.093). Support for the hypothesis that self-reported overall compliance based on one item is higher than that based on WHO-5 items was found for physicians (M=87.2 vs. 84.1%, p=0.041; nurses: 84.4 vs. 85.5%, p=0.296). Exploratory analyses showed that this effect was confined to orthopedic/trauma surgeons (89.9 vs. 81.7%, p=0.006).
Conclusions: Results indicate stronger overestimation among physicians. Overestimation is a psychological mechanism that may be used in behavior change techniques such as "incompatible beliefs" and "feedback on behavior" to draw attention to discrepancies between current behavior and relevant self-images.

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Short-wave ultraviolet light-based surface disinfection using light-emitting diodes – efficiency and limitations in clinical environment

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Background: UV-C irradiation is a promising method for the eradication of microbial loads in water, in air and on surfaces. There are many data available on the reduction of microbial loads from UV-C radiation. However, these data are often not standardized with regard to the detection method, and many factors that influence the UV-C efficiency have not yet been investigated in detail. Therefore, in this study, we examined the suitability of UV-C-based surface disinfection for use in hospital environments under different conditions.
Method: Test carriers were inoculated with the microbes, air-dried, and then irradiated at different wavelengths. Subsequently, the surviving microorganisms were recovered from the test surface via a washing step and plated on agar plates for quantification. The efficacy of UV-C surface treatment was tested on four different organisms that were either dried on a carrier surface or in liquid solution. In addition, the influences of soiling, microbial loads, surface material, and the wavelength of radiation on the disinfection performance were investigated.
Results: UV-C treatment of *E. coli*, *S. aureus*, *G. stearothermophilus* spores, and *C. albicans* at a dose of 15,000 J/m2 revealed that *E. coli* and *S. aureus* were the most susceptible to UV-C radiation, followed by *C. albicans*. All three showed inactivation of > 5 log10 levels. The spores showed a higher resistance to UV-C, with a reduction of 3 log10 levels. *E. coli* on a dry surface shows a significantly reduced UV-C sensitivity (> 2 log10 levels) compared to that of *E. coli* isolates in a liquid environment. The components of full medium also led to a reduced UV-C efficiency compared to that of buffered solutions in which the microorganisms were dissolved (> 5.5 log10 lower inactivation of *E. coli)*. We were able to demonstrate that the

microbial load and the texture of the carrier surface have a major influence on the UV-C disinfection efficiencies.
Conclusion: This study showed that UV-C is generally a suitable method for surface disinfection, but only under certain conditions. The surfaces should be smooth and as free as possible from contamination, and an excessive microbial load should be avoided. The areas in which complementary UV-C treatment in the hospital environment is useful must therefore be carefully considered.

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Scaling individual determinants of compliance to prevent surgical site infections – results from the WACH-study based on the COM-B-model
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Question: Tailoring of interventions to promote compliance to prevent nosocomial infections implies to gain an a priori understanding of determinants of compliance. One approach is the **COM-B** model, which states that **B**ehaviour is influenced by **C**apabilities, **O**pportunities and **M**otivation [1]. In the WACH-study (German Clinical Trials Register ID: DRKS00015502), a COM-B-questionnaire was developed to assess individual and environmental determinants of compliance with surgical site infection-(SSI-)preventive measures. The aim of this analysis was to determine the reliability and construct validity of the respective scales. Explorative analyses scrutinized associations of the COM-factors with self-reported compliance.
Methods: A questionnaire survey was conducted among physicians and nurses in nine surgical clinics (general/visceral or orthopedics/trauma surgery) of six non-university hospitals. COM-factors relating to self-reported SSI-preventive compliance were assessed via 18 items using 7-point-Likert-scales. To test for construct validity, a confirmatory factor analysis using robust FIML-estimation was conducted. Reliability was determined by McDonalds ω and Bollens ω*. To test for measurement equivalence, a multiple group confirmatory factor analysis using robust FIML-estimation was conducted. Associations with average self-reported compliance rate across 26 measures were explored by multiple regressions.
Results: Ninety-three physicians (response rate: 28.4%) and 225 nurses (30.4%) participated in the survey. A three-factor-model representing capabilities, motivation and planning skills as individual determinants of compliance showed excellent model fit ($X^2 = 111.3$, $df = 51$, $p < .001$, RMSEA = .065, SRMR = .039, CFI = .95). Reliability was confirmed for capabilities (ω*=.891), motivation (ω=.861), and planning (ω=.898). A four-factor-model, extended by opportunities as environmental determinants, showed good model fit ($X^2 = 220.2$, $df = 110$, $p < .001$, RMSEA = .060, SRMR = .048, CFI = .95), but the opportunities factor did not show satisfactory reliability (ω*=.720). Partial residual measurement equivalence was shown for the three-factor-model. Associations with self-reported compliance were found among nurses for capabilities and planning skills ($p<0.001$).
Conclusions: Results indicate that the WACH COM-scales are reliable and valid measures to assess individual determinants of SSI-preventive compliance. Thus, they may be used in processes of tailoring interventions which target compliance with SSI-prevention. Whether the finding that COM-associations with self-reported compliance were confined to nurses indicate better match of the model with this group or higher compliance overestimation among physicians remains to be determined. Also, future research on how to reliably and validly measure external determinants is needed.
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Anti-adhesive effects of microstructured titanium surfaces created by ultrashort pulsed direct laser interference patternig
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Introduction: Titanium is one of the most commonly used materials for the production of orthopedic implants. However, titanium-based implants are also a common cause of orthopedic implant infections (OII), which may pose a severe risk for the patient. The heat-induced formation of a titanium dioxide (TiO2) layer and ultrashort pulsed direct laser interference patterning (DLIP)-based microstructuring of the titanium surface in the lower µm range are both known to improve the healing process and the integration of the implant into the bone. However, the impact of both surface modifications on bacterial adhesion to titanium has not been addressed yet. In order to fill this gap, we studied here the impact of heat treatment and DLIP on the surface topography of titanium and its effects on the binding capacities of the OII-associated bacterial pathogens *Staphylococcus aureus* and *Escherichia coli*.
Methods: Titanium surfaces were first heat-treated for two hours at 450°C to ensure a solid TiO2 (Rutile) layer formation and were subsequently microstructured by DLIP. Surface topographies of all processing steps were characterized by scanning force microscopy (SFM), scanning confocal microscopy, and scanning electron microscopy, and tested for their bacterial binding capacities by classical microplate-based adhesion assays for *E. coli* and SFM-based single-cell force spectroscopy (SCFS) for *S. aureus*.
Results: We found that a TiO2 layer produced by heat treatment already reduced the capacity of the bacteria to stick to the surface, which could be further decreased when the heat-treated titanium surfaces were microstructured by DLIP to create a sinusoidal surface topography with a 3 µm spatial period. SCFS experiments with *S. aureus* demonstrated a significantly lower adhesion force of individual bacterial cells on this kind of structured substrate surface compared to the untreated surface, and microplate-based adhesion assays revealed a minimum number of adherent *E. coli* cells on the structured and heat-treated surface.
Conclusions: Heat treatment and DLIP created a titanium surface that displayed reduced binding properties for the bacterial model organisms *E. coli* and *S. aureus*. Our findings may help to create novel titanium-based implants that display a reduced risk for OII.

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Testing the activity of antimicrobial examination gloves under realistic conditions
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Background Antimicrobial materials or antimicrobial surfaces are advertised for many hygiene-relevant areas as a measure for infection prophylaxis. However, the efficacy of such antimicrobial surfaces has not been sufficiently investigated. In this study, the antimicrobial activity of an examination glove with light-activated antimicrobial properties against Gram-positive microorganisms was investigated. Method In a standardized experimental set-up, the antimicrobial properties of the glove were tested with different test sub-jects (n=10) in comparison with conventional examination gloves from the same manufacturer. The gloves were contaminated by a realistic contamination through a standardized activity of the test persons for construction with building blocks, which were contaminated on the surface with a dried Acinetobacter baumannii outbreak strain as well as E. faecium ATCC 6057. After the standardized activity, the gloves were held with the palm side open and facing upward for 10 minutes in the light present in the room and the grade of contamination was determined by quantitative

culture. The light intensity at the position of the hands was measured during all experiments. Results The light intensity in each experiment was significantly above the limit specified by the manufacturer for the activa-tion of antimicrobial properties (500 lx). The mean values for experiments with antimicrobially active and non-active gloves were 955 and 935 lx, respectively. As expected, the gloves showed no efficacy against Acinetobacter baumannii. Against E. faecium, the gloves showed only very low antimicrobial activity with a reduction factor 4 log10 within 5 minutes for Gram-positive microorganisms demonstrated for the product in a standard test procedure (ASTM D7907) could not be confirmed in a realistic experimental test set-up even after 10 minutes of light expo-sure. The effectiveness against Gram-positive microorganisms should be further investigated in the reality of patient care with more varying light intensities.

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Understanding the role of biofilm and surface properties in bacterial adhesion to fabrics – a step towards curbing nosocomial infections

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Introduction: Nosocomial infections are a major concern in hospitals and transmitted mainly through fabrics used in the healthcare settings. Interactions between bacteria and fabrics depend upon the surface characteristics of microbes and fabrics. In this study, some important bacterial surfaces as well as fabric characteristics were examined. Bacterial adherence may lead to the biofilm formation by production of exopolysaccharides (EPS). Further, correlation between EPS production and biofilm formation among different bacterial strains was also assessed.
Materials and Methods: Four common nosocomial infection causing bacteria *viz; Acinetobacter calcoaceticus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* along with common hospital fabrics like polyester, cotton, polyester-cotton (70:30) blend and silk were selected for the study. All fabrics were scoured to remove dirt and autoclaved. Areal density (g m-2) of each fabric was measured as per ASTM D3776. EPC and PPC were counted using pick glass. Surface roughness of fabrics was measured using Kawabata Evaluation System- (KES-FB4). Bacterial surface charge was analyzed using dynamic light scattering (DLS). Biofilm formation was analyzed qualitatively as well as quantitatively, and correlated with EPS production by the bacterial strains.
Results: Maximum (12.8 g m-2) areal density was observed for blend and minimum (4.5 g m-2) for silk fabric. The surface roughness of blend fabric was highest (3.1±0.4 µm) and silk had least roughness (1.6 ±0.2 µm). Highest hydrophobicity was shown by *Pseudomonas aeruginosa*, followed by *Acinetobacter calcoaceticus*, *Staphylococcus aureus*, and *Escherichia coli*. Highest zeta potential (-27.7 mV) was observed for *Escherichia coli* and lowest for *P. aeruginosa* (-6.6 mV). A similar trend was observed for cell surface charge with *E. coli* exhibiting the highest and *P. aeruginosa* being least. It was observed that *P. aeruginosa* and *S. aureus* were strong biofilm producers, as compared to *A. calcoaceticus* and *E. coli* the latter two being moderate biofilm producers. Further, biofilm formation correlated well with EPS production as *P. aeruginosa* produced maximum EPS (0.11 ± 0.4 g l-1) of viscous nature and *E. coli* produced minimum EPS (0.020 ± 0.8 g l-1) of non-viscous nature. FTIR characterization of EPS revealed the presence of different functional groups like C-H stretching alkane, and O-H stretching alcohol among all four bacterial strains.
Discussion: The present study clearly indicates that transmission of nosocomial infections depends on a number of factors including bacterial surface properties, ability to produce EPS and fabric properties. This information may help mitigate bacterial infections in hospital settings by designing newer fabrics used in hospitals. As minimum biofilm formation was observed on silk, thus, the use of silk in healthcare settings would be helpful for the management of cross-contamination of nosocomial infections.

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Impact of intensified contact precautions while treatment of hematopoietic stem cell transplantation recipients during aplasia

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Introduction: Bacterial infections are a major complication for patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Therefore, protective isolation is considered crucial to prevent nosocomial infections in this population. Here, the impact of intensified contact precautions on environmental contamination and the occurrence of bloodstream infections (BSI) in patients on a HSCT unit was compared between two contact precaution measures.
Methods: A 2-year retrospective observational study was performed. In the first year, strict contact precaution measures were applied (i.e. protective isolation, the use of sterile PPE by healthcare workers and visitors and sterilization of linen and objects that entered the patient's room). After one year, contact precautions were reduced (i.e. no use of sterile PPE, no sterilization of linen and objects that entered the patient's room). Environmental contamination in randomly selected patient rooms was monitored by sampling six standardized environmental sites in the respective patient treatment units. In a before-and-after study, the number of BSI episodes of those patients, who were accommodated in the monitored rooms was compared.
Results: In total, 181 treatment units were monitored. Most prevalent microorganisms in the patient environments were coagulase-negative *Staphylococci* (74.6%, n=135), *Bacillus* spp. (35.9%, n=65), *E. faecalis/E. faecium* (14.4%, n=26) and *S. aureus* (10.5%, n=19), showing no significant difference in the contamination of anterooms and patient's rooms between both groups. A total of 168 febrile patients were followed for the occurrence of BSIs during the entire study period (before: 84 patients, after: 84 patients). The total count of patients with BSI episodes showed a higher incidence in the period with reduced contact precautions (30/84 vs. 17/84, p = 0.025). The cause of this increasing number of bloodstream infections can be traced back to BSI episodes with only skin and environmental bacteria (17/84 vs. 5/84, p = 0.006), while the number of BSI episodes with common species of true BSI remained constant (13/84 vs. 12/84, p = 0.828).
Conclusions: The impact of maximal barrier measures on patients' outcomes remains controversial. Adequate infection control and prevention bundle strategies including contact precautions, disinfection strategies and distinct antimicrobial initiatives are necessary to prevent nosocomial infections in aplastic HCST recipients, instead.

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The number of Vancomycin-resistant *E.faecium* (VREfm) bloodstream infections begins to surpass those caused by Vancomycin-sensitive *E.faecium* (VSEfm) – a molecular study of VRE epidemiology

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Objective: VRE blood stream infections (BSI) are a problem of rising significance in Germany and Europe. Our study aims at clarifying some open questions about VREfm BSI to improve the efficiency of infection prevention measures: 1. whether BSIs caused by VREfm add on to those caused by VSEfm or whether only the proportions change. 2. whether patient groups at highest

risk for VREfm or VSEfm BSI are distinct. This knowledge could help to develop strategies that address the most vulnerable patient groups specifically. 3. whether comparing the molecular epidemiology of VREfm isolates causing BSI to that of screening isolates can identify certain "high risk clones" that are responsible for a higher proportion of clinical infections. In that case infection control measures could focus on them. We therefore evaluate the local epidemiology and clinical characteristics of VREfm BSIs over a four-year period.

Methods: This retrospective analysis includes all first VREfm and VSEfm blood culture (BC) isolates of every patient at a university hospital collected during the years 2018-2021. Basic patient characteristics and clinical outcomes are assessed (work in progress). For all VRE isolates whole genome sequencing (WGS) was performed and cgMLSTyping and detection of resistance genes with SeqSphere (Ridom)/NCBI AMR Finder Plus carried out.

Results: We documented 128 patients with VREfm vs. 221 with VSEfm BSI over the study period. In 2018 the number of patients with VSEfm BSI was more than twice as high as for VREfm BSI (53 vs. 21), but the relations changed gradually until in 2021 VREfm BSIs outnumbered those caused by VSEfm (48 vs. 44). Until now, WGS data with sufficient sequencing quality were available for 112/128 VREfm BC isolates (work in progress). VanB genotype was present in 57%, vanA in 38% and both vanA and vanB in 4% of isolates. MLST analysis detected nine different sequence types with ST117 (45; 40%), ST80 (37; 33%) and ST1299 (19; 17%) being most common. CgMLST found five complex types (CTs) to be most significant (overall: 79/112: 71%; ST80/CT1065: 28; ST1299/CT1903: 15; ST117/CT5130: 15; ST117/CT71: 11; ST117/CT3247: 10), the other 26 CTs were detected only sporadically. Interestingly, we found only ST80/CT1065 to be frequent over the whole study period, while the other most prevalent CTs changed over time. The comparison of the prevalent CTs of VRE BC isolates of 2020 with those of all first VRE isolates from any specimen (47% rectal screening isolates) from the same year (poster presented at ECCMID 2022, Rath et al.), revealed a similar distribution of CTs.

Discussion: We found a worrisome increase in the number of VREfm BSIs while the number of yearly VSEfm BSIs showed no clear trend. The rise in VREfm BSI was triggered by a few and changing dominant CTs. No VREfm high risk clones could be identified. Impact of clinical parameters on VRE complex types is currently assessed.

Technical Hygiene (StAG HY/FG PR)

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Safe, effective, and cost-efficient air cleaning for populated rooms and entire buildings based on the disinfecting power of vaporised hypochlorous acid

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Introduction: Pathogen-carrying aerosol particles are recognized as important infection carriers, particularly indoors. This infection route is often underestimated yet represents the infection course that has been least systematically countered to date. Current indoor safety measures (e.g.: distancing, masks, filters) provide only limited protection. Inhalation of vaporized hypochlorous acid (HOCl) was recently shown to be safe and effective in prevention and even in reduction of symptoms of already infected individuals. This study investigated if the disinfecting strength of HOCl in suspensions can also be demonstrated for a novel facility-wide air-disinfection concept utilizing vaporized HOCl.

Material and Method: The biocidal effect of HOCl was quantified by a series of standard suspension deactivation tests, performed according to the methods of CEN Technical committee 216: EN 1276, 13624, and 14476. For in-air tests we injected aerosolized bacterial suspensions into controlled lab chambers preloaded with vaporized HOCl at different concentration levels - all well below of regulated limits (5-30%). Concentration of "free active chlorine" was determined with a special gas sensor system controlling the amount of vaporized HOCl via a special device.

Results: In suspension experiments we found sufficient efficacies for all studied organisms at minimum concentrations of 200 ppm HOCl. The suspension test results demonstrate that the sensitivity to HOCl being approximately in the same concentration range for all organisms, with vaccinia virus at the lower limit, i.e., even slightly more sensitive overall. Vaccinia virus is considered a surrogate virus for all enveloped viruses in the tests of CEN TC 216, i.e., with sufficient efficacy against vaccinia virus, efficacy against all enveloped viruses (which includes SARS-CoV-2) can be assumed.The in-air measurement set-up allowed to follow microbe deactivation by HOCl interaction. The deactivation rate increases with the HOCl concentration. For the investigated Gram-negative species (Pseudomonas fluorescence, E. coli) we found an almost linear relationship between HOCl concentration and bacterial deactivation rate.

Discussion: The measured effect is very important considering the chemical and structural similarity of the envelope of Gram-negative and coronavirus lipid envelopes. Our results support the assumption that airborne enveloped viruses can be progressively deactivated with increasing HOCl gas concentrations.We could confirm the disinfecting power of HOCl in suspensions and determined the high efficacy of vaporized HOCl to disinfect atmospheres of populated indoor places at safe and non-irritant levels. The investigated microbes provide a model system for infectious particles, including enveloped viruses (e.g.: Coronavirus). Our early results suggest that HOCl based air-cleaning for populated rooms may become a potential alternative to incumbent approaches and should be further evaluated.

Fig. 1

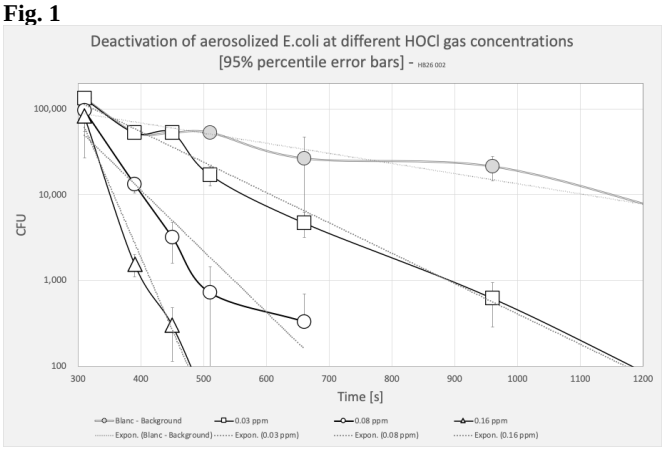
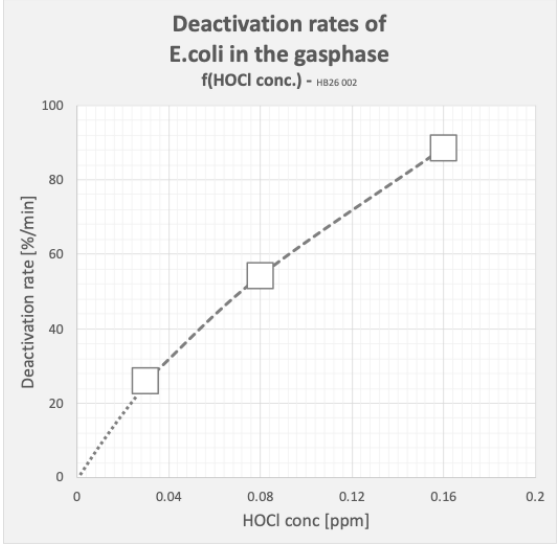


Fig. 2



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***In vitro* determination of the virucidal effect of UVC irradiation of different wavelengths on SARS-CoV-2 and other respiratory viruses**

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Background: In December 2019, the novel coronavirus SARS-CoV-2 became a pandemic and led to global problems e.g. social distancing, short time work, psychological stress, etc. Besides the socio-economic burdens, the disease also caused serious health damages.
These symptoms range from mild respiratory symptoms such as fever or fatigue up to life threatening symptoms such as respiratory distress or pneumonia. In addition to SARS-CoV-2, there are also other respiratory diseases in livestock which play an important economic role.
Therefore, technical innovations are being investigated for their use to inactivate viruses in areas, where chemical disinfectants cannot be used. In the present study, we analysed the vulnerability of a broad spectrum of different viruses to UVC irradiation of 230 and 270 nm on various surfaces. It has already been investigated in other studies, that UVC at 254 nm show an inactivating effect on bacteria. UVC is absorbed by RNA and DNA and leads to a photochemical fusion of two base pairs into covalently linked dimers. These dimers block the function of reading and duplicating enzymes. Because there is no life without DNA, this effect concern all life forms and it cannot develop resistance in microorganisms.
Material and Methods: In our study, we are using germ carriers with different surfaces (glass, plastic and stainless steel). After application and drying of the viruses, the germ carriers are irradiated in specific doses. In the following step, the germ carriers are rinsed off with medium and the collected supernatant will be used for virus titration. Untreated prepared germ carriers serve as reference. Efficient inactivation is defined as a reduction of a minimum of four log levels between treated and untreated samples.
Results and Conclusion: In the present study, we demonstrated that all of our tested viruses could be sufficiently inactivated by 270 nm. Thus, UVC with a wavelength of 270 nm is an effective method to inactivate viruses on surfaces. Furthermore, we determined the antimicrobial effectivity of UVC of 230 nm and thereby its therapeutically usability e.g. for medical treatment of infected skin. Thus, we used a maximum dose of 80 mJ/cm2 that was shown to have a tolerable damaging effect on skin (Glaab et al., 2021, Sci Rep.). Thereby, we only achieved the targeted four log level inactivation for poliovirus (vaccine strain).

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Complete disinfection of the surfaces of a neonatal incubator using ozone without major disassembly

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Introduction: Neonatal incubators can be involved in transmission of microorganisms and subsequent infections. Therefore, reprocessing of incubators is a key aspect for infection prevention in neonatology. In this study we investigated the effectivity of a standardized no touch disinfection process using ozone for incubator disinfection.
Methods: In a Babyleo® IncuWarmer (Dräger) contaminated (>106 cfu *Enterococcus faecium*) ceramic tiles or stainless steel targets were placed beside the fan impeller (p1), in the warm air duct (p2), on the top of the air duct cover (p3), beneath the x-ray tray (p4) as well as on the top of the mattress tray (p5). At positions p1, p2, p3 and p5 both materials were placed. At position p5 only stainless steel targets fitted in the remaining gap. Additionally, a contaminated incubation chamber filter was investigated. Using the SterisafeTM pro instrument standard disinfection cycles (80 ppm ozone; 90 % RH; 60 min) were carried out with a none-disassembled incubator (only removal of the heating mattress and the silicone seals as well as opening of doors and flaps).
Results: In five independent experiments mean reduction rates of >6 log10 were reached at positions p1, p2, p3 and p5 for both surfaces. At position p4 a mean reduction rate of 3.1 log10 was observed. However, in individual experiments the contaminated targets were moved while removing the x-ray tray, indicating that the targets were jammed in the gap and ozone might failed to reach the target surface. For the filter a mean reduction rate of >6 log10 was observed. No visible alterations of the materials of the Babyleo® IncuWarmer and the removed silicone seals as well as the heating mattress after a total of 20 cycles of ozone disinfection.
Conclusions: Ozone might be an effective disinfectant for the reprocessing of the Babyleo® IncuWarmer without complete disassembly of the incubator. However, the x-ray tray should be removed and processed separately to reach fully effective disinfection. Necessary investigations with regard to the compatibility of materials and electronics of the incubator are still in progress, so no conclusive statements on possible effects can be made in this regard.

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Generation of an artificial organ model as a test soil for cleaning of medical devices

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Introduction: The cleaning efficacy of surgical instruments is evaluated, among other things, by using test soils. Authorized soils are regulated in DIN 15883, whereas a large variety in composition can be observed between individual countries. For example, in the Netherlands a mixture of, amongst others, albumin and muscin is used, whereas in Germany defibrinated sheep blood, egg yolk and semolina are used for the same instrument classes. This leads to heterogeneous results evaluating the cleaning efficacy. Even using one test soil different results were obtained[1]. Overall, several aspects of the test soils are problematic: comparability, reproducibility and the application itself, which is mostly manually on a non-moving instrument. This does not reflect the experience, since instrument movements causes the relocation of contaminates to less accessible instrument regions. Therefore, we wanted to develop a reproducible, standardizes test soil which can be used in a test routine with simulated instrument movements to obtain a more reliable test contamination.
Material/ Method: We defined gelatine as the scaffold supplemented with ions, proteins, carbohydrates and fatty acids. The concentration of individual components does not correspond to a specific organ or tissue. Data here is limited and varies between publications. Therefore, the selected parameters are only an approximation of the body average. In order to achieve a realistic contamination, the instrument movement for contamination is first carried out in gelatine which can be modified with blood. Process-adapted usability tests were used to evaluate the reproducibility, resource requirements and safety of the artificial organ model.
Results: The reproducibility of the gel production was verified by numerous usability tests. The gel strength can be adjusted according to the fabric strength to be simulated via the mass fraction of gelatin. The measured values of a series for gel strength of the modified hydrogels never deviated from each other by more than 0.1 N on average. It was found that different gel compositions, in particular varying salt concentrations, and the ambient temperature influence the gel strength. Higher strength at higher temperatures (body temperature) could be achieved with higher mass fractions of hydrogel. Experiments have shown that the gel is suitable for moving instruments in it and that it is also suitable for soiling RF instruments. When energy was supplied to the instrument, the characteristic deposits and discolourations formed.

Discussion: The new test soil has better comparability, reproducibility and authenticity compared to the previous soils. The possibility of partial automation, the mapping of motion effects and energy outputs lead to more realistic test scenarios.

Literature

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334/HYPRP

Bacterial biofilm formation on nano-copper added PLA suited for 3D printed face masks

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Introduction: The COVID-19 Pandemic leads to an increased worldwide demand for personal protection equipment (PPE) in the medical field, such as face masks. New approaches to satisfy this demand have been developed, and one example is the use of 3D printing face masks. Reusable 3D printed mask also have a positive impact on the environment due to decreased littering. However, the microbial load on the 3D printed objects is often neglected. The face, especially the area around the mouth, is a source of many microorganisms such as bacteria like Pseudomonas aeruginosa. Therefore, the demand for antimicrobial properties has also found its way into the area of 3D filaments. Some companies already list 3D filaments provided with antimicrobial properties in their product range. For this study, we have chosen the suspected antimicrobial Plactive™ filament, with a nano-Copper additive, by Copper 3D. We analyzed the bacterial growth and the biofilm-forming potential of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* on suspected antimicrobial Plactive™ PLA and non-antimicrobial Giantarm™ PLA with biological and physical techniques.

Material/method: To characterize bacterial adhesion and the biofilm-forming potential scanning electron microscopy (SEM), Confocal scanning electron microscopy (CLSM) and colony-forming unit assays (CFU) were performed. Three bacterial species were selected for the biological assays. The strong biofilm producer Pseudomonas aeruginosa, isolated from a domestic washing machine, Staphylococcus aureus (DSMZ 24167), and Escherichia coli XL1-blue strain (Stratagene/Agilent, San Diego, CA, USA).

Results: By using SEM and CLSM, attached bacteria could be observed on both tested 3D printing materials. Gram-negative strains P. aeruginosa and E. coli reveal a strong uniform growth independent of the tested 3D filament. SEM analysis demonstrated a multilayered biofilm for P. aeruginosa and E. coli with already typical features of the initial maturation stages during biofilm growth. CFU results of *P. aeruginosa* could reveal a stressed induced growth reaction on Plactive™ PLA. Only for Gram-positive *S. aureus* a strong growth reduction on Plactive™ PLA could be detected.

Discussion: The results clearly indicate that the different membrane compositions of Gram-positive and Gram-negative bacteria could be the origin of differences in antimicrobial activity. The postulated antimicrobial Plactive™ PLA does not affect the bacterial growth and biofilm-forming potential of Gram-negative bacteria species. These results also indicate that reusable masks, while better for our environment, may pose another health risk.

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Impact of temperature and ultrafiltration on *Legionella* sp. and aquatic bacteria in a potable water emulator

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Introduction: Pathogenic bacteria in biofilms of potable drinking water pipelines, like *Legionella pneumophila*, can pose significant health risks to end users. Legionellae can be aerosolized by showers and once inhaled it can cause severe pneumonia. Depending on the general health status of patients, infections can be fatal. To prevent growth of legionellae 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 and an upstream ultrafiltration device (UF) affect the amount of legionellae and the total cell counts (TCC) in a contaminated emulation module.

Material/Methods: We constructed an emulation module 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 consists of 4 temperature periods (45,50,55,60°C), lasting 28 days each. Every 7th day samples were taken. Between the influent and the emulator an UF was implemented, which was proposed to reduce the inflowing TCC and numbers of legionellae.

The presence of legionellae was culturally determined and additionally investigated by means of qPCR. Flow cytometry was used to measure the TCC and distinguish intact (ICC) and damaged (DCC) cells.

Results: The legionellae concentration in cold water samples remained at a similar level throughout the experiment. No differences were detected between the numbers before (b-)UF and after (a-)UF. After 8 weeks, the TCC at the influent and b-UF were at the same level, while a-UF they were reduced and remained by about 1 log unit.

Water temperatures of 45°C and 50°C had no effect on the legionellae and TCC in the emulator. Adjusting the water temperature to >50°C, the number of legionellae detected via culture and qPCR decreased within the circulation and distal pipes. This reduction was also observed for the TCC.

In all water and biofilm samples, more ICC than DCC were measured. In contrast to the water samples, the TCC of the biofilms increased with higher water temperatures. Still, the number of legionellae decreased with rising temperatures.

Discussion: The detectable legionellae were low and impermanent in the inflowing system. Due to fluctuations around the detection limit within samples of the influent, b- and a-UF a definite statement concerning the effectiveness of the UF regarding a reduction of legionellae cannot be made.

Increasing the water temperature to >50°C led to a reduction of *Legionella* sp. within the circulation. The raised temperature 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 2 log units. The microorganisms within the biofilms seemed less affected by rising water temperatures, as the TCC increased.

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Composition of the extracellular matrix in the biofilm of a carpapenemase-producing *Klebsiella pneumoniae* strain with increased tolerance to disinfectants

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Introduction: Nosocomial outbreaks of carbapenemase-producing *Klebsiella pneumoniae* (CPKP) are a major concern in healthcare facilities worldwide. In some cases, duodenoscopes have been identified as a source of transmission. In the biofilm of a CPKP strain obtained from a duodenoscopy-associated outbreak we found an increased tolerance towards the disinfectant peracetic acid (PAA) (in press). Since the amount and composition of

extracellular matrix (ECM) have been shown to be linked to increased antimicrobial resistance in biofilm-associated bacteria, we assessed the composition of ECM in biofilm of the CPKP outbreak strain in comparison with a carbapenem-susceptible *K. pneumoniae* type strain.
Methods: We investigated the biofilm architecture of the CPKP outbreak strain and the *K. pneumoniae* type strain (ATCC 13883) by scanning electron microscopy (SEM). Using confocal laser scanning microscopy (CLSM) imaging we performed staining for DNA (Syto60) and protein (Sypro Orange).
Results: CLSM imaging of biofilm of the outbreak strain showed compact, dense, strand-like biofilm aggregates embedded in an extremely protein-rich ECM.The compact aggregates of the outbreak strain were also visible in SEM and confirmed the strand-like appearance as well as the abundance of ECM material. There were numerous sedentary separate bacteria of the outbreak strain with a larger number of surface-adhering filaments. In contrast, the type strain showed a dominant signal for DNA-specific staining when compared to the outbreak strain in CLSM; SEM imaging of biofilm of the type strain showed larger, more voluminous multilayer cell aggregates covered by a blanket-like ECM. While the type strain also showed adhering solitary bacterial cells, these featured a considerably lower amount of adhesive filament structures when compared with the outbreak strain.
Discussion: Here, we show the co-incidence of increased tolerance to PAA-disinfection and a protein-rich ECM in the biofilm of a CPKP strain that had caused a nosocomial outbreak linked to contaminated duodenoscopes. The outbreak strain with decreased susceptibility towards PAA (Brunke et al., *Antimicrob Resist Infect Control*, in press) showed very compact biofilm aggregates with a striking abundance of protein in its ECM. PAA is commonly used in for the reprocessing of duodenoscopes. It is an oxidizing agent and has also protein-fixating properties. The high protein content of ECM of the outbreak strain might represent a mechanism contributing to withstand the disinfection by PAA. Further investigation aims to perform semi-quantitative analysis of these observations and to include other ECM components such as lipids and carbohydrates. The significance of these observations in relation to increased tolerance to disinfectants needs to be systematically analysed in future studies with more CPKP isolates.

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The effect of transition metals and metal oxides on the survival and biofilm formation of Pseudomonas aeruginosa
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Introduction: For thousands of years, people have been fighting bacteria with various agents. Nanoparticles were among the first agents used by mankind to fight infections. With the discovery of organic antibiotics in the 20th century, a new era of combating against microorganisms began. Nowadays antibiotics are the standard method to eliminating microorganisms. Antibiotics access components and pathways of prokaryotes that are not present in eukaryotes. Nevertheless, microorganisms become resistant to antibiotics through constant mutations and information exchange. Pseudomonas aeruginosa and Staphylococcus aureus are among the bacteria responsible for numerous clinical infections and also cause considerable damage in industry due to their strong biofilm formation. These two microorganisms mentioned above belong to most biofilm-forming and multi-resistant bacteria, which makes the combating against them quite complicated. In terms of antibacterial control, nanoparticles provide an effective alternative to antibiotics for fighting bacteria. Several studies have investigated the biotoxicity of transition metal nanoparticles (d-block), and they show promising results. In this work, we investigate the sensitivity of P.aeruginosa and S.aureus in the presence of certain nanoparticles from the d-block of the periodic table.
Material and Methods: To determine the minimum inhibitory concentration (MIC), we cultivated the bacteria on agar plates with different concentrations of nanoparticles for 24h at 37°C. In addition, we performed colony forming units (CFU) over 32 hours to quantify the survival rate of the bacteria. Then we used confocal laser scanning microscopy (CLSM) to investigate the ratio of live and dead bacteria and their relationship to the biofilm in the medium. The characteristics of the nanoparticles and their distribution in the biofilm and on the bacterial surface were studied using electron scanning microscopy (SEM).
Results: The results of the CFU show that the nanoparticles of metals are more toxic than the metal oxides. The metal oxides are toxic to S.aureus in high amounts. P.aeruginosa, on the other hand, can survive and form a biofilm even at high concentrations of metal oxides. The nanoparticle of metals show a very strong antibacterial effect on S.aureus after a few hours. Surprisingly, P.aeruginosa can survive even in high concentrations on nanoparticles. The SEM results confirm the removal of nanoparticles by P.aeruginosa, and the CLSM images show that P.aeruginosa can exhibit a resistance effect against metal nanoparticles thanks to the formation of a strong biofilm.
Discussion: We were able to show through this work that some nano-sized transition metals can be very toxic to bacteria, even in small amounts. However, the tested strain of P.aeruginosa is able to survive through cluster formation and suppression of the stress factor even in higher concentration of nanoparticles.

Surveillance of Nosocomial Infections and MDROs (StAG HY/FG PR)

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Report of the National Reference Centre for multidrug-resistant gram-negative bacteria on carbapenemases in Germany in 2021
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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 6860 isolates were investigated for carbapenemases at the National Reference Centre in 2021. Carbapenemases were found in 1765 *Enterobacterales* strains, 358 of *P. aeruginosa* and 293 of *A. baumannii*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 495), VIM-1 (n = 288), KPC-2 (n = 241), NDM-1 (n = 218), OXA-181 (n = 116), OXA-244 (n = 110) NDM-5 (n = 107) and KPC-3 (n = 84). In *P. aeruginosa*, VIM-2 was the most frequent carbapenemase (n = 243), followed by GIM-1 (n = 36) and NDM-1 (n = 23). OXA-23 was again the most frequent carbapenemase in *A. baumannii* (n = 197), followed by OXA-72 (n = 94) and NDM-1 (n = 32). Isolates producing more than one carbapenemase were again detected with increasing frequency: In total, 110 isolates of Enterobacterales produced multiple carbapenemases with the combination of NDM-1/OXA-48 (n = 35) beeing the most prevalent one.
Conclusions: A variety of different carbapenemases is constantly detected in Germany. The molecular epidemiology in Germany 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 significant rise of OXA-244 and NDM-5 has come to an end, while the number of isolates producing more than one carbapenemase is still increasing.

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Simulation of a genomic real-time transmission surveillance in a tertiary care hospital

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Background: Early detection of pathogen transmission events is challenging in the hospital setting and requires stringent monitoring of patient occupancy and reliable strain typing methods. Whole genome sequencing (WGS) has become the gold standard for bacterial typing, whereas Fourier-transform infrared (FTIR) is a fast technique that can achieve comparable results at least for some pathogens.
The aim of this study was to evaluate a simulated real-time transmission surveillance in a tertiary care hospital by combining epidemiologic data with these strain typing techniques. The performance of FTIR spectroscopy was also compared to WGS for different bacterial species.
Methods: All bacterial isolates (n=1543) of *Staphylococcus aureus*, *Stenotrophomonas spp.*, *Acinetobacter spp.*, *Pseudomonas spp.* and *Enterobacterales* (excluding *Escherichia coli*) were collected from routine microbiologic diagnostics for two months. Hospital occupancy data was constantly screened by an algorithm for potential patient-to-patient transmissions, which was defined as identification of the same bacterial species in specimens from two patients that occupied the same room at the same time. Bacterial isolates involved in these potential transmissions were subjected to both FTIR spectroscopy and WGS for strain typing to verify or exclude transmission.
Results: Screening of epidemiologic data revealed 103 potential patient-to-patient transmissions involving 94 isolates of six different bacterial species, but only 32 (31.1 %) of these cases were confirmed by WGS. Overall, FTIR spectroscopy exhibited a sensitivity of 84.4 % and specificity of 93.5 % for transmission detection when compared to WGS. The performance of FTIR spectroscopy was highly species-dependent: While the concordance of the two methods was high for *Enterobacter cloacae* (sensitivity 93.3 %, specificity 100 %) and *Klebsiella pneumoniae* (sens. 76 %, spec. 100 %), the agreement was rather low for *Staphylococcus aureus* (sens. 50 %, spec. 84 %). For *Pseudomonas aeruginosa* none of the suspected transmissions were confirmed neither by WGS nor by FTIR spectroscopy. For *Acinetobacter spp.* and *Stenotrophomonas spp.* the agreement of both methods was 100 %, but the number of suspected transmissions was very small.
Conclusions: We present a feasible and straightforward tool for transmission surveillance by combining epidemiologic information with FTIR spectroscopy and WGS for strain typing. The performance of the former as a strain typing technique is highly species-dependent and therefore has to be determined for each bacterial species individually.

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Outbreak analysis of vancomycin-resistant Enterococcus faecium isolates in an internal intensive care unit using cgMLST and split k-mer method in combination

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Background: In Germany, the increasing occurrence of vancomycin resistant Enterococcus faecium (VREfm) is associated with the presence of dominant strain types as ST117 or ST80 [1]. Although whole genome sequencing (WGS) using core genome MLST (cgMLST) analysis is considered as a high-resolution method to elucidate VREfm outbreaks, its power to discriminate VREfm isolates with similar genomes, like ST117 isolates, is limited. The objective was to investigate a VREfm outbreak with cgMLST and split k-mer analysis (SKA) in combination.
Methods: This outbreak report includes n=60 VREfm isolates obtained from an internal intensive care unit (ICU) from 01-2021 to 04-2022. The isolates were collected from patient samples (n=53; patients n=51) and environmental samples (n=7). Infection control professionals investigated epidemiological data. All isolates underwent WGS using Illumina Nextera XT Library Prep and Illumina MiSeq System with 250-cycle paired end chemistry. For sequence data analysis, we used the cgMLST-approach with Ridom SeqSphere+ (published cgMLST task template with default threshold ≤20) and SKA following the published study from Higgs and colleagues (threshold 7 SNPs) [2, 3].
Results: The cgMLST analysis of all isolates showed that 75% (n=45) belonged to ST117, 22% belonged to ST80 (n=13) and 3% (n=2) to ST78. The overview of the different clusters regarding cgMLST and split k-mer analysis is shown in figure 1. SKA determined clusters with smaller size and more singletons, which is concordant with the local epidemiology as identified by infection control professionals. The cgMLST approach assigned more isolates to one cluster without any obvious epidemiological link.
Discussion: Using WGS we identified a polyclonal VREfm outbreak in an internal ICU and we were able to elucidate the outbreak at a higher discriminatory level and with stronger accordance to epidemiological links with SKA in comparison to cgMLST analysis regarding the dominant strain types ST117 and ST80. Nevertheless, cgMLST is an appropriate starting point for outbreak analysis, and both approaches should be considered for routine investigations.
Figure 1. Cluster overview of all VREfm isolates (n=60) using cgMLST and split k-mer analysis (SKA).
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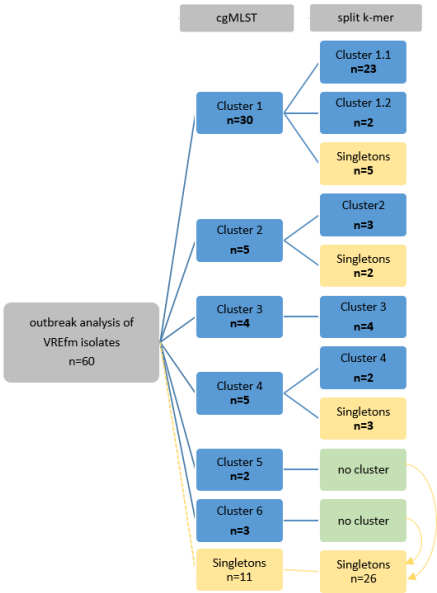
Fig. 1



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Bacterial transmissions from sink drain to patients in a neonatal intensive care unit – a retrospective analysis and evaluation of the current German guideline

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Introduction: Transmission of Gram-negative bacteria from water systems to patients in health care facilities is a feared complication. Hence, the current German national guideline on hygiene requirements for wastewater-carrying systems recommends avoiding the implementation of washbasins in units comprising immunocompromised patients that have an increased risk of acquiring infections. When a new neonatal intensive care unit (NICU) in our tertiary-care hospital was planned, we performed a retrospective evaluation of two nosocomial clusters with Gram-negative pathogens to determine the extent of potential risks from water diverting systems in the present NICU and to evaluate the current national guideline for this patient clientele.
Material/method: During 2017 to 2022, two clusters of Gram-negative bacteria, namely one *Klebsiella oxytoca* and one *Pseudomonas aeruginosa* cluster, occurred on the NICU uncovered by clinical routine screening according to the German national screening guidelines for neonates and microbiological breastmilk surveillance. Subsequent infection control investigations included sampling of washbasins; the detected pathogens were subjected to whole genome sequencing (WGS) to determine their genetic relatedness using core genome cg multilocus sequence typing (cgMLST).
Results: In 2017, *K. oxytoca* was isolated from six patient samples during routine microbiological screening and additionally from environmental sampling of washbasins in two separate rooms. cgMLST showed that all six isolates formed a genetic cluster with either identical or closely related genotypes that differed in ≤ 4 alleles. In 2021/22, *P. aeruginosa* was isolated from seven patients and two samples of breastmilk originating from two different individuals. During the subsequent environmental sampling, *P. aeruginosa* was found in five samples that were collected from four different washbasins in separate rooms. Again, cgMLST identified two clusters, of which one was comprising genetically closely related environmental and breastmilk isolates, whereas the other cluster only consisted of closely related washbasin isolates.
Discussion: Wastewater systems in health care facilities treating immunocompromised patients can serve as a relevant source of especially Gram-negative pathogens. Our observations underline that also implementation of sink drains in a NICU may have negative effects on patients" safety. Construction planning should concentrate on the avoidance of washbasins in patient rooms when redesigning sensitive areas like a NICU. In those areas, the use of waterless care for patients is preferable for hygienic reasons.

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Identification of VRE reservoirs in patient environment

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Question: Vancomycin resistant enterococci (VRE) are responsible for many nosocomial infections worldwide. In Germany, approximately 1000-4000 deaths are attributed to infections associated with multidrug resistant organisms (MDRO) per year (Gastmeier et al., DMW 2016, 141(6):421-426) . Since their first detection in Europe in the late 1980s, prevalence of VRE as well as other MDROs has steadily increased. Besides person to person transmission, contaminated surfaces and especially the patient environment in hospitals are important reservoirs for VRE as they are able to survive on surfaces up to several months. Therefore, we aimed to identify reservoirs of VRE in two wards of a tertiary care hospital in Germany.
Methods: We investigated the environmental contamination with VRE in an intensive care and a haemato-oncologic ward of a tertiary care hospital in Germany. In our screening, we enclosed 30 areas, the bulk being high-touch locations. These locations were regularly screened at first bi-monthly and later monthly over the course of half a year. Screening was conducted with Poly-Wipes in randomly selected patient rooms occupied by non-VRE patients, right or shortly after rooms were cleaned by service personnel. The findings were graphically illustrated using Tableau (Tableau Software; Seattle, USA) and regularly communicated to healthcare and cleaning personnel on the respective wards. Samples were typed using multi-locus sequence typing.
Results: In total, out of 539 screened points VRE could be detected in 46 cases. Some of the locations have been screened positive for VRE repeatedly. VRE were most frequently detected in commode chairs followed by emergency- and others carts. Comparison of both wards yielded less VRE detections on the intensive care ward (9 of 239 screened locations) than on the normal care ward (37 out of 300 screened locations). Fortunately screening points in close patient environment were mainly uncontaminated. Interestingly, multi-locus sequence typing exhibited that there are two different dominating VRE sequence types, which are separately existing on the two wards.
Conclusions: Although transmission via contaminated surfaces is well documented in literature, data on exact locations is sparse. Out of a user perspective Poly-Wipes were better suitable in comparison to contact plates, especially when curved surfaces were screened. Here, we could illustrate the most VRE contaminated locations at an intensive care and a heamato-oncologic ward. These points emerged mostly as objects with ambiguous cleaning responsibility. Consequently, at some of these points, responsibilities for disinfection could be updated and optimized.

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Germany's burden of disease of healthcare-associated bloodstream infections due to vancomycin-resistant *Enterococcus faecium* between 2015-2020

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Background: Healthcare-associated infections and antimicrobial resistance pose a substantial threat to population health and health systems worldwide. In Germany, a rise of vancomycin-resistant *Enterococcus faecium* (VREF) isolates in healthcare-associated bloodstream infections (HA-BSIs) was observed. Therefore, we estimate the incidence of VREF positive blood cultures and calculate the annual burden of disease in disability-adjusted life years (DALYs) for HA-BSIs due to VREF between 2015 and 2020 in Germany.
Methods: We used data from the German *Antibiotic Resistance Surveillance* (ARS) system to estimate the incidence per 100,000 inhabitants. A reported VREF blood culture isolate was counted as a proxy of HA-BSI. Copy strains and recurring isolates from the same patient within three months were excluded from the analyses. Antibiotic susceptibility testing for vancomycin was defined according to the laboratories, using CLSI and EUCAST classifications. The incidence was estimated based on the total observed isolates in ARS, taking the coverage of ARS and the cluster effects into account. Incidences were stratified by age, sex, and region. These incidence estimates were used as an input for the *Burden of Communicable Disease in Europe* (BCoDE) toolkit to calculate the attributable DALYs per 100,000 inhabitants for the study period.
Results: A total of 3,417 VREF blood culture positive isolates were observed within ARS between 2015 and 2020. The estimated incidence of VREF HA-BSIs per 100,000 inhabitants increased from 1.4 (95% UI: 0.8-1.9; n = 1,115 isolates) in 2015 to 2.9 (95% UI: 2.4-3.3; n = 2,345 isolates) in 2020. The calculated DALYs per 100,000 inhabitants due to VREF HA-BSIs increased from 8.5 (95% UI: 7.3-9.7; YLD = 0.9, YLL = 7.6) in 2015 to 15.6 (95% UI: 14.6-16.6; YLD = 1.6, YLL = 14) in 2020. The current highest burden and increasing trends are detected in the north-eastern

region (5.1 [95% UI: 4.6-5.6]; YLD = 0.5 YLL = 4.6) and south-eastern region (5.5 [95% UI: 4.9-6.1]; YLD = 0.6, YLL =4.9), compared to the lower DALYs in the southwestern region (2.2 [95% UI: 1.9-2.2]; YLD = 0.2, YLL =2), north-western region (1.5 [95% UI: 1.2-1.8]; YLD = 0.1, YLL =1.4) and the western region (1.5 [95% UI: 1.3-1.6]; YLD = 0.2, YLL =1.3) within the observed period. The most affected age groups are the 65-69-year-old and neonates accounting for 13.8% and 2.9% (43.8% in the younger age groups < 30 years) of the total burden, respectively. Men are more at risk of HA-VREF-BSIs in comparison to women with a shared burden of 66.8% vs. 33.2%, respectively.

Discussion: The increasing DALYs due to HA-BSIs due to VREF require targeted prevention to address its adverse impacts, especially in Germany, where the incidence strongly increased. In particular, elder hospitalized men and neonates may be the target groups. A better understanding of its increase and regional differences requires continuous monitoring to improve prevention and control measures.

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SARS-CoV-2 surveillance in an inpatient setting

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Introduction: During a pandemic, ongoing adjustments of hygiene measures to the respective situation in the inpatient setting are necessary. The systematic collection and interpretation of data from SARS-CoV-2 patients (surveillance) can be used, among other things, as a basis for adaptation. Surveillance should be carried out with as little time as possible and automatically.

Materials and methods: The derivation of coding data of all hospitalized SARS-CoV-2 patients as well as the evaluation of nosocomial or brought infection, patient age and intensive care takes place automatically. According to an internal definition, the classification in nosocomial cases is automated from day 6 and as community transmission on day 1 to 3. Cases with diagnostics on day 4 and 5 are classified manually according to admission diagnosis and symptoms. Cases in a likely epidemiological context are assigned to a cluster with a corresponding Cluster-ID.

Results: In the period from 16.03.2020 to 31.03.2022, a total of 1,991 patients were hospitalized at the University Hospital (Fig. 1), whereby 83% of the patients had an community transmission infection according to the internal definition. Over 50% of patients with nosocomial infection belonged to a cluster. Stay in the intensive care unit after initial detection as well as the nosocomial proportion of SARS-CoV-2 patients varied in the individual phases (Fig. 2).

Fig. 1: Epidemiologic curve of inpatient SARS-CoV-2 cases by calendar week (CW)

Fig. 2: Nosocomial proportion, proportion of COVID-19 patients treated in intensive care in the individual phases according to Robert Koch- Institute (RKI)[1]

Discussion: Currently, there is no uniform definition for nosocomial SARS-CoV-2 infections in Germany. Thus, according to the internal definition, there was probably an overcoverage. If the case definition of ECDC[2] is chosen, the rate for definite healthcare-associated (> day 14 after admission) decreases to 6% in the present surveillance. In particular, if hospital hygiene measures are derived from the surveillance, such as an extension of the testing regime, a definition that is as precise as possible is essential in order to avoid drawing false conclusions. In the individual phases, there were different proportions of nosocomial infections and stays in the intensive care unit, which presumably depended on the prevailing variant of concern (VoC) and required additional measures. Surveillance provides a quick overview of the situation and the possible need for appropriate measures.

References:

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2. European Centre for Disease Prevention and Control (ECDC): Surveillance definitions for COVID-19

Fig. 1

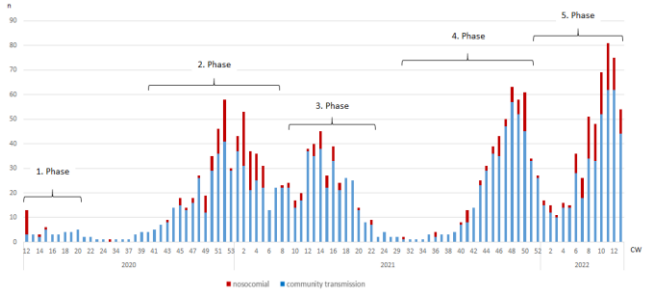

Fig. 2

	1. Phase	2. Phase	3. Phase	4. Phase	5. Phase
CW	10-20/2020	40/2020-8/2021	9-23/2021	31-51/2021	52/2021-13
VoC			Alpha	Delta	Omikron
Proportion of NI %	27,3	20,4	10,6	12,2	21,6
Proportion of ICU-Patients	20,5	12,5	15,6	9,6	1,8

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Nasal MRSA colonisation of veterinary staff in equine clinics – current situation and use of barrier precautions

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Introduction: MRSA, in particular LA-MRSA CC398, has become a widely disseminated nosocomial pathogen in equine clinics1. The comparatively high prevalence of MRSA colonization of veterinary teams (involving all members of the staff) was the reason for this study. Here we report on the dynamics of this colonization and on the use of barrier precautions.

Materials and methods: From 2018- 2020 nasal MRSA colonization of veterinary team was recorded in 17 equine hospitals which are located all over Germany. In parallel, participants were educated about basic hygiene regimens and the use of barrier precautions (surgical face-masks and disposable gloves). Sampling, bacteriological diagnostics, molecular typing methods (*spa*-typing, for selected isolates Multi-Locus Sequence Typing (MLST), whole genome sequencing (WGS), and antibiotic susceptibility testing (evaluation by EUCAST) was performed as described previously2.

Results: The average initial MRSA-prevalence of 18% dropped to 14% and finally to 12% with differences between individual clinics. Four of these clinics were particularly successful: 27,8% à11,8%; 25% à0,0%; 12,5% à0,0%; 10% à 0%. Molecular typing attributed 85% of the isolates to the animal hospital associated subpopulation of LA-MRSA CC398, while 15% corresponded to LA-MRSA CC398 known from livestock. High resolution WGS based typing of the isolates from a large clinic revealed that there was not an endemic situation with one particular strain but rather continually introduction of MRSA. Barrier precautions revealed as effective for reduction of MRSA carriage. Among the veterinary assistants 71% reported wearing of gloves for relevant activities, 51% used face-masks. The use of these precautions was less frequently reported from veterinarians (29% and 64% respectively). In 4 of the clinics we observed a rise of the prevalence in 2021 although barrier precautions were not used less frequently. This observation points to deficiencies in regimens of basic hygiene. Eradication of nasal MRSA carriage by means of mupirocin ointment was successful for 20 among 24 persons, for 4 persons an additional treatment was necessary.

Conclusions: Although we observed a reduction of nasal MRSA colonization during the course of the study there is still need for optimization with respect to basic hygiene regimens into which the use barrier precautions must be embedded and to recording of MRSA positive patients at admission.

Funding: The study was performed within the framework of the German Research Network on Zoonotic Infectious Diseases;

funded by the German Federal Ministry of Education and Research (BMBF): project #1 Health-PREVENT, grant no. 01KI2009F.
References: 1 Cuny C. et al. *One Health* 2015; 2: 11-17. 2 Cuny C. et al. Microorganisms 2022; PMID: 35456729.

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The hidden secrets of *Serratia marcescens* in the hospital sewage system

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Background *Serratia marcescens* was considered non-pathogenic to humans for many years. Nowadays, it is accepted that *S. marcescens* is an important pathogen of nosocomial infections and is regularly detected in the inanimate environment during outbreaks. In this study, carbapenemase producing clinical isolates were compared with isolates from siphons and hospital wastewater for more than five years.
Methods Samples were collected from sink traps in ICU rooms (October 2016 to January 2022) and from the hospital main sewer line (in November 2019 and February 2021). The presence of *S. marcescens*, resistant to third-generation cephalosporins, was analyzed by culturing on selective agar with subsequent identification of β-glucosidase-producing isolates by mass spectrometry (Biotyper, Bruker, Bremen, Germany), and whole genome sequencing was performed for representative isolates (n=169). Furthermore, carbapenem resistant clinical isolates were included. Clonal relatedness was investigated by automated analysis of an *ad hoc* core genome (cgMLST, 3177 alleles) using SeqSphere+ software (Ridom, Münster, Germany). Clonal relatedness was defined as a difference of maximum 10 alleles. Resistance gene analysis was performed by ResFinder software (Center for Genomic Epidemiology, Copenhagen, Denmark).
Results Five clonally closely related lineages (KL1-KL5) were identified to persist for prolonged periods at different locations in the sewage system. KL1, KL2, and KL3 were associated with nosocomial cases. KL1 isolates (n=73) were detected in siphons, wastewater, and four patients. At initial detection in a siphon and subsequent in two patients in 2016, KL1 isolates were positive for CTX-M-9, OXA-48, SHV-12, and VIM-2. Over time, KL1 isolates were observed with CTX-M-15, OXA-1, and OXA181, which were subsequently detected twice in patients. In wastewater, one isolate each was identified with an additional GES-5 or OXY-2-8-like gene. KL2 isolates (n=64) were identified exclusively in siphons and one patient. Within KL2, variable detections of β-lactamases, including an OXA-48 or OXA-181, also appeared during the course. KL3 isolates (n=16) were identified in siphons, wastewater, and one patient in 2016. As of 2019, individual isolates showed newly acquired VIM-2 or GES-5. Isolates of KL4 (n=14) were detected exclusively in siphons and showed no newly emerging β-lactamases. Isolates of KL5 (n=8) were detected in siphons and wastewater and showed no acquisition of β-lactamases.
Conclusions The data collected show that single clonal lines of *S. marcescens* persist for long periods of time in the wastewater system of a hospital. In addition to the continuous release of these carbapenemase-positive clones into the municipal sewage system, retransmission from siphons to patients may occur in rare cases. The data of this study indicate that relevant horizontal gene transfer may already occur in the draining wastewater pathways starting from the siphon.

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Resistance rates in uncomplicated urinary tract infections in Germany within the REDARES project

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Background: Uncomplicated urinary tract infections (UTIs) are common bacterial infections in outpatient care and are usually treated with empiric antibiotic therapy. The relevant S3 Guideline gives recommendations on the choice of antibiotic and suggests to take the local resistance situation into account. Current local resistance data are often not available, since microbiological diagnostics are primarily recommended for complicated UTIs.
Frequently occurring recurrent UTIs are often categorized as uncomplicated UTIs, but these are characterized by higher levels of resistance compared to non-recurrent UTIs.
Question: How high are the current resistance proportions of *E. coli* in uncomplicated UTIs in Germany?
Methods: Using a cross-sectional study design, the resistance proportions of *E. coli* to antibiotics recommended as first (fosfomycin, nitrofurantoin, nitroxolin, pivmecillinam, trimethoprim) respectively second-line (cotrimoxazole, ciprofloxacin, cefpodoxime, levofloxacin, ofloxacin, norfloxacin) in the S3 guideline for community-acquired uncomplicated urinary tract infections were determined in Germany (divided into 5 regions). UTIs in women over 18 years of age without other relevant concomitant diseases were considered uncomplicated. These were compared with uncomplicated recurrent UTIs (2 or more episodes of UTIs within the last 6 months or use of an antibiotic in the last 2 weeks). Urine samples were taken and physicians completed a short questionnaire including the frequency of occurrence of the UTIs. Species identification and antimicrobial susceptibility testing were performed by automated systems. The results were interpreted according to EUCAST standards.
Results: A total of 8 laboratories and 138 general practitioners distributed over 4 regions participated in the study. One region was excluded because not enough study participants could be recruited. 2,996 patients were included in the analysis. Overall, 1,816 (60.0%) patients (mean age 55 years; range 18-98) had a positive urine culture with ≥10^3 CFU/ml. *E. coli* was identified in 1,070 (58.9%) of these samples. Of these, 648 were non-recurrent and 422 were recurrent UTI.
Resistance proportions of *E. coli* in non-recurrent UTIs throughout all of Germany were 14.1% (trimethoprim) and 11.4% (cotrimoxazole), respectively, and less than 10% for all other antibiotics studied. For recurrent UTIs resistance proportions were higher. These differences were significant for trimethoprim (21.8%) and cotrimoxazole (20.1%), but not for all other antibiotics. Resistance proportions for all examined antibiotics are presented in Table 1.
Conclusions: Accurate data should be made available to the treating physicians. Knowledge of local resistance data is essential for evidence-based antibiotic prescribing. There is a substantial difference in resistance of *E. coli* between non-recurrent and recurrent UTIs.

Fig. 1

	Uncomplicated non-recurrent UTI				Uncomplicated recurrent UTI		
	Antibiotic	Resistance proportion	95%-CI	resistant/n	Resistance proportion	95%-CI	resistant/n
First-line antibiotics	Mecillinam	4.26%	2.71-6.63%	18/423	5.08%	3.11-8.22%	15/295
	Fosfomycin	0.87%	0.37-2.01%	5/577	0.80%	0.27-2.32%	3/376
	Nitrofurantoin	0.15%	0.03-0.87%	1/647	0.71%	0.24-2.08%	3/420
	Nitroxolin	0.68%	0.23-1.98%	3/441	1.06%	0.36-3.07%	3/283
	Trimethoprim	14.06%	11.58-16.97%	90/640	23.24%	19.43-27.55%	96/413
Second-line antibiotics	Cefpodoxime	4.62%	3.04-6.95%	21/455	8.36%	5.73-12.05%	25/299
	Norfloxacin	8.21%	5.11-12.91%	16/195	11.22%	6.38-18.99%	11/98
	Ofloxacin	9.52%	5.26-16.65%	10/105	4.55%	1.26-15.13%	2/44
	Levofloxacin	7.48%	5.54-10.02%	40/535	11.68%	8.66-15.56%	39/334
	Ciprofloxacin	6.82%	5.12-9.03%	44/645	12.35%	9.54-15.84%	52/421
	Cotrimoxazole	11.44%	9.21-14.12%	74/647	20.95%	17.33-25.10%	88/420

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Detection of intra-host transmissions of carbapenemase-harbouring plasmids in a hospital setting using long-read whole genome sequencing

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Background: Multidrug-resistant gram-negative bacteria (MDRGN) are a global challenge in today's health care systems. Especially carbapenem resistance limits therapeutic options and challenges infection control measures. In Germany, *bla*$_{OXA-48}$ and *bla*$_{VIM-1}$ are the two most common carbapenemases in MDRGN. Both genes can be transmitted by plasmids via horizontal gene transfer. Here we illustrate, how plasmids and their transmission can be identified in routine hospital screening samples based on long-read-sequencing data.
Methods: At our hospital, all carbapenem resistant MDRGN are sequenced via long-read sequencing. We analyzed datasets of carbapenem resistant *Enterobacterales* collected during a 24 month period from 2020 to 2021 for potential plasmid transmission. All isolates were detected as multidrug resistant as part of routine testing of clinical or screening samples at our hospital. Samples were sequenced on a PacBio® Sequel IIe system. Next, raw sequences were assembled *de novo* and analyzed using the SMRT® Link software suite v.10.1 with default parameters. Antimicrobial resistance genes and their location were determined using target gene sets for antimicrobial resistance based on the NCBI AMRFinderPlus. To predict the plasmid replicon type the respective contigs were checked for complete circularization and uploaded to PlasmidFinder v.2.1. For annotation, we used the online Pipeline dfast and performed BLASTn.
Results: We identified an identical *bla*$_{OXA-48}$-carrying lncL plasmid in a *Klebsiella pneumoniae* and a *Serratia marcescens* isolate sampled from one patient and a *bla*$_{VIM-1}$-carrying IncN2 plasmid in a *Klebsiella michiganensis* and an *Escherichia coli* isolate sampled from another patient, respectively. The IncL plasmid shows a length of 62,812 bp and the IncN2 of 53,293 bp. The *bla*$_{OXA-48}$ is flanked by IS4-family transposases. Next to the *bla*$_{VIM-1}$, we found further multiple antimicrobial resistance genes. Both plasmids show > 99.9% identity in BLASTn analysis and were identified in different bacterial species, strongly suggesting horizontal gene transfer. All of the four bacteria displayed a clinical relevant carbapenem resistant phenotype in accordance to EUCAST breakpoints.
Conclusions: These two cases describe the intra-host transmission of an IncN2 and an IncL plasmid harboring clinically relevant carbapenemases. Their horizontal transfer among different species are one of the most critical events in the spread of antimicrobial resistance. Yet, the impact of horizontal gene transfer on transmissions of carbapenemases in the hospital is unknown. Our cases illustrate how long-read sequencing technology can be supportive as a diagnostic tool to identify and monitor resistance plasmids in the hospital setting. Further studies should investigate this approach and develop algorithms for automated data analysis.

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Antibiotic susceptibility and molecular characterisation of *Staphylococcus aureus* in North-Central Nigeria

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Introduction: *Staphylococcus aureus* (*S. aureus*) is a leading cause of hospital- and community-acquired infections worldwide. The disease prognosis of *S. aureus* is associated with antibiotic resistance and virulence gene carriage and is lineage-specific. There is a paucity of data on *S. aureus* clonal diversity based on regions in Nigeria. Active surveillance and characterization of *S. aureus* isolates are important for adequate infection prevention and control (IPC) measures. This study describes the first report on the antibiotic susceptibility, virulence gene content, and clonal structure of clinical *S. aureus* isolates in North-Central Nigeria.
Methods: Two hundred and twelve *S. aureus* isolates (n=212) from various clinical samples at the University of Ilorin Teaching Hospital (a tertiary health care institution), North-Central Nigeria, were characterized. The susceptibility to antibiotics was determined by Vitek-2. Molecular characterization included PCR detection of methicillin resistance (*mecA*) and Panton-Valentine leukocidin (PVL) genes and staphylococcal protein A (*spa*). One isolate each from the predominant *spa* types was selected for whole-genome sequencing.
Results: All the isolates were susceptible to daptomycin, fosfomycin, fusidic acid, linezolid, mupirocin, rifampicin, teicoplanin, tigecycline, and vancomycin. Also, 99 (46.7%) and 113 (53.3%) isolates were confirmed as methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA), respectively. Other resistance rates are as follows: trimethoprim/sulfamethoxazole (72.6%; n=154), tetracycline (38.2%; n=81), gentamicin (28.3%; n=60), levofloxacin (26.8%; n=57), erythromycin (16.5%; n=35), and clindamycin (1.9%; n=4). The prevalence of the PVL gene was 60.4% (n=128), with similar rates observed among the MSSA (53%; n=68) and MRSA (47%; n=60) isolates. Overall, fifty-eight *spa* types were identified. The dominant *spa* types were t355-ST152 (18.4%; n=39), t091-ST789 (9.4%; n=20), t657-ST772 (9.4%; n=20), t4690-ST152 (7.5%; n=16), and t084-ST15 (5.2%; n=11).
Conclusions: We observed a high burden of MRSA and PVL-positive *S. aureus* at the tertiary institution in North-Central Nigeria. ST152 *S. aureus* is the major clone in this region. These observations call for action on active surveillance and IPC measures in health care institutions in North-Central Nigeria.

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Characterisation of ESBL-producing *Klebsiella pneumoniae* and *Enterobacter cloacae* isolated from poultry meat legally and illegally imported from third countries into the EU

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Question: About 500,000 tonnes of poultry meat is imported into the EU yearly, approximately half of which from Brazil. Poultry meat can serve as a vehicle for multidrug-resistant and ESBL-producing *Enterobacteriaceae*, which can spread widely due to the international trade. Localization of resistance genes on mobile genetic elements may facilitate their dissemination. *Enterobacter cloacae* and *Klebsiella pneumoniae* are a main cause of nosocomial infections worldwide which pose health hazards because of limited therapeutic options. The aim of the study was to determine genotypic characteristics, such as the antimicrobial resistance profile and the occurrence of mobile genetic elements, of *K. pneumoniae* and *E. cloacae* isolated from poultry meat imported into the EU.
Methods: In this study *E. cloacae* isolates (n = 3) and *K. pneumoniae* isolates (n = 3) originating from poultry meat either legally imported from Brazil or illegally from Egypt between 2014 and 2015 were examined. Species identification was performed by MALDI-TOF. Antimicrobial susceptibility to 22 antibiotics/antibiotic combinations and ESBL confirmatory test by broth microdilution were performed and evaluated according to Clinical and Laboratory Standards Institute (CLSI) standards. Isolates were screened for antibiotic resistance genes, virulence-associated genes and mobile genetic elements, such as integrons and plasmids, and pulsed-field gel electrophoresis (PFGE) was performed.
Results: All studied isolates were ESBL-producers and resistant to 3 or more classes of antimicrobials. All *K. pneumoniae* and one *E. cloacae* carried *bla*$_{SHV}$ genes (SHV-2, -12, -27). Two *E. cloacae* showed an ESBL phenotype but were negative for screened ESBL

genes. One *K. pneumoniae* from Egypt carried *bla*$_{SHV-27}$ and *bla*$_{CTX-M-14}$. Up to 10 different resistance genes in one isolate were detected, most mediating resistance to sulfonamides, trimethoprim, quinolones and tetracyclines. Class 1 integrons were detected in all *K. pneumoniae*, which harboured *dfrA12* and *aadA2* in two and *dfrA1* in the third. In two *K. pneumoniae*, the virulence-associated genes *entB and mrkD* were detected and in one *entB*, *mrkD* and *kfu*. PFGE showed the highest similarity within each species was 60%. Presence of plasmids was detected in all *K. pneumoniae.*

Conclusions: Examined *K. pneumoniae* and *E. cloacae* may pose a consumers risk because of their multidrug-resistance and, related to *K. pneumoniae*, their virulence-associated genes. Accumulation of resistance genes within these species is frequently reported but few data are known which ESBL genes are mainly found in poultry meat from Egypt and Brazil. Combination of *bla*$_{SHV-27}$ and *bla*$_{CTX-M-14}$ detected in one *K. pneumoniae* is not commonly reported. Furthermore, multi locus sequence typing (MLST) and conjugation experiments with *K. pneumoniae* will be implemented. Collected data can contribute to detect genotypic characteristics of these isolates originating from poultry meat from Egypt and Brazil.

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Technical and medical evaluation of an algorithm for the digital surveillance of multidrug-resistant organisms (MDRO) and blood stream infections on the intensive care unit (ICU)

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Introduction: The surveillance of hospital-acquired infections in Germany is usually conducted via manual chart review; this, however, proves resource intensive and is prone to a certain degree of subjectivity. Documentation based on electronic routine data may present a worthy alternative to manual methods, which is what we analyzed in order to conduct this study. We compared the data derived via conventional clinical documentation and manual chart review to data that was derived algorithmically from electronic routine data originating from the laboratory and the hospital information system in order to validate the algorithm.

Materials/Methods: Data was obtained from five university medical center ICUs, with a total of 99 beds, over a period of 12 months. Clinical data was collected by the infection prevention and control team according to the Protection against Infection Act (IfSG); documentation thereof was carried out in the electronic hospital information system according to the ICU-KISS module"s protocol provided by the National Reference Center for the Surveillance of Nosocomial Infections (NRZ). Algorithmically derived data was generated via an algorithm originally designed and used in the EFFECT study; ward-movement data was linked with microbiological test results, generating an anonymous data set that allows for evaluation as to whether or not an infection was nosocomial/ICU-acquired. Descriptive statistics were used to indicate frequencies, percentages and measures of central tendency and dispersion (median along with IQR).

Results: The compiled data contains information from 6,460 patients. 12/14 MRSA cases, 35/48 VRE cases and 73/95 MDRGN (multidrug-resistant gram-negative bacteria) cases were classified as ICU-acquired by both manual chart review and the EFFECT algorithm. 85% of ICU-acquired sepsis cases identified via manual chart review (and therefore in line with ICU-KISS criteria) were considered ICU-acquired bacteremia by means of the EFFECT algorithm. Most discrepancies between the two sets of data were due to differing definitions regarding the patients" time at risk for acquiring either an MDRO or bacteremia.

Discussion: The overall correlation between manual chart review and algorithmically generated data regarding ICU-acquired infections was high. This study not only suggests but also shows that hospital infection surveillance based on electronically generated routine data is a worthwhile and sustainable alternative to the resource-intensive and time-consuming method based on manual chart review.

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Benefits of whole genome sequencing for MRSA infection control in a rural hospital in Germany

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multi-resistant pathogen associated with nosocomial and community-acquired infections, with increased risk for the patient"s outcome as well as an infection source for other patients in a hospital. Therefore, early MRSA detection and implementation of prevention strategies are required. However, regular diagnostic assays cannot distinguish between sporadic and outbreak strains. Whole genome sequencing (WGS) allows detailed genotyping of MRSA strains, identification of resistance genes as well as traceability of MRSA-clusters.

Methods: In 2021, we performed a retrospective survey in a 620-bed secondary care hospital and analyzed the potential benefits of regular WGS typing of MRSA isolates. A total number of 15.602 patients with increased MRSA-risk were screened by selective culture and positives confirmed by MALDI-ToF, PBP2a presence and antibiotic resistance testing. For 180 MRSA positive patients detailed genotyping analysis of isolates was done by WGS. Core-genome multilocus sequence typing (cgMLST) with cluster analysis, outbreak detection, and resistance genes analysis were performed with SeqSphere+ software (v 8.3.4).

Results: 180 MRSA results were obtained and assigned to the following sequence types: 38% CC398, 14% CC22, 9% CC225, 4% CC5/CC45/CC9, respectively, and others. Phylogenetic analysis confirmed that the majority of isolates were unrelated despite predominant sequence types. Few outbreaks with almost identical strains (< 12 allele differences) could be confirmed. One suspected MRSA outbreak in the neonatology, encompassing 7 persons (1 parent, 6 infants incl. 2 pairs of twins) could be subdivided into 2 independent clusters without direct transmission. Moreover, two MRSA clusters originating from nursing homes were without direct link in the hospital. These cases were hospitalized at non-overlapping time points but could be assigned to clonal clusters originating in the same long-term care-facility. Besides outbreak analysis, WGS showed that all regional livestock-associated MRSA CC398 isolates had characteristic mutations in fosfomycin resistance genes, *glpT* and *murA*. However, these mutations did not yet confer phenotypic resistance to fosfomycin.

Discussion: Regular WGS provides information on the predominant MRSA lineage, which was shown here for LA-MRSA in a secondary care hospital in a rural area. Overall, analysis was able to proof the effectivity of the implemented transmission control under routine conditions and to discriminate suspected hospital associated outbreaks. Additionally, regular WGS of hospitalized patients can identify transmissions outside the hospital, as shown here by the detection of various clonal MRSA clusters in different nursing homes. Multiple benefits of regular MRSA WGS typing for management of infections control will become more evident as soon as onsite testing with availability of results within few days can be implemented.

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Patients with multidrug-resistant organisms feel inadequately informed about their status – adverse effects of contact isolation

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Question: Contact isolation of patients with multidrug-resistant organisms (MDRO) is an essential element of infection prevention strategies in hospitals. However, this practice may be associated with adverse side effects. This study is the first to assess mental health and well-being variables among isolated patients compared to control patients in a German cohort.
Methods: We conducted a matched case-control study among N = 267 patients admitted to a tertiary care hospital in Germany. Their levels of anxiety, depression, loneliness, and dissatisfaction with their hospital experience were assessed using a questionnaire. Additionally, among isolated patients, it was evaluated how well they felt informed about their MDRO-status.
Results: In our cohort, patients under contact isolation were significantly more dissatisfied than control patients but did not show higher levels of anxiety, depression, and loneliness. A large proportion of patients felt insufficiently informed about their MDRO-status. This lack of information was the strongest predictor of dissatisfaction among isolated patients.
Conclusion: These findings underline the importance of adequate patient communication. It is essential for patients" well-being to receive timely, relevant, and understandable information about the background and consequences of their infection or colonization with MDRO.

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Molecular epidemiology, clinical course and implementation of specific hygiene measures in hospitalised patients with *Clostridioides difficile* infection in Brandenburg

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Introduction: Over the past 20 years, the incidence and severity of *C. difficile* infections (CDI) has increased worldwide, and the disease has become one of the most common hospital-acquired infections. Some strains of *C. difficile* are referred to as hypervirulent, such as RT027. Currently, published data on the prevalence and distribution of different strains in hospitals in Northeast Germany is scarce. Furthermore, little is known about follow up on patients with CDI from this region, or within what time frame specific hygiene measures were applied after laboratory confirmation of the diagnosis. This work aimed to evaluate the molecular epidemiology of *C. difficile* strains, the clinical course of the disease, and the time of initiation of specific hygiene measures in patients with CDI in a hospital in Brandenburg, Germany.
Material/Method: The study was conducted as an analytic prospective epidemiologic study. Fecal samples from hospitalized patients diagnosed with CDI in a large tertiary hospital in Brandenburg from October 2016 to October 2017 were included. Clinical information about patients, clinical outcome, and hygiene measures was collected. Patients with CDI were followed up for 30 and 90 days after laboratory confirmation of the diagnosis. The pathogens were isolated and identified as toxigenic *C. difficile*. Thereupon, ribotyping was performed.
Results: A total of 112 patients were diagnosed with CDI and (median age 77,6 years) met the inclusion criteria for the study. The overall prevalence of CDI was 0.28 per 100 patients, whereas the incidence density was 0.35 per 1000 patient days. The infection could be designated as nosocomial in 92,1% of patients according to the ECDC criteria. The recurrence frequency within 30 and 90 days after CDI diagnosis was 15.7% and 18.6%, respectively. The lethality in the study population was 21.4% and 41.4% within 30 and 90 days of diagnosis. Among the 99 *C. difficile* isolates that were available for further analysis, 22 different *C. difficile* ribotypes were detected. The most frequent ribotypes were RT027 (31.3%), RT014 (18.2%), and RT005 (14.1%). Barrier or single-room isolation was initiated in 95.7% of CDI patients. These measures were performed in half of the patients on the same day as the onset of diarrhea. The switch to sporicidal surface disinfectants occurred in 42% of cases with confirmed CDI.
Discussion: Our study shows that CDI is prevalent among hospitalized patients in this region. The vast majority of cases were healthcare-associated. The 30 and 90 days lethality after diagnosis of CDI was unexpected high, although comparable data from this region is missing. This was associated with a high percentage of RT027 among the clinical isolates. This data highlights the importance of rapidly initiated and specific hygiene protocols to prevent and control CDI and the need to improve CDI's molecular surveillance at the local and national level.

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The epidemiology and mortality of antibiotic resistance in clinical infections in Germany – a systematic review and meta-analysis

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Question: Antibiotic resistance is of great public health concern worldwide. This study is a comprehensive summary of the epidemiology and mortality of antibiotic resistance in clinical infections in Germany.
Methods: We conducted a systematic review and meta-analysis of published German studies, including data from the national *Antibiotic-Resistance-Surveillance* (ARS) of the Robert Koch-Institute (PROSPERO ID: CRD42022306576). Primary outcomes were (i) antibiotic resistance proportions in bacterial isolates from clinical infections (study publication date: 2016-2021) and (ii) the associated all-cause mortality (2010-2021). Time trend analyses and the comparison of resistance proportions in the hospital *vs.* outpatient sector were performed using ARS data (2014-2020).
Results: Out of 3226 unique records identified in the systematic literature search, 60 studies and data from the ARS database were included. Between 2014 and 2020, there was an increase of vancomycin resistance proportions in *Enterococcus faecium* (VRE) isolates from bloodstream infections (odds ratio [OR]: 1.18, $p<0.0001$) with a pooled proportion of 34.9%. In contrast, methicillin resistance in *Staphylococcus aureus* (MRSA) infections decreased in the same period (OR: 0.89, $p<0.0001$) and the pooled resistance proportion was 7.9%. All-cause mortality in MRSA and VRE infections were higher compared to infections with susceptible strains (OR: 2.29 and 1.69, respectively). Carbapenem resistance was low (<4%) in infections with *K. pneumoniae*, *A. baumannii*, and *E. coli* and moderate in *Enterobacter* spp. (5-9%), but relatively high (17%) in *P. aeruginosa* isolates. Moderate third-generation cephalosporin resistance proportions (10-11%) were observed in *K. pneumoniae*, *P. aeruginosa* and *E. coli* isolates but the reported third-generation cephalosporin resistance proportion in *Enterobacter* spp. was 19%. Fluoroquinolone resistance in Gram-negative pathogens was moderate to high (5-25%) and a rising trend was observed in *P. aeruginosa* (OR: 1.22, $p<0.0001$, pooled resistance proportion: 25%). In. *E. coli*, the most frequent cause of urinary tract infections, high resistance proportions were observed for trimethoprim (25%), penicillins (51%) and penicillins + β-lactamase inhibitors (31%), while resistance against nitrofurantoin and fosfomycin was low (≤2%). Importantly, nationwide ARS surveillance data show that antibiotic resistance proportions in clinical isolates from patients treated in hospitals are higher compared to resistance proportions in isolates from the outpatient sector.
Conclusion: Major bacterial pathogens isolated from clinical infections frequently harbour antibiotic resistances and are often associated with increased all-cause mortality. Continuous efforts in surveillance and infection prevention and control as well as antibiotic stewardship are needed to limit the spread of antibiotic resistance in Germany.

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Reserve antibiotics – exemption from the benefit assessment according § 35a SGB-VA successful pull mechanism

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Introduction: Due to an amendment to §35a SGB V, reserve antibiotics are exempted from the benefit assessment of the Federal Joint Committee (G-BA). This means that for antibiotics classified by the G-BA as reserve antibiotics according to §35a, the additional benefit is considered proven, without pharmaceutical companies having to prove it through corresponding studies. This is a pull mechanism that is intended to promote the development and market introduction of new antibiotics against multi-resistant bacterial pathogens (MRB).

The amendment includes the following tasks for the Robert Koch Institute (RKI), which are to be implemented in cooperation with the Federal Institute for Drugs and Medical Devices (BfArM):

1. Development of criteria for the classification of an antibiotic as a reserve antibiotic
2. Development of a non-exhaustive list of MRB
3. Statement on the quality-assured use of a reserve antibiotic, considering the effects on the resistance situation in the event of classification as a reserve antibiotic.

Method: The criteria were defined after consultation of experts using the DELPHI method, including the WHO-AWaRe categorization and subsequent comments by representatives of national reference centers, relevant professional societies and other external institutions.

The World Health Organization"s Pathogen Priority List (WHO PPL) provided the basis for the selection of pathogens for the pathogen list to be compiled. Further criteria were defined for adaption to the national context. The basis for this data was the *Antibiotic-Resistance-Surveillance* system (ARS) as a continuously ongoing surveillance system. The focus was on the proportion of resistances and the development of trends. In addition, other parameters were considered including mandatory reporting and clinical relevance.

Results: The main criteria for classification as a reserve antibiotic were determined as efficacy against relevant MRB and limited therapy options. The classification is carried out using an indicator list and a flow chart.

With the exception of fluoroquinolone-resistant non-typhoidal *Salmonella* and vancomycin-resistant *Staphylococcus aureus*, the pathogen list includes all WHO PPL pathogens as well as *Burkholderia-cepacia* complex and *Stenotrophomonas maltophilia*, being relevant in Germany.

A first statement on quality-assured use was prepared in the consultation process for Cefiderocol.

Conclusion: By amending §35a SGB V and its implementation it is now possible for the G-BA to classify new antibiotics as reserve antibiotics on the basis of criteria and a non-exhaustive list of MRB. This accelerates and facilitates the market introduction of new antibiotics and thus represents an important incentive for investment in their development.

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Surveillance of multidrug-resistant bacteria within a newborn unit of a Kenyan Tertiary hospital

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Introduction: Neonatal mortality in sub-Sahara Africa, including Kenya, remains at a high level. Severe newborn infections with multidrug-resistant organisms (MDRO), and especially carbapenem-resistant organisms (CRO), substantially contribute to this problem. This study represents the initial phase of a joint effort of Kenyan and German clinical scientists and aims at assessing colonization prevalence of MDRO and pinpointing transmission events in a newborn unit at a Kenyan tertiary hospital. Besides routine microbiology, whole genome sequencing (WGS) and hospital surveillance data were used to assess antimicrobial resistance genes and phylogenetical relatedness of 160 isolates.

Methods: During a four-month study period, a total of 1,097 swabs, deriving from 300 mother-baby pairs as well as samples from the hospital environment were taken and assessed using routine clinical microbiology methods. Of the 288 detected MDRO, 160 isolates were then molecularly characterized using whole genome sequencing (WGS) and bioinformatic analysis.

Results: A high prevalence of MDRO in maternal vaginal swabs was found (15%; n=45/300, including 2% CRO; n=7/300). Likewise on admission, MDRO detection rate for neonates was in a similar range (16%; n=48/300, including 3% CRO; n=8/300). However, a threefold increase for MDRO was observed until discharge (44%, n=97/218) and an even fivefold increase for CRO (14%, n=29/218). MDRO were also identified in environmental samples (11%; n=18/164, including 2% CRO; n=3/164). Molecular characterization revealed that among CRO, K. pneumoniae harboring blaNDM-1 (n=20) or blaNDM-5 (n=16) were most frequent. WGS analysis further unveiled 20 underlying phylogenetically related transmission clusters (including five CRO clusters).

Conclusion: This study characterizes MDRO and CRO found in a Kenyan NBU during a four-month period. Rather than a single outbreak scenario, data indicate independent transmission events. Especially, the CRO rate among newborns attributed to spread of NDM-type carbapenemases is worrisome. Das diesem Bericht zugrundeliegende Vorhaben wurde mit Mitteln des Bundesministeriums für Bildung, und Forschung unter dem Förderkennzeichen 01KA1772 gefordert. Die Verantwortung für den Inhalt dieser Veröffentlichung liegt beim Autor.

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Emergence and polyclonal dissemination of NDM-5-producing *Escherichia coli* in Germany

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Introduction: The emergence and rapid dissemination of carbapenemase-producing Enterobacterales (CPE) pose a major threat to public health. Although prevalence of CPE in Germany is comparatively low, increasing numbers of CPE, specifically in *Escherichia coli*, were detected since 2013. Besides the rapid spread of OXA-244-producing *E. coli*, we also observed an increase of *E. coli* isolates producing the New Delhi metallo-β-lactamase 5 (NDM-5). As part of our ongoing molecular surveillance program, we here provide a detailed investigation of NDM-5-producing *E. coli* in Germany. We used whole genome sequencing (WGS) to characterise the genomic epidemiology of NDM-5-producing *E. coli* isolates circulating in Germany during 2013 and 2019.

Material and methods: Between 2013 and 2019, a total of 325 non-duplicate NDM-5-producing *E. coli* isolates were identified including 224 isolates with information on hospital origin which were subjected to Illumina whole-genome sequencing. Analyses of 222 successfully sequenced isolates included multilocus sequence typing (MLST), core genome (cg)MLST and single-nucleotide polymorphism (SNP)-based analyses. Several isolates were also sequenced using ONT long-read technology to generate hybrid assemblies of full-length blaNDM-5 plasmid sequences which along with publicly available data were used for reference-based mapping.
Results: A total of 43 different sequence types (ST) were identified, of which ST167, ST410, ST405 and ST361 were the most prevalent accounting for more than half of the isolates (52.7%). Results of cgMLST revealed many genetically distinct clusters, particularly among isolates of ST167, ST410, ST405 and ST361/10042. Phylogeographic SNP analyses identified sporadic cases of nosocomial transmissions on a small spatial scale. However, large clusters corresponding to clonal dissemination of ST167, ST410, ST405 and ST361 strains were found in different regions from Germany that were collected in consecutive years. Detailed sequence analyses indicate a predominance of NDM-5-encoding IncF- and IncX3-type plasmids.
Conclusions: Here, we show that the increase of NDM-5-producing *E. coli* in Germany was to a large extent due to isolates belonging to the international high-risk clones ST167, ST410, ST405 and ST361. Of particular concern, we provide evidence for the dissemination of strains of these epidemic clones on a supra-regional scale. We further showed the dissemination of the blaNDM-5 gene via IncF- and IncX3-type plasmids among non-clonal *E. coli*. Although detailed epidemiological data are lacking, available information suggest community spread of NDM-5-producing *E. coli* in Germany, highlighting the importance of an integrated surveillance system in the One Health context.

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Use of Cefuroxime in the outpatient sector in Germany, 2019/2020
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Background: Cefuroxime is a 2nd generation cephalosporin classified by WHO in the "watch" category of the Essential Medicines List's AWaRe system. It can be prescribed for respiratory tract infections like bronchitis, sinusitis, tonsillitis and pharyngitis, otitis media, urinary tract infections, uncomplicated infections of the skin and soft tissue, as well as early stage Lyme disease. While it is generally NOT recommended as first line treatment option and has potentially many unwanted adverse effects, Cefuroxime continues to be commonly prescribed to outpatients in Germany.
Methods: Provided by the National Association of Statutory Health Insurance Funds (GKV-Spitzenverband), we analysed data on all antibiotic prescriptions filled in pharmacies in 2019 and 2020 by medical specialty, patient age and region with a focus on Cefuroxime. To compare federal states and age groups, we used public health insurance membership data. All data were analysed in Stata®17.
Results: A total of 3,728,842 Cefuroxime prescriptions were filled in 2019 and 2,555,566 Cefuroxime prescriptions in 2020, a reduction that is in line with the overall decrease in antibiotic prescriptions in the outpatient sector during the pandemic. Most prescriptions were issued by primary care physicians (1.875.238 in 2019; 1.212.755 in 2020), in internal medicine (661.464; 442.566), ear nose and throat (ENT) (355.725; 212.683) and urology (197.631; 189.014).
Cefuroxime represented a large proportion of all J01 prescriptions with an average proportion of 11.0% across all specialties in 2020, with surgery (24.9%), orthopaedics (22.5%), ENT (21.5%), ophthalmology (21.2%), anaesthesiology (16.2%), primary care (12.9%), internal medicine (12.6%) and urology (12.5%) above average.
Persons in age groups <15 years received 1,209, persons aged 15-79 years 3,575 (range 3,049-4,380) and persons ≥80 years 5,892 (range 5,313-7,216) Cefuroxime prescriptions per 100,000 population in 2020.
By federal state, Cefuroxime prescriptions ranged from 2,256 in Bremen, 2,478 in Berlin and 2,901 in Brandenburg to 4,105 in Saarland, 4,555 in Hesse and 4,852 in Rhineland-Palatinate per 100,000 population.
Conclusion: While Cefuroxime is in general not recommended as a first line antibiotic, it continues to be a frequently prescribed drug in the outpatient sector with large regional differences. Antibiotic stewardship activities at regional level and by medical speciality can further improve outpatient care and adherence to guidelines. Surveillance of antibiotic use in the outpatient sector needs to be strengthened to include diagnostic data and to provide targeted feedback to prescribers.
Limitations: Information on diagnosis and patient sex was not available.

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Detection of multiresistant Gram-negative pathogens in international travellers during the COVID-19 pandemic – a single-centre experience
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Introduction: During the COVID-19 pandemic and the consecutive lockdowns, a considerable decrease in the detection of many notifiable diseases and multiresistant pathogens was reported from Germany and elsewhere. International travel is a known risk factor for acquisition of intestinal colonisation with such bacteria. Here, we present data from a single travel clinic pertaining to colonisation with multi-resistant Gram-negative bacteria (MRGN) before and after travel, and report associated risk factors.
Methods: Between July 2021 and March 2022, outgoing international travellers presenting to a travel clinic in southwest Germany were enrolled. All participants provided one stool sample before their travel, and another sample three weeks after their return. Additionally, they answered a clinical questionnaire regarding general hygiene behaviour, use of disinfectants and potential risk factors for colonisation. A chromogenic agar plate medium (CHROMagar ESBL agar; Mast Diagnostica) was used to screen for MRGN, and suspicious colonies were subjected to matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry for identification. Antimicrobial susceptibility testing was performed using the MicroScan WalkAway system. We considered bacteria as MRGN whenever they carried extended-spectrum beta lactamases (ESBL) or showed a phenotypic resistance pattern as 3MRGN or 4MRGN, according to a definition put forth by the respective German committee (KRINKO). Stool samples were also analysed microscopically for the presence of intestinal parasites and with culture methods for *Clostridioides difficile*.
Results: Complete data were available from 200 participants. There were more female than male or diverse participants (53.5% vs. 46.0% vs. 0.5%) and the average individual age was 43 years. The pre-travel prevalence of 3MRGN was 3.0% (n=6; 5 x *Escherichia coli*, 1 x *Klebsiella pneumoniae*), which increased to 7.5% post-travel (n=15; all *E. coli*). Individuals with pre-travel 3MRGN carriage reported use of antibiotics in the preceding 6 months more frequently. Most 3MRGN-colonised travellers had returned from South Asia (n=7), followed by West Africa and Central Asia (n=2 each). Neither 4MRGN nor *C. difficile* carriage was found.

Discussion: Our observations confirm an increased rate of intestinal colonisation with multiresistant Gram-negative bacteria following international travel. However, this effect was not as pronounced as in similar studies, which had been conducted before the COVID-19 pandemic. A 3MRGN pattern of *E. coli* was the most frequently encountered phenotype, while no carbapenemase-producing bacteria or 4MRGN were detected. Further research should explore potential links between improved personal hygiene behaviour linked to the COVID-19 pandemic and a reduced risk of acquiring multiresistant pathogens during international travel.

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Dissemination of OXA-48-producing *Citrobacter* species in Germany is mediated by high-risk clonal lineages ST19 and ST22 with atypical plasmid support

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Introduction: Carbapenem-resistant Enterobacterales pose a global medical health threat according to the WHO. Carbapenem resistance is mainly caused by carbapenemases, bacterial enzymes that hydrolyze carbapenems and other beta-lactam antibiotics. OXA-48 is the most prevalent carbapenemase in Germany, Western Europe, North Africa and the Middle East. It is most often encoded on a transferable 63 kb IncL plasmid. OXA-48 has been mainly identified in *Escherichia coli* and *Klebsiella pneumoniae* in the past, but a significant increase of OXA-48-producing *Citrobacter freundii* has been observed recently. Here, we investigated the dissemination of OXA-48-producing *Citrobacter* spp. in Germany.
Methods: A total of 82 OXA-48-producing *Citrobacter spp*. isolates were collected over a 10-year period from clinical isolates of more than 20 hospitals from all parts of Germany. Isolates were genome sequenced employing short-read (Illumina) and long-read (Oxford Nanopore) technologies to generate hybrid assemblies. These were used to analyze phylogeny, resistome, plasmidome and genetic support of OXA-48. Antimicrobial susceptibility to 16 antibiotics was evaluated using antibiotic gradient strips and broth microdilution. Horizontal gene transfer of $bla_{\text{OXA-48}}$ harbouring plasmids was evaluated by liquid mating assays.
Results: Of the 82 *Citrobacter* isolates, 73 belonged to the *C. freundii* complex, whereas 6 were C. *koseri* and 3 *C. amalonaticus*. Almost 30% of all isolates were recovered from urine samples, which is in contrast to other Enterobacterales species which are mainly recovered from rectal swabs. OXA-48 *sensu stricto* was the most frequent carbapenemase, but OXA-48 variants OXA-162 and OXA-181 emerged since 2018 (n=9). Among the 73 *C. freundii* isolates, 33 belonged to ST19 and ST22, but showed no linkage to place and time of isolation. The other *Citrobacter* isolates were genomically highly diverse and consisted of 30 different ST. Meropenem, ceftazidime-avibactam and colistin were the most active antibiotics, whereas resistance rates of >50% were recorded for other cephalosporins, aminogylcosides, fluoroquinolones and cotrimoxazole, which was supported by the presence of respective resistance genes. All OXA-48 carbapenemases were plasmid encoded but the genetic support was highly diverse. Strikingly, 40% of all OXA-48 plasmids were IncF and IncL types of 27 to 78 kb sizes and differed substantially from the epidemic 63 kb IncL plasmid. Conjugation rates of the 63 kb IncL OXA-48 plasmid to different acceptor strains was high, ranging from 10^{-1} to 10^{-4}. Eleven plasmids could not be conjugated and four displayed conjugation rates below 10^{-4}. This was referred to insertions of IS elements or deletions in distinct transfer genes or genes with yet unknown functions.

Pathogenesis of Zoonotic Infections (FG LM/FG ZO)

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Gene expression of *E. coli* O104:H4 strain C227/11Φcu during long-term persistence in environmental soil samples

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Introduction: Several outbreaks caused by enterohemorrhagic *Escherichia coli* (EHEC) were attributed to the consumption of non-heated vegetables. Especially, agricultural soils have been suggested as a reservoir for human pathogens such as EHEC, and may pose a contamination source for edible plants. Previous studies showed that EHEC are able to survive for several weeks in soil, and that the survival is depended on different abiotic and biotic factors. However, genetic factors that enable the long-term survival of EHEC in environmental soil have been investigated only scarcely so far. In the current study, we performed differential transcriptome analysis of *E. coli* O104:H4 strain C227/11Φcu incubated in the soil environment for up to four weeks under sterile and non-sterile conditions.
Methods: Soil microenvironments containing autoclaved alluvial loam (AL) were inoculated with strain C227/11Φcu to a final inoculum of 109 CFU/g soil and incubated at 4°C for up to 4 weeks. RNA was isolated after 0, 1 and 4 weeks and used for transcriptome analysis. A further transcriptome analysis of C227/11Φcu was performed. The strain was exposed for 24 h to minimal medium and non-autoclaved AL soil or autoclaved AL soil suspensions in a dialysis tube at 4°C to investigate differences between sterile and non-sterile conditions.
Results and conclusion: The differential expression analysis showed that C227/11Φcu responds to the soil environment and changes its transcriptional profile. We found an enhanced expression of genes for e.g., stress response, heavy metal resistance, ABC transporter and metabolism. In the soil environment, bacteria are exposed to different stresses, such as temperature changes or nutrient starvation. Upregulation of stress response mechanisms allow the adaption to sublethal environmental conditions. In addition, EHEC strain O104:H4 C227/11Φcu showed adaption to low nutrient availability in the soil environment by metabolic changes in the utilization of alternative C- and N-sources.

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Peroral clove essential oil treatment ameliorates acute campylobacteriosis – results from a preclinical murine intervention study

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Introduction: *Campylobacter jejuni* infections pose progressively emerging threats to human health worldwide. Given the rise in antibiotic resistance, antibiotics-independent options are required to fight campylobacteriosis. Clove essential oil appeared as a promising candidate since it has been shown to exert antimicrobial activity against *C. jejuni* and other bacterial pathogens mainly by disruption of the outer membrane and by the production of intracellular ROS in the bacteria.
Methods: Since the health-beneficial effects of clove have been known for long, we here analyzed the antimicrobial and immune-modulatory effects of clove essential oil (EO) during acute experimental campylobacteriosis. Therefore, microbiota-depleted interleukin-10 deficient (IL-$10^{-/-}$) mice were perorally infected with *C. jejuni*strain 81-176 and treated with clove EO via the drinking water (100 mg per kg body weight per day) starting from

day 2 post-infection until the end of the observation period, whereas the placebo control mice exclusively received autoclaved drinking water.
Results: On day 6 post-infection, lower small- and large-intestinal pathogen loads could be assessed in clove EO as compared to placebo treated mice. Although placebo mice suffered from severe campylobacteriosis as indicated by wasting and bloody diarrhea, clove EO treatment resulted in a better clinical outcome and in less severe colonic histopathological and apoptotic cell responses in *C. jejuni* infected mice. Furthermore, lower colonic numbers of macrophages, monocytes, and T lymphocytes were detected in mice from the verum versus the placebo cohort that were accompanied by lower intestinal, extra-intestinal, and even systemic pro-inflammatory cytokine concentrations.
Conclusion: In conclusion, our preclinical intervention study provides first evidence that the natural compound clove EO constitutes a promising antibiotics-independent treatment option of acute campylobacteriosis in humans.

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Leaky gut through the paracellular leak pathway induced by *Campylobacter jejuni* is rescued by resveratrol

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Introduction: *Campylobacter jejuni* infection leads to gastroenteritis and sequelae like irritable bowel syndrome. Campylobacteriosis by *C. jejuni* represents the most common foodborne zoonosis and bacterial diarrheal disease worldwide. Bioinformatics predictions for potential protective compounds in campylobacteriosis, raised the question whether the polyphenol resveratrol is efficient in alleviating intestinal epithelial dysfunction induced by *C. jejuni*.
Method: Experimental infection and treatment studies were performed in human epithelial HT-29/B6 cell culture and an abiotic IL-10$^{-/-}$ mouse model. For assessment of epithelial electrical resistance and paracellular marker fluxes, Ussing chamber experiments were performed on mouse colon and cell monolayers. In parallel, molecular analysis was made by immunostainings for confocal microscopy and Western blotting.
Results: Transepithelial electrical resistance (TER) was decreased and the paracellular marker flux of fluorescein (332 Da) increased in *C. jejuni*-infected HT-29/B6 cell monolayers (Permeability$_{fluorescein}$ at 48 h post infection: 0.24 ± 0.01 ·10^{-6} cm/s in control versus 0.76 ± 0.09 ·10^{-6} cm/s under *C. jejuni* (P<0.01) versus 0.40 ± 0.07 ·10^{-6} cm/s under resveratrol treatment and *C. jejuni* (P<0.05), one-way ANOVA with Bonferroni correction, n = 10). Concomitantly in confocal microscopy, the tight junction (TJ) proteins occludin and claudin-5 were re-distributed off the tight junction domain of the epithelial cells. In parallel, an increased induction of epithelial apoptosis was observed. Both changes contribute to barrier dysfunction and opening of the paracellular leak pathway. The recovery experiments with resveratrol treatment revealed a functional improvement of the disturbed epithelial barrier in both models in vitro and in vivo. Under resveratrol treatment, TJ localization of occludin and claudin-5 was restored in the paracellular domain of HT-29/B6 cells. Moreover, resveratrol decreased the ratio of apoptotic epithelial cells. In a stimulation experiment with pro-inflammatory cytokines, we could show that resveratrol antagonized the barrier-impairing action of interferon-γ in the inflamed leaky gut.
Discussion: In conclusion, the molecular and cellular effects of resveratrol are sufficient to improve epithelial barrier function of the intestine, alleviating the leaky gut phenomenon. The induction of the leak pathway by *C. jejuni* and the restoration of barrier function by resveratrol shows its potential as a preventive or therapeutic supplement for mitigating the leaky gut-associated campylobacteriosis.

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Novel chamber separation cell migration assay for endothelial infection analyses of *Streptococcus pneumoniae* under physiological flow conditions

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Introduction: *Streptococcus pneumoniae* (the pneumococcus) causes systemic infections in humans. Pneumococci produce several pathogenicity factors, such as the cytotoxic pore-forming toxin pneumolysin, which mediates vascular injury during pneumococcal infection. Here, we present a novel cell culture infection technique, which we used to determine the impact of pneumococci and purified pneumolysin on endothelial cell migration, cell morphology, and bacterial attachment during infection.
Material/method: We used specific inserts to generate a defined cell-free area in the monolayer of primary endothelial cells (HUVEC) for the so-called chamber separation cell migration assay (CSMA). The CSMA enabled the analysis of time courses of live cell infection studies with pneumococci. Additionally, differential immunofluorescence staining followed by confocal microscopy visualizes the effects of bacterial cell attachment.
Results: Incubation of HUVEC with wild-type pneumococci significantly reduced the velocity of cell migration and significantly inhibited endothelial gap closure. Similar results were determined by employing purified pneumolysin protein, which identifies this cytotoxin as a potent inhibitor of endothelial cell migration. With the aim to analyse the effect of pneumococcal infection on endothelial wound healing under physiological flow conditions, we combined the CSMA with a microfluidic pump system, which enables the application of defined shear stress values, thereby mimicking the vascular blood flow. Comparable to infection under static conditions, circulating pneumococci prevented endothelial gap closure at a defined shear stress of 10 dyn/cm^2.
Discussion: Concluding, the novel combined *in vitro* infection technique provides valuable insights into the effect of pneumococci on endothelial cell migration and barrier integrity. Therefore, the results obtained confirm the excellent suitability of the developed CSMA for the analysis of streptococcal infection in the vasculature during systemic disease progression.

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