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ABSTRACTS

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EHEC01.06

Shiga toxin 2a A- and B-subunits are not produced in a 1:5 ratio in enterohemorrhagic *Escherichia coli*

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Introduction: Shiga toxins (Stx) of enterohemorrhagic *Escherichia coli* (EHEC) are AB_5 protein toxins consisting of a single enzymatically active A-subunit and a pentamer of non-covalently linked B-subunits. The description of Stx as an AB_5 protein and the observation that A-subunits without corresponding B-subunits also intoxicate eukaryotic cells, led to the question whether the subunits are produced by the bacteria cells in a 1:5 ratio or whether the A-subunit is produced in excess revealing free A-subunits released in the bacterial environment.

Aim: The aim of this study was to investigate a possible molecular background for the occurrence of free Stx2a A-subunits in a foodborne *E. coli* O113:H21 strain, and five clinical EHEC strains of serotypes O157:H7/H-, O26:H11, O103:H-, and O104:H4.

Material, Methods and Results: Transcriptional analysis of the Stx2a subunit gene expression showed that the A-subunit gene was expressed on average 1.90 times stronger than the gene encoding the B-subunits, indicating the presence of free A-subunits. By use of native polyacrylamide gel electrophoresis and subsequent Western blot analysis, free A-subunits were indeed detectable in the culture supernatants of all six strains. To investigate whether the ratios observed after transcription are in a similar range as the amount of subunit proteins present in the culture supernatants after translation, a quantitative ELISA specific for StxA2a and StxB2a was established. The quantification of the subunits by use of ELISA revealed two groups of strains with StxA2a:StxB2a subunit ratios of 1.10 and 4.63.

Summary: The results of this study demonstrate that free A-subunits might occur and can be released from the bacterial cells. Further analysis considering transcriptional and translational regulation and the role of free A-subunits for disease development will be conducted in future.

EHEC01.08

Interaction of Shiga Toxin 2a A-subunits with HeLa cells: characterization of binding and uptake

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Introduction: Shiga toxins (Stx) are the major pathogenicity factors of enterohemorrhagic *Escherichia coli* (EHEC). Stx are AB5 toxins, the A-subunit of which inhibits eukaryotic protein biosynthesis. The B-subunits are responsible for binding to the receptor globotriaosylceramide (Gb3). The A-subunit alone has been shown to exhibit toxic effects on different cell lines. This raises the question of the binding and uptake mechanism of the A-subunit in absence of the corresponding B-subunits.

Aim: The aim of the current study is to investigate the binding, uptake and intracellular transport of the Shiga toxin 2a A-subunit (StxA2a) in HeLa cells in the absence of its corresponding B-subunits.

Materials & Methods and Results: For detection of the binding to HeLa cells, StxA2a was labelled with the fluorescence dye ATTO-647N. HeLa cells were intoxicated with StxA2a-647N and binding and uptake was investigated with a confocal Laser Scanning Microscope (cLSM900). To analyse whether the A-subunit follows the same retrograde pathway as the Stx holotoxin, co-localization of StxA2a-647N with the endoplasmic reticulum was examined using the cholera toxin B-subunit, labelled with Alexa-Fluor 594. The results of this study demonstrate that the Stx2a A-subunit can bind and be taken up in HeLa cells in absence of the corresponding B-subunits. Moreover, we could show that StxA2a co-localizes with the endoplasmic reticulum.

Summary: The results of this study demonstrates that the A-subunit follows a similar retrograde pathway than the Stx holotoxin.

EHEC01.09

The impact of naturally occurring *rpoS* polymorphisms on virulence gene expression in EHEC O104:H4

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Question: Polymorphism acquisition in the gene coding for the stationary-phase sigma factor RpoS is a well-described adaptive mechanism that enables *Escherichia coli* (*E. coli*) and other Proteobacteria to survive in diverse natural conditions. We showed recently that RpoS acts as a repressor of virulence gene expression in enterohemorrhagic *E. coli* (EHEC) of serotype O104:H4, which caused the largest recorded outbreak of food-borne infections in Germany in 2011 with 4,000 cases of acute gastroenteritis, and >50 deaths. More specifically, we identified a laboratory-acquired single nucleotide polymorphism (SNP) in the start codon of *rpoS*, which was associated with enhanced virulence gene expression. The goal of this project was to screen our collection of EHEC O104:H4 outbreak isolates for naturally acquired mutations in *rpoS* and explore their impact on virulence.

Methods: Whole genome sequencing data of EHEC O104:H4 outbreak isolates were analysed with SeqSphere+. Different *rpoS* alleles were expressed heterologously in EHEC O104:H4 Δ*stx2 ΔrpoS*. The impact on virulence genes was determined by Western blots of total protein lysates and secreted proteins. To measure RpoS functionality we performed catalase assays by adding H_2O_2 on plated cells and recording the generation of air bubbles. Lastly, biofilm formation was quantified by staining statically grown cultures with crystal violet.

Results: We identified two SNPs introducing a premature stop codon (829C>T) or a non-synonymous substitution (836G>T) in the C-terminal region of *rpoS*, which is known to influence transcription initiation on relaxed DNA templates. Next, we demonstrated that RpoS abundance was decreased in strains expressing the mutated *rpoS* alleles, but also that the premature stop codon led to a truncated protein variant. Correspondingly, we observed that RpoS-regulated catalase activity was completely inactivated in isolates with *rpoS* mutations. We could also show that abundance of aggregative adherence fimbriae type I was significantly higher in isolates with *rpoS* mutations, correlating with an increased aggregation phenotype in liquid cultures and an increased biofilm formation. Lastly, we confirmed that production of other

virulence factors such as SepA, a serine protease autotransporter, and Aap, a dispersin, was also upregulated.

Conclusions: Our findings demonstrate that naturally acquired *rpoS* mutations result in increased virulence of pathogenic EHEC O104:H4, thus further expanding our understanding of the role of RpoS as a master regulator of virulence.

EHEC01.11
Integrated Genomic Surveillance (IGS) of STEC/EHEC infections in Germany at NRC for salmonella and other bacterial enteric pathogens
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Introduction: Shiga toxin-producing *E. coli* (STEC), including the subgroup of enterohemorrhagic *E. coli* (EHEC), are important bacterial pathogens which cause diarrhea and the severe clinical manifestation hemolytic uremic syndrome (HUS). Genomic surveillance of STEC/EHEC is a state-of-the-art tool to identify infection clusters and to extract markers of circulating clinical strains, such as their virulence and resistance profile for risk assessment and implementation of infection prevention measures. In 2020 started a BMG-founded program for establishment of IGS for these microorganisms. The aim of the project was characterization of the clinical STEC population in Germany and creating of a reference data set for STEC/EHEC molecular surveillance and detection of infection clusters.

Methods: From 2020 to 2024 2,498 STEC isolates, including 55 of known HUS association, were analyzed by PCR-based virulence gene analysis, antibiotic susceptibility testing and whole genome sequencing including bioinformatic analysis.

Results: Major serogroups in all clinical STEC analyzed were O26, O146, O91, O157, O103, O128, O145 and O146. HUS-associated strains were dominated by O26, O145, O157, O111, and as singletons O76, O80 and O153. *stx1* was less frequently and *stx2* or a combination of *stx, eaeA* and *ehxA* were more frequently found in HUS-associated strains. Predominant *stx* gene subtypes in all STEC strains were *stx*1a (24 %) and *stx*2a (21%) and in HUS-associated strains were mainly *stx*2a (69%) and the combination of *stx*1a and *stx*2a (12.8%). Genomic analyses revealed also strains harboured the *stx*2g subtype detected among the top ten STEC serogroups in animals and foods (O187:H25) and *stx*2e subtype (O63:H6 and O125:H6) increasingly. Furthermore, novel O-antigen gene clusters (RKI6 – RKI11) and strains of serovars O45:H2 and O80:H2 showing multidrug resistance were detected. By means of phylogenetic analysis, 751 isolates (30%) were assigned to 300 infection clusters (threshold allelic distance < 10 AD) including two to ten isolates.

Conclusions: The implemented surveillance tools now allow to comprehensively define the population of clinical STEC strains including those associated with the severe disease manifestation HUS reaching a new surveillance level in Germany. Therefore, the integration of epidemiological data and data of the competent food authorities on national and international level leads to highly efficient control strategies positively impacting public health.

EHEC01.12
Insights into Shiga toxin-producing *Escherichia coli* of serogroup O187
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Shiga toxin-producing *Escherichia coli* (STEC) of serogroup O187 are among the top ten detected STEC serogroups in animals and foods during the years 2015 and 2022 in Germany. Among these, serotype O187:H28 is most prevalent followed by O187:H52. STEC O187 were isolated from human clinical samples in Germany for the first time in 2017. In addition, STEC O187:H28 were isolated from human clinical specimen in Denmark during the years 2014 to 2022 and sequence data available from Enterobase and NCBI suggest that STEC of O187:H28 and O187:H52 are widely distributed. Also, genome sequences of additional serotypes of the O187 serogroup are published in public repositories.

Here, we investigated 141 STEC isolates from food and human samples from Germany and Denmark using whole genome sequencing. In addition, 220 available genome data of *E. coli* serogroup O187 from public repositories were included in the study. We determined the presence of virulence associated genes including genes for the different Shiga toxin subtypes as well as antibiotic resistance genes. Clustering analyses were conducted using core-genome multi-locus sequence typing and also pan-genome analyses were carried out.

Strains were derived from human (41.0 %), animal (20.8 %), food (31.0 %) and the environment (3.6 %) and were isolated from 19 countries worldwide. Genomic analyses revealed 11 different serotypes and 25 MLST sequence types. Shigatoxin genes were identified in O187:H28 and O187:H52 serotypes, only. O187:H28 strains mainly harboured the *stx2g* subtype whereas in O187:H52 *stx1c* was predominantly identified. In contrast, antimicrobial resistance genes were mainly detected in non-O187:H28/H52 strains. STEC O187:H28 were especially isolated from wild animals, meat products from wild animals and flour but also from humans. STEC O187:H52 were especially isolated from farm animals and respective meat products as well as humans. Pan-genome analyses of the core-genomes of O187:H28 and O187:H52 strains revealed 289 genes specific to O187:H28 and 70 genes specific to O187:H52, indicating a higher variability in genomes of O187:H52 strains. Permanova analyses of O187:H52 strains based on the pan-genome identified three subclusters which could be explained by pathotype and matrix/isolation source.

In summary, *E. coli* strains of serogroup O187 are distributed worldwide and were isolated from a variety of matrices. *Stx* genes were only identified in serotypes O187:H28 and O187:H52 showing differences in identified *stx*-subtype but also in the geographic distribution and the isolation source indicating different evolutionary settings for those lineages. However, STEC of both serotypes were isolated from human clinical samples indicating an impact on human health.

EHEC01.13
Prevalence estimation of the Shiga toxin gene *stx2* in the

healthy human microbiome using publicly available metagenomes

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The *stx2* gene, encoding Shiga toxin 2, is a major virulence factor of enterohemorrhagic *Escherichia coli* (EHEC). Expression of *stx2* is associated with the development of hemolytic uremic syndrome (HUS) in humans, and its molecular detection has become a proxy for identifying high-risk EHEC strains in both diagnostic and surveillance contexts. However, little is known about the background prevalence of *stx2* in the microbiomes of healthy individuals, independent of symptomatic infection. Such information is essential for improving risk assessment, source attribution, and the interpretation of positive diagnostic signals in both clinical and environmental samples.

We screened >10,000 publicly available human-gut metagenomes—curated for host age (0–98 yr), health status (healthy, non-diarrhoeic, diarroheic), and socio-geographic context (all inhabited continents, all human-development strata)—with a rapid k-mer–based pipeline. *stx2* DNA was detected in ≈ 2–3 % of samples. This prevalence remained remarkably stable across all age categories, healthy and non-diarrhoeic conditions, and the full range of geographic and developmental backgrounds.

Since *stx2* is typically phage-encoded and not necessarily expressed, its presence alone does not imply pathogenic potential. In ongoing work, we are investigating the co-occurrence of *stx2* with other *E. coli*-associated pathogenicity factors such as *stx1* (Shiga toxin 1), *eae* (intimin), *ehxA* (enterohemolysin), and *espP* (serine protease), which could help clarify whether - and in which microbiomes - *stx2*-positive signals are part of broader virulence gene profiles. While the available data make it difficult to associate *stx2* with specific organisms or mobile elements, its detection remains relevant. Detecting *stx2* in healthy individuals has important implications for diagnostics, public health surveillance, and understanding the distribution of virulence genes in the human microbiome.

EHEC01.17

Prevalence and characteristics of Stx2e-encoding *Escherichia coli* (STEC-2e) isolates from weaned piglets in German pig farms

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Oedema disease is a severe and often fatal infectious disease of piglets usually observed within two weeks after weaning. The causative *Escherichia coli* (EDEC) strains are characterized by carriage of genes encoding Shiga toxin subtype Stx2e and F18 fimbriae. The aims of the study were to estimate the prevalence of STEC-2e on German pig farms and to determine the characteristics of current STEC-2e strains.

In a cross-sectional study, 99 pig farms in Germany were examined. Faecal and oral fluid samples were collected in five pens of each farm and subsequently screened for STEC-2e by culture and PCR methods. Among the STEC-2e isolates, representative isolates were selected for whole genome sequencing (WGS) analysis ensuring that at least one isolate per STEC-2e-positive farm, pen and sample type was included. WGS was carried out on the Illumina platform yielding sequence fragments of 2 x 150 bp, with a total targeted 100-fold coverage. WGS data were examined using the tools ResFinder and VirulenceFinder (CGE website). Phenotypic resistance to 17 antimicrobial agents was determined by broth microdilution according to CLSI standards.

STEC-2e (n = 237) were isolated from 24.9 % of the pens on 47.5 % of the farms investigated. WGS analysis of 100 selected STEC-2e isolates revealed that 40 % encoded for F18 fimbriae and thus fulfilled both EDEC criteria. Genes for *E. coli* enterotoxins were additionally detected in 37.5 % of these isolates which classified them as STEC/ETEC (enterotoxigenic *E. coli*) hybrids. Fifty-five STEC-2e isolates carried at least one of the 40 resistance genes discovered in total with 1.8 % to 65.5 % of these isolates having the respective gene. Among the five most frequently detected genes, three (*aph(6)-Id*, *aph(3")-Ib*, *aadA1*) conferred resistance to aminoglycoside antibiotics but none to gentamicin. Most isolates (43 %) encoded for resistance to beta-lactam antibiotics and four isolates even carried genes of extended-spectrum beta-lactamases, but none for carbapenemases. Phenotypically, even 51 % of the isolates displayed resistance to ampicillin but only 1 % showed resistance to gentamicin. The *mcr-1* and *mcr-4* genes were detected in 7 % and 1 % of isolates, respectively, all of which were phenotypically resistant to colistin. A total of 34 % of STEC-2e isolates proved phenotypically non-susceptible to at least three antimicrobial classes and were regarded as multidrug-resistant.

Our data suggest that STEC-2e occur frequently in weaned piglets in Germany and divide into several viro- and resistotypes. Among these, STEC/ETEC hybrids may cause post weaning diarrhoea thus masking signs of oedema disease and leaving the true health threat to diarrheic piglets not correctly recognized. Since oedema disease itself is rarely an indication for antimicrobial chemotherapy, frequent occurrence of resistance and resistance genes in STEC-2e may reflect high selective pressure by antimicrobials administered to piglets with different objectives.

EHEC01.18

Shiga toxin-encoding *Escherichia coli* from South American camelids in Germany – prevalence, *stx* gene subtype distribution and strain characterization

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Introduction: South American camelids (SAC), popular in Europe, frequently kept with other livestock species and in close contact with humans, represent a potential reservoir for transmission of epizootic and zoonotic bacteria to livestock and humans. However, knowledge on bacterial pathogens in SAC, such as Shiga toxin-encoding *Escherichia coli* (STEC), is too sparse for drafting appropriate monitoring and preventive medicine programs.

Objective: Investigate the presence of STEC in SAC in German herds and analyze isolates for their virulomes and resistomes.

Material & Methods: Fecal samples from SAC were plated on Gassner agar, the resulting colonies washed off and

cleared lysate aliquots tested for *stx*1/2 gene presence and subtype by PCR. Positive samples were re-plated , colonies picked and DNA extracted from *stx*-positive isolates, subjected to whole-genome-sequencing and bioinformatic analysis.

Results: Fecal samples were taken from 20 animals each at 4 different time-points in 10 and then 9 herds. In total, 716 fecal samples from 442 animals were tested for the presence of STEC. The prevalence of *stx*-positive samples for all ten holdings over the four rounds of sampling was 32.4% (232/716). This resolved into prevalences of 6.42% (46/716), 18.44% (132/716) and 7.54% (54/716) for *stx1*-, *stx2*- and *stx1/stx2*-positive samples, respectively. The corresponding animal prevalence for the presence of an *stx*-gene was higher with 42.3% (187/442). We did not detect a difference in prevalence between alpacas (43.1% positive; 125/290) and llamas (41.1% positive; 62/151. The herd prevalence diverged widely from 0% (0/20) to 95% (19/20) *stx*-positive animals in a herd over all sampling rounds and holdings. In sampling round A, 9 of 10 herds tested positive, while all herds were positive in sampling rounds B to D. The *stx*-gene subtypes identified were predominantely *stx1c* (78%; 78/100) and *stx2b* (77%; 144/186), considered to be of low risk for causing severe disease. Herds were transiently PCR-posiitive for *stx* subtypes *1a*, *2c*-*2g*. Multiple *stx2* subtype signals were detected in 13% of the samples (25/186). In 27 *stx2*-positive samples, a subtype could not be assigned. Five isolates were obtained from the last sampling round from five different farms and short-read whole genome sequenced. Three share the genoserotype O87:H16, the phylogroup A and the sequence type 2101. One has the genoserotype O151:O118:H16, the phylogroup B1 and the sequence type 56 and the remaining isolate has the genoserotype O76:H19, the phylogroup B1 and the sequence type 675.

Summary: The herd prevalences determined, although differing widely between farms, are similar to prevalence ranges published for cattle, small ruminant and camelid herds. We did not observe seasonality in *stx*-gene presence. The STEC isolates identified so far and those from a previous cross-sectional study in Central Germany suggest low pathogenic potential. Still, EFSA classifies all STEC strains as potential human pathogens.

EHEC01.19

Bacteriophages MM-1 and MM-2 target enterohemorrhagic *Escherichia coli* and *Salmonella enterica* strains with high efficiency

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) are important human pathogens causing various serious human diseases such as bloody diarrhea, hemorrhagic colitis and the hemolytic-uremic syndrome (HUS). In recent years, the consumption of minimally processed products, such as fresh produce, was increasingly associated with outbreaks of *E. coli* O157:H7 involving the development of HUS. Furthermore, the treatment of EHEC infections with antibiotics is controversially discussed due to the potential induction of phage-borne Shiga toxins (Stx). Alternatives, such as the use of strictly lytic phages, are considered as powerful tools for the biocontrol of EHEC.

Aim: The aim of this study was the isolation and phenotypic as well as genomic characterization of virulent phages against EHEC. Since it is only poorly investigated, whether bacterial lysis caused by phages may trigger the SOS-mediated *stx* induction immediately before cell death, putative *stx2a* expression after phage infection was also investigated by performance of qRT-PCR.

Results: In this study, the two tailed bacteriophages vB_EcoS_MM-1 (MM-1) and vB_EcoS_MM-2 (MM-2) were isolated form a local sewage water treatment plant using *E. coli* O157:H7 prototype strain EDL933 as host. Oxford Nanopore Sequencing revealed that the two phages were closely related to each other and belong to the genus *Tequintavirus* with a genomic size of appr. 118,000 bp of linear dsDNA. Host range analysis showed that MM-1 and MM-2 are not limited to serotype O157:H7/H-, but also infected important clinical EHEC, food-borne STEC and *Salmonella enterica* serotypes. In addition, MM-1 and MM-2 displayed stability at a broad temperature (4°C to 60 °C) and pH range (pH 3 to 12) combined with a short latent period of appr. 15-20 min and a burst size of appr. 90 PFU mL-1. They effectively inhibited the growth of EDL933 at all applied MOIs of 0.1,1 and 10. Infection with a MOI of 1 was most efficient with a growth reduction of appr. 97 % and appr. 93 % already after 2 h after infection for MM-1 and MM-2, respectively. Furthermore, analysis of the *stx2a* expression after phage infection revealed no upregulation of the *stx2a* expression after infection of *E. coli* O157:H7 strain EDL933 with both phages at all applied MOIs of 0.1, 1 and 10 compared to the non-infected culture.

Conclusion: This study highlights that phages MM-1 and MM-2 are promising and effective biocontrol agents not only against EHEC, but also STEC and *Salmonella enterica* serotypes, for further use in food safety applications and phage therapy.

WB01.01

Reformen des Medizinstudiums – Wie geht es weiter?

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Seit Jahren wird in der Bundesrepublik über eine Reform des Medizinstudiums diskutiert. Eigentlich sollte diese Diskussion schon 2020 in die Verabschiedung einer neuen Ärztlichen Approbationsordnung (ÄApprO) münden - was aber aktuell in weite Ferne gerückt zu sein scheint.
Unabhängig von einer neuen ÄApprO gibt es aber viele Möglichkeiten, Reformen auch im Rahmen der aktuell gültigen ÄApprO umzusetzen. Der Vortrag gibt einen Überblick über die bundesweite Diskussion bzgl. möglicher Reformen, den Stand der Bearbeitung des Nationalen Kompetenzbasierten Lernzielkatalogs Medizin (NKLM) und die Rolle des 'LOOOP-Ausbildungsforschungsnetzwerks'.

WB01.02

"Schwere Infektionen auf der Intensivstation: Fünf Tage live einen Patienten, die Infektionsdiagnostik und antiinfektive Therapie verfolgen": Ein interdisziplinäres und interprofessionelles Lehrkonzept

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Hintergrund: Schwere Infektionen wie der septische Schock werden regelhaft auf Intensivstationen behandelt und haben auch in Industrienationen wie Deutschland eine anhaltend sehr hohe Mortalität von fast 50%. Im Rahmen des Wahlpflichtcurriculums an der Universitätsmedizin Frankfurt hat das Institut für Medizinische Mikrobiologie und Krankenhaushygiene zusammen mit der Klinik für Anästhesiologie und Intensivmedizin sowie dem Institut für Pathobiochemie ein interdisziplinäres und interprofessionelles Lehrangebot im klinischen Studienabschnitt entwickelt. Wesentliche Lernziele sind ein Grundverständnis für die Infektionsdiagnostik auf Intensivstation und im Labor, für eine rationale Antibiotikatherapie, für krankenhaushygienische Aspekte sowie für die komplexe interprofessionelle Zusammenarbeit verschiedener Berufsgruppen.

Aufbau: In der einwöchigen ganztägigen Lehrveranstaltung begleiten ca. acht Studierende in Kleingruppen einen Patienten in klinischen Visiten (inkl. ABS-Visiten) auf einer operativen Intensivstation. Die Untersuchungsgänge der von den Studierenden entnommenen Patientenproben werden in einem diagnostisch orientierten Laborpraktikum von der Probengewinnung bis zur Befunderstellung in der Medizinischen Mikrobiologie und zum Teil in der Pathobiochemie eigenständig durchgeführt. Die Veranstaltung wird durch Seminare (Sepsis, virale Infektionen auf Intensivstation, schwere Infektionen bei Reiserückkehrern, Dosierungen/Interaktionen von Antiinfektiva, *whole genome sequencing* multiresistenter Erreger u.a.) interdisziplinär begleitet. Durch einen Wahltag in beteiligten Bereichen (Krankenhaushygiene, Krankenhausapotheke, Krankenhausseelsorge, Physiotherapie, Krankenpflege) wird das interprofessionelle Wissen vertieft. Die Leistungsüberprüfung erfolgt anhand einer abschließenden strukturierten Patientenvorstellung inklusive Reflexion und Therapievorschläge. Die Veranstaltung wurde mit einem Fragebogen von den teilnehmenden Studierenden evaluiert.

Ergebnisse: Alle Teilnehmenden waren der Meinung, dass durch die angenehme Lernatmosphäre und den Aufbau der Veranstaltung die komplexen Krankheitsbilder transparenter wurden, das Verständnis für den Stellenwert von Infektionen auf Intensivstation gefördert und die Veranstaltung zum Durchdenken der diagnostischen Abläufe und Behandlungskonzepte motiviert hat.

Fazit: Dieses innovative Lehrformat ermöglicht den Studierenden wertvolle Einblicke in das komplexe Zusammenspiel der unterschiedlichen medizinischen Disziplinen und Professionen auf einer Intensivstation unter besonderer Berücksichtigung der Medizinischen Mikrobiologie und Krankenhaushygiene. Im Verlauf einer derartigen Lehrveranstaltung verdichteten sich bei den Studierenden die Kenntnisse in der anspruchsvollen Diagnostik und Behandlung von Patienten mit schweren Infektionen auf der Intensivstation.

WB01.03

Deliberate use of misinformation to uncover learning gaps in infection prevention/control and infectious diseases among clinical phase medical students – a mixed methods approach

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Question: Video-based teaching positively affects knowledge acquisition and skills development. However, widespread digital platforms could easily foster the circulation of misinformation (MI), especially within Infection Prevention & Control (IPC) and Infectious Diseases (ID). Thinking outside the box, we investigated the potential of MI to uncover IPC/ID learning gaps and improve critical thinking among medical students.

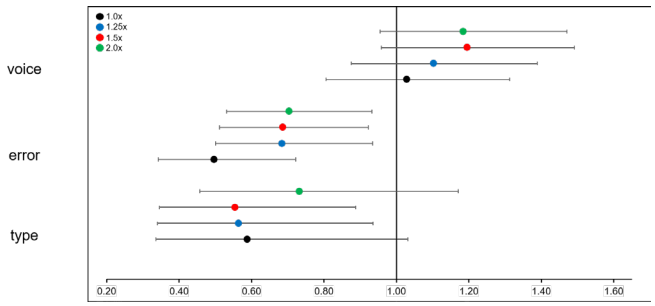
Methods: We prepared four teaching-videos covering the following IPC/ID topics: placing a peripheral venous catheter (PVC), tick-borne encephalitis (TBE), outbreak management (OM) and proper use of personal protective equipment (PPE). Of those, only TBE and PPE contained deliberately placed MI. TBE and PVC had a descriptive voice cover, while the other two displayed background music. We designed an online-questionnaire (n = 83 single- or multiselect items) covering video-contents, targeting third-year medical students (monocentric). Watching the videos was not mandatory for answering the items. Pooled items were the units of observation. The outcome event was answering a given item correctly (= hit) with the frequency of hits being inversely proportional to the level of learning gaps. The odds for hit-achievement was quantified by multi-variable adjusted logistic regression. The questionnaire was followed by an evaluation form with open and close answer opportunities.

Results: We received 111/365 responses (30%) totaling 7,724 completed items (81% hits). Seriously viewing the videos resulted in a 25% (95%-CI 11—41%) higher odds for hit achievement. Correct item-selection did not require extended time when the respective video was viewed (p=0.296), indicating that hits were not biased by time. When videos were viewed, items from MI-videos displayed lower hit-odds as compared to non-MI video items (OR = 0.57, 95%-CI 0.39—0.83). Expectedly, the same effect size showed higher uncertainty around the neutral effect point when videos were omitted (OR = 1.17, 95%-CI 0.91—56%). Taken together, the omission-normalized hit-odds was 50% lower for items from MI-videos (OR = 0.50, 95%-CI: 28–66%). Similar observations were made independent of maximal video playback-speed (Fig. 1).

The subsequent evaluation revealed that our videos increased attention (~ 60% of participants), interest in IPC/ID (~ 68%) and strengthened existing knowledge (~ 70%). Most importantly, they were found to support critical thinking skills by the majority of participants (~62%). Among potential disadvantages mentioned in the evaluation was the risk of memorizing wrong information instead of the legitimate ones.

Conclusions: Videos containing deliberately placed misinformation could therefore in principle help to uncover learning gaps, which might otherwise go undetected using traditional teaching videos. Potential ramifications such as memorizing misinformation should be considered in order to further improve this approach.

Fig. 1



WB01.04
Lehre in Umweltmedizin durch einen Train-the-Trainer-Ansatz an der Universität Witten/Herdecke

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Einleitung: Das Fach Umweltmedizin/-hygiene hat als präventionsmedizinisches Fach eine besondere Stellung in der Approbationsordnung für Ärzt:innen. Nur 12 von 40 medizinischen Fakultäten verfügen über Lehrstühle für Hygiene und Umweltmedizin in Deutschland. Es gilt jedoch für alle medizinischen Fakultäten eine Vielzahl von prüfungsrelevanten komplexen und übergreifenden Themen zu vermitteln, die eine große didaktische Herausforderung darstellen. An der Universität Witten/Herdecke (UW/H) wird seit 2006 ein innovatives Lehr- und Prüfungskonzept nach dem Train-the-Trainer-Ansatz praktiziert.

Ziele: Hier stellen wir vor, wie die detaillierte Erarbeitung aller wesentlichen Themenfelder auf aktuellem Forschungsstand durch Studierenden-Gruppen alle teilnehmenden Studierenden auf den gleichen Wissenstand in der Umweltmedizin bringt.

Materialien & Methoden: Im Sommersemester 2024 wurden 16 Themenfelder von insgesamt 126 Studierenden (6. Sem., Jg. 53) bearbeitet. Dabei wurden die Studierenden so angeleitet, dass sie ein gewähltes Thema auf akademischem Niveau entwickeln, um ihre Mitstudierenden anhand von Referaten in diesem Thema zu unterrichten. Das Abnehmen der Gruppen-Referate im Plenum des Seminartages zugleich als zentrale Prüfungsveranstaltung, bei der das umweltmedizinische Lehrpersonal als Jury und als akademisches Korrektiv fungiert. Der intendierte Wissenszuwachs wurde im Pre/Post -Vergleich mit 80 Fragen im True/False-Format ermittelt.

Ergebnisse: Den Wissenstest vor dem Seminartag (Pre-Test) bearbeiteten 80 Studierende (63.45% des Jg. 53) freiwillig und anonym mit im Durchschnitt 46.13±10.92 korrekten Antworten, den Post-Test (N=77, 61.4%) mit 49.68±10.63 korrekten Antworten. Ein gutes Vorwissen besteht in den Bereichen Feinstaub durch Holzverbrennung (3.48 ± 1.46) sowie Passivrauchen (3.06 ± 1.57). Die geringsten Kenntnisse zeigen sich hingegen bei den Themen Klimawandel in Verbindung mit Infektionserkrankungen (0.78 ± 1.11) und PFAS (0.69 ± 1.27). Weitere Wissenslücken mit medizinischem Bezug sind insbesondere bei den Themen Arzneimittel im Abwasser und CO_2 in Innenräumen erkennbar.

Zusammenfassung: Der Train-the-Trainer-Ansatz hat sich an der UW/H für die Vermittlung umweltmedizinischer Inhalte bewährt und kann als didaktisches Konzept weiterempfohlen werden. Bei aktuellen umweltmedizinischen Themenfeldern muss auch bei Medizinstudierenden von geringerem Vorwissen ausgegangen werden.

WB01.05
Dynamics of bacterial outbreaks and antimicrobial resistance using whole genome sequencing: a student hands-on course

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Introduction: Whole genome sequencing (WGS) is shaping infectious disease diagnostics, bacterial outbreak investigation, and antimicrobial resistance (AMR) surveillance. To prepare future healthcare professionals for this genomic era, an innovative course—launched in the summer term of 2023 at the Medical Faculty of University of Cologne, Germany— introduces students to the principles and applications of WGS in microbiology and outbreak investigation.

Materials and Methods: The course combined lectures, microbiology lab work, and applied case studies. Students investigated a cluster of carbapenem-resistant *Acinetobacter baumannii* (CRAb) and the spread of KPC-2 and OXA-162 carbapenemases in Enterobacterales within a hospital. Practical work included antimicrobial susceptibility testing, PCR and lateral flow assays for detecting AMR genes, and genomic analysis using core genome/classical MLST, real-time sequencing with the Oxford Nanopore MinION platform and user-friendly bioinformatics tools enabled bacterial species identification, resistance and plasmid profiling. Finally, students were introduced to genome assembly and command line tools.

Results: During the course, the students successfully sequenced and analysed bacterial genomes, identified resistance determinants and, by interpreting phylogenetic relationships, identified the transmission of a *bla*$_{\text{NDM-1}}$- and *bla*$_{\text{OXA-23}}$-positive CRAb in the internal medicine department of a German hospital. Furthermore, participants investigated the spread of *bla*$_{\text{KPC-2}}$ and *bla*$_{\text{OXA-162}}$ using long-read sequencing and identified multi-genera outbreaks triggered by stable or unstable plasmids and the contribution of horizontal gene transfer in the spread of AMR. Through hands-on activities, participants gained insight into how WGS informs clinical decision-making and hygiene measures to control bacterial spread. The course also fostered interdisciplinary thinking by linking genomic data to microbiological and clinical concepts, such as research on phage therapy, and by demonstrating the application of WGS data to hospital hygiene. In addition to medical students, the course is open to undergraduate, graduate and postgraduate students from other faculties, such as Biophysics and Biology, as well as healthcare professionals. So far, the course counts 45 participants.

Discussion: This course effectively introduced the participants to cutting-edge genomic tools and their relevance in modern healthcare. Participants gained both technical expertise and conceptual knowledge by combining WGS with conventional microbiology and outbreak case studies. The

accessibility of the MinION platform further proved that WGS could be implemented in real-time and in clinical settings. Overall, the course fostered the knowledge of the participants on genomic data and emphasized the importance of genomics in combating the spread of infectious diseases and AMR.

WS01.01

47-year-old immunosuppressed patient with multiple abscesses in liver and spleen

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The patient suffers from multiple myeloma and has been undergoing chemotherapy for approximately six months. He develops agranulocytosis (DD metamizole-induced) and develops pulmonary aspergillosis caused by *Aspergillus fumigatus*. Liposomal amphotericin B is administered with 204 mg/day for a body weight of 77 kg.

Subsequently, the patient develops fever up to 38.8°C despite treatment with liposomal amphotericin B and meropenem. Infection parameters remain largely unremarkable. PET-CT imaging reveals multiple abscesses in the liver and spleen.

Abscess aspirates from the liver and spleen show aseptate hyphae on blankophor staining, and PCR is positive for *Rhizomucor* spp. Unfortunately, sequencing does not allow species differentiation. Cultures remain negative.

Consequently, the liposomal amphotericin B dosage is increased to 710 mg/day for a body weight of 71 kg, and posaconazole 300 mg/day is added. Surgical resection is deemed not feasible due to the multiple pattern of the lesions.

Retrospective analysis of stored serum and plasma samples from the patient using a Mucorales-specific PCR detected Mucorales DNA in serum as early as 24 days before the initial detection in the liver and spleen aspirates. Since the initiation of mucorales-targeted therapy, PCR results from serum samples have been negative.

The patient is eventually discharged on posaconazole suppression therapy.

Follow-up approximately one year later shows that the abscesses remain visible but have decreased in size compared to previous findings. The patient is due to commence a new chemotherapy cycle, with anticipated renewed immunosuppression. Monitoring of his mucormycosis is conducted with weekly serum PCRs and regular imaging of the abscesses. Under posaconazole therapy, the abscesses remain stable in size despite chemotherapy, and the patient is subsequently discharged on posaconazole.

This case covers questions regarding differential diagnoses of newly occurring lesions in immunosuppressed patients, the diagnostics to be requested in such cases, antifungal dosing, (new/alternative) diagnostic options for mucormycosis, and the management of mucormycosis.

WS01.02

Therapeutic approach with sulbactam/durlobactam on a multidrug-resistant *Acinetobacter baumannii* complex infection in a paediatric intensive care unit

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Introduction: Nosocomial infections caused by multidrug-resistant (MDR) *Acinetobacter baumannii* complex represent a growing global challenge in intensive care units, often leading to severe complications, especially among immunocompromised and critically ill patients. Therapeutic options are limited, particularly in paediatric patients. Here, we report the case of an infant admitted to the paediatric intensive care unit (PICU) due to a severe respiratory infection caused by an OXA-24-producing *Acinetobacter baumannii* complex with viral co-infections, posing a significant therapeutic challenge.

Case description: A two-year-old child with a history of tracheostomy and percutaneous endoscopic gastrostomy (PEG) was transferred to the PICU of our university hospital for extracorporeal membrane oxygenation (ECMO) due to a severe respiratory infection acquired abroad. The patient had previously been diagnosed with an infection caused by OXA-24-producing *Acinetobacter baumannii* complex, along with respiratory co-infections with adenovirus and herpes simplex virus-1. Initial antimicrobial treatment with meropenem, teicoplanin, and clarithromycin was adjusted to cefiderocol, ampicillin/sulbactam, cidofovir, and aciclovir following consultation with the antibiotic stewardship team. Given the patient's critical status and the lack of improvement under the aforementioned therapy, a therapeutic approach involving sulbactam/durlobactam was discussed and implemented. Susceptibility testing for sulbactam monotherapy revealed a minimum inhibitory concentration (MIC) of 32 µg/ml. Sulbactam/durlobactam was requested from the manufacturer. This drug lacks official approval in Germany, and there are no prescribing information for paediatric dosing. Therefore, the dosage was determined based on the reference range for high dosage treatment with sulbactam in ampicillin/sulbactam (50-80 mg/kg/day in divided doses every 6 hours) and experimental blood level monitoring. Cefiderocol and ampicillin/sulbactam were replaced by sulbactam/durlobactam (recommended dosage: 400 mg sulbactam/400 mg durlobactam in 4 single doses infused over 3 hours), accompanied by drug monitoring after the first and before and after the third and fourth dosage, respectively. Despite optimised antimicrobial therapy, the patient"s condition further deteriorated, with worsening respiratory and haemodynamic instability. Ultimately, the patient died due to respiratory insufficiency.

Summary: This case underscores the importance of early microbiological diagnostics, resistance testing and individualised antimicrobial therapy in managing MDR infections. Additionally, it highlights the urgent need for further research on novel antimicrobial agents, their optimal dosing and pharmacokinetic monitoring, particularly for use in paediatrics.

WS01.03

A rare case of soft tissue infection following a Brazilian Butt Lift

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Background: Medical tourism and aesthetic procedures performed under non-standardized conditions present a growing challenge for infectious disease diagnostics and treatment. We report a rare case of a soft tissue infection caused by *Mycobacterium fortuitum* in a patient following a cosmetic gluteal augmentation procedure ("Brazilian butt lift") performed abroad.

Case Presentation: A 43-year-old woman presented to the outpatient plastic surgery clinic of our institution (Klinikum rechts der Isar, Munich) several months after undergoing a Brazilian butt lift in Ecuador. Autologous fat had been harvested from the abdominal region and injected into both gluteal areas. The patient complained of persistent tenderness in the left buttock region, without systemic symptoms such as fever. Laboratory workup showed only mildly elevated inflammatory markers (CRP 0.7 mg/dL). The patient"s medical history was unremarkable aside from bronchial asthma, treated with salmeterol, fluticasone, and salbutamol.

Magnetic resonance imaging (MRI) of the pelvis revealed a 7 x 3.5 x 9 cm abscess formation in the left subcutaneous gluteal fat, with multiple confluent abscess cavities. Additional encapsulated fat deposits were seen bilaterally (up to 6 cm in diameter), without signs of active infection. A CT-guided drainage was performed, and fluid samples were sent for bacterial and fungal cultures. Although no specific request for mycobacterial culture was made, *Mycobacterium fortuitum* was successfully cultured on blood agar after five days of incubation.

Antimicrobial susceptibility testing guided treatment with imipenem/cilastatin, amikacin, trimetoprim / sulfamethoxazole and levofloxacin. The patient was followed up in our infectious diseases outpatient clinic, with gradual clinical improvement under ongoing therapy.

Discussion: *Mycobacterium fortuitum* is a rapidly growing nontuberculous mycobacterium (NTM) of the *M. fortuitum* complex, ubiquitous in soil and water. While most commonly associated with pulmonary disease, it can also cause skin and soft tissue infections, particularly after cosmetic procedures, tattoos, or trauma. Diagnosis is often delayed due to atypical clinical presentation and slow culture growth. In our case, extended incubation on routine media facilitated microbiological identification despite no initial suspicion of NTM.

This case underscores the importance of considering rare pathogens in the differential diagnosis of postoperative infections, particularly in the context of procedures performed under potentially inadequate hygienic conditions abroad. It also highlights the diagnostic value of extended culture protocols in suspected soft tissue infections of unclear origin.

Conclusion: With increasing numbers of patients seeking aesthetic procedures abroad, awareness of NTM infections, including *M. fortuitum*, is critical. Prompt recognition and targeted antimicrobial therapy are essential for successful outcomes.

WS01.04

Lymphogranuloma venereum in a patient on HIV pre-exposure prophylaxis: diagnostic challenges

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Background: Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by invasive genotypes (L1–L3) of *Chlamydia trachomatis*. It is prevalent in several countries in Africa, Asia and South America and only occurs sporadically in Europe, mostly affecting men who have sex with men (MSM). LGV can present with a range of clinical manifestations, including inguinal lymphadenopathy, proctitis and genital ulcers.

Case Presentation: A 51-year-old man presented to the infectious disease outpatient clinic with a painful, swollen lymph node in the left inguinal region. He also reported pruritus of the penis without discharge approximately ten days before. He had been taking HIV pre-exposure prophylaxis (PrEP) with TDF/FTC 245/200mg for the past 16 months due to sexual risk exposure. He attends regular follow-up visits and was treated for rectal chlamydia and N. gonorrhoeae infection one year ago.

Clinical examination showed a swollen lymph node of approximately 5 cm on the left side. Swabs were taken from urethra and rectum, and a multiplex PCR for sexually transmitted pathogens (STI panel) was performed. PCR analysis of the urethral swab detected *Chlamydia trachomatis* later identified as LGV by MOMP-genotyping, the rectal swab was positive for *Chlamydia trachomatis* genotype F, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. In addition, inguinal ultrasonography revealed a reactive lymph node with signs of abscess formation. According to the guidelines, the patient was treated with doxycycline 100 mg twice daily for 21 days. At a follow-up visit after 4 months, he was asymptomatic and the STI screening yielded negative results for *Chlamydia trachomatis*.

Discussion: This case highlights several clinically and diagnostically relevant aspects in the context of STI management in a patient on HIV pre-exposure prophylaxis (PrEP). Multiplex PCR for STIs can be a useful tool for detecting sexually transmitted infections; however, it may also lead to findings of uncertain clinical relevance, such as the detection of *Mycoplasma hominis* or *Ureaplasma urealyticum*,

which are often found to be part of the commensal flora. In cases with suggestive clinical findings, such as inguinal lymphadenopathy in this patient, genotyping of *Chlamydia trachomatis* becomes particularly important, as detection of LGV strains guides treatment duration and approach.

*B.T. Schleenvoigt and M. Baier contributed equally to this work.

WS01.05

Challenges in diagnosing atypical infections including neoehrlichiosis in a lymphoma survivor following Chimeric Antigen Receptor (CAR) T cell therapy

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Introdcution: The therapeutic landscape in the treatment of refractory lymphoma has changed significantly in recent years mainly due to CAR T cells. With these new options arise new challenges like rare and difficult-to-diagnose infections due to sometimes profound and lasting immunosuppression.

Case: We present the case of a 56-year-old male with diffuse large B-cell lymphoma (DLBCL) in April 2015. First-line treatment consisted of six cycles of R-CHOP, two cycles of R-MTX, and consolidative radiotherapy of the initial gluteal bulk. Due to relapse 36 months later, the patient received R-DHAP twice and high-dose R-BEAM followed by autologous stem cell transplantation. Persistent splenic foci led to a splenectomy in September 2019 which also revealed the presence of DLBCL. Subsequently, the patient received CD19 CAR-T cell therapy in September 2019 and went into a still ongoing complete remission.

The patient experienced hypogammaglobulinemia and a mild SARS-CoV-2 infection in April 2022. An atypical pneumonia 2 month later deteriorated the general well-being. Only by bronchioalveolar lavage persisting SARS-CoV-2 infection was diagnosed and paxlovid led to a rapid recovery. From February 2024, increasing fatigue and night sweats occurred. There was no relapse by CT-scan but blood analysis by flow cytometry revealed expanded atypical $CD8^+$ T cells that were partially CD8low and numerically elevated γδ T cells. Despite suspicion of a T cell lymphoma, the patient refused a bone marrow biopsy. The atypical T cell populations further expanded and the symptoms progressed to continuous cyclic fever, strong night sweats and progressive weight loss. Mucosal HSV-1 reactivation was detected, but treatment with acyclovir did not lead to an improvement. In July 2024, comprehensive diagnostic workup did not show any pathologic finding. By metagenomic sequencing of cell-free DNA from plasma using the DISQVER platform (Noscendo GmbH, Germany) *Neoehrlichia mikurensis could be detected. Neoehrlichia mikurensis is* a tick-borne, intracellular bacterium that rarely causes symptomatic infections in immunocompromised individuals with prolonged fever, systemic inflammation and sometimes fatal outcome . Treatment with doxycycline led to rapid resolution of all symptoms with a gradual decline of *Neoehrlichia mikurensis* DNA in plasma.

Conclusion: This case highlights the challenges of immune impairment after CD19 CAR T cell therapy in heavily pretreated DLBCL patients and the potential of metagenomic sequencing of cell-free DNA. In fever of unknown origin, also rare infections have to be considered. Metagenomic analysis of cell-free DNA can be a valuable tool for identification of mostly unexpected infectious agents.

WS02.01

Linking Candida albicans protein kinases to cytotoxity – characterisation of Crk1 functions during epithelial invasion

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Microbial signal transduction pathways regulate adaptation to changing environmental conditions and facilitate the success of both commensal and pathogenic during interactions with the host. Most of these pathways are regulated by protein kinases. The opportunistic fungal pathogen Candida albicans exists as a harmless commensal on mucosal surfaces of most humans, but can also cause superficial to invasive infections upon certain circumstances. Both life styles require a complex network of signalling pathways.

The C. albicans genome was predicted to encode 108 protein kinases, yet nearly 50% remain uncharacterised. We aim to dissect the role of C. albicans protein kinases during the transition from commensal to pathogen.

We used in vitro intestinal epithelial cell (IEC) models to investigate the role of fungal protein kinases and to study their impact on the pathogenicity of C. albicans. First, we screened a library containing individual C. albicans mutants lacking each of the identified protein kinase genes for their ability to damage IEC. Mutants displaying altered IEC cytotoxicity were further tested for their growth and morphology phenotypes. Surprisingly, a mutant in which CRK1 was deleted caused increased IEC cytotoxicity, despite slower growth and reduced hyphal length as compared to the parental wild type. To dissect the role of CRK1 in pathogenicity, we monitored metabolic fitness by using Biolog Phenotypic Microarrays, and stress resistance by spot dilution assays, quantified the damage capacities towards other cell types, and quantified the adhesion, invasion and translocation potential.

Infection of various epithelial cell lines in different cell culture media revealed that the increased damage potential of crk1Δ/Δ is cell type- and media-dependent. Accordingly, metabolic profiling and spot dilution assays showed that CRK1 is important for metabolic adaptations and resistance to cell wall stress. When investigating the stages of IEC infection, we found that adhesion of crk1Δ/Δ to IEC was slightly reduced, whereas invasion was significantly increased. In contrast, the ability to translocate through an IEC barrier in a transwell assay was reduced.

Collectively, we used in vitro models to investigate the role of protein kinases for C. albicans pathogenicity and identified the protein kinase gene CRK1 as being critical for the regulation of processes linked to medium- and host cell type-specific cytotoxicity.

WS02.02

Free ISG15 dampens neutrophil hyperactivation by *C. albicans*

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Vulvovaginal candidiasis (VVC) is one of the most common fungal infections affecting women in their reproductive years. The interplay between the immune system and the virulence mechanisms of C. albicans drives the pathogenesis of VVC, leading to a hyperinflammatory response, neutrophil recruitment and activation as hallmarks of the infection. Type I interferon signalling and stimulation of interferon-stimulated genes (ISGs) has been identified as a common signature of early vaginal epithelial cell responses to infection with Candida species. This response improves epithelial resistance to Candida infections. While a myriad of ISGs are regulated by interferon signalling, ISG15 warrants further exploration given its role as both an intracellular and extracellular mediator controlling certain viral and bacterial infections.

We investigated the localisation of ISG15 during C. albicans infection of vaginal epithelial cells (VECs). Upon infection, we observed an intracellular accumulation of ISG15 using fluorescence microscopy. Our measurements of intracellular freeISG15 showed a decrease at 3 h post-infection, without an increase in release, which suggests conjugation to other proteins. Silencing of ISG15 mRNA expression was associated only with slightly decreased epithelial damage, compared to infection with non-targeting siRNA. This could imply a subtle role for ISG15 in epithelial resistance to infection. Knowing the localisation of ISG15 determines its function, we used recombinant ISG15 to study its effects on neutrophil function. We observed that ISG15 modulates neutrophil function by dampening IL-8 release, ROS production while increasing NETosis and improving the neutrophil lifespan. However, C. albicans clearance was not negatively impacted.

Collectively, our data suggest that while ISG15 is expressed within vaginal epithelial cells upon fungal infection, its secretion may be tightly regulated or dependent on additional factors. Extracellular ISG15 in the context of neutrophil effector mechanisms could act in an effector-function dependent manner, either activating or dampening the anti-fungal response. Thus, ISG15 may play a role in dampening the inflammatory responses driving immunopathology in VVC, which warrants its investigation in patient cohorts.

WS02.03

***Wohlfahrtiimonas chitiniclastica*: a potential disruptor of wound healing and glucose metabolism in diabetic foot ulcers**

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Introduction: The worldwide increase in multidrug-resistant bacteria is drawing attention to previously underestimated pathogens and their impact on known infections. *Wohlfahrtiimonas chitiniclastica* has been lately identified in chronic, non-healing diabetic foot ulcers (DFUs), highlighting its emerging role in these infections.

Question: The aim of this study was to investigate the cellular and metabolic interactions of *W. chitiniclastica* in a diabetic wound environment.

Methods: To explore host-pathogen interactions, an epithelial cell line and monocyte cell line THP-1 cells were infected with *W. chitiniclastica.* Gene expression profiling was conducted. Furthermore, human neutrophils were isolated then infected with *W. chitiniclastica* to assess reactive oxygen species (ROS) production. To mimic diabetic conditions, infections were performed under varying glucose levels, followed by PCR for cytokine analysis. To assess potential effects on wound healing, a scratch assay was performed on epithelial cells infected with *W. chitiniclastica* for 24 hours, using a Keyence BZ-X800 microscope for analysis.The glucose metabolism of *W. chitiniclastica* was assessed by culturing the bacterium in media containing varying concentrations of glucose, followed by monitoring of its growth over time.

Results: Our results demonstrated that *W. chitiniclastica* had no significant effect on epithelial cells; however, it elicited a pronounced activation of THP-1 monocytes, characterized by elevated cytokine production. Notably, cytokine expression in THP-1 cells was attenuated under high-glucose conditions. Moreover, ROS production was generally reduced across all *W. chitiniclastica* strains, with the exception of one isolate. Surprisingly, the scratch assay revealed a significant delay in cell line closure in the presence of all *W. chitiniclastica* isolates or their supernatants. Additionally, all strains demonstrated robust growth in different glucose concentration environments, with no significant differences observed. This growth behaviour was comparable to that of "non-fermenting" bacterial species.

Conclusion: These findings suggest that *W. chitiniclastica* modulates immune responses in a glucose-dependent manner, potentially enabling immune evasion in the inflammatory environment of DFUs. *W. chitiniclastica* isolates significantly delayed wound closure, indicating a potential role in impaired chronic wound healing. Furthermore, *W. chitiniclastica´*s ability to grow in hyperglycaemic conditions without classical fermentation indicates oxidative glucose metabolism and a survival advantage.

WS02.04

Pneumococcal serine protease expression and their role in pathogen-host interactions

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Introduction: *Streptococcus pneumoniae* colonizes the upper nasopharynx asymptomatically, but can become pathogenic upon external triggers thereby causing severe infections. Serine proteases such as HtrA, PrtA, SFP, and CbpG are known to influence pneumococcal colonization and adherence. However, their expression, regulation and functional roles remain incompletely understood.

Objectives: This study investigated growth-phase-dependent expression and function of serine proteases in *S. pneumoniae* TIGR4 and EF3030 under nutrient-limited conditions. We

assessed protease expression, and investigated their potential substrates, and contribution to adherence and virulence using *ex vivo* and *in vivo* models.

Methods: TIGR4 (serotype 4) and EF3030 (serotype 19F) were cultivated in optimized chemically-defined medium (CDM+), mimicking nutrient-restricted conditions. RNA from early, mid, and late log-phase cultures was isolated and Northern blots performed for *serine proteases*. Proteomics was applied to compare wild-type and mutants for expression dynamics and protein localization and the bacterial morphological was visualized via electron microscopy. Murine lung slices were used for adherence studies and the *Galleria mellonella* infection model for *in vivo* virulence studies. Purified SFP and PrtA2 were incubated with epithelial monolayers to assess barrier disruption.

Results: Our proteome analysis revealed dynamic shifts in protein abundance across growth phases, notably in metabolism, stress response, competence, and virulence. EF3030 in CDM+ retains the ability to take up foreign DNA during the later stages of growth, with increased levels of ComEA, ComGA, and DprA. Serine proteases most probably influence pilus abundance and adherence, with lower levels of type1 pilus proteins RrgA, B and C. HUNTER N-terminomics analysis with platelet rich plasma (PRP) identified PrtA2-mediated cleavage of host ECM proteins such as fibronectin, vitronectin, and thrombospondin. While not significantly enhancing virulence, PrtA2 and SFP caused epithelial detachment, suggesting a role in host barrier disruption. The *serine protease* knockout strain exhibited reduced attachment in our *ex vivo* model. HtrA and PrtA were both detected extracellularly and in cell pellets of EF3030 and TIGR4Δ*cps*. SFP, only present in the TIGR4Δ*cps* but not 19F genetic background, was only detected in the supernatant fraction by proteome analysis.

Summary: Our findings demonstrate that serine proteases in *S. pneumoniae* module host interaction by promoting adherence and disrupting epithelial integrity. While not major virulence factors, SFP and PrtA2 play most likely key roles in early colonization and immune evasion.

WS02.05

Bupivacaine hydrochloride has antiviral and antimycotic properties during co-infection with influenza A viruses and *Aspergillus fumigatus in vitro*

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Respiratory infections are among the most common and consequential diseases worldwide that are associated with significant mortality. Infections with influenza A virus (IAV) and secondary infections caused by bacteria such as *Staphylococcus aureus* or opportunistic pathogens like the saprotrophic mold *Aspergillus fumigatus* often result in severe clinical outcomes, especially in the case of co-infections. The availability of suitable treatments is limited. Drug repurposing, which involves the use of already approved compounds for new therapeutic targets, enables the rapid and cost-effective development of alternative treatment options and represents a promising strategy. Previous studies have shown that local anaesthetics such as lidocaine and procaine act against viral, fungal and bacterial infections. Based on these findings, bupivacaine hydrochloride (bupivacaine), a structurally related local anaesthetic, was investigated in this study for its antimicrobial properties.

To explore these effects, *in vitro* experiments were performed using IAV-infected and IAV/*A-fumigatus* co-infected Madin-Darby Canine Kidney II (MDCK II) cells and Calu-3 human lung adenocarcinoma cells. First cytotoxicity assays were performed on the host cells to confirm tolerated concentrations. Possible antifungal effects of bupivacaine against *A. fumigatus* were analysed in a human cell-free environment. To investigate antiviral effects, MDCK II or Calu-3 cells were infected with IAV for 24 h with or without bupivacaine treatment at various non-toxic concentrations. In addition, Calu-3 cells were co-infected with IAV and *A. fumigatus* and treated with bupivacaine for 9 h. The antimicrobial effects of bupivacaine were analysed using various methods, including plaque assays, Western blotting, absorbance measurements, and immunofluorescence microscopy. The results indicate that bupivacaine inhibits the growth of *A. fumigatus* in a dose-dependent manner. Moreover, treatment with this local anaesthetic led to a significant reduction in IAV titers and to a reduced expression of viral proteins. Preliminary results from co-infection experiments indicate an inhibitory effect of bupivacaine against both tested pathogens.

In summary, bupivacaine showed both antiviral and antifungal effects *in vitro* at concentrations below the cytotoxic threshold. These results indicate a potentially expanded range of treatments for viral and fungal infections. Further investigations are required to clarify the underlying mechanisms and to validate these effects in more complex infection models.

WS02.06

The role of the pAA plasmid in mediating interaction and invasion of extraintestinal cell linages

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Introduction: Pathogenic Escherichia coli strains have traditionally been classified based on their ability to cause intestinal or extraintestinal infections, such as urinary tract infections or bloodstream infections. However, recent studies have revealed that some E. coli strains can cause diarrhea and extraintestinal infections within the same patient. One such strain belongs to the enteroaggregative E. coli (EAEC) pathotype, typically associated with diarrheal diseases, but has also been increasingly found in extraintestinal infection sites. This observation has raised important questions about whether EAEC-specific virulence factors might also contribute to extraintestinal pathogenicity.

EAEC pathogenicity is attributed to specific virulence factors encoded by the aggregative adherence plasmid (pAA), which plays a key role in the development of diarrheal disease. While these factors have a central role in defining the EAEC pathotype, the specific functions of most pAA-encoded genes and the genetic diversity among pAA plasmids remain poorly understood. Additionally, this topic has not been studied in the context of extraintestinal infections. Goals: To investigate whether the presence of pAA plasmids could enhance extraintestinal virulence. Materials and methods: The pAA plasmid from three EAEC strains, isolated from symptomatic urinary tract infections (HSP60, LSC52, and UPEC100), was transferred to non-pathogenic E. coli strain MG1655 through conjugation. The isogenic strains with and without pAA plasmids were quantitatively evaluated and compared for their capacity to interact with and invade eukaryotic cells from different sources. The tests were conducted on four extracellular-derived cell lineages: A549 (lung), T24 (urinary

bladder), EA.hy926 (endothelium), and HK-2 (kidney), along with one intestinal epithelial cell line, Caco-2 cells.

Results: Our preliminary findings indicate that the acquisition of different pAA variants leads to distinct phenotypic outcomes. In some cases, the presence of specific pAA variants increases the capacity of E. coli MG1655 to adhere to and invade cells derived from extraintestinal sources but not from intestinal sources. Conclusions: These results suggest that pAA plasmids may play a broader role in E. coli pathogenicity beyond their established roles in diarrheal disease. They could contribute to the emergence of hybrid strains capable of causing diverse infections.

Further characterization of the genetic content of the pAA plasmids will be critical to understanding their role in shaping the E. coli virulence. Additionally, it may provide new insights into the virulence and emergence of hybrid pathotypes that can potentially cause both intestinal and extraintestinal infections.

WS03.01

Antimicrobial activity of a nitroxoline conjugate against Candida spp.

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Introduction and goal: Nitroxoline is an approved drug with highly potent, broad-spectrum antimicrobial activity against major human pathogens, including multidrug-resistant strains. The clinical use, however, is limited to uncomplicated urinary tract infections due to insufficient organ distribution and cytotoxicity at higher doses. We have addressed these shortcomings by developing a nitroxoline conjugate (NC) within the framework of the EU funded project "Targeted Nitroxoline Delivery for Treatment of Multidrug-resistant Pathogens" (TANDEM). Here, we present data on *in vitro* and *in vivo* antifungal activity of NC.

Material & Methods: The antifungal activity of NC was assessed against 35 *Candida* spp. including 30 different species, e.g. *Candida albicans, Candida glabrata and Candida auris.* The minimum inhibitory concentrations (MIC) of NC, nitroxoline (N) and conjugate (C) were determined using the broth microdilution method. The mode of action, i.e. fungicidal or fungistatic, of the anti-infective NC was determined by time-kill kinetics. Fungicidal activity was defined as a ≥ 3 log killing after 24 h. *In vivo* antifungal activity of the NC against *Candida albicans* was assessed via *Galleria mellonella* infection model.

Results: The MIC data obtained confirm the broad antimicrobial *in vitro* activity of nitroxoline against *Candida* spp. Additionally, the NC showed comparably good antimicrobial activity. The NC MIC50/90 and MIC range in mg/L were 4, 8 and 1-32 for *Candida* spp. The mode of action for the NC was determined to be fungicidal for *Candida albicans*. In the *Galleria mellonella* infection model, both Nitroxoline and the NC exhibited good in vivo activity, as evidenced by a significantly higher survival rate in treated larvae compared to the untreated, infected controls.

Conclusions: Our data indicate a promising approach in the fight against infections due to *Candida* spp. Further experiments are needed to investigate the toxicity and antimicrobial activity of NC *in vivo.*

WS03.02

RamanBioAssay™ platform for rapid identification and antimicrobial susceptibility testing of bacteria

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Antibiotic resistance is a growing problem in modern medicine (Mohsen Naghavi et al. 2024). As a result, the treatment of infectious diseases is becoming increasingly complicated, with serious medical and financial consequences (Fongang et al. 2023; Dadgostar 2019). Identification of causative bacteria and subsequent antimicrobial susceptibility testing are important tools to mitigate these implications (Jim O'Neill 2016). Every hour that passes without effective antibiotic treatment increases the risk of complications or death (Gaieski et al. 2010; Buehler et al. 2016).

The RamanBioAssay™ is a hygienic and flexible multi-well platform for rapid bacteria identification and antimicrobial susceptibility testing compatible with bacteria relevant for infectious diseases. Various sample origins with increasing complexity were tested: laboratory control strains, patient isolates and real patient blood cultures. Special emphasis was placed on blood cultures as samples of great clinical importance, as bloodstream infections require timely and precise treatment. Bacteria, which have been separated from the blood culture matrix, interact with various antibiotics in different concentrations for a brief period of 90 min. Molecular changes caused by the antibiotic can be probed by spontaneous Raman spectroscopy, which enables rapid phenotypic antimicrobial susceptibility testing (Schröder et al. 2017). Raman is a label-free and non-destructive technique that investigates the vibrational states of the sample and thus its molecular composition (Sato et al. 2024).

The spectroscopic results are translated into resistograms using statistical data analysis. As different bacteria have different spectral fingerprints, the identification of bacteria in conjunction with phenotypic antimicrobial susceptibility testing is possible without the need for additional measurements or instrumentation. The test turnaround time from bacterial sample to result is ≤ 3.5 h which qualifies the RamanBioAssay™ as a rapid diagnostic platform.

WS03.03

A prospective comparison of five methods for rapid antimicrobial susceptibility testing (RAST) for Gram-negative bacteremia

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Introduction: Sepsis, a medical emergency resulting from a dysregulated host response to infection, causes life-threatening organ dysfunction and is a major global contributor to infection-related mortality. Timely administration of effective, targeted anti-infective therapy is crucial for patient

survival. Conventional antimicrobial susceptibility testing (AST) requires a subculture of the positive blood culture (BC) followed by additional 16-24 h of incubation time, which delays the initiation of targeted therapy. Therefore, rapid AST (RAST), which significantly reduces time to result, can improve patient management and, hence, the clinical outcome.

This prospective study aimed to evaluate and compare five different systems for RAST.

Materials & Methods: 110 BCs from three laboratories positive for Gram-negative rods were analyzed via five different methods providing AST results: a multiplex PCR yielding species ID and results for ESBL and carbapenemase coding genes (BioFire BCID2 [bioMérieux]), two automated platforms for MIC determination (dRAST [Quantamatrix] and Specific Reveal [bioMérieux]), and disk diffusion according to the EUCAST RAST methodology. This analysis was performed in parallel using conventional incubation and the automated BD Kiestra system (Becton Dickinson), with results for up to three different incubation periods (4, 6, and 8 h). VITEK 2 and, upon indefinite results, gradient strip or broth microdilution testing were applied to determine the reference results.

Results: Results were obtained for 93 non-duplicate isolates, with the majority being *Escherichia coli* and *Klebsiella pneumoniae*. The time to result ranged from 1-9 h. The proportion of valid analyses was much higher in automated test systems, as these support testing of a much broader range of species compared to the agar-based RAST, which offers breakpoints for only few species.

The PCR assay demonstrated high sensitivity and specificity for species identification and detection of ESBL-type resistance.

The overall error rates ranged from 4-10 % for the different phenotypic assays. The high error rates of the agar diffusion-based assays were primarily attributed to results in the area of technical uncertainty. The automated systems for MIC determination yielded fewer errors in total, but higher rates of major and very major errors.

For penicillin antibiotics, a small proportion of false-susceptible results was obtained with all culture-based methods. Particularly piperacillin / tazobactam testing was challenging (both false-resistant and false-susceptible results). For cephalosporins and carbapenems, all test systems demonstrated good results, with the exception for testing of *Proteus mirabilis*, which was tested false-resistant in multiple samples by one assay.

Conclusion: All test systems for rapid AST demonstrated strong performance but also specific weaknesses, for which awareness is necessary.

WS03.04

Efficacy of Cefiderocol in combination with Xeruborbactam vs. Taniborbactam against Cefiderocol-resistant NDM-producing Pseudomonas aeruginosa

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Introduction: Elevated cefiderocol MICs have been reported in NDM-positive Gram-negative bacteria. In high-income countries, carbapenem resistance in *P. aeruginosa* is typically driven by porin loss and efflux mechanisms. In contrast, NDM-producing *P. aeruginosa* is more prevalent in low- and middle-income countries, where colistin is often used as a last-resort treatment despite its toxicity. Recently developed boronate-based β-lactamase inhibitors, taniborbactam and xeruborbactam, have shown inhibitory activity against all β-lactamases, including MBLs. Phase 1 clinical trials evaluating cefiderocol in combination with xeruborbactam (NCT06547554) are currently ongoing. Preliminary results suggest that xeruborbactam reduces the cefiderocol MIC in Enterobacterales. However, their efficacy against NDM-producing *P. aeruginosa* clinical isolates has not been evaluated.

Objective: To evaluate the activity of cefiderocol in combination with taniborbactam and xeruborbactam against cefiderocol-resistant, NDM-positive *P. aeruginosa* clinical isolates from Nigeria and Vietnam.

Methods: A total of 46 *bla*$_{NDM}$-positive *P. aeruginosa* isolates recovered from clinical specimens were included in the study. Cefiderocol MICs were determined using broth microdilution in iron-depleted cation-adjusted MHB, both with and without the addition of 4 µg/mL of either taniborbactam or xeruborbactam. Dipicolinic acid (DPA) was used to confirm the role of NDM in cefiderocol resistance. Two *bla*$_{NDM}$-positive, cefiderocol-resistant non-*Pseudomonas* Gram-negative isolates were included as external comparators to confirm the activity of xeruborbactam and taniborbactam in our testing. Hybrid whole genome sequencing and mRNA expression were performed to investigate resistance mechanisms.

Results: Cefiderocol resistance (MIC > 2 mg/L) was observed in 22/46 isolates (47.8%). All isolates harboured the *bla*$_{NDM-1}$ gene. DPA restored susceptibility in all resistant isolates, confirming NDM as the primary resistance mechanism. Taniborbactam restored cefiderocol susceptibility in all but one isolate (X273), which had multiple *bla*$_{NDM-1}$ copies and a 3–4-fold increase in mRNA expression. Xeruborbactam had no effect on cefiderocol MICs.

Conclusion: Our findings confirm that NDM activity is the primary cause of cefiderocol resistance in P. aeruginosa. Taniborbactam effectively restored susceptibility in almost all isolates. However, xeruborbactam was ineffective against P. aeruginosa, emphasising the importance of conducting species- and mechanism-specific evaluations when developing β-lactam/β-lactamase inhibitor combinations.

Table 1: Susceptibility testing of cefiderocol and its combinations with either xeruborbactam (XER), taniborbactam (TAN) or dipicolinic acid (DPA), for the studied NDM-positive *P. aeruginosa* isolates and NDM-positive non-Pseudomonas quality control strains.

Fig.1

P. aeruginosa isolates	MIC (mg/L)			
	cefiderocol	cefiderocol + XER	cefiderocol + TAN	cefiderocol + I
X273	32	32	16	0.5
X351	1			
X444	0.5			
X445	8	8	2	0.5
X474	4	4	1	0.5
X503	4	8	0.5	0.25
X549	2			
X583	4	4	2	0.5
X734	4	4	0.5	0.25
X888	4	4	1	0.5
X951	0.06			
X2041	2			
X010_19	4	4	0.5	0.25
X109_19	4	4	0.5	0.25
X123_19	8	8	1	0.5
X132_19	4	4	0.5	0.25
X159_19	2			
X193_19	8	8	2	0.25
X201_19	8	8	1	0.125
X202_19	16	16	2	0.5
X204_19	8	8	1	0.25
X205_19	4	4	2	1
X230_19	4	4	0.5	0.06
X235_19	2			
X239_19	1			
X257_19	0.25			
X263_19	0.25			
X265_19	8	8	2	0.25
X274_19	1	1	0.25	<=0.03
X302_19	2			
X303_19	2			
X306_19	4	4	1	0.25
X309_19	4	4	0.5	0.5
X312_19	1			
X314_19	0.5			
X324_19	2			
X331_19	2			
X332_19	0.06			
X338_19	8	8	0.5	0.5
X341_19	0.25			
X344_19	1			
X352_19	2			
X359_19	4	4	0.5	0.5
X361_19	1			
X362_19	0.125			
X363_19	0.5			
Non-*Pseudomonas* control isolates				
Klebsiella pneumoniae Klpn003	8	2	2	1
Enterobacter cloacae etcl_1	4	1	0.5	0.25

WS03.05

First detection of a phenotypically difficult-to-detect vancomycin-resistant Enterococcus faecium clone linked to a localised outbreak in south-eastern Austria starting in 2024

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Background: *Enterococcus faecium* has become a critical nosocomial pathogen, largely due to the increasing prevalence of vancomycin-resistant strains (VREfm), driven by the dissemination of vanA- but also vanB-mediated resistance. These strains are associated with limited treatment options and increased mortality rates. VREfm can spread via colonisation and contaminated hospital environments, making infection control challenging. Rapid detection and genomic surveillance are essential to contain outbreaks and guide effective therapy – especially when unusual phenotypic resistance patterns or localised transmission clusters are observed.

Objectives: This study aimed to investigate an outbreak caused by a novel VREfm strain with an unusual resistance phenotype, which was challenging to diagnose with different assays applied in routine diagnostics.

Materials and methods: We phenotypically analysed isolates collected between 2024 and 2025 in Styria, Austria, and compared automated susceptibility testing (VITEK 2 [AST P655], bioMérieux), disk diffusion according to EUCAST, MIC gradient strip assay (ETEST, bioMérieux), and broth microdilution (Sensititre [EUENCF], Thermo Scientific) using standard conditions and prolonged incubation. VanB-type resistance was confirmed by molecular analysis (Xpert vanA/vanB, Cepheid). Complementarily, whole-genome sequencing (WGS) via nanopore sequencing (Oxford Nanopore Technologies) was performed. The National Reference Centre for Staphylococci and Enterococci of the Robert Koch Institute, Wernigerode, Germany, provided additional control and reference strains, as well as sequence data from their genomic databases.

Results: All 29 non-duplicate isolates were obtained from patients with clinical infections, with 83% of them having direct contact to one surgical facility. Automated susceptibility testing consistently indicated resistance to vancomycin (MICs of 8mg/dl), while teicoplanin was reported susceptible. In contrast, disk diffusion implied susceptibility to glycopeptides. Broth microdilution, representing the gold standard in susceptibility testing of enterococci, confirmed elevated MICs only after prolonged incubation.

These isolates all belong to a newly emerged complex type (CT), for which occurrence of vancomycin resistance has not yet been reported. Although isolated over a period of more than one year, all strains exhibited a maximum of 5 allelic differences in cgMLST analysis based on 1,423 loci, suggesting ongoing circulation of a genetically stable clone, which differs from the closest local control strain by over 150 alleles.

Conclusion: The observed emergence of VREfm at a defined surgical context, showing a unique ST/CT profile, indicating a previously unrecognised clonal lineage in South-Eastern Austria not only underscores its clinical and hygienic significance, but also reveals a possible diagnostic gap, as current screening strategies and conventional AST-methods may fail to reliably detect this strain.

WS03.06

Bacterial membrane vesicles as a potential surrogate for an impaired gut barrier integrity

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Background: In critically ill or immunosuppressed patients, a bacteraemia is oftentimes the consequence of an impaired intestinal barrier integrity. Thus, bacteria are capable of translocating from intestine to surrounding tissue or bloodstream. As a significant number of bacteria are known to produce membrane vesicles (MV), the present study investigates whether MV can serve as an indicator of impaired intestinal barrier function ("leaky gut") in critically ill patients.

Methods: A total of 18 male and female wild-type C57BL/6 mice (aged between 9–13 weeks) were randomly assigned to one of three groups: laparotomy (= sterile inflammation), caecal ligation and puncture (= sepsis) or no intervention. After 18 h, MV were isolated from plasma using ultracentrifugation. Faeces-derived MV were isolated by a combination of ultracentrifugation and density gradient centrifugation. The nanoparticle tracking analysis was used to assess the concentration and size of the MV. The proportion of plasma- and faeces-derived MV was analysed using fluorescently labelled anti-CD63 for mouse-derived MV and anti-LTA antibodies for bacterial MV in FACS analysis.

Results: Following sterile inflammation, the concentration of in blood increased by 48 % (3.4×10^8 particles/mL) due to elevated levels of murine and Gram-positive bacterial MV. The overall MV were 28 % smaller (108.4 ± 54.9 nm) than those of control mice (2.3×10^8 particles/mL, 150.9 ± 46.7 nm) in terms of size, whereas the size of faeces-derived MV increased by 58 % (189.6 ± 61.9 nm).

In septic mice, MV both in size and concentration in plasma and faeces were increased. A 4-fold increase in MV concentration (9.2×10^8 particles/mL; control: 2.3×10^8 particles/mL) and a 25 % increase in size (186.7 ± 25.1 nm) were detected. An enhanced proportion of Gram-positive bacterial MV accounted for the higher MV load in plasma of septic mice. In faeces, an elevated MV concentration of 52 % (3.3×10^8 particles/mL; control: 2.2×10^8 particles/mL) and a 17 % increase in MV size (140.6 ± 74.2 nm, control: 119.9 ± 49.6 nm) were measured.

Conclusion: Bacterial MV translocation is elevated in septic and sterile inflammatory mice, indicating a loss of gut barrier integrity in both conditions. Thus, MV detection in plasma serves as a promising tool for early detection of patients suffering from leaky gut syndrome. Further translational research is needed to verify present findings in human.

WS04.01
IL-36 subfamily cytokines from the IL-1 family may potentiate inflammation during RVVC

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Vulvovaginal candidiasis (VVC) is a yeast infection of the vaginal mucosa, most commonly caused by *Candida albicans*. Compared to other *Candida* infections, VVC is unique in that the disease severity is predominantly driven by the host inflammatory responses rather than a compromised immunity. VVC pathogenesis is generally steered by the complicated interplay between fungal pathogenicity and concurrent protective and dysregulated inflammatory responses.

When assessing cytokine levels in vaginal lavages of RVVC patients, we found significantly higher interleukin (IL)-36β (IL-1F8) levels in women with RVVC compared to asymptomatic women. As members of the huge IL-1 family, cytokines of the IL-36 subfamily have been reported to activate neutrophil-mediated inflammation, contributing to numerous inflammatory diseases.

We found that vaginal epithelial cells (VECs), the first barrier of vaginal defense, and monocyte-derived macrophages did not release IL-36 cytokines upon *C. albicans* infections. While systematically exploring various cell types of the vaginal niche, vulva cells were identified as a potential source of IL-36 cytokines during *C. albicans* infection. To shed light on the role of IL-36 during VVC, VECs were infected with *C. albicans* in the presence of IL-36β or IL-36γ. The presence of these cytokines potentiated the release of alarmin IL-1α upon encountering *C. albicans*, suggesting the epithelial cells can elicit stronger downstream proinflammatory responses. Meanwhile, neutrophil chemoattractant IL-8 and survival factor GM-CSF were induced by IL-36β/γ irrespective of infection, conjecturing that IL-36 stimulations can lead to both the recruitment and activation of neutrophils. As RVVC is characterized by a dysfunctional neutrophil-mediated fungal clearance, we explored how IL-36 cytokines directly, as well as the IL-36-activated VECs, impact the recruitment and effector functions of neutrophils. We observed increased neutrophil recruitment to *C. albicans*-infected and IL-36-activated VECs. However, the presence of IL-36 did not guarantee more effective neutrophil mediated fungal clearance, particularly when stimulated by the *C. albicans*-infected and IL-36-activated VECs.

Collectively, these data suggest that IL-36 family cytokines have to be considered in RVVC inflammatory pathology given their potential to amplify epithelial responses to *C. albicans*-infection and impact neutrophil anti-*C. albicans* functions.

WS04.02
TIR-domain-containing Protein C (TcpC) of uropathogenic *E. coli* CFT073 as regulator of innate immune checkpoints

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Urinary tract infections are one of the most common community acquired infection worldwide affecting approximately 150 million people each year. Uropathogenic *E. coli* are responsible for the vast majority of UTI. In order to infect the lower and upper urinary tract they express a wide variety of virulence factors. One of these virulence factors is TcpC, a Toll/interleukin-1 receptor domain-containing protein produced by various *E. coli* strains of the phylogenetic group B2 including CFT073. Studies have shown that TcpC is able to inhibit TNFα and IL-1β release of mouse macrophages during an infection with *E. coli* CFT073. It is suggested that TcpC is able to inhibit cytokine release of these cells by binding to specific proteins of the TLR4 signaling cascade and the NLRP3 inflammasome.

We now report that TcpC is able to stimulate cytokine release of immune and epithelial cells during infection. Human monocytes and human bladder epithelial cells release higher amounts of proinflammatory cytokines after infection with TcpC producing CFT073 strains compared to a TcpC knockout. Differentiation of monocytes to macrophages abrogates this TcpC dependent effect. Infection of T24ΔTLR4 cells suggest that exclusively TLR4 is responsible for a proinflammatory reaction. Furthermore, infection of T24ΔMyD88 cells suggests that the TcpC induced stimulation of proinflammatory cytokines is MyD88-independent. THP-1 cells treated with conditioned medium in which TcpC was overexpressed at different levels showed, that TcpC inhibited cytokine release after stimulation with LPS at low levels of induction. Deletion of the TIR-domain of TcpC leads to a loss of the inhibitory capabilities showing that it is crucial for the function of the protein in this context.

During an infection of monocytes with CFT073, the proinflammatory response is increased compared to the TcpC knockout strain whereas treatment with culture supernatants containing TcpC inhibit the proinflammatory response of monocytes. In summary, we conclude that the TcpC induced inhibition versus stimulation of release of proinflammatory cytokines may depend on the direct contact between CFT073 and eukaryotic cells. Since TcpC is able to bind to TLR4 and MyD88 we think it affects myddosome formation after stimulation of cells with LPS which may be further influenced by the direct contact of the bacterium to the immune cells.

WS04.03

Bactericidal/permeability-increasing protein promotes the immune response towards Mycobacteria

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The neutrophil-derived bactericidal/permeability-increasing protein (BPI) is well known for its bactericidal activity towards Gram-negative bacteria, neutralization of LPS and enhancement of the immune response towards lipoproteins of Gram-positive bacteria. Elevated serum levels of BPI and upregulated gene expression in peripheral blood of tuberculosis (Tb) patients indicate an additional role of BPI in mycobacterial infection. Since a detailed implication of BPI in Tb is not yet described, we aimed to characterize the influence of BPI in infection with *Mycobacterium tuberculosis* (Mtb). According to our data, BPI interacts with preparations of the Mtb cell envelope glycolipids PIM6 and ManLAM. Thereby BPI provokes a pronouncedly enhanced reaction of human peripheral blood mononuclear cells (PBMCs) towards either stimulus in form of increased secretion of the cytokines GM-CSF and TNF in a TLR2-dependent manner. Flow cytometry and single-cell RNA sequencing revealed that myeloid dendritic cells, classical and intermediate monocytes are the cellular subsets most responsive to the boosting effects of BPI. The enhanced response of myeloid cell populations also fosters the downstream T-cell response regarding secretion of IFN-γ and IL-17A. Interestingly, the affected cytokines are key factors in the well-orchestrated host-defence against mycobacteria. Based on these results, we define BPI as a soluble pattern recognition receptor for perception of mycobacterial pathogen associated molecular patterns (PAMPs).

WS04.04

Iron regulatory proteins 1 and 2 have opposing roles in regulating inflammation in bacterial orchitis

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Introduction: Acute bacterial orchitis (AO) is a prevalent cause of intrascrotal inflammation, often resulting in sub- or infertility. A frequent cause eliciting AO is uropathogenic *Escherichia coli* (UPEC), a gram negative pathovar, characterized by the expression of various iron acquisition systems to survive in a low-iron environment. On the host side, iron is tightly regulated by iron regulatory proteins 1 and 2 (IRP1 and -2) and these factors are reported to play a role in testicular and immune cell function; however, their precise role remains unclear.

Methods: To investigate the role of iron in UPEC infection, we used an epididymo-orchitis mouse model with WT, *Irp*1$^{-/-}$, and *Irp*2$^{-/-}$ mice, as well as *in vitro* infections of bone marrow-derived macrophages (BMDM) isolated from WT and *Irp*1$^{-/-}$ mice. UPEC-induced pathology in testes was analyzed using histological, cellular, and molecular techniques. The impact of iron availability on UPEC was also assessed using standard microbiological methods.

Results: Here, we show that the absence of IRP1 in UPEC induced orchitis results in less testicular damage and a dampened immune response. Compared with infected wild-type (WT) mice, testis of UPEC-infected *Irp*1$^{-/-}$ mice showed impaired ERK signaling. Conversely, IRP2 deletion led to a stronger inflammatory response. Notably, immune cell infiltration varied by genotype. Unlike WT and *Irp*2$^{-/-}$ mice, *Irp*1$^{-/-}$ testis showed no increase in monocytes and neutrophil numbers following infection. Instead, *Irp*1$^{-/-}$ mice had elevated levels of F4/80^{+}CD206^{+} macrophages, a subset linked to anti-inflammatory and tissue repair functions. Despite these differences, bacterial loads were comparable across all genotypes. We further explored how iron availability affects UPEC pathogenicity and found that UPEC invaded *Irp*1$^{-/-}$ BMDM more efficiently than WT BMDM. Additionally, IRP1 deletion altered the expression of virulence factors related to bacterial invasion and vacuole formation in macrophages.

Conclusions: Increased immune cell infiltration and inflammation caused by UPEC infection induced severe damage in testis of *Irp*2$^{-/-}$ mice. However, IRP1 deletion in mice showed a protective effect by reducing the inflammatory response to infection, which could be related to changes in pathogenicity of the microbe.

WS04.05

Macrophage-dependent interferon-γ signaling regulates local tissue perfusion, tissue oxygenation and antimicrobial control

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Low levels of oxygen (O2) prevail in infected or inflamed tissues. Low lesional O2 levels promote the survival of the intracellular parasite *Leishmania* (*L.*) *major*, whose control depends critically on the ability of macrophages to produce high levels of NO through the O2-dependent antimicrobial enzyme type 2 nitric oxide synthase. However, the mechanism leading to local O2 deficiency in the infected lesion is unknown. The focus of this study is to understand the regulation of oxygenation and perfusion in *L. major*-infected tissues.

Using non-invasive optical imaging of O2 and LASER speckle perfusion measurements in a C57BL/6 (healer) / BALB/c (non-healer) mouse model, we observed a substantial increase in tissue perfusion in C57BL/6 mice, but not in BALB/c mice, which was paralleled by normalization of O2 levels and clinical resolution of disease. Furthermore, T and B cell-deficient (Rag2-KO) C57BL/6 mice showed a non-healing course without an increase in perfusion and no normalization of tissue O2, which could be rescued by adaptive T cell transfer.

Global transcriptomic analysis revealed an enrichment of interferon-γ-dependent signaling in lesional endothelial cells, fibroblasts, T cells and myeloid cells. We tested the contribution of interferon-γ signaling in mice deficient for IFNGR2 in their myeloid compartment. Compared to controls, mice were unable to improve tissue perfusion and showed rapid progression of the lesion.

We show that tissue perfusion serves as a driver for O2 normalization and lesion healing. In addition to their antimicrobial role, T cells and myeloid cell-dependent interferon-γ signaling contribute to an appropriate immunovascular tissue response, which is critical for infection control and resolution.

WS04.06

Streptococcus pneumoniae primes and activates the NLRP3 inflammasome in cardiomyocytes during pneumococcal pneumonia

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Introduction: *Streptococcus pneumoniae*, a Gram-positive, α-hemolytic pathogen, is associated with severe invasive diseases mainly in elderly and infants. These diseases include meningitis, pneumonia, and sepsis. Severe pneumococcal pneumonia with bacteremia is frequently associated with cardiac complications like heart failure. Possible causes are the robust inflammatory response and the direct or indirect interactions of the bacteria with the heart. Whereas the mechanisms underlying pneumococcus-induced cardiomyopathy are elusive, pro-inflammatory cytokines, such as interleukin-1β (IL-1β), are suspected to play a central role. The NLRP3 (NLR family pyrin domain containing 3) inflammasome is a key regulator of IL-1β activation, and its role in septic cardiomyopathy has been previously demonstrated. However, whether NLRP3 inflammasome activation contributes to cardiac injury during pneumococcal infection is still unknown.

Aim: This study tests the hypothesis that activation of the NLRP3 inflammasome in the heart contributes to cardiac injury.

Material and Methods: In our *in vivo* model for *S. pneumoniae*-induced heart failure female CD-1 mice (10–12 weeks) were intranasally infected with *S. pneumoniae* TIGR4*lux* (6×10^7 bacteria, n=24) or PBS (n=15) for 72 h. Further, the interaction of pneumococci with cardiomyocytes was also analyzed *in vitro* using neonatal rat ventricular cardiomyocytes. Cytotoxicity was assessed using a BioLegend LDH-Cytox™ Assay Kit. Heart tissue and cell lysates were analyzed for changes in mRNA and protein expression of inflammatory components using qRT-PCR and Western blot, while hematoxylin and eosin staining and immunohistochemistry were performed to assess immune cell infiltration in heart slices.

Results: We observed elevated expression of pro-inflammatory markers (*Il6*, *Socs3*, *Tnfa*) along with increased mRNA and protein levels of key NLRP3 inflammasome components (NLRP3, caspase-1, GSDMD, and *Il1b*) in heart tissue. Activation of the NLRP3 inflammasome was confirmed by the cleavage of caspase-1 and GSDMD, indicating inflammasome assembly and pyroptosis. Histological analysis revealed immune cell infiltration. Furthermore, both priming and activation of the inflammasome were observed *in vitro* in cardiomyocytes, resulting in pyroptosis.

Conclusions: Our findings demonstrate that pneumococcal pneumonia primes and activates the NLRP3 inflammasome in the heart, suggesting a mechanistic link between *S. pneumoniae* infection and cardiac complications. Targeting the NLRP3 inflammasome may offer a novel approach to prevent or mitigate cardiac dysfunction during pneumococcal pneumonia. Further studies are warranted to explore this pathway as a potential therapeutic target.

WS05.01

Automated detection of healthcare associated infections using hospital routine data

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Background: Healthcare-associated infections (HAIs) affect approximately 6% of hospitalized patients in Europe. Surveillance and reporting of these infections place a significant burden on infection prevention and control (IPC) teams—consuming 30–50% of their working hours at the studied university medical center, despite being limited to intensive care units. This project, conducted in collaboration with two partners from the healthcare and chemical industries, aims to address this challenge by developing an automated, scalable surveillance system.

Goals: The primary objective is to develop an automated detection system for HAIs using hospital routine data. This approach seeks to extend the surveillance beyond intensive care units to the entire hospital, while improving the specificity of HAI detection through a standardized, easily implementable method. A key aim is to reduce the workload of the IPC team on manual surveillance, enabling them to focus on critical activities such as staff training, hygiene audits, and personalized consultations.

Methods: The system utilizes routine data structured through Germany"s Diagnosis Related Group (G-DRG) system. The legal basis of the G-DRG system is established in § 17b of the Hospital Financing Act (KHG), which defines its essential characteristics. Data inputs include ICD diagnosis codes, OPS procedure codes, complication and comorbidity levels (CCL), as well as demographic and hospitalization data. Building upon the IQTIG NWIF module, suspected HAI cases are validated through manual patient chart reviews, allowing for continuous refinement of the detection algorithm. Starting with the identification of surgical side infections (SSI), we use the results from one of our previous studies (2018-2020) as a baseline to calibrate the algorithm. The analysis includes 2,459 patients of an orthopaedic trauma surgery department, who were part of HAI surveillance of the IPC team.

Results: Preliminary findings indicate that the algorithm achieved a sensitivity of 92.3% and a specificity of 91.1% after only a few evaluation cycles. The number of false positives was 214, and false negatives 5. Compared to the basic algorithm we were able to improve the performance (Sensitivity: 65.7%, Specificity: 93.7%, positive predicted value: 16.5%). These results demonstrate that our approach has the potential to improve further as more cases are evaluated. IPC resources and the high quality of manual documentation were key factors contributing to this early success.

Conclusion: Automated HAI surveillance using routine data is a promising method to support IPC teams. Despite current limitations in the number of validated cases, the approach shows potential for broader application across infection types. The system may ultimately support the retro- or prospective identification of high-risk patients based on coding patterns—enabling targeted, individualized infection prevention strategies and infection control outcomes.

WS05.02
Beyond KISS: exploring the concordance of conventional central line associated bloodstream infection (CLABSI) surveillance with fully automated hospital-onset bacteraemia (HOB) surveillance
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Background: In recent years, automated hospital-onset bacteraemia (HOB) surveillance has been proposed as a novel surveillance metric. However, its ability to meaningfully differentiate between wards with high and low infection rates has not been yet investigated in the German healthcare context.

Aim: To compare a method for fully automated HOB detection to manual central line associated bloodstream infection (CLABSI) surveillance using the methods of the intensive care component of the German national surveillance system (ITS-KISS).

Methods: We compared yearly ITS-KISS CLABSI cases per 1000 central line days to HOB rates per 1000 patient days from ten different intensive care units (ICU) at a university hospital over a seven-year period (2018-2024). KISS data were gathered through prospective routine surveillance. HOB rates were generated retrospectively through the application of the automated HOB surveillance algorithm jointly developed by the PRAISE network (1). CLABSI in KISS are primary BSI that occur in patients with central lines. Conversely, HOB entails bacteraemia and fungaemia irrespective of underlying aetiology and presence of a central line. In this analysis, the ability of both surveillance methods to distinguish ICU with high infection rates (outliers) was compared and the ITS-KISS method was defined as the reference standard. ICU were considered outliers if their annual CLABSI or HOB rates exceeded the 75th percentile of all included rates.

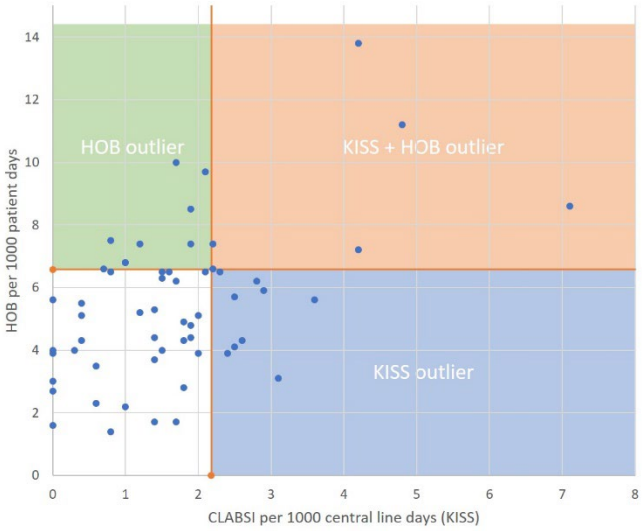
Results: A total of 58 ITS-KISS infection rates (yearly CLABSI rate in an ICU) were available, of which 6 were identified as outliers by both systems. In 8 cases, the automated HOB system identified outliers not flagged as such by KISS, whereas in 9 cases, KISS identified outliers not flagged by the HOB algorithm. In 35 cases, the rates were below the 75th percentile in both systems (Fig. 1). When taking the ITS-KISS method as a reference standard to identify ICU outliers, this results in a sensitivity of 40.0%, specificity of 81.4%, positive predictive value of 42.9%, negative predictive value of 79.5%, and an overall accuracy of 70.7% of the HOB algorithm.

Discussion: Alike conventional surveillance, HOB can be used to benchmark and compare infection rates from different wards. The moderate agreement with the ITS-KISS method to identify ICU outliers shown in our study likely reflects differences in underlying aetiologies of the respective metrics, and indicates that HOB may not replace conventional CLABSI surveillance, but rather enhance it. Further investigations on the matter should aim to include more data from different institutions, and consider other types of infection also collected in ITS-KISS and subtypes of HOB (e.g., common commensal HOB).

Fig. 1: Distribution of yearly CLABSI and HOB rates from ten different ICU between 2018 and 2024. Orange lines: 75%-percentile of CLABSI rates (vertical lines) and HOB rates (horizontal lines).

Ref. 1): DOI: 10.1101/2024.09.16.24310433

Fig. 1



WS05.03
Estimating sepsis incidence in Germany using two extrapolation approaches
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Background: Sepsis is a life-threatening consequence of infections, leading to acute organ dysfunction and potentially resulting in shock, multi-organ failure, and death. It was estimated that sepsis caused 49 million cases and 11 million deaths worldwide in 2017, making it a leading cause of mortality. In Germany, sepsis incidence was estimated 370 per 100,000 population in 2015. However, underestimation of sepsis is likely due to under-ascertainment. This study aims to estimate and compare sepsis incidence and trends in Germany while enhancing comprehension of available data sources.

Methods: Two independent data sources covering 2017–2022 were retrospectively analysed: laboratory-based data from the Robert Koch Institute's antimicrobial resistance surveillance system (ARS), and statutory health insurance data from AOK, Germany's largest health insurance provider. Each dataset covers approximately 30% of hospitalised patients in Germany. In ARS, positive blood cultures were used as a proxy for bloodstream infections (BSI) and served as a lower bound estimate, BSIs were adjusted for false positive isolates due to potential contamination (e.g. only 1/3rd of *Staphylococcus epidermidis* were counted). A stratified random sampling approach was used to extrapolate the number of BSI cases to the German population. For insurance claims data, a complex set of ICD-10 codes was applied to

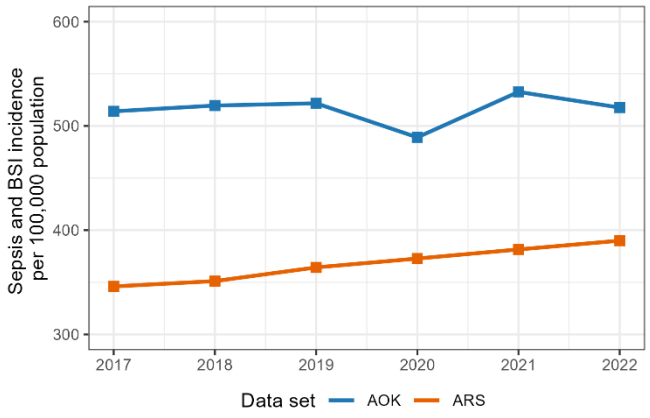
identify bacterial sepsis cases. The number of cases was subsequently extrapolated to the national level by age group and sex.

Results: For 2022, we estimated the number of sepsis cases 434,000 based on insurance data and 327,000 as the lower bound from BSIs. These estimates align with previous findings. Over time, the incidence remained relatively stable (Figure 1). A decrease in insurance claims-based incidence during the COVID-19 pandemic coincided with the change to the sepsis-3 definition. In both datasets, incidence was higher in males, especially in the youngest and oldest age groups. The pathogen spectrum showed that 70.9%–73.7% of BSI cases were due to gram-positive pathogens, with *Staphylococcus aureus* (20.1%) and *Staphylococcus epidermidis* (17.7%) being the two leading causative pathogens. Among gram-negative pathogens, *Escherichia coli* (50.7%) and *Klebsiella pneumoniae* (11.2%) constituted the main pathogens.

Discussion/Conclusion: This study provides a novel approach to estimate sepsis incidence in Germany by combining laboratory surveillance with insurance claims data. The consistency of findings over time highlights the robustness of the applied methods. Despite methodological challenges, the combined approach offers a promising way to improve sepsis surveillance, incorporating both routine health claims data and microbiological data. The persistently high incidence indicates the urgent need for enhanced prevention and treatment efforts.

Figure 1 Estimation of sepsis and BSI incidence using data from ARS and AOK

Fig. 1



WS05.04

A German-wide analysis to assess the impact of incidence on nosocomial SARS-CoV-2 infections

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The spread of SARS-CoV-2 and other infections transmitted via respiratory particles within hospitals remains a crucial concern for patients, healthcare workers, extending beyond pandemics. Assessing the risk of nosocomial infections and implementing measures against them is a central task for any infection prevention and control (IPC) department and the hospital governance. The aim was to assess the impact of weekly community SARS-CoV-2 incidence on risk of nosocomial SARS-CoV-2 infections (nosocomial infections).

The data was gathered through a web-based electronic system "webKess" as part of the COSIK Surveillance module, 37 hospitals (bed size 51–1,700) actively submitted data [1]. In addition, regional incidences were obtained from the official repository provided by the Robert Koch Institute. A generalised linear model (GLM) with a Poisson distribution was used to estimate the impact of incidence on nosocomial infections, using a nationwide dataset collected between 03.2020 and 04.2023.

The regression with an offset on patient-days indicated that regional incidence was significantly associated with an increase in nosocomial infection density (β=. 000778; SE=.000027, p<0.01). A likelihood-ratio analysis showed that both variables contributed equally to the explained deviance. Furthermore, the GLM predicted values follow a linear distribution up to a regional incidence of 110 positive SARS-CoV-2 cases per 100,000 inhabitants beyond which the trend becomes exponential (Figure 1).

The link between community incidences and nosocomial risk is known [2, 3]. However, the extent of this effect on the COVID-19 pandemic has not been quantified. Our findings indicate that an increase in community incidence from 10 to 110 SARS-CoV-2 cases per 100,000, is associated with ~7% rise in nosocomial infection density. Furthermore, community incidence impacts nosocomial infections to a similar extent as patient-days, underscoring the importance of monitoring incidence rates to more accurately assess nosocomial infection risk. In addition, the analysis shows a possible tipping point on incidences of 110 positive SARS-CoV-2 cases per 100,000 inhabitants. This study design could be used to inform transmission risk for other respiratory infections and contribute to further estimate the additional burden for IPC strategies.

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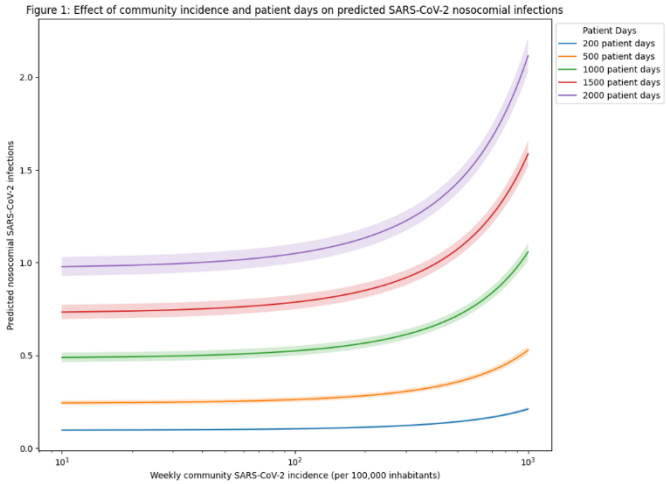
Fig. 1



WS05.05
Fungal priority pathogens: surveillance of fungi in Germany from 2019-2023

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Introduction: In 2022, WHO published the fungal priority pathogens list. We explored the frequency and resistance patterns of fungi in the past five years from the critical und high priority group that are relevant for Germany based on data from the German Antimicrobial Resistance Surveillance System (ARS).

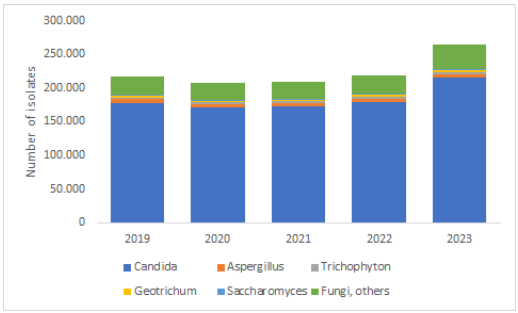
Methods: ARS collects routine microbiology data on pathogen identification and antimicrobial susceptibility testing from voluntarily participating laboratories. For trend analysis, only institutions continuously participating from 2019 to 2023 are included. We analyze frequency of first isolates per patient and quarter and resistance proportion against fluconazole (FLU), micafungin (MIF), Amphotericin B (AMB) and Voriconazole (VOR) with corresponding 95% confidence intervals (CI) in all specimen-types.

Results: From 2019-2023, 1,053,963 fungal isolates were identified in 472 hospitals (about 31% of German acute care hospitals) and 12,900 practices (about 17% of German outpatient care sites). The yearly number of fungal detections increased from 2019-2023 by 21% (n=205,442 in 2019 vs. n=247,911 in 2023), but without considerable change in distribution of identified genera. The predominant genus is *Candida* spp. (87%, n=915,618), mainly *C. albicans* (83%, n=577,050), *C. glabrata* (syn. *Nakaseomyces glabratus,* 18%, n=127,412), *C. tropicalis* (5%, n=31,301) and *C. parapsilosis* (4%, n=28,595). Resistance against MIF was 0.2% and 0.4%; against FLU 0.9% and 32.1% in *C. albicans* and *C. glabrata*, respectively. Resistance against FLU was 2.1% and 4.7%. in *C. tropicalis* and *C. parapsilosis, respectively.* Reported AMB resistance in *C. albicans* increased from 0.3% (95% CI: 0.2%-0.5%) in 2019 to 1.1% (95%CI: 0.9-1.2%) in 2023. *Aspergillus* spp. were reported less frequently (2%, n=24,814), with *A. fumigatus* being the most prevalent species (66%, n=14,897). Resistance against VOR was 1.2% (n=10 resistant isolates).

Conclusion: In Germany, we observe an increase of fungal detections within the critical and high-priority group since 2019. This could be influenced by higher awareness and changes in testing schemes. Not all data are consistent according to expert microbiology review, indicating that antifungal susceptibility testing in clinical routine is still challenging and requires optimization. Quality of fungal susceptibility testing and the increase of *C. albicans* resistance against AMB should be further investigated. The wide spread of *C. albicans* and other *Candida* species of high priority as well as changing resistance proportions underline the importance of continuous surveillance of these pathogens.

Fig.1. Number of first fungal isolates by genus, all specimen-types, ARS, Germany, 2019 – 2023

Fig. 1



WS05.06
GENTRAIN – genome-based infection chain analysis to increase digitalization level in the public health sector

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Background: Monitoring the dynamics of pathogen spread by public health authorities is a core element for infection prevention during pandemics but also in inter-pandemic phases. Infection management measures that are adaptable to individual circumstances can be highly advantageous for tracing local outbreak scenarios. One example of this is genome-based or integrated infection chain analysis. This concept combines methods of classic contact tracing with genomic sequence data from molecular pathogen analysis. This enables high resolution infection chain management within the general population or in diffuse local outbreak scenarios.

Methods: During the SARS-CoV-2 pandemic, we successfully demonstrated applications for digitally supported infection chain analysis, showing its valuable potential (Walker et al. 2022). The aim of the GENTRAIN project (GENetic TRAcing of INfection Chains, funded by the European Union) is to develop digital applications for the use of genomic-based infection chain analysis by public health authorities in Germany for further relevant pathogens beyond SARS-CoV-2. In order to contribute to a sustainable increase in digital development, the project primarily aims to enhance the dimensions of data interoperability, software and data management as well as the area of employee competence within the German public health authorities.

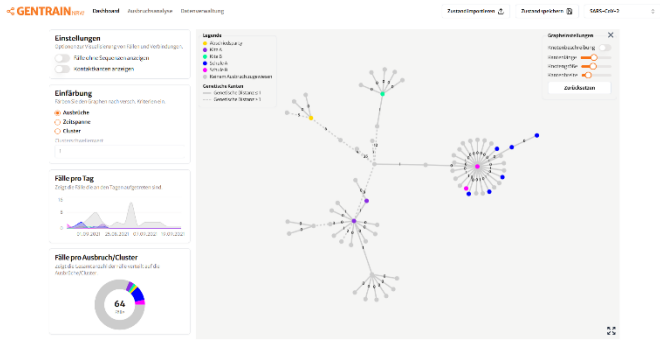
Results: The project expanded the tools of integrated genomic surveillance to analyze genomes of other public-health relevant pathogens (e.g. antibiotic-resistant bacteria).

For sustainable improvement of data management capacities in public health authorities, we identified and describe several key factors. For instance, adaptation of existing software is essential with special regard to needs and capacities of local public health authorities. Availability of interfaces for routine data exchange is highly significant, as well as ongoing training for public health personnel.

Conclusions: The utilization of this potential by public health authorities is essential, especially for predicted pandemic situations and the threat of increasing antibiotic resistance. Perspectively, this will contribute to more precise, evidence-based and faster reporting systems to support political decisions in terms of preventive measures - both at municipal and state level. To further strengthen these developments, additional interfaces (e.g. with ISGA) are planned, as well as the integration of further pathogens relevant to public health authorities. Moreover, targeted training sessions and workshops are being scheduled to promote sustainable implementation and user competence.

Walker A, et al. German COVID-19 OMICS Initiative (DeCOI). Characterization of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection Clusters Based on Integrated Genomic Surveillance, Outbreak Analysis and Contact Tracing in an Urban Setting. Clin Infect Dis. 2022 Mar 23;74(6):1039-1046. doi: 10.1093/cid/ciab588

Fig. 1



WS06.01
Genotypic characterization of clinical *Staphylococcus aureus* isolates from bone and joint infections

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Question: *Staphylococcus aureus* is a common pathogen of bone and joint infections including prosthetic joints and other foreign materials and is involved in recurrent and/or persistent infections. Although colonization with *S. aureus* is recognized as a risk factor for certain *S. aureus* infections, there is sparse information on genotypic features of colonizing strains and clinical isolates recovered from patients with bone and joint infection. Therefor, the presented study amied to characterize and compare those isolates, and identify possible molecular pattern linked to this clinical presentation.

Methods: Patients screened for colonization with *S. aureus* (MRSA and MSSA) in an 18-month period at Heidelberg University Hospital were prospectively followed up for infections caused by *S. aureus*. Isolates were cultured from tissue specimen of bone and joints and, if available, the respective nasal isolates. Genomes were analyzed by whole genome sequencing and antimicrobial susceptibility was tested by microdilution.

Results: 47 patients were included in the study, from which 147 *S. aureus* isolates were analyzed. A total of 25 (53%) patients were colonized with *S. aureus* and according to the clonal complex, 64% of the patients colonized had clonally related isolates from the nose pointing to an endogenous infection. 17 patients had recurrent and/or persistent infections and in 14 cases, prosthetic material was involved. The most frequent clonal complexes identified were CC5 (n=23/147), CC30 (n=22/147 and CC15 (n=20/147) with ST30 (n=20/147), ST15 (n=16/147) and ST182 (n=10/147) being the most frequent MLST types. 5 Isolates were positive for Panton-Valentine-Leucocidin. 140 isolates were available for antimicrobial susceptibility testing and showed phenotypic resistance to oxacillin in 5% (n=7), and penicillin resistance in 80% (n=110). No resistance towards vancomycin, teicoplanin, daptomycin, linezolid or trimethoprim/sulfamethoxazole was observed. Clindamycin resistance was detected in 13% of isolates (n=18), erythromycin resistance in 37% (n=51) and moxifloxacin resistance in 20% (n=27) of isolates.

Conclusions: *S. aureus* isolates from bone and joint infections are mainly MSSA and display heterogeneous genotypes that are found in the epidemiological landscape in Germany. Endogenous infections are frequently observed. Resistance to penicillin was common, while resistances to erythromycin, moxifloxacin and clindamycin were observed in part of the isolates.

WS06.02
Identifying bacterial transmission clusters and genetic determinants using a large-scale genomics surveillance dataset

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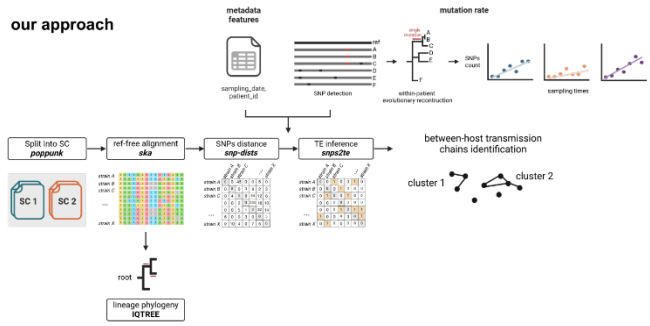
Whole-genome sequencing (WGS) enables high-resolution analyses of bacterial transmission clusters. Attempts to identify genetic links rely on a predefined single nucleotide polymorphism (SNP) threshold. However, in datasets spanning long periods of time, genetic relationships might be underestimated. Here we establish molecular clock rates for *E. coli*, *K. pneumoniae* and *P. aeruginosa* sequence clusters (SC) based on patient-specific phylogenetic trees reconstructions. Mutation rates allow us to infer strain relatedness over time and identify transmission clusters. Some clusters persist for months, showing signatures of hidden transmission unseen by predefined SNP thresholds or epidemiological links, suggesting the involvement of intermediate hosts or reservoirs. Epidemic strains are genetically and phylogenetically distinct and are associated with increased morbidity and mortality. We use genome-wide association studies (GWAS) to understand how epidemic strains emerge from non-epidemic strains and identify the genetic variants associated with transmissibility in the three bacterial species. Heritability estimates show that genetic determinants explain 49-75% of the observed variance. Finally, we evaluate the trade-offs of transmissibility. We examine how epidemic strains repeatably evolve during successful infection. We observe a loss in viruelence

associated genes during an outbreak of *K. pneumoniae* SC34/ST86, likely associated to the loss of a virulence plasmid, occurring in multiple patients. Together, these findings highlight the importance of utilizing alternatives to SNP thresholds where clock rates are variable and samples collection are extended over time, and emphasize the need for dynamic frameworks to address pathogen transmissions and guide targeted interventions.

Fig. 1



WS06.03

Evidence for early circulation of the M1UK sublineage of *Streptococcus pyogenes* in Germany, 2015–2023

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Background: Several European countries have reported a rise in invasive Group A Streptococcus (GAS) infections, particularly linked to the toxigenic *emm*1 sublineage M1UK. In Germany, historical molecular data are limited due to the absence of systematic molecular surveillance.

Methods: We performed whole-genome sequencing (WGS) on 189 *Streptococcus pyogenes* isolates collected between January 1, 2015, and May 31, 2023, at University Medical Center Carl Gustav Carus, TU Dresden. Clinical data were extracted from patient records. M1UK sublineage identification was based on 27 characteristic single nucleotide polymorphisms (SNPs). A Bayesian coalescent analysis estimated the evolutionary timescales of the M1UK clade in Germany.

Results: The most common *emm* type was *emm*1 (33%, 63/189), followed by *emm*12, *emm*4, and *emm*89. Of the 63 *emm*1 isolates, 31 (49%) were M1UK. No significant associations were found between clinical outcomes and M1UK or M1global genotypes. Although a post-pandemic shift favoring M1UK was observed, our analysis indicates that M1UK had already been circulating in Germany by 2017. The estimated most recent common ancestor dates to 2012 (95% highest posterior density: 2009–2015), with a stable effective population size over time.

Conclusion: Our findings confirm the pre-pandemic circulation of M1UK in Germany. While the clinical impact of M1UK remains unclear, integrating clinical data with high-resolution molecular surveillance may improve early detection of emerging high-risk clones.

WS06.04

Prospective whole-genome sequencing uncovers factors influencing bacterial transmission in neonates

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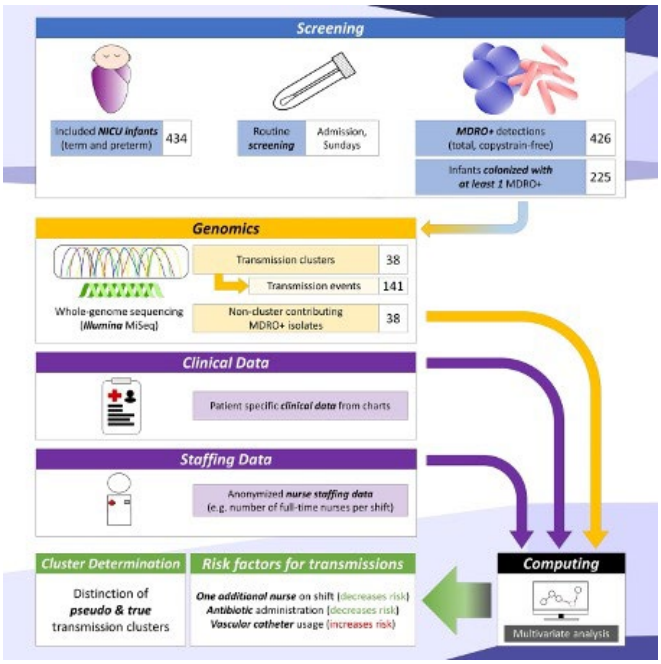
Infants in intensive care units are at risk of inter-individual transmission of bacteria with multidrug-resistance and/or epidemic potential (MDRO+), which may precede invasive infections. Here we combined established culture-based bacterial screening with whole genome sequencing (WGS) for granular transmission analysis. We found that WGS reliably distinguished true transmission clusters from pseudo-clusters and outperformed amplified fragment length polymorphism typing in precision. Overall, half of the study participants were colonized with ≥1 MDRO+, and one-third of colonizations were resulted from transmissions. Integration of genomic transmission data with granular patient, clinical and nurse staffing data enabled a complex multivariate analysis that revealed factors influencing a patient"s risk for becoming part of a transmission cluster. One additional nurse per shift and prior antibiotic usage lowered the risk, while catheter usage increased it. Thus, WGS enables data-driven infection prevention and control measures that can prevent MDRO+ infections and outbreaks interventions.

Fig. 1



WS06.05

KITA-LOVE: pilot environmental surveillance of respiratory pathogens in German daycare centers

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Introduction: Daycare centers are key transmission hubs for respiratory infections due to close-contact dynamics, immature immune systems, and variable hygiene practices.

However, their potential as sentinel surveillance environments is still largely unexplored. The COVID-19 pandemic has highlighted the necessity for proactive, non-clinical monitoring systems to facilitate early outbreak detection in community settings.

Goals: KITA-LOVE (Kindergarten Infektionsüberwachung Tröpfchen Ausbreitung: Luftwegerkrankung und optimale Vorbereitung in der Epidemie) is designed to evaluate the feasibility of utilizing molecular profiling of air and surface microbiomes in daycare centers. The objectives of this initiative are to:

(1) detect circulating respiratory pathogens,

(2) assess the influence of environmental conditions on sampling protocols, and

(3) investigate the potential of daycares as early indicators of broader community infection trends.

Materials & Methods: A longitudinal environmental sampling campaign was implemented in four daycare centers in Münster, Germany. Using Coriolis µ air samplers, monthly air collections were conducted in three different rooms per Kita (150 L/min for 10 minutes each) to maximize spatial coverage. Sampling frequency increased during seasonal infection peaks. Evaporation tests under varying temperatures and humidity assessed sample loss and informed medium selection (e.g., water, saline, preservation buffer). Samples underwent dual DNA/RNA extraction, followed by viral detection using the NxTAG® Respiratory Pathogen Panel + SARS-CoV-2 and bacterial profiling via 16S rRNA sequencing. Surface samples were collected using Polywipes (MWE) for parallel analysis.

Results: The optimization of sampling techniques revealed an average volume loss of 20% at temperatures between 21–24°C, escalating to ~50% after three cycles; however, the use of buffered media effectively mitigated evaporation. Notably, viral particles were successfully recovered, with Human Bocavirus detected in several air samples. Surface sampling revealed higher microbial diversity, suggesting that this method complements the detection of sedimented or persistent organisms. Nucleic acid concentrations from air remained low (~2–3 ng/µl), requiring further refinement for reliable metagenomic workflows.

Summary: KITA-LOVE demonstrates both the technical and conceptual viability of implementing environmental pathogen surveillance in daycare settings. Early results support the potential of Kitas as key sentinel sites. The insights gained regarding sample preservation and microbial recovery will serve as a framework for future research. The final outcomes will aim to integrate microbial and epidemiological data to evaluate the predictive value and inform public health strategies.

WS06.06

Investigating the dynamics of the environmental microbiome and its associated resistome in veterinary clinics

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Antimicrobial resistance (AMR) is an emerging global health threat, driven by widespread antibiotic use in both human and veterinary medicine. Hospitals are acknowledged as major reservoirs for AMR bacteria, contributing to their persistence and further spread. This is reflected in the high incidence of healthcare-associated infections with multidrug-resistant pathogens, not only in humans but also in companion animals such as dogs and horses. However, data on AMR in veterinary clinics remain limited compared to human healthcare. Understanding the establishment and evolution of microbial communities and their associated resistome in veterinary clinics is essential for AMR control and risk assessment.

To investigate these dynamics, we monitored the relocation of two large veterinary hospitals: an equine clinic (HC) and a small animal clinic (SAC), both moving from decades-old facilities into newly constructed buildings. We aimed to determine (1) how microbiome and resistome evolve in the new clinics once operational, and (2) whether they are newly established or transferred from the former sites.

Environmental samples were collected at five time points: in the old clinic, in the unoccupied new building, and on days 14, 90, and 120 after opening. Using electrostatic dust wipes, 2505 samples were obtained from functional areas (floors, drains, sinks, and human/animal contact surfaces). Microbiome characterization was performed using 16S rRNA sequencing (352 DNA-extracts), while shotgun sequencing (163 DNA-extracts) was used to assess the resistome. The sequencing data were analyzed bioinformatically and statistically using AMR++ and R.

Microbiome composition (β-diversity) changed significantly between the old and new clinics and over time. However, variables "clinic" and "timepoint" explained only a small fraction of the variation (R^2 values from PERMANOVA using Bray-Curtis dissimilarity), suggesting no simple transfer but a dynamic and clinic-specific community development. In the HC, all six ESKAPE pathogens were consistently detected in all samples with species-level assignments, along with other clinically or zoonotically relevant taxa, and even low zoonotic pathogens like Shigella spp. In contrast, pathogen detection in the SAC was substantially lower, though key ESKAPE members were still present. Resistome profiles also differed significantly post-opening. The most frequently observed AMR genes in both clinics conferred resistance to aminoglycosides, sulfonamides, tetracyclines, and β-lactams. Over time, resistomes showed a trend toward convergence, suggesting ongoing adaptation.

This study provides new insights into the temporal development of the microbiome and resistome in veterinary clinics. These findings can support improved AMR surveillance and risk assessment in animal healthcare environments, with potential relevance to public health.

WS07.01

Investigation of a multicentre outbreak of NDM-1/OXA-48-producing *Klebsiella pneumoniae* in a German city between December 2023 and December 2024

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Background: Carbapenemase-producing *Klebsiella pneumoniae* (Kp) are a public health threat due to carbapenem resistance and are notifiable in Germany.

Between 12/2023 and 04/2024, an outbreak of Kp sequence type 147, producing NDM-1 and OXA-48 carbapenemases, occurred in one hospital, where person-to-person transmission was suspected. After the declared end of the outbreak, the same strain type was detected in another hospital.

Objective: Assess epidemiological links and contain further spread.

Methods: We searched for cases in the affected hospitals and nationally by comparing outbreak sequences with the sequences within the German integrated surveillance system. We defined outbreak cases as individuals colonized or infected with OXA-48/NDM-1 Kp since December 2023 with a direct epidemiological link or with isolates clustering genetically (≤15 allelic differences in core genome multilocus typing). Hospitalisation histories and outpatient links between cases were collated from hospital records and interviews with cases or relatives conducted in 2025 by public health authorities using a standard questionnaire. We compared cases by demographics, hospitalisation and outpatient links.

Results: We detected 14 cases across three hospitals in the same city between 30.12.2023 and 04.12.2024; For 13 cases, clustering isolates were obtained. The median age was 51 years (range: 0-77); three cases were female. All cases had stayed in an ICU or NICU and six died. Eleven cases had overlapping stays in hospital 1 implicated in the initial outbreak, further two cases in hospital 1, one case from hospital 2 had no overlap (see Figure1). Detailed data on hospitalisation and outpatient links from interviews was available for six cases; four from hospital 1 and two from hospital 2. We did not identify any concurrent hospital stays or transfers between the three hospitals. In total, the cases were admitted to at least 32 wards across 12 hospitals, 10 of which in the city. No common outpatient exposures were identified.

Conclusion: Despite a comprehensive retrospective investigation, epidemiological links may have been missed due to the time elapsed since hospitalisation and limited interview data. Transmission pathways between hospitals remained unclear, although gaps in hospitalisation data of cases and transfer or re-admission of intermediary, undetected cases appear plausible. There were no signals for spread in undetected outpatient settings. Hospitalisation series of patients across multiple facilities might increase the patients" risk of colonization or infection with resistant pathogens and the risk of inter-hospital spread. These findings highlight the importance of admission screening, informed by assessing medical histories (e.g. stay in risk countries) and discharge summaries in receiving facilities.

Timely cluster detection may be improved through rapid, more comprehensive exchange of isolates and epidemiological data between institutions.

Fig. 1

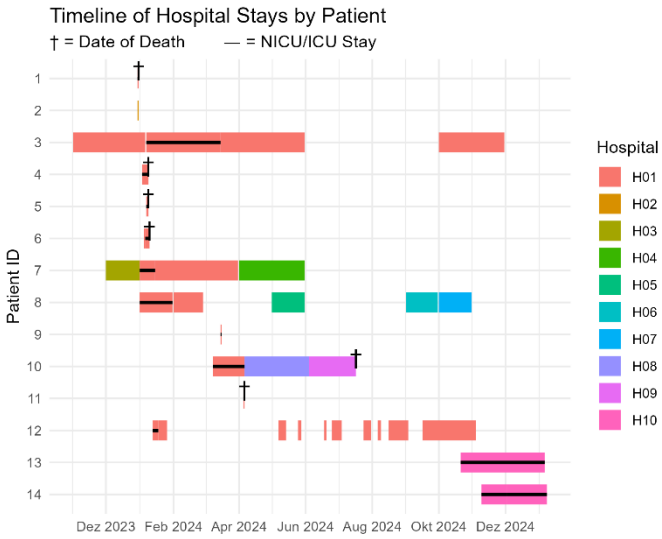
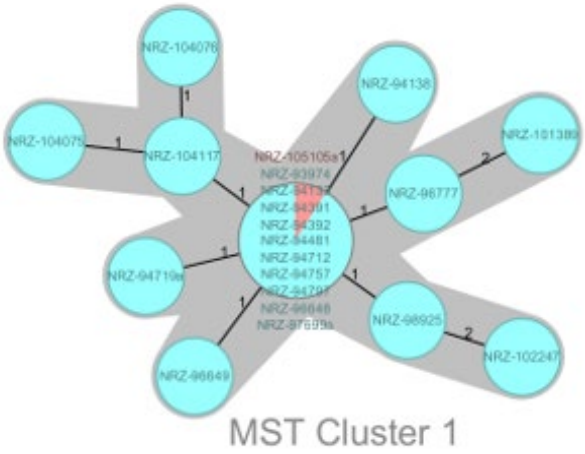


Fig. 2



WS07.02

Differential translocation potential of VRE-derived membrane vesicle

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Background: Enterococci are part of the normal gut microbiota. However, vancomycin-resistant *Enterococcus faecium* (VREfm) are a significant cause of bloodstream infections in critically ill and immunosuppressed patients with an impaired gut barrier integrity. VREfm are classified upon the Multilocus Sequence Typing (MLST) scheme, which results in the designation of sequence types (ST). In Germany, the predominant ST involved in VREfm infections are ST80 and ST117, replacing the formerly prevalent ST17 and ST78. ST1299 seems to be an upcoming new sequence type. There is evidence that membrane vesicles (MV) derived from gut microbes play a crucial role during an impaired gut barrier integrity status. Especially for Efm derived MV, the characteristics remain to be fully elucidated. The objective of this study is to ascertain Efm derived MV of different ST with and without antibiotic treatment, and the translocation potential of Efm MV through an intact gut barrier.

Methods: A total of 20 VREfm strains, isolated from blood cultures and comprising ST17, ST78, ST80, ST117 and ST1299, and three control strains (*E. coli* DSM498, vancomycin-susceptible Efm and Efm DSM20477) were employed in the investigations. Strains were cultivated in Lysogeny Broth (LB) in the absence of antibiotics, and in LB supplemented with subinhibitory concentrations of vancomycin (18 µg/mL) and linezolid (10 µg/mL). Following isolation and purification by ultracentrifugation and Optiprep® density gradient, size and concentration of MV were measured using nanoparticle tracking analysis. The efficiency of MV translocation was determined in an *in vitro* Caco-2 cell culture model. In a transwell assay, tight Caco-2 monolayers (transepithelial electrical resistance [TEER] > 400 Ω cm2) were exposed to MV of different VREfm ST and control strains.

Results: In the absence of antibiotic treatment, VREfm released MV ranging in size from 40 to 266 nm, with ST18 and ST117 identified as high-releasing strains. Antibiotic treatment differentially affected MV production: The administration of vancomycin resulted in a marginal increase in MV concentration, while linezolid led to a decrease. The present research examined the translocation rates of both VREfm strains and their MV through a dense Caco-2 cell layer. For VREfm strains, translocation rates of 1.4 ± 1.2 % were observed, with a 60-fold higher translocation potential of their MV (60.8 ± 7.7 %). Here, we identified strains with low-translocating (ST18, ST117) and high-translocating (ST78, ST80, ST1299) potential for MV.

Conclusion: MV derived by clinically relevant VREfm harbour intestinal translocation potential in an *in vitro* cell model of an intact gut barrier, with so far unknown consequences for host health. However, further experiments are necessary to elucidate the underlying translocation mechanisms and the role of MV during disease progression.

WS07.03

Epidemiological characterization of genetic clusters in the integrated genomic surveillance reflects different transmission patterns of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in Germany, 2023-2024

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The incidence of notifiable carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) and *Escherichia coli* (CP-Eco) in Germany increases. While CP-Kp is considered a nosocomial pathogen, community transmission of CP-Eco has been implicated in previous studies. In the Integrated Genomic Surveillance, we combine sequence and notification data. We extensively detect genetic clusters among CP-Kp and CP-Eco, indicative of distinct chains of transmission. We aimed at comparing clusters for both pathogens epidemiologically to infer potentially different transmission patterns.

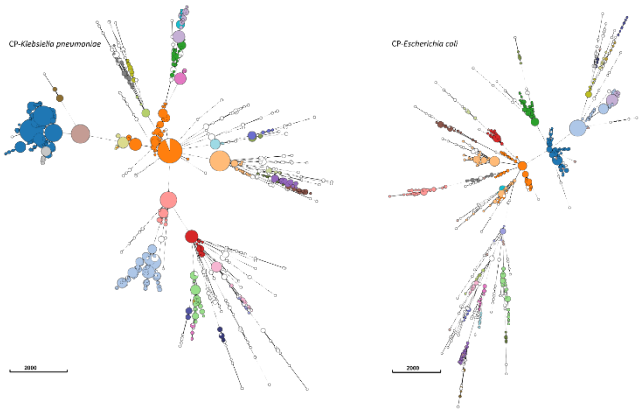
We included cases, i.e. individuals infected or colonized with CP-Kp and CP-Eco, confirmed by isolates submitted to the National Reference Centre between January 1st, 2023-July 1st, 2024. Isolates were matched to notifications probabilistically and Illumina whole genome-sequenced. Clusters were defined as ≥2 isolates with ≤15 and ≤10 allelic differences for CP-Kp and CP-Eco, respectively, in core genome multilocus sequence typing. When categorizing clusters by cases" exposure locations, we excluded clusters without isolate-notification matches. Cluster analyses based on pairwise case comparisons required ≥2 matches.

For CP-Kp and CP-Eco, we detected 255 and 169 clusters containing 1,578 and 711 cases. The median (interquartile range (IQR); maximum) of cases/cluster were 3 (2-5; 103) and 2 (2-4; 54), respectively. Larger clusters were more frequent for CP-Kp (p=0.04). Excluding 19/255 CP-Kp and 15/169 CP-Eco clusters without matches, 30/236 (13%) CP-Kp and 8/154 (5%) CP-Eco clusters were linked to cases from Ukraine (p=0.02). Exposure in other foreign countries was known for cases in 45/236 (19%) CP-Kp and 16/154 (10%) CP-Eco clusters (p=0.03). No cases with exposure abroad were found in 161/236 (68%) CP-Kp and 130/154 (85%) CP-Eco clusters. Among these clusters without exposure abroad, 111/161 CP-Kp and 74/130 CP-Eco clusters had ≥2 matches. These occurred across more than one federal state in 59/111 (53%) CP-Kp and 55/74 (74%) CP-Eco clusters (p<0.01). CP-Kp and CP-Eco clusters had similar median durations (IQR) between sampling the first and last case: 147 (29-304) and 141 (41-319) days (p=0.65). For CP-Kp and CP-Eco, 12/111 (11%) and 2/74 (3%) clusters contained cases notified as part of locally detected outbreaks (p<0.05).

Both species cause many clusters, partially large and protracted. Transmission abroad is particularly implicated for CP-Kp but clusters that are not linked to exposure abroad predominate for both species. Among the latter, CP-Eco clusters are more dispersed and less frequently identified as part of an outbreak, potentially reflecting more frequent community transmission. Intensified cluster investigations might thus be particularly relevant for CP-Eco to better understand and stop transmission.

Figure: Minimum spanning trees of CP-Kp and CP-Eco, Germany, January 2023-July 2024. Branches >100 allele differences shortened. Colored by 30 most frequent sequence types.

Fig. 1



WS07.04
A temporary goodbye? VRE clearance and the risk of recolonization

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Introduction: Vancomycin-resistant enterococci (VRE) are multi-drug resistant pathogens that call for appropriate infection control measures in hospitals. This includes extensive hygienic measures including isolation, use of personal protective equipment (PPE) and thorough disinfection in order to prevent the transmission of VRE. Even after the supposedly loss of VRE carriage, patients are at risk of a subsequent recolonization with VRE. As a result, extended hygiene measures in the clinical setting are sometimes only lifted after a delay, when multiple negative follow-up swabs are at hand.

Goals: The aim of this study was to analyze the rate of VRE recolonization of previously colonized hospitalized patients.

Methods: We analyzed data collected from routine VRE diagnostic in anal swabs from adult patients in the period from January 2019 to June 2024. The study included patients with a documented history of VRE in whom no VRE was detected in a series of at least two consecutive anal swabs and in whom a further swab was performed. The first VRE-negative anal swab was used to calculate the duration of negativity in days. The period ended either with a VRE-positive anal swab (recolonization) or with the last anal swab in the patient's data set, which was hence negative (clearance).

Results: In total 284 patients were included and followed over a median observation time of 402 days (IQR: 167–801 days). Of the patients analyzed, 75% (n=212, 95% CI: 70-80%) remained VRE negative and therefore showed a persistent clearance of VRE colonization during the examined period with a median observation time of 252 days (IQR: 77-569 days) counting from the beginning of clearance. Recolonization with VRE occurred in 25% (n=72, 95% CI: 20-30%) of the patients examined after a median time period of negativity of 55 days (IQR: 34-179 days). Of the 72 patients in whom a VRE recolonization was found, 76% (n=55, 95% CI: 66-86%) experienced it in the first six months after the initial negative VRE swab. In 86% (*n*=62, 95% CI: 78-94%) of the patients, a recolonization was found within the first twelve months after the first negative VRE swab. Therefore, only 3.5% (*n*=10, 95% CI: 1.4-5.7%) of all patients experienced a recolonization later than twelve months after the first negative VRE swab. Following another period of negativity, 10 out of the 72 patients were recolonized again. Furthermore, 22 exhibited clearance, while 40 demonstrated no additional series of two consecutive VRE-negative anal swabs.

Conclusion: In the majority of cases, patients remained VRE-negative after two consecutive negative anal swabs. Recolonization was only observed in a small proportion of patients, with the majority of these cases occurring within the first twelve months after the first negative swab.

WS07.05
Persistence of ESBL-producing *Escherichia coli* colonization in rehospitalized patients: a retrospective cohort study

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Introduction: Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-EC) are increasingly relevant in hospital settings due to their resistance to commonly used antibiotics and their ability to colonize the gut for prolonged periods. Persistent colonization raises the risk of transmission and infection, particularly during re -admissions. Understanding colonization persistence is essential for infection control and screening policies, especially in settings with high patient turnover or vulnerable populations.

Objectives: This retrospective study aimed to assess the persistence of ESBL-EC colonization among patients who were tested positive during a hospital stay and re-admitted later. We analyzed the proportion of patients who remained colonized and evaluated whether factors such as age, length of hospital stay, ICU admission, and underlying diagnoses were associated with persistence.

Materials and Methods: We included all patients with a positive ESBL-EC culture during a hospital stay and at least one later re-admission between 2018 and 2024 in a university hospital. Persistent colonization was defined as a positive result of a rectal screening swab at re-admission; otherwise negativity was assumed. Data collected included patient age, sex, length of hospital stay, ICU care status, and ICD-10-coded diagnoses. Statistical analysis was conducted using group comparisons.

Results: Among all included patients (n = 228), approximately 54% were still colonized at re-admission, while 46% showed no further ESBL-EC detection. This distribution was independent of the time elapsed between hospital stays (approx. 6 month average, up to 45 month until re-admission, P = 0,88). The median first hospital stay in the persistent group was 14.1 days, compared to 18.3 days in the negativ group (P = 0.9). Patients who only visited normal wards (no ICU) were slightly more likely to be positive on re-admission (P = 0.27). Notably, patients with diagnoses categorized under chapter K of the ICD-10 (Diseases of the digestive system) showed a significantly higher rate (P= 0.009) of persistent colonization upon re-admission compared to other diagnostic groups.

Summary: Roughly half of the patients previously colonized with ESBL-EC remained positive upon re-admission, independent of time between the stays. The duration of hospital stay and ICU treatment did not significantly influence persistence. However, diagnoses within the ICD-10 K category were associated with a significantly higher rate of persistent colonization, suggesting that gastrointestinal conditions may play a role in long-term carriage. These findings emphasize the relatively high probability of ESBL-EC

persistence in readmitted patients and its possible correlation with diseases of the digestive system. The inclusion of diagnostic data has provided important insights into potential risk profiles and could better guide future populations for screening ESBL-producing E. coli.

WS07.06

Serratia marcescens in pediatric patients, typing options and epidemiological background – how medical microbiology and hospital hygiene benefit from each other

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Introduction: Clusters of nosocomial infections are adverse events that jeopardize patient safety and their favorable therapy outcome.

The detection of increased nosocomial transmissions should result in an intensification of basic hygiene, supplemented by e.g. additional measures of barrier care, isolation and/or cohorting. In this way, nosocomial infections following colonization and, in particular, outbreaks of infection should be prevented as far as possible.

Between September and December 2024, 14 pediatric patients were either found to be colonized or infected with *Serratia marcescens*.

Aim: The aim was to document transmission events of Serratia marcescens promptly and with the greatest possible reliability in order to optimize outbreak management and to prevent further transmissions by means of targeted observations, environmental investigations and hygiene measures.

Methods: Epidemiological data were collected to investigate the outbreak. For a timely assessment of the outbreak situation, the *Serratia marcescens* patient strains detected during the outbreak were typed using PFGE and the strains were preserved for later evaluation of the typing results using WGS.

Results: The PFGE showed one main pattern with the same clonal origin and two unrelated isolates. WGS confirmed the near-monoclonal origin of the outbreak. The immediate measures had been made by intensifying observations, environmental investigations and increasing hygiene measures escpecially hand hygiene precautions. As in many other outbreaks this outbreak was also multifactorial in nature and cannot be attributed to a single source.

Summary: Direct coordination between medical microbiology and clinical hygiene is essential for successful outbreak management in order to secure specimens and complement each other methodically. The typing results confirm nosocomial transmissions. The hygiene measures introduced immediately prevented further transmissions and contained the outbreak.

WS08.01

Metabolites of _Acinetobacter baumannii_ and _Klebsiella pneumoniae_ trigger the development of acute-on-chronic liver failure

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Introduction: Acute-on-chronic liver failure (ACLF) is the result of an imbalance within the gut-liver axis, culminating by the translocation of bacteria and bacterial compounds from the gut barrier to the liver through the portal vein. Balanced liver cirrhosis can be driven to an acute liver failure with high mortality rates, especially by infections with multidrug-resistant bacteria.

Material and Methods: Caco-2 (gut) cells were infected with various strains of *Acinetobacter baumannii* and *Klebsiella pneumoniae*, followed by targeted and untargeted metabolite profiling of cell culture supernatants. Selected metabolites were assessed *in vitro* for hepatocytotoxic effects in HepG2 liver cells. Metabolomics analysis of serum samples of two independent ACLF patient cohorts were applied to validate the *in vitro* findings and assess clinical relevance.

Results: We identified distinct metabolic signatures in *A. baumannii* (19 metabolites) and *K. pneumoniae* (15 metabolites) infected Caco-2 cells supernatants, with comprehensive metabolite profiling. Four key metabolites from each pathogen were prioritized for further experiments: *A. baumannii*-α-ketoglutarate, indoleacetic acid, p-coumaric acid, uridine; *K. pneumoniae*-desthiobiotin, N8-acetylspermidine, N-acetylglutamine, ß-pinene. Hepatocyte toxicity on HepG2 cells was demonstrated using Caco-2 cell culture supernatants, metabolite mixtures, and individual metabolites. Notably, elevated levels of α-ketoglutarate, N-acetylglutamine, indoleacetic acid, and N8-acetylspermidine were validated in ACLF patient blood samples.

Discussion: Our findings demonstrate that pathogen-derived metabolites with hepatotoxic properties likely contribute to the progression of organ failure in ACLF patients. These metabolites may serve as biomarkers of disease progression in the context of bacterial infections and as potential therapeutic targets.

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WS08.02

CD101 amplifies anti-colitogenic functions when expressed on T cells and anti-bacterial properties when expressed on neutrophil granulocytes

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Introduction: Disruptions of immune cell-specific metabolic networks and inter-cellular communication circuits accompany many immune-mediated and infectious diseases. However, hardly any biomarkers have been identified that reliably indicate tissue-specific disease activities or pathological changes within metabolic and molecular networks of individual immune cell subsets. We have observed that inflammatory and infectious stimuli prominently influence the expression of the Ig-like molecule CD101 that is found on different lymphocyte and myeloid cell subsets. Thus, CD101 is a promising marker molecule for cell-type specific alterations occurring in immune-mediated and infectious diseases. However, the metabolic and molecular targets of CD101 within individual cells have not been explored yet.

Goals: To characterize the cell-specific functions of CD101, we compared the course of disease in conventional and conditional CD101 knockout mice before and after application of dextran sulfate sodium (DSS) or intestinal infection with *Salmonella* Typhimurium (*S.* Tm). Moreover, we studied the regulation of CD101 expression in in patients with inflammatory bowel disease (IBD).

Materials & Methods: We assessed cellular and intraluminal metabolism, myeloid immune cell responses, the course of bacterial infection, and the severity of intestinal inflammation in mouse models of acute and chronic *Salmonella*-induced colitis using conditional CD101-knockout mice crossed to CD11c Cre, Foxp3 Cre, LyzM Cre and Cx3Cr1 Cre mice.

Results: Following *Salmonella* infection, myeloid cell subsets became transiently CD101-negative while T cells maintained their CD101 expression. The expression of CD101 by regulatory T cellsTregs was sufficient for the CD101-mediated amelioration of intestinal pathology in both models. CD101 inhibited the FoxP3-independent expression of IL-2Ra and promoted IL-2 signalling in Tregs. Importantly, CD101-expressing neutrophils, but not other CD101-expressing myeloid cell subsets restrained *Salmonella* infection *in vitro* and *in vivo*. CD101-intrinsic and -extrinsic mechanisms contributed to the control of bacterial replication and systemic spread. The CD101-dependent containment of *Salmonella* infection depended on the concomitant expression of the *immune responsive gene 1* (*Irg-1*) and of the nicotinamide adenine dinuceotide phosphate oxidase (*Nadph oxidase*) and promoted the accumulation of their products, itaconate and reactive oxygen species (ROS) in neutrophils.

An increased circulation of intestinal microbial antigens in the sera of IBD patients correlated inversely with the distribution of CD101 expression on myeloid cells, mirroring the suppression of CD101 in mice following *Salmonella* infection.

Summary: Depending on the experimental or clinical setting, CD101 restrains inflammatory insults or bacterial infections due to cell type-specific modulation of metabolic, immune-regulatory, and anti-microbial pathways.

WS08.03

A *pbpB1* mutation causing β-lactam resistance in clinical *Listeria monocytogenes* isolates

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Introduction: Listeriosis is a severe foodborne infection and associated with high mortality. Treatment is based on ampicillin, amoxicillin or penicillin, often combined with gentamicin, but meropenem is also used occasionally. β-lactam resistant *Listeria monocytogenes* isolates are infrequently described but the mechanism of resistance is not known.

Methods: A clinical *L. monocytogenes* isolate with suspected β-lactam resistance was collected from a German listeriosis patient. Resistance profiling, whole genome sequencing, comparative genomics and genetic experiments were used to identify the causative DNA polymorphism. Spontaneous ampicillin resistant suppressors of *L. monocytogenes* reference strain EGD-e were selected and their genomes sequenced.

Results: A W428R substitution near the active site of penicillin binding protein B1 (PBP B1) was identified as the cause of ampicillin, amoxicillin and meropenem resistance. Further clinical *L. monocytogenes* isolates with similar resistance profiles were found by searching the genome database of the German consultant laboratory for *Listeria* for *pbpB1 W428R*-positive isolates. Furthermore, the same mutation was selected for in strain EGD-e during cultivation in the presence of ampicillin.

Conclusions: *L. monocytogenes* can develop β-lactam resistance by a specific substitution in PBP B1, likely selected for during β-lactam treatment. Antibiotic susceptibility testing should be considered an important part of adequate listeriosis therapy.

WS08.04

Unravelling the infection dynamics of *Candida albicans* clinical isolates on gut epithelial cells

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Candida albicans is a common commensal of the human gastrointestinal tract, but under certain conditions, it can shift to a pathogenic lifestyle, causing disease. Although epithelial cell responses to *C. albicans* infection have been well characterized, the activation of programmed inflammatory cell death pathways—such as pyroptosis and necroptosis—remains unexplored. Additionally, most insights derive from studies using the *C. albicans* reference strain SC5314, known for its robust filamentation, adhesion, and cytotoxicity. However, clinical isolates from various niches (oral, gut, vaginal, blood) show wide variation in virulence traits such as hyphal formation, invasion, and candidalysin secretion. Therefore, conclusions based solely on SC5314 may overlook key aspects of the interactions between *C. albicans* and host epithelial cells. To better understand these interactions, we

employed a panel of *C. albicans* clinical isolates and mutants lacking key virulence factors. Cytotoxicity screen were performed at 6, 24, and 48 hours post-infection and strains causing high damage were selected for further study. To monitor host responses, we employed reporter fluorescent lines: a mitochondrial redox sensor (mt-roGFP2) for redox status, a caspase-3/7-mRuby3 reporter for apoptosis, and an ASC-LmCerulean reporter for inflammasome activation allowing precise temporal tracking of redox status, apoptosis, and ASC specks through live cell imaging.

ROS production, apoptotic activity, ASC speck formation, adhesion, and invasion varied across *C. albicans* clinical isolates, supporting the need to study a broader range of strains. For instance, mt-roGFP2 reporter revealed strain-specific mitochondrial ROS responses, with distinct strains (SC5314, C24, and C227) showing strong oxidative shifts. The apoptosis reporter indicated increased caspase-3/7 activation at late time points in strains C127, C227, and C634. Surprisingly, C227 induced high ROS and apoptosis levels despite low adhesion and invasion, while CEC3943 showed low ROS but comparable invasion to SC5314.

These findings highlight that while classical virulence traits contribute to host-pathogen interactions, they do not fully explain the diversity in host cell death responses observed across *C. albicans* strains. By using live-cell reporters as precise and dynamic tools, we were able to uncover subtle, strain-specific differences in host responses that might be missed by conventional assays.

WS08.05

Shiga toxin breaks the epithelial barrier and induces inflammation in a human 3D gut-on-a-chip model

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Question: Enterohemorrhagic *E. coli* (EHEC) are intestinal pathogens that can cause hemorrhagic colitis and the life-threatening hemolytic uremic syndrome (HUS). The cardinal virulence factor of EHEC is Shiga toxin (Stx), which is a potent AB5 toxin that irreversibly inhibits eukaryotic protein synthesis thus leading to host cell death. There are two immunologically distinct Stx types, Stx1 and Stx2, that are further divided into the subtypes Stx1a,c,d and Stx2a-g,i, with Stx2a being the one most often associated with severe clinical course and the development of HUS. Animal models have been crucial for studying EHEC-host interactions; however, none of the currently available ones fully reflects EHEC pathogenesis in humans. Here, we evaluated the potential of a human 3D gut-on-a-chip model to serve as a suitable system to study EHEC virulence by monitoring the effects of bacterial culture supernatants and purified Stx on the epithelial barrier, inflammation and cell viability.

Methods: Caco-2 and HT29-MTX intestinal epithelial cells were cultured against an extracellular matrix (ECM) in an OrganoPlate® 3-lane 40 plate (Mimetas, Oegstgeest, Netherlands) under continuous medium perfusion. Leak-tight intestinal tubules were treated with sterile filtered bacterial supernatants or purified Stx1a or Stx2a for 48 hours (h). Epithelial barrier integrity was assessed by monitoring the translocation of fluorophore-labeled dextran from the tubule lumen to ECM channel. Inflammation was determined using an IL-8 ELISA and cell viability using a WST-8-based assay.

Results: Bacterial culture supernatants of Stx2a-producing EHEC O104:H4 but not EHEC O104:H4 *Δstx* caused disruption of the epithelial barrier and increased IL-8 secretion in the 3D model after 48 h of treatment, suggesting that Stx2a is the virulence factor responsible for these phenotypes. Accordingly, the *E. coli* K-12 strain MG1655 transduced with the Stx2a phage from EHEC O104:H4 and thus producing Stx2a acquired also the ability to break the epithelial barrier and cause strong inflammation in this model system. Interestingly, at this time point no loss in the epithelial cell viability associated with the presence of Stx2a in bacterial supernatants was detected. Experiments with purified toxin confirmed that both Stx1a and Stx2a could alone cause the effects on epithelia barrier integrity and inflammation. Ongoing experiments with bacterial supernatants of clinical EHEC isolates expressing different Stx1 and Stx2 subtypes should reveal their virulence potential in the model.

Conclusions: We could show that Stx1a and Stx2a can break the epithelial barrier and cause inflammation but no detectable cytotoxicity within 48 h of treatment in our 3D gut-on-a-chip model. These results serve to validate this human *in vitro* 3D model as a suitable system to study EHEC virulence potential and provide valuable insights into EHEC course of infection.

WS08.06

TisB-mediated ATP reduction primarily affects prophage induction in *Salmonella* Typhimurium, reconsidering the role of ATP in antibiotic persistence

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Introduction: Antibiotic persistence is a phenomenon whereby non-resistant bacteria survive lethal concentrations of antibiotics and reemerge post-therapy, resulting in treatment failure and chronic infections [1]. Several mechanisms have been described by which persisters can survive exposure to antibacterial agents, including toxin/antitoxin modules [1], activation of stress responses [2], prophages [3], and low ATP levels [4].

The bacterial toxin TisB is a short peptide that causes proton leakage across the inner membrane after induction via the SOS response, leading to a reduced ATP concentration and an increased fraction of persisters [2].

Aims: The involvement of prophages in antibiotic persistence, particularly those under the control of the SOS response, has been largely overlooked. Our aim in this study was to investigate the connection between ATP levels (*tisAB* expression), prophage induction, and persister cell formation.

Materials and Methods: *Salmonella* serovar Typhimurium strain ATCC 14028 harbours four SOS response-inducible prophages, designated Gifsy-1, Gifsy-2, Gifsy-3, and ST64B. Antibiotic killing assays were performed with the wild type and a *tisAB* mutant. Prophage induction was determined via RT-qPCR and FACS. In addition, we tested the prophage-free counterparts to determine persister cell formation following ciprofloxacin treatment (8x MIC). To study the effect of *tisAB* deletion on RecA binding to DNA, we used a RecA fusion with mScarlet-I.

Results: Deletion of *tisAB* reduced the fraction of persister cells after treatment with ciprofloxacin. Furthermore, the SOS

response was increased in the *tisAB* mutant, as well as the ATP concentration and prophage induction following drug treatment.

Summary: Exposure of bacteria to ciprofloxacin results in DNA damage, which leads to the induction of the SOS response, including *tisAB* and prophages. The induction of *tisAB* can interfere with a strong SOS response and the activation of prophages. This interference protects a small subpopulation of the wild type from prophage-mediated lysis, resulting in increased persister cell formation following ciprofloxacin treatment.

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WS09.01

Post-translational modifications in response to hypoxia in the filamentous fungus *Aspergillus fumigatus*

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Introduction: The filamentous fungus *Aspergillus fumigatus* is an opportunistic human pathogen, which can cause multiple diseases ranging from life-threatening invasive pulmonary aspergillosis in immunocompromised patients to chronic, noninvasive forms of infection and allergies. The ability of the fungus to sense and adapt to low environmental oxygen concentrations is an important virulence trait. The transcription factor *SrbA* is the central regulator of the fungal hypoxia stress response. Less is known about the changes on the protein level and direct and indirect oxygen-regulated post-translational modifications, e.g. phosphorylation and cysteine thiol modifications.

Aims: To get more insights into hypoxic adaptation of *A. fumigatus* on the protein level, we performed quantitative proteomics, phospho- and thio redox-proteomics analyses by comparing fungal mycelium grown under either normoxic or hypoxic conditions.

Material and Methods: Proteins were extracted from mycelium and analyzed after tryptic digest by LC-MS/MS. Phosphopeptides were enriched using a TiO_2/ZrO_2 solid phase extraction protocol, while the identification of oxidative thiol modification was determined by the OxICAT technology.

Results: We identified in total 5136 proteins, of which 318 proteins and 1674 phosphopeptides showed significantly different abundance upon hypoxia (fold change >4). In particular proteins involved in mitochondrion organization, amino acid metabolism, and lipid metabolic processes increased in abundance under hypoxia. The phosphoproteomic data indicated further that the mitotic cell cycle and autophagy processes were differentially regulated under hypoxic growth. Indeed, phosphopeptides derived from proteins of the Atg1 signaling complex, which is known to initiate autophagosome formation, showed drastic changes in phosphorylation under hypoxia. The OxICAT method revealed 44 cysteine-containing peptides with a decreased and 36 peptides with an increased level of thiol oxidation under hypoxia. A cysteine in the osmotic stress regulating mitogen-activated protein kinase SakA showed a most drastic level of thiol oxidation under hypoxia.

Summary: Phosphoproteomics of *A. fumigatus* revealed an altered regulation of autophagy under hypoxic growth conditions, while redox proteomics uncovered a hypoxia-sensitive cysteine in the MAP-kinase SakA. Initial experiments have started to investigate the impact of autophagy on hypoxic growth in *A. fumigatus.*

WS09.02

Sucrose enables *C. albicans* intestinal colonization

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Candida albicans is a frequent colonizer of the human gut. Increased colonization is one risk factor for disseminated infection, often mediated by antibiotic treatment. In contrast, the effects of diet on intestinal *Candida* colonization are less well studied. We observed that the addition of sucrose to the drinking water of C57BL/6 mice facilitated intestinal colonization with *C. albicans*. To identify the responsible mechanisms, we investigated the consequences of sucrose supplementation and different diets (fiber-rich grain-based diet, fiber-poor purified diet) on *Candida* colonization and microbiome composition. In SPF mice, a purified diet facilitated stable *C. albicans* colonization. Sucrose supplementation elevated the fungal burden in mice fed grain-based chow to the level of the purified diets. The highest fungal density was reached by combining sucrose supplementation of drinking water with a purified diet. In contrast, sucrose supplementation did not further increase fungal load in germ-free mice fed a purified diet, suggesting that the maximum level of colonization was reached. Microbiome analysis of murine feces showed that mice fed a grain-based diet displayed no changes of their microbiome after supplementation of sucrose in the drinking water. Mice fed purified diets showed clear changes in bacterial composition characterized by reduced α-diversity. This points to purified diets, which are artificial and low in fiber, having a negative impact on the microbiome diversity while sucrose addition alone does not. Sucrose seems to have direct positive effects on *C. albicans* colonization ability. In accordance with that, a *C. albicans* mutant, unable to hydrolyze sucrose, displayed a fitness defect in competition with the corresponding wildtype in mice given a grain-based diet. This highlights *C. albicans* ability to effectively use disaccharides from grains available in the murine gut. A *C.*

albicans mutant, unable to transport sucrose, only showed fitness defects under high sucrose conditions in competition with bacteria. Quick uptake of sucrose gives *C. albicans* a fitness advantage under these circumstances. In conclusion, purified diets negatively affect the microbiome composition and promote increased *C. albicans* colonization. Sucrose alone does not change the microbiome but directly supports *C. albicans* fitness. The fungus displays fitness disadvantages if sucrose utilization genes are knocked out. Therefore, the effect of diet content should be considered in patients at risk for disseminated candidiasis, taking into account the fiber- as well as the sucrose content.

WS09.03

Bacterial and fungal diversity in laboratory mice originating from different vendors and the influence on *Candida albicans* colonization

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The gastrointestinal microbiota plays a crucial role in the host's health and disease, notably by mediating colonization resistance against pathobionts such as the fungus *Candida albicans*. Consequently, microbial dysbiosis induced by antibiotic treatment is a significant risk factor for candidiasis.

To identify bacterial candidates responsible for mediating colonization resistance against *C. albicans*, we examined natural variations in the microbiota of laboratory mice from different breeding facilities. Fecal samples from 20 C57BL/6 colonies were analyzed using 16S rRNA and ITS gene sequencing. Based on differences in taxonomic composition and microbial burden, five colonies were selected for subsequent colonization experiments with *C. albicans*. After antibiotic treatment and oral inoculation with *C. albicans*, fecal samples were collected at different time points to assess fungal burden in the gut and shifts in the intestinal microbiome.

Despite prior variations in bacterial and fungal compositions, all five mouse cohorts exhibited similar patterns of *C. albicans* colonization. Antibiotic treatment resulted in increased fungal colonization. Interestingly, sucrose supplementation in drinking water was sufficient to support stable and substantial *C. albicans* colonization.

Our findings suggest that significant microbiota variations in breeding colonies of laboratory mice do not necessarily affect colonization resistance. However, fecal microbiome analysis revealed changes in bacterial composition not only associated with *C. albicans* colonization but also significantly influenced by housing conditions and sucrose supplementation throughout the course of the experiments. This dataset will be used for further analysis to identify bacterial candidates involved in colonization resistance.

WS09.04

Exploring the antibacterial mechanism of *Candida albicans'* hyphal-specific toxin using the model organism *Escherichia coli*

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The opportunistic fungal pathogen *Candida albicans*, poses a significant risk to human health by causing superficial infections and severe systemic infections under certain predisposing conditions. Recently it was demonstrated that hypha formation plays a crucial role in facilitating *C. albicans* gut colonization in hosts with either an undisturbed gut microbiota or carrying specific bacterial populations. In this niche, hypha-competent *C. albicans* outcompeted yeast-locked mutant cells. This phenotype was primarily attributed to the expression of *ECE1*, encoding candidalysin (CaL) - the first (ribosomal) peptide toxin identified in a human pathogenic fungus – and seven additional non-candidalysin Ece1 peptides. While it is well known that CaL directly inflicts damage to human cells, we propose that Ece1 may also act on bacteria of the human microbiota during commensalism.

We explored the effects of Ece1 on co-colonizing bacteria by assessing the susceptibility of selected bacteria from different body sites to Ece1 peptides. Our screening revealed that CaL influences the growth of several members of the microbiota. To further understand the mechanism of CaL-mediated antibacterial activity, we focused on the model organism *Escherichia coli*. With fluorescent labeling of CaL and protoplastation experiments, we were able to show that the peptide binds the surface of *E. coli* and that bacterial membranes are susceptible to CaL-mediated lysis, respectively. Using genetic tools, we identified the lipopolysaccharide (LPS) component of the bacterial membrane as particularly important for the defense of these bacterial cells against CaL. By analyzing the transcriptional response of wild-type *E. coli* and an LPS-defective mutant strain by RNA-seq, we found a specific transcriptional stress response of the bacteria upon CaL-stimulation.

Our study underlines the antibacterial activity of CaL and gives mechanistic insights. Further, our results demonstrate the importance of this multifunctional fungal factor that likely evolved in *C. albicans* to improve fungal fitness during gut commensalism or polymicrobial infections through inter-kingdom competition.

WS09.05

***ECE1* in closely related non-*albicans Candida* species**

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The yeast *Candida albicans* is both a common commensal colonizer of mucosal surfaces, in particular the gut, but also an opportunistic pathogen of humans. One major pathogenic

trait is the production of invasive filaments, which is associated with the strong induction of the *ECE1* gene. The encoded Ece1 pre-pro-protein is processed into smaller peptides, including candidalysin (CaL) – a secreted pore-forming peptide toxin, well characterized as a key factor for hypha-associated host cell damage. Thereby, candidalysin facilitates *C. albicans* virulence during superficial and systemic infections, however, it was also found to be relevant for commensal gut colonization. While orthologous *ECE1* genes are found in the closely related pathogenic species *C. dubliniensis*, *C. tropicalis*, and *C. viswanathii*, several related non-pathogenic species including *C. maltosa* and *C. sojae* also possess it.

In this study we aimed to characterize these non-*albicans Candida* species with regard to the role of *ECE1* in their niche and microbial environment. Using *in silico* analysis of *ECE1* sequences, we found a strong conservation of Ece1, including processing sites and predicted protein structure. These findings suggest that this protein evolved from a common ancestor of these species and was retained as an important fitness factor.

Non-*albicans Candida* species were phenotypically characterized, revealing differences for fungal growth under various temperature, nutrient and stress conditions as well as for filamentation. None of the species matched *C. albicans*" cytotoxicity during *in vitro* infection of human epithelial cells. However, synthetic versions of the non-*albicans* candidalysins clearly showed cytotoxicity against human as well as bacterial cells. Fluorescent transcriptional reporter strains were used to characterize expression conditions for *ECE1* in these non-*albicans* species to further shed light on the functional role of Ece1 for each species and its associated environmental condition.

Our observations clearly suggest a multi-functional role of candidalysin/Ece1 beyond *C. albicans* host infections and highlight the evolution within *Candida* species in the context of their environmental training ground.

WS09.06
Multi-reporter cell lines for detection of cell death types in infection

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Most microbial pathogens can cause the death of their host cells. These host cells, however, die in a multitude of ways. Some are inflammatory, such as necroptosis and pyroptosis, and some are not, such as apoptosis, which is decisive for the course of infection. The tools currently available to detect cellular stress and cell death lack the necessary spatial and temporal specificity to distinguish in real time between the diverse cellular events which lead to a cell"s death.

Addressing this, we are creating a fluorescent multi-reporter cell line to detect cellular stress and death pathways in parallel. This will allow to detect and distinguish these events in real-time by fluorescence microscopy. Cell lines are created *via* lentiviral transduction from a mix-and-match set of reporters, which are simultaneously active in a single cell line. We have created fluorescent reporters for redox status as an indicator of stress using a roGFP2 fluorophore and for apoptosis using a switch-on fluorophore for live detection of caspase 3/7 activation. A reporter using a bi-molecular fluorescence complementation (BiFC) system for live detection of pyroptosis is currently being validated. All reporters work with non-overlapping spectra, which allows us to create "multi-reporter", to simultaneously monitor different types of cell stress and death pathways during infection. We have validated and tested our multi-reporter with chemicals (LPS & raptinal), but also pathogens (*Candida albicans* & *Salmonella enterica*). To leverage the ability for real-time spatial detection of cell death in infections, we are implementing single reporter cell lines in a gut-on-chip system, a 3D *in vitro* model that mimics the dynamic and physiological conditions of the human gut.

Our expanding set of reporter cell lines will allow us to monitor cellular stress and differentiate between different host cell death pathways that are induced during fungal and other microbial infections. Importantly, it now makes it possible to follow the state of host cells in a temporally and spatially resolved manner. These reporters will, therefore, help to answer many of the open questions on host-pathogen interactions during infection.

WS10.01
Dynamics of catheter-associated biofilm microbiome communities in liver transplant recipients

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Introduction: Biliary tract infections, particularly cholangitis, significantly contribute to morbidity and mortality after liver transplantation. Often linked with bacteremia, these infections are increasingly difficult to treat due to rising antibiotic resistance. This study explores how surgical methods and biofilm-associated microbial communities on biliary catheters influence post-transplant biliary tract infections

Objectives: This study aimed to determine the impact of biliary reconstruction techniques on cholangitis incidence and to characterize the microbial communities of biliary catheters, focusing on Enterobacteriaceae and Enterococcaceae. It further assessed how antibiotic resistance and biofilm composition affect infection risk.

Materials & Methods: In a prospective pilot study involving 34 liver transplant patients, biliary catheters were analyzed postoperatively using SEM, 16S rRNA sequencing, and selective microbial cultures. Cholangitis occurred in 26.5% of patients, significantly associated with bilio-digestive anastomosis ($p<0.001$), longer ICU stay ($p=0.001$), and intraoperative bile culture positivity ($p<0.001$).

We established an in vitro model using patient-derived isolates and microbiomes to examine biofilm structure,

antibiotic resistance, and species interactions on various catheter materials.

Results: Duct-to-duct anastomosis showed a lower risk of cholangitis and microbial colonization. Enterobacteriaceae dominated catheter biofilms, while Enterococcaceae were enriched during infection. In the in-vitro assay, the same balanced ratio between Enterobacteriaceae and Enterococcaceae was observed, closely mirroring both biofilm and infection-associated distributions. Within the biofilm, Enterobacteriaceae formed the structural backbone, facilitating the integration of nosocomial and drug-resistant pathogens, including bloodstream-derived Enterococcus species. Interestingly ESBL-producing Enterobacteriaceae (bla_SHV-positive) formed more robust, drug-resistant biofilms. Enterococci from bloodstream infections integrated into biofilms, which were less responsive to antibiotics than planktonic cultures. All catheter materials supported biofilm formation.

Summary: Surgical technique significantly influences the risk for biliary tract infections, with duct-to-duct anastomosis limiting biofilm formation. Biofilms act as reservoirs for pathogens that mirror clinical isolates, reinforcing the need for strategies targeting microbial communities rather than individual species. These findings support biofilm-focused therapeutic and surgical planning to reduce post-transplant infections.

WS10.02

Gut microbiome strain transmission within families from the LoewenKIDS study

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Question: The human gut microbiome is home to thousands of microbial species and trillions of microbial cells. Far from being static, our gut microbiomes are shaped from birth throughout life by continuous microbial acquisition and transmission, affecting health beyond infectious diseases. However, the interpersonal gut microbiome transmission dynamics - especially within households - remains underexplored.

Methods: Here, we analyze a cohort of 534 individuals from 141 households of the German LoewenKIDS study, each consisting of a mother (mean age 41.65 ± 5.18), a father (44.16 ± 5.24) and one to four children (8.32 ± 2.68). A stool sample was collected from each individual and analysed using deep shotgun metagenomic sequencing to investigate how household structure shapes microbiome strain transmission.

Results: Consistent with previous findings, household members share significantly more species than unrelated individuals. Using strain-level data, we demonstrate that 91% of shared species include strains transmitted within households. Notably, the strain-sharing rate (defined as the number of shared strains divided by the number of shared species) varies by relationship types: siblings share 60% of strains, followed by mother-child (35%), father-child (25%), and spousal pairs (25%). Furthermore, transmission efficiency varies across microbial species, with distinct species preferentially transmitting between specific family members. Ongoing analyses explore the longitudinal microbiota composition of children within these households to pinpoint the time points in life when distinct groups of microbes are first observed, thereby clarifying colonization dynamics.

Conclusions: In summary, our study suggests that cohabitation is a key driver of gut microbiome transmission, modulated by both relationship type and microbial species. These findings highlight the importance of understanding transmission dynamics in the context of microbiome-associated noncommunicable diseases.

WS10.03

Harnessing multi-omics machine learning signatures for precision diagnosis and risk stratification of *Helicobacter pylori*-induced gastritis

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Introduction: *Helicobacter pylori* colonizes approximately half of the global population, with outcomes ranging from asymptomatic infection to gastritis, peptic ulcers, and gastric cancer. The ability to predict *H. pylori*-carriage and disease progression remains limited, complicating decisions about eradication therapy. This study utilizes a multi-omics machine learning (ML) approach to identify signatures distinguishing pathogenic from benign colonization, aiming to enhance diagnostic precision.

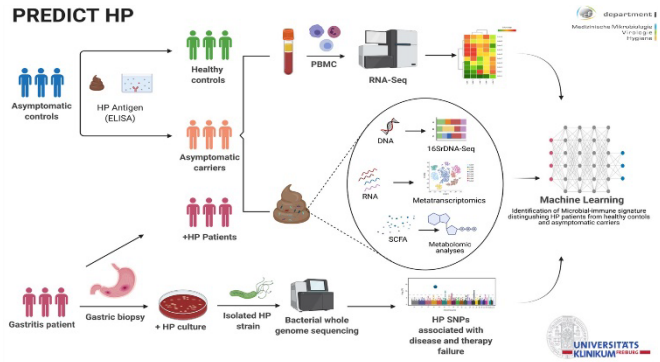
Methods: A cohort of 230 individuals was recruited, including 160 *H. pylori*-negative individuals, 42 positive asymptomatic controls, and 28 patients with gastritis. Stool samples underwent 16S rDNA sequencing, bacterial RNA-seq, and metabolite profiling, while peripheral blood samples were analyzed for host transcriptomic changes. Whole-genome sequencing of 13 clinical *H. pylori* strains isolated from patients" gastric biopsies was performed. Data integration followed FAIR-compliant workflows, and ML models were developed using random forests with feature selection to identify infection-specific signatures.

Results: Two distinct gastritis related *H. pylori* variant groups were identified, differentiated by the presence of the *cag* pathogenicity island, a key virulence factor. Transcriptomic analysis revealed significant immune-related gene expression changes and upregulation of various immune regulatory pathways in *H. pylori*-induced gastritis patients compared to healthy individuals. ML models integrating stool 16SrDNA sequencing and host blood transcriptomics data achieved an ROC-AUC of 0.8 in predicting gastritis patients from *H. pylori*-positive and -negative healthy individuals, with smaller feature sets retaining predictive power.

Conclusion: Our findings demonstrate the potential of multi-omics ML models for accurately diagnosing *H. pylori*-induced gastritis. This approach could facilitate earlier detection and personalized risk assessment, ultimately improving clinical

decision-making and patient outcomes. Future work will fo cus on validating these models in larger cohorts.

Fig. 1



WS10.04

First insights into microbial changes within an inflammatory bowel disease family cohort study

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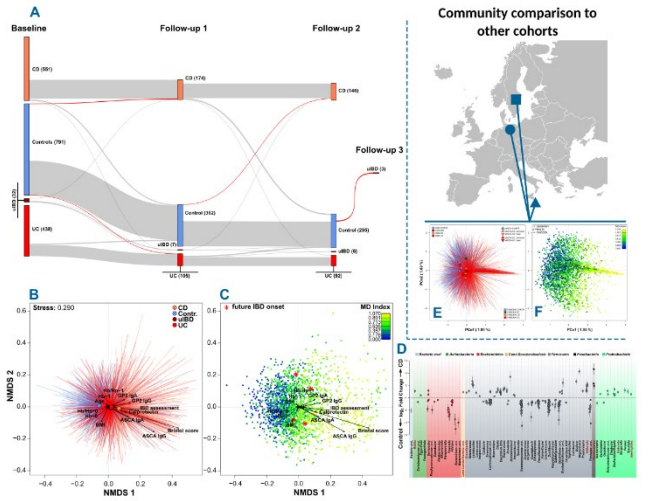
The prospective Kiel Inflammatory Bowel Disease (IBD) Family Cohort Study (KINDRED cohort) was initiated in 2013 to systematically and extensively collect data and biosamples from index IBD patients and their relatives (e.g., blood, stool), a population at high risk for IBD development. Regular follow-ups were conducted to collect updated health and lifestyle information, to obtain new biosamples, and to capture the incidence of IBD during development (Fig. 1). By combining microbial community data collected at successive time points with extensive anthropometric, medical, nutritional, and social information, this study aimed to characterize the factors influencing the microbiota in health and disease via detailed ecological analyses.

Using a dysbiosis metric based on the German KINDRED cohort, we identified strong and generalizable gradients within and across different IBD cohorts, which corresponded strongly with IBD pathologies, physiological manifestations of inflammation (*e.g.,* Bristol stool score, ASCA IgA, ASCA IgG), genetic risk for IBD, and risk of disease onset. Anthropometric and medical factors influencing transit time strongly modify bacterial communities. Various *Enterobacteriaceae* (*e.g., Klebsiella sp.*) and opportunistic *Clostridia* pathogens (*e.g., C. XIVa clostridioforme*), characterize in combination with ectopically colonizing oral taxa (*e.g. Veillonella sp.*, *Cand. Saccharibacteria sp.*, *Fusobacterium nucleatum*) the distinct and chaotic IBD-specific communities (Fig. 1).

Our findings demonstrate broad-scale ecological patterns which indicate drastic state transitions of communities into characteristically chaotic communities in IBD patients. These patterns appear to be universal across cohorts and influence physiological signs of inflammation, display increased resilience, but show only limited heritability/intrafamily transmission.

Figure 1: (A) Flow of individuals through sampling time points (current data freeze), with IBD-onset patients highlighted as red connections (N=7). (B) Non-Metric Multidimensional scaling plots of community composition show a clustering of samples by IBD pathology and a strong gradient of dysbiosis level (C) and further highlight the extent and direction of significantly correlated physiological markers of inflammation with community composition and the position of onset cases in the community distribution (red-*). (D) Differential abundance patterns of bacteria in KINDRED with respect to IBD pathology (oral bacteria in red). (E) Principle Coordinate Analysis of community differences between IBD pathologies and (F) dysbiosis levels across independent cohorts (KINDRED-Germany, Sweden, Malta), which shows common clustering patterns.

Fig. 1



WS10.05

Major secondary bile acid producing bacteria of human gut microbiota

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Bile acids (BA) are largely involved in the host metabolism and a dysregulation of the total BA pool is associated with various diseases. Gut microbiota play an important role in shaping the BA pool by catalyzing their deconjugation and metabolizing primary bile acids (PBA) into secondary bile acids (SBA). The main pathway for SBA formation is the synthesis of deoxycholic acid (DCA) and lithocholic acid (LCA) from cholic acid (CA) and chenodeoxycholic acid (CDCA), respectively, via 7α-dehydroxylation. We recently identified that this function is predominantly carried out by three taxa, namely *Clostridium scindens,* a *Dorea ssp*. and a novel and yet uncultured bacterium belonging to the family of Oscillospiraceae. However, research on SBA formation exists

only for the model species *C. scindens* while the other two taxa remain poorly characterized. Our aim was the ecophysiological characterization of those bacteria and the verification of their 7α-dehydroxylation capacity using *in vitro* and *ex vivo* approaches.

Target bacteria were grown with different PBA concentrations in pure culture *in vitro* in order to verify their SBA synthesis capacity. Furthermore, selected fecal samples were incubated *ex vivo* under different nutritional conditions. 7α-dehydroxylation capacity was confirmed *in vitro*. The main product from CA was DCA for all strains, whereas in the case of CDCA *C. scindens* produced 3-keto-CDCA and LCA in equal amounts, while *Dorea ssp.* predominantly metabolized CDCA to LCA. Increasing concentrations of PBA resulted in reduced bacterial growth, as observed by a lower growth rate and a lower final OD. *Dorea ssp.* was more affected by PBA than *C. scindens* in terms of growth rate, whereas final OD was similar for both taxa; SBA-formation rates were higher in the former.

We tested a broad array of complex carbon sources and identified a xylan derivate and a specific type of resistant starch to be the ideal components for stimulating growth of SBA-forming bacteria within whole fecal communities *ex vivo*. Furthermore, the addition of PBA resulted in up to 60-fold higher final growth of the target bacteria and they displayed similar growth dynamics as other community members. Transcriptomics analyses from samples taken during exponential growth are ongoing in order to get detailed insights on BA transformation on a molecular level in both pure cultures and whole fecal communities.

The experiments verified 7α-dehydroxylation capacity of the major SBA-producing bacteria *in vitro*. Furthermore, we provide important knowledge on the ecophysiology of those bacteria grown in both pure culture and within whole fecal communities. The results lay the groundwork for investigating BA transformation in clinical contexts focusing on diseases characterized by an altered bile acid metabolism.

WS11.01

Blood culture volume, guideline adherence and pathogen yield of 35,433 paediatric blood cultures in Germany

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Background: Blood cultures (BCs) are essential for diagnosing bloodstream infections in children. While few studies have shown a positive correlation between blood culture volume (BCV) and pathogen yield in children, paediatric guidelines on the collection of BCs—particularly the sampling volume—vary significantly.

Objectives: The aim of this study was to quantify paediatric BCVs in Germany, to assess guideline adherence, and to analyse factors influencing BCV and pathogen yield.

Methods: This prospective, multicentre, observational study was conducted across 20 paediatric centers in Germany from 4/1/2023 to 6/30/2024. Data collected included patient age, sex, paediatric subspecialty, type and weight of blood culture bottles (BCBs), site of venous puncture (peripheral/central), species-level pathogen identification, time-to-positivity (TTP), total number of BCs, patient days, and internal guideline per center. Patient weight was approximated using percentiles. BCV was calculated based on bottle weight after inoculation and the average weight for each bottle type. Pathogens versus contaminants were determined based on bacterial species and site of venous puncture. Adherence to guidelines

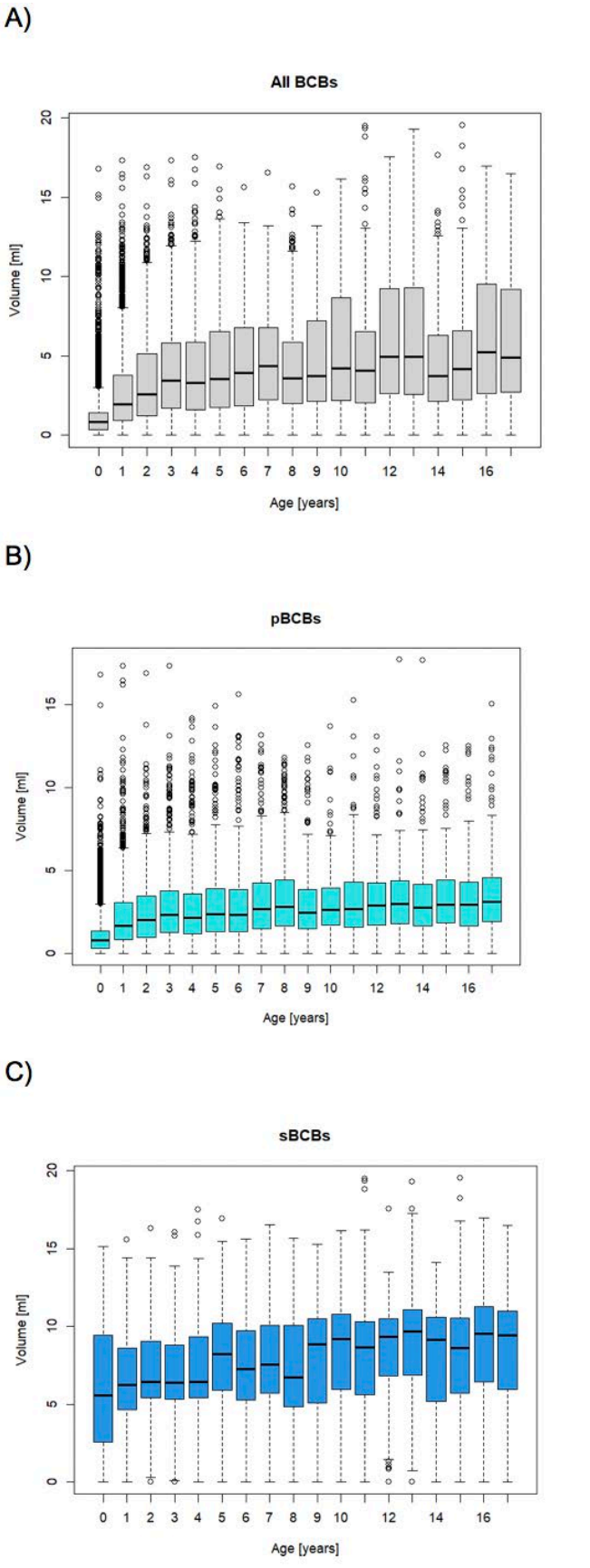
was assessed based on internal guidelines (general paediatrics) and a German national guideline (oncology).

Results: 35,433 weighed BCs were included. The median BCV was 2.61mL, with the median peripheral and central BCV being 1.36mL and 5.33mL. The highest median BCV was recorded in Oncology (5.34mL), and the lowest median BCV was found in Neonatology (0.67mL). Median BCVs increased with age, reaching their peak in schoolchildren for paediatric BCBs and in adolescence for standard BCBs (figure). Ten of the 20 study centers had an internal guideline considering BCV. Adherence to internal guidelines (General Paediatrics) was 51.2% (6,334/12,373), adherence in Oncology was 70.1% (5,290/7,546). In 1,727 positive BCs, 1,793 bacterial species were identified at the species level (monomicrobial: 1,665, two species: 58, three species: 4). The distribution of microorganisms varied depending on inoculated BCVs, site of venous puncture, and subspecialty. Althoughpositivity rate did not increase with rising BCVs, the proportion of probable pathogens increased significantly (< 1 ml: 1.9%; 5-10 ml: 4.0%; p-value: <0.001). TTP was available for 491/1,793 BCBs from monomicrobial detections. The median TTP for probable contaminants was longer than for probable pathogens (20.47h vs. 12.45h; p=<0.0001).

Conclusion: BCVs of children in Germany are low, and guideline adherence is limited. Higher BCVs enhance pathogen detection reliability. This supports the implementation of standardized paediatric guideline in order to improve diagnostic accuracy.

Fig.1: Boxplot of inoculated volumes of all 35,433 weighed blood culture bottles (A), 27,498 paediatric blood culture bottles (B) and 7,935 standard blood culture bottles (C).

Fig. 1



WS11.02

Quantifying inadequate single blood culture pairs: a data-driven quality indicator for diagnostic stewardship

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Introduction: The collection of only a single pair of blood cultures is considered inadequate in nearly all indications for blood culture collection in adult patients. Consequently, the number of blood culture pairs per indication has been recommended as a quality indicator, but is challenging to determine without algorithmic data analysis.

Aim: We describe an approach to operationalize blood culture collection episodes in order to determine the rate of single blood cultures. Additionally, we assessed how the chosen temporal threshold used to define a new episode impacts this rate.

Materials and Methods: Microbiological laboratory data covering a six-year-period from a university hospital were available in CSV format and analyzed using standard Python packages. We defined a blood culture collection episode as a group of temporally adjacent blood culture pairs, where the time difference between consecutive samples does not exceed a predefined threshold (e.g., two days). A new episode was defined whenever this threshold was exceeded. The number of blood culture pairs per episode served as the numerator; the total number of episodes served as the denominator.

Results: After excluding pediatric patients, the dataset included 85,936 blood culture pairs from 23,030 adult patients. Relative to a two-day threshold, the rate increased 1.09-fold when the threshold was reduced to one day. In contrast, increasing the threshold to three, four, and five days resulted in rate decreases to 0.96-fold, 0.94-fold, and 0.93-fold, respectively.

Conclusion: For surveillance and quality control, it is extremely useful to distinguish between metrics that count indications for blood culture collection and those that count the number of blood culture pairs per indication. The concept of a *blood culture collection episode* enables this distinction. Blood cultures repeated after a longer time interval may represent control cultures (e.g., in *Staphylococcus aureus* bacteremia) or new indications (e.g., treatment failure) and must be differentiated from the recommended initial collection of two or more pairs. We consider a threshold of two days a reasonable choice to define a new episode. However, given the potential variability, we quantified the effect of different thresholds on the resulting rate.
Importantly, this metric is intended for aggregated analyses e. g. at the ward level and not for assessing individual patient care. We propose the blood culture collection episode as a practical operationalization to enable algorithmic assessment of the rate of inappropriate single blood culture pairs and advocate its inclusion as a diagnostic stewardship metric for quality monitoring in blood culture diagnostics.

WS11.03

Rapid antimicrobial susceptibility testing of Enterobacteriaceae based on MALDI-TOF mass spectrometry against different antibiotics

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Introduction: The increasing prevalence of multidrug-resistant microorganisms underscores the critical need for rapid antimicrobial susceptibility testing (AST) to inform timely and effective treatment decisions. In this study, we assessed the performance of a research-use-only MALDI-TOF MS-based assay (MBT FAST, Bruker, Germany), which utilizes the direct-on-target microdroplet growth assay (DOT-MGA) methodology. This approach enables phenotypic AST by detecting bacterial growth directly on MALDI targets following short incubation periods.

Materials and Methods: A total of 256 *Enterobacteriaceae* isolates, encompassing diverse species and resistance profiles, were tested against multiple antibiotics of various antimicrobial classes. Each isolate was incubated in 6-µl microdroplets of cation-adjusted Mueller-Hinton broth, both with and without antibiotics at two-fold serial dilutions, within a humidity-controlled chamber (MBT FAST Shuttle, Bruker) at 35°C for a duration of six hours. Following incubation, the broth was removed using an absorptive pad facilitated by a research-use-only prototype device (MBT FAST Stamp, Bruker). Subsequent MALDI-TOF MS analyses were conducted using the MALDI Biotyper Sirius system (Bruker). For comparative analysis, the broth microdilution method (MICRONAUT, Bruker) served as the reference standard for determining minimum inhibitory concentrations (MICs).

Results: The assay demonstrated validity (successful detection of the growth control) in 96.2% of tests. Essential agreement (EA) defined by ISO 20776-2 as MIC results within ±1 two-fold dilution step of the reference method, was observed at the following rates: amoxicillin/clavulanic acid 94.5%, amikacin 98.4%, aztreonam 97.3%, ceftazidime/avibactam 96.1%, ceftazidime 96.9%, cefepime 94.9%, ciprofloxacin 94.9%, colistin 91.8%, ceftriaxone 97.7%, cefotaxime 96.9%, gentamicin 95.7%, imipenem 93.0%, levofloxacin 99.6%, meropenem 87.5%, meropenem/vaborbactam 93.4%, piperacillin/tazobactam 92.2%, trimethoprim/sulfamethoxazole 96.9%, temocillin 94.5%, tobramycin 96.5%. The overall EA across all tested antibiotics was 95.2%.

Summary: The research-use-only MBT FAST workflow delivers accurate AST results within a significantly reduced timeframe. By leveraging the widely implemented MALDI-TOF MS platform, this method provides a practical and cost-efficient solution for rapid AST. The method may offer a seamless integration into routine clinical microbiology workflows, enhancing timely therapeutic decision-making,

provided that it may be made available as an IVD device in the future.

WS11.04

Mechanisms of false-susceptible results for vancomycin in semi-automated antimicrobial susceptibility testing in *Enterococcus faecium*

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Introduction: Enterococci, particularly vancomycin-resistant *Enterococcus faecium* (VRE), are increasingly important healthcare-associated pathogens. The mainstay for detection of VRE is phenotypic antimicrobial susceptibility testing (AST) from clinical isolates defined as a pure culture derived from a clinical specimen [1]. We evaluated the ability of the semi-automated BD Phoenix AST system (Heidelberg, Germany), EUCAST disk diffusion, and VITEK2 (bioMérieux, Nürtingen, Germany) to detect VRE and sought to uncover the mechanism of false susceptible results for vancomycin in *E. faecium*.

Material/method: 318 clinical *E. faecium* isolates were tested in parallel with semi-automated AST BD Phoenix system, EUCAST disk diffusion, and *vanA*/*vanB* PCR. All isolates were additionally tested using VITEK2. Isolates, which showed discrepant results between the different methods, were further investigated by broth microdilution and whole-genome sequencing (WGS).

Results: A total of 42% strains were VRE. Very major error (VME) rate of the BD Phoenix system was 14%, VME rate of the VITEK2 system was 9%, and VME rate of EUCAST Disk diffusion was 1%. Both isolates missed by EUCAST disk diffusion lacked vanS/vanR regulator genes, as shown by WGS. In several phenotypically homogenous clinical VRE isolates missed by semiautomated AST methods, there were different fractions with one vancomycin-resistant and one vancomycin-susceptible portion. When a mixture of different proportions of VRE and VSE was subjected to VITEK2 and Phoenix AST, there was evidence that the semi-automated system failed to detect vancomycin resistance.

Discussion: Our results show that semi-automated AST does not reliably detect vancomycin resistance in *Enterococcus faecium*. Relying solely on *vanA/B* PCR results can lead to false vancomycin resistance prediction, while EUCAST disk diffusion was able to detect all VRE with high accuracy.

1. Wiedmann, M., *Molecular subtyping methods for Listeria monocytogenes.* J AOAC Int, 2002. **85**(2): p. 524-31.

WS11.05

Early detection of ampicillin susceptibility in *Enterococcus faecium* with MALDI-TOF MS and LightGBM

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Background: *Enterococcus faecium* is an important pathogen that can cause severe infections. Although ampicillin is a well-tolerated antibiotic with potent activity against enterococci, it is often ineffective in *E. faecium* due to widespread resistance. As a result, clinicians often use vancomycin empirically, inadvertently selecting drug-resistant bacteria. MALDI-TOF mass spectrometry, the gold standard for bacterial identification, may also serve to detect ampicillin susceptibility. This approach enables rapid optimisation of antimicrobial therapy while minimising resistance development.

Methods: A subset of the MS-UMG database [1], comprising 1136 ampicillin-resistant and 158 susceptible *E. faecium* spectra, was used to develop LightGBM models in Python with five-fold nested cross-validation. For external validation, MALDI-TOF spectra of 382 ampicillin-resistant and 49 susceptible *E. faecium* isolates were collected from the Technical University of Munich (TUM) microbiology laboratory [2].

Results: The model showed good performance in identifying ampicillin-susceptible isolates within the MS-UMG dataset, achieving a positive predictive value (PPV) of 0.911 (SD 0.092) and a moderate sensitivity of 0.671 (SD 0.150) at a threshold of 0.5 (see Figure 1). The area under the precision-recall curve (AUPRC) was 0.902 (SD 0.029), indicating strong overall model performance. Ampicillin-resistant isolates were identified with high confidence, achieving a negative predictive value (NPV) of 0.956 (SD 0.019) and a specificity of 0.989 (SD 0.012). External validation on the TUM dataset suggested reasonable model transferability with an AUPRC of 0.812.

Conclusion: This study demonstrates that LightGBM models can reliably identify ampicillin-resistant *E. faecium* isolates from MALDI-TOF spectra. Susceptible isolates can be detected with moderate confidence, but still require confirmation through additional tests, such as PCR. SHAP analysis revealed a single dominant spectral feature across models, suggesting biological relevance (see Figure 2). In the future, larger MALDI-TOF spectra datasets will allow the development of more robust machine learning models.

1 Park, Y. et al. MS-UMG: MALDI-TOF Mass Spectra and Resistance Information on Antimicrobials from University Medical Center Göttingen. DOI: 10.5281/zenodo.13911744; 2 Weis, C. et al. Direct antimicrobial resistance prediction from clinical MALDI-TOF mass spectra using machine learning. DOI: 10.1038/s41591-021-01619-9

Figure 1. Performance metrics of LightGBM models for predicting ampicillin susceptibility in *E. faecium* isolates, using five-fold nested cross-validation on the MS-UMG dataset and external validation on the TUM dataset.

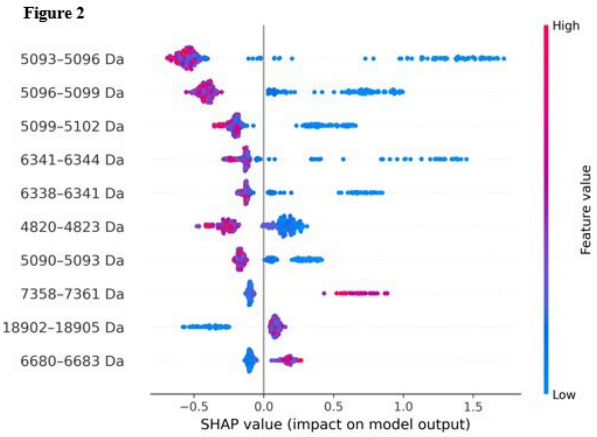
Figure 2. SHAP (Shapley Additive Explanations) analysis showing the 10 most impactful MALDI-TOF spectral features for the prediction of ampicillin susceptibility in *E. faecium* isolates for the first outer fold of a five-fold nested cross-validation model (MS-UMG dataset).

Fig. 1

Figure 1

Metric	Mean	SD
Five-fold nested cross-validation (MS-UMG dataset)		
PPV	0.911	0.092
Sensitivity	0.671	0.150
NPV	0.956	0.019
Specificity	0.989	0.012
MCC	0.750	0.073
AUPRC	0.902	0.029
External model validation (TUM dataset)		
PPV	0.299	
Sensitivity	0.959	-
NPV	0.993	-
Specificity	0.712	-
MCC	0.443	-
AUPRC	0.802	-

Fig. 2



WS11.06

Quinolone-*N*-oxides kill multidrug resistant *N. gonorrhoeae* by unleashing the endogenous zeta toxin

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Gonorrhea is a major sexually transmitted disease and the emergence of multidrug-resistant *Neisseria gonorrhoeae* heralds a global health threat. Here, we identify an unexpected vulnerability of gonococci towards quinolone *N*-oxides released by *Pseudomonas aeruginosa*. Gonococcal growth was abrogated by 2-nonyl-4-quinolone *N*-oxide (NQNO) *in vitro*, whereas NQNO did not impair growth of commensal *Neisseriae* or viability of human cells. While NQNO poisoned the electron transport chain of *Neisseria gonorrhoeae*, the exquisite sensitivity of this pathogen was due to release of the endogenous zeta toxin from its cognate antitoxin. Chemical modification yielded improved NQNO derivatives with nanomolar potency (MIC 500 nM) and lack of resistance development. Topical application of NQNO or derived compounds in a humanized mouse model of vaginal infection, prevented colonization by multi-drug resistant *N.gonorrhoeae* and cleared the pathogen from the vaginal tract. Our findings chart an unexplored route to selective killing of bacterial pathogens by endogenous toxins and provide strong impetus to exploit NQNO derivatives as potent treatment against multidrug-resistant gonococci.

WS12.01

Indoor water systems as reservoirs for clinically relevant non-tuberculous mycobacteria in Germany

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Non-tuberculous mycobacteria (NTM) are environmental bacteria increasingly recognized as causes of severe disease, particularly in vulnerable individuals. Yet, key reservoirs and routes of human exposure remain insufficiently characterized. In this study, we investigated the presence and genetic diversity of NTM in 102 indoor water samples collected from private households and a hospital in Frankfurt am Main, Germany. Isolates were characterized via whole genome sequencing, drug susceptibility testing, and core genome multi-locus sequence typing (cgMLST) to assess potential links with nearly 3,000 global clinical and environmental NTM isolates.

NTM were isolated from 19.5% of households and 14.7% of samples, with a higher recovery from shower water (29.3%) compared to tap water (9.2%). Eight different species were identified, including one putative novel species. Rapid and slow-growing NTM were dominated by *M. chelonae* and *M. chimaera*, respectively. None of the isolates were resistant to macrolides or aminoglycosides. Several isolates from household water showed high genetic similarity to clinical strains from Germany and abroad. Strikingly, we detected a *M. abscessus* strain belonging to the dominant circulating clone DCC3 — which is one of the most frequently isolated clones from NTM patients globally — in a shower water sample. Additionally, we describe for the first time dominant circulating clones of *M. chelonae*.

These findings reinforce the relevance of indoor water systems as reservoirs for clinically important NTM and emphasize the need for targeted One Health interventions to minimize exposure, particularly among at-risk populations.

WS12.02

Impact of hydrogen peroxide-based disinfection of dental chair units on *Legionella* species and other waterborne microorganisms

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Microorganisms in dental chair unit (DCU) waterlines play a major role in increasing infection risk for patients and staff. Regular rinsing can reduce bacterial loads in DCUs as suggested by the KRINKO recommendation. For infection prevention reasons, the annual determination of the total bacterial counts and the investigation for presence of *Legionella* species in DCUs is recommended. DCUs are equipped with disinfection programs, whereby disinfectants are used consisting of an active combination based on hydrogen peroxide (H_2O_2).

In this study, we investigated the impact of DCU disinfectants on the species composition and quantity of bacteria with emphasis on *Legionella* species.

Water samples from DCUs, containing disinfectants based on H_2O_2, were collected. After photometric determination of the H_2O_2 concentration, aliquots were plated on R2A and GVPC agar following incubation. In order to reduce oxidative stress and to inactivate H_2O_2, catalase was added to an aliquot prior plating. The total colony forming units (cfu) were counted and calculated as cfu per mL. Bacterial colonies suspicious for *Legionella* species were isolated and identified on species level by MALDI-TOF biotyping.

Out of 3,789 water samples of 459 dental practices, 36.4% were *Legionella* positive with predominance of *L. anisa* (97.89%). *L. pneumophila* was detected very rarely. The reasons for *L. anisa* dominance may be diverse, especially in consideration of disinfection. Our results demonstrated an increased tolerance to H_2O_2 treatment. In a 6-hour treatment with the high concentration of 200 mg/L H_2O_2, the cell count decreased from an initial cell concentration of 7 $\log_{10}$/mL to a 3 $\log_{10}$-level, while *L. pneumophila* showed no growth after just 3 hours under these conditions. *L. anisa* was even more stable at lower H_2O_2 concentrations.

The H_2O_2 concentration spectrum in DCUs was very wide, ranging from 0 to 182 mg/L. Only few microorganisms could be cultivated as accompanying flora after the specified incubation period of 48 h. Extended incubation resulted in a significant increase in cell numbers. When present in the water sample, bacteria could still be determined even at a H_2O_2 concentration of 141 mg/L. The addition of catalase to the samples containing bacteria tended to result in a slightly higher recovery rate than with directly plated samples.

In conclusion, more than one third of the examined DCUs proved *Legionella* contamination indicating an increased infection risk, whereby *L. anisa* was predominant. This species tolerated increased H_2O_2 concentrations and survived prolonged incubation, indicating higher stability in DCUs treated with low doses of H_2O_2-based disinfections. Inactivation of H_2O_2 by catalase may induce a re-cultivation state of stressed and dormant bacteria in DCUs to avoid under-detection. Regular microbiological analyses combined with H_2O_2 concentration determinations could provide comprehensively plausible results regarding contamination of a DCU.

WS12.03

Isolation and characterization of wastewater-derived plasmid-specific bacteriophages for the reduction of plasmid-carrying enterobacteria in wastewater

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Conjugative plasmids are one of the most relevant sources for antimicrobial resistance (AMR) genes. When pathogenic bacteria take up such plasmids, they can accumulate AMR genes, which poses a serious threat for human and veterinary health, as the therapeutic options are becoming more and more limited. Wastewater, derived from communal, clinical, industrial or agricultural sources, consists of multi-bacterial communities including human and veterinary pathogens, such as enterobacteria. As these bacteria often carry various conjugative plasmids, wastewater is a hot spot for the dissemination of AMR genes. Bacteriophages, targeting the sex pilus of the conjugational apparatus encoded on conjugative plasmids, might therefore be a useful tool to specifically target the plasmid-bearing bacterial populations in various environments, thereby reducing the plasmid load in bacterial populations. In order to learn more about *Escherichia coli* and plasmid-specific bacteriophage diversity in environmental samples we investigated various wastewater sources with respect to resistance profiles and plasmid content of the bacteria. Moreover, we screened the same samples for novel plasmid-specific bacteriophages and characterized them with molecular biological methods. We show that wastewater sources contained not only bacteria harboring conjugative (AMR) plasmids, but also a substantial amount of plasmid-specific bacteriophages. Thus, the appearance of conjugative (AMR) plasmids and plasmid-specific bacteriophages in wastewater goes hand in hand. Finally, from a One Health perspective, we investigated if wastewater treatment with plasmid-specific bacteriophages can lead to an overall reduction of antimicrobial resistances in these diverse bacterial populations at the lab-scale. Therefore, our results should help to explore future applications of plasmid-specific bacteriophages, including decontamination and reduction of AMR in wastewater of various origin, thus helping to limit the spread of conjugative (AMR) plasmids and to maintain current therapeutic options in human and veterinary health care.

WS12.04

Down the drain: tracking multidrug-resistant pathogens in hospital plumbing

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Introduction: Hospital wastewater systems are increasingly recognized as critical reservoirs for multidrug-resistant organisms (MDROs), particularly in high-risk areas such as hematology-oncology wards. Despite established infection control protocols, the role of wastewater infrastructure in nosocomial transmission remains underexplored.

Objectives: This study aimed to investigate transmission routes of Verona Integron-encoded Metallo-beta-lactamase (VIM)-producing gram-negative bacteria—primarily *Pseudomonas aeruginosa* and *Enterobacter cloacae*—on a hematology-oncology transplant unit, to identify the wastewater system as a potential reservoir, and to evaluate the effectiveness of a targeted intervention bundle.

Materials & Methods: Between March 2023 and March 2024, five patients tested positive for VIM-producing *P. aeruginosa* (four colonizations, one infection). Weekly environmental screening began in December 2023 across 14 single-patient rooms at three sites per wet area (sink siphon, shower siphon, toilet). Additionally, removed siphons from six rooms were systematically sampled using Polywipes™. Species were identified via culture, VITEK, and eazyplex® PCR. Whole-genome sequencing (WGS) and cgMLST were used to assess clonal relationships.

Results: Clonal links between patient and environmental isolates from toilet drains (Cluster 1) and environmental-only isolates from sinks and showers (Cluster 2) were confirmed. Notably, while routine swab-based screenings often yielded negative results, the in-depth analysis of removed siphons revealed up to five VIM-producing species per unit, highlighting a significant underestimation of contamination by conventional methods. To address this, a comprehensive

intervention bundle was implemented after the ninth week of a 40-week surveillance period, including post-discharge siphon replacement, intensified cleaning protocols, and antibiotic stewardship. Following implementation, no further VIM-positive patient cases were detected over the subsequent eight months. Environmental detection rates also declined significantly.

Conclusion: Wastewater infrastructure in high-risk hospital areas can serve as both a reservoir and transmission vector for MDROs. Routine swab-based surveillance may fail to detect significant contamination, as demonstrated by the high sensitivity of deep siphon sampling using Polywipes™. This study emphasizes the need for enhanced diagnostic strategies and sustainable structural interventions to effectively control wastewater-associated transmission pathways in healthcare settings.

WS12.05

Prevalence of multiresistant *Enterobacterales* in the Hamburg City sewage system and surface waters

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Introduction: In metropolitan areas considerable quantities of wastewater are introduced from various sources and support the formation of complex microbial populations. As part of the newly established collaborative research project Molecular Monitoring of Bacterial Biodiversity in The Water Cycle (MOMOBIO) a combination of culturomics and PCR-based assays is being used to investigate the occurrence of 3rd Gen. Cephalosporin or carbapenem resistant *Enterobacterales* (3GCRE or CRE) as well as resistance genes spatially and temporally along the Hamburg City wastewater system.

Methods: In a first sampling period in 2023 eight different water samples were taken from a hospital, a wastewater treatment plant (WWTP) and various surface waters. Presence of 3GCRE or CRE were quantified using selective media. Whole genomes were sequenced for representative pure isolates and clonality of the isolates (cgMLST and classical MLST) and their resistance genes were analyzed using seqsphere (Ridom, Münster, Germany). In addition, total DNA of the samples were analyzed by quantitative PCR (qPCR) for various resistance genes.

Results: 3GCRE were detected in 7/8 water samples, ranging from 4x102 to 1.45x107 cfu/L. Growth of CRE was not observed in surface water while in wastewater samples CRE ranged from 3.0x102 cfu/L to 4.15x106 cfu/L. A total of 248 isolates were sequenced (133 *Escherichia coli*, 58 *Klebsiella* species, 39 *Citrobacter* species, 11 *Serratia* species, and 7 *Enterobacter* species). In *E. coli* 50 different classical MLST sequence types could be determined, including a prominent cluster of 25 ST2179 isolates from hospital wastewater and the high risk clone ST617, that was detected multiple times in two different samples. Carbapenemase genes could be detected in 39 *Citrobacter*, 20 *Klebsiella*, 13 *E. coli* and 4 *Enterobacter* isolates from the WWTP influent and the hospital wastewater within different STs of the individual species. Quantitative PCR revealed the presence of all tested carbapenemase genes (*bla*OXA-48, *bla*VIM, *bla*NDM, *bla*KPC, *bla*GES) in the hospital wastewater and in both WWTP influents, with *bla*GES being the most frequent gene. The genes *bla*GES and *bla*OXA-48 could be also identified in two and one surface water bodies, respectively.

Conclusion: Results from the first sampling period revealed the presence of 3GCRE in all but one of the samples and of CRE in wastewater samples. In addition, in two samples of surface waters carbapenemase genes were detected by qPCR. The widespread detection of culturable 3GCREC and CRE as well as the presence of antibiotic resistance genes in surface waters indicates the relevance of monitoring of the urban water body. In the planned upcoming sampling periods of the MOMOBIO project the combinatorial analysis will be extensively expanded and supplemented by further methods such as in-depth metagenomics and ecological modeling.

WS12.06

What ends up in agriculture? – a long-term study on the use of sewage sludge

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Background: In Germany, over 1.5 mio tons of dry sewage sludge are produced yearly. Sewage sludge is usually incinerated, but in rural areas it might be used as fertilizer in agriculture. By law sewage sludge must be limed or long term stored before use as fertilizer. However, only few data is present about remaining bacteria and bacterial resistance genes. Therefore, the effectiveness of liming and long term storage was investigated in this study to enable future risk assessment.

Methods: Six rural sewage treatment plants (STP) with different treatment processes (3x dewatering + liming, 1x only dewatering, 2x no dewatering or liming) were sampled monthly for one year. By applying resuspended samples to selective media, third-generation cephalosporin-resistant *Enterobacterales* (3GCRE) were quantified. Representative pure isolates were sequenced by whole genome sequencing and analyzed with regard to their clonality (cgMLST and classical MLST) and resistance determinants. In addition, total DNA was extracted from each sewage sludge sample and the occurrence and frequency of numerous resistance genes was analyzed using quantitative PCR (qPCR).

Results: Growth of 3GCRE was observed in 2 of 36 limed samples and in 33 of 36 unlimed samples (maximum of 1.05x106 cfu/g dry weight). A total of 529 isolates were identified as *Enterobacterales*, of which 362 isolates of the most common species *E. coli* (n=308), *K. pneumoniae* (n=38) and *K. oxytoca* (n=16) were sequenced. A high genetic diversity according to cgMLST as well as MLST could be observed with 69, 11 and 9 different classical STs for *E. coli*, *K. pneumoniae* and *K. oxytoca*, respectively. In *E. coli*, 20 STs were identified that were present in a STP at 2 or more time points. In addition to common, ubiquitous STs such as ST131, animal associated clones were also identified multiple times. Potential high risk clone STs ST88 and ST361, associated with pig farming, were identified several times in one STP with a pig farm in its catchment area. Analysis of the resistance genes revealed extended spectrum beta-lactamases (ESBL) genes in 97.3 % of all strains, with *bla*CTX-M-15 (n=161) being most common. The presence of carbapenemase genes was also confirmed by qPCR in 49 of 61 available DNA

samples, including the DNA of limed sludge samples, with blaGES (n=45) being most common.

Conclusion: The study confirmed the relevance of liming sewage sludge. Individual clonal lineages detected at different time points indicate permanent entry or long-term retention. In addition, individual clones could possibly be traced back to input from animal husbandry. Using q PCR carbapenemase genes were identified in the majority of samples, both in the unlimed and limed samples. Therefore the agricultural application of sewage sludge, as it is currently used in rural areas, should be critically questioned with regard to the One Health concept.

WS13.01

The influenza A virus promotes fungal growth of *A. fumigatus* via direct interaction *in vitro*

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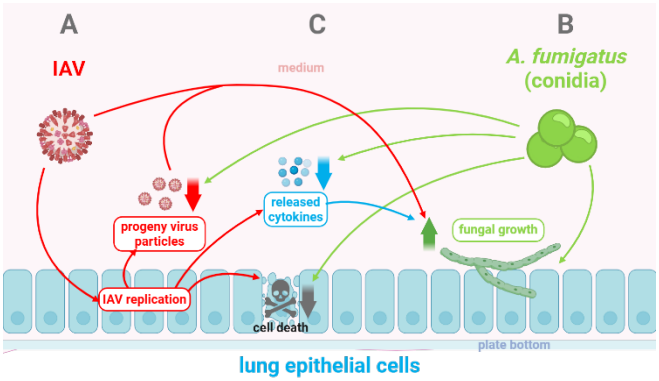
Severe disease progression and death during influenza A virus (IAV) infections are often associated with secondary bacterial infections. However, superinfections with opportunistic pathogenic fungi, particularly *Aspergillus* species, also occur, and are associated with increased morbidity and mortality compared to influenza virus infections alone. The clinical aspects of influenza-associated pulmonary aspergillosis have been well described in recent years. Nevertheless, prior to this project, *in vitro* studies to analyze the fatal synergism of the two pathogens on a molecular level did not exist.

For these reasons, we established an *in vitro* co-infection model to investigate pathogen-host and pathogen-pathogen interactions during the complex infection of lung epithelial cells (e.g. Calu-3 cells) with influenza A virus (IAV) and *Aspergillus fumigatus* conidia. Remarkably, our results reveal a reduction in viral load, as well as lowered viral protein and mRNA expression levels in the presence of *A. fumigatus*. Consistent with these findings, IAV-induced cytokine expression and host cell death were also diminished during co-infection. Interestingly, at the same time, IAV infection appeared to promote hyphal growth of *A. fumigatus*, compared to single infection conditions. Using immunofluorescence and scanning electron microscopy, we demonstrated that purified IAV particles can directly interact with the fungal surface and influence fungal growth.

In summary, we present a complex infection model that enables the investigation of the interaction of different pathogens with each other as well as host-pathogen interactions. Our findings offer initial insights into the complex interplay between IAV and *A. fumigatus* and their combined impact on the host at the molecular level.

Figure 1: *Graphical representation of the proposed host-pathogen-pathogen and pathogen-pathogen interaction during co-infection of lung epithelial cells with influenza A virus (IAV) and Aspergillus fumigatus in vitro.* (A) Single infection of lung epithelial cells with IAV leads to the release of progeny viruses and the induction of host responses, including cytokine secretion and cell death. (B) In contrast, during single infection with *A. fumigatus*, conidia undergo germination and hyphal growth. (C) Co-infection with both pathogens results in reduced IAV titers, diminished cytokine release and inhibition of IAV-induced cell death. Conversely, the presence of extracellular IAV particles promotes the growth of *A. fumigatus* by interacting with the fungal surface. (Legend) Red arrows indicate the effect of IAV on lung epithelial cells and *A. fumigatus*. Blue arrows indicate the effect of IAV-induced host cell response on *A. fumigatus*. Green arrows indicate the effect of *A. fumigatus* on the viral replication and the virus-related host cell response. Created with biorender.com.

Fig. 1



WS13.02

Human neutrophils maintain an antimicrobial extracellular RNA landscape upon *Aspergillus fumigatus* challenge

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Extracellular RNAs (exRNAs) are now recognized as potent bidirectional interkingdom effectors in plant and insect systems, but the repertoire and function of exRNA in defense against human fungal pathogens like *Aspergillus fumigatus* remains limited. Here, using small RNA-seq, we reveal a diverse neutrophil exRNA-ome capable of promoting antifungal immunity against *A. fumigatus*. Intriguingly, we observed the extracellular microRNA (miRNA) pool to be enriched for immune-regulatory and antimicrobial let-7 family sRNAs but impervious to infection status, suggesting that neutrophils produce and maintain an antimicrobial RNA environment independent of stimulus and seemingly despite well-characterized *A. fumigatus* immune repressive factors like dihydroxynaphthalene-melanin and/or gliotoxin. Cross-kingdom miRNA target prediction and exRNA ex vivo delivery experiments demonstrated that predicted putative fungal targets are modulated across kingdoms, with experiments in progress to assess miRNA:mRNA interactions. This regulation appeared independent of fungal argonaute proteins linked to RNA interference, but we did detect host argonautes in the extracellular fraction, suggesting host RNA binding proteins may contribute to cargo delivery. Unlike the miRNAs, extracellular tRNA fragments (tRFs) did display some alterations in response to infection, possibly providing an additional arm to antifungal immunity and offering potential as biomarkers for detection of fungal infection. Future investigations will be required to assess the influence of the tRFs on host defense and immune coordination. In conclusion, our study provides a much-needed first step towards understanding the complex host extracellular RNA landscape in response to a deadly human fungal pathogen.

WS13.03

Investigation of infection dynamics and host-pathogen interactions through establishment of a lung-on-chip model for invasive mucormycosis

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Invasive mucormycosis (IM) is a critical pulmonary infection induced by inhalation of Mucorales spores—primarily *Lichtheimia corymbifera* (*L.cor*), *Mucor circinelloides*, and *Rhizopus arrhizus*. These inhaled spores localise in the lung alveoli, subsequently germinating and disseminating through the bloodstream. The infection predominantly manifests as a breakthrough infection (BTIs) in immunocompromised patients post-invasive aspergillosis (IA), leading to high mortality rates of up to 60-90%.

Macrophages serve as the first line of defence against pulmonary infections. Through in vitro experiments and the development of a lung-on-chip model, we intend to analyse macrophage response to mucoralean infections. The kinetics and response time of macrophages during phagocytosis of spores were assessed by co-culturing macrophages with lung epithelial cell lines H441 and A549 through live-cell imaging facilitated by confocal laser scanning microscopy. Additionally, macrophages are co-infected with both *Aspergillus fumigatus* (*A.fum*) and *Lichtheimia corymbifera* to simulate a breakthrough infection scenario, allowing us to explore pathogen preference and macrophage response under these conditions. These prevalences will then be replicated in a lung-on-chip configuration, which represents the next level of complexity. It consists of a multi-tissue setup comprising both vascular endothelial and lung epithelial cells separated by a porous membrane. This system effectively mimics the lung microenvironment for analysing host-pathogen infection and angioinvasion of hyphae.

Presently, it has been observed that an increase in the phagocytosis of fungal pathogens corresponds to a reduction in the motility of the macrophages. Nevertheless, the macrophages continue to function actively throughout the course of infection. In the co-infection setup, both *A.fum* and *L.cor* spores appear to be subjected to phagocytosis at comparable rates; however, the germination rates of the fungi differ. Under lung-on-chip experimental conditions, *L.cor* demonstrates germination within five hours.

While traditional disease modelling relies on mouse models, these remain constrained in advancing diagnostic and therapeutic strategies for IM. In contrast, lung-on-chip models provide a platform for in-depth evaluation of host-pathogen interactions, facilitating the visualisation of these interactions and the calculation of the underlying kinetics. It also enables the assessment of diagnostic markers, making it an effective setup for testing antifungal therapies against IM.

In this presentation, first results of the interplay between spores and phagocytes as well as endo-/epithelial tissue will be presented and discussed in the light of angioinvasion and dissemination of IM.

WS13.04

Bacterial signal peptides are key players for innate immune cell recruitment against bacteria

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Introduction: Formylated peptides in supernatants of bacterial cultures represent one of the oldest pathogen-associated molecular patterns (PAMPs) known in the field of innate immunity. Moreover, it is well recognized that these peptides are capable of inducing a potent immune activation via formyl peptide receptors (FPRs).

Hypothesis & Aim: We proposed that N-terminal fragments of bacterial signal peptides, which are required for the protein export via the secretory protein translocation machinery, could be the source and origin of the formylated peptides in bacterial supernatants. Also, there are many aspects about formylated peptides that are not well understood yet. This includes their precise biosynthetic origin, their release mechanism as well as their biological significance in comparison to other bacterial PAMPs. In addition to that, we wanted to investigate which effect these peptides would have on innate immune cell behavior.

Methods & Results: To find out whether signal peptides are the FPR activators in bacterial supernatants, we tested different bacterial supernatants with and without specific protease and filtration treatments on transiently transfected HEK293T cells. This revealed that all bacterial supernatants are detected by FPRs and that the activators are peptides, small in size and formylated. By using HPLC, size exclusion chromatography and mass spectrometry on *E.coli* and *S.aureus* supernatants, we could show that FPR activators occur naturally in large numbers and are extremely heterogenous. Moreover, we could *de* novo sequence, identify and also quantify several formylated peptides in *E.coli* and *S.aureus* supernatants that originate from bacterial signal peptide fragments. Many of the identified peptides could robustly and sensitively activate FPRs up to low picomolar concentrations. Furthermore, we demonstrate that the release mechanism of formylated peptides in *E.coli* is SecA-depended. Of note, we found that for primary isolated innate immune cells the signal peptide detection is essential to trigger parts of important inflammatory responses such as cell migration and interleukin release that could not be substituted by other PAMPs.

Conclusion: Signal peptides are the source of formylated peptides in all bacterial supernatants. They orchestrate a non-redundant function of host innate immunity that mediates

chemotaxis and pro-inflammatory responses against invading bacteria.

WS13.05

Streptolysin S beyond cell lysis: a novel mediator of platelet activation by *Streptococcus pyogenes*

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Background: *Streptococcus pyogenes* (group A Streptococcus, GAS) is a human pathogen responsible for a wide range of diseases, from mild superficial infections to severe invasive conditions such as streptococcal toxic shock syndrome (STSS). STSS is characterized by several clinical manifestations, including thrombocytopenia, which is associated with poor prognosis. GAS produce several virulence factors, including streptolysin S (SLS), a peptide toxin best known for its hemolytic and cytolytic activities. To date, GAS-induced platelet activation has been primarily attributed to serotype-specific interactions involving M protein. This study aimed to investigate the impact of SLS on platelet dysregulation, with particular emphasis on elucidating its underlying mechanism.

Methods: Human platelets were infected with GAS strain 5448 and its isogenic mutant deficient in SLS (Δ*sagA*). Platelet activation was assessed by measuring surface expression of CD62P using flow cytometry. Calcium chelators and chemical receptor antagonists were used to identify involved receptors. All experiments were performed in the presence of IVIG.

Results: In all experimental settings, the use of IVIG markedly improved platelet viability. The GAS strain 5448 strongly induced platelet activation, whereas its isogenic Δ*sagA* mutant failed to trigger a response. This activation was driven by SLS-mediated influx of extracellular Ca^{2+}, as demonstrated by its inhibition upon calcium chelation. Furthermore, platelet activation was significantly reduced by suramin and NF449, both antagonists of purinergic P2 receptors.

Conclusion: Our findings reveal a new role of SLS in contributing to the severity of GAS infection. Although IVIG treatment significantly improved platelet viability, it failed to adequately suppress platelet activation due to the non-immunogenic nature of SLS. These results underscore the urgent need to develop complementary therapeutic strategies that specifically target SLS-mediated signaling pathways on platelets.

WS13.06

Cytosolic sodium accumulation is a common danger signal triggering NLRP3 inflammasome activation

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Detecting noxious molecules within the endo/lysosomal system is essential for maintaining cellular integrity. Here, we identify acute cytosolic sodium accumulation as a key trigger for NLRP3 inflammasome activation. Large clostridial toxins, as well as monosodium urate and silica crystals, rapidly elevate cytosolic sodium by promoting its release from the endo/lysosomal system, triggering concurrent potassium efflux and extracellular sodium influx. This rise in cytosolic sodium disrupts endocytic trafficking by collapsing the sodium gradient across endosomal membranes, leading to peripheral redistribution and activation of NLRP3. Importantly, non-particulate stimuli, including nigericin, also activate NLRP3 by this sodium-dependent mechanism. These findings position cytosolic sodium as a key downstream effector of potassium efflux and a central signal for NLRP3 activation across diverse stimuli.

WS14.01

30 years of national reference centers and consultant laboratories in Germany – a review and a look into the future

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In the past three decades, the German national reference centers (NRC) and consultant laboratories (CL) were faced with various threats posed by pathogens to public health in Germany and worldwide. Their crucial role within public health systems was especially evident during the COVID-19 pandemic and their sustained funding is crucial in both pandemic and non-pandemic times. Here, we provide an overview of the national reference laboratory network, challenges dealt with in the past and an outlook for the future of German NRC and CL.

WS14.02

Outbreak report: invasive *Haemophilus influenzae* serotype b (Hib) infections among persons with history of substance use

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Introduction: Invasive *Haemophilus influenzae* serotype b (Hib) infections are rare and typically sporadic in Germany due to general Hib vaccination in childhood. However, we report an ongoing outbreak of Hib among adult substance users in Northern Germany.

Methods: The National Reference Laboratory for Meningococci and *H. influenzae* (NRZMHi) is typing all invasive *H. influenzae* isolates by slide agglutination and PCR. Clusters of disease are identified through spatio-temporal

analysis and reported to local health authorities. Whole genome sequencing (WGS) is performed on all isolates suspected to be part of the outbreak. Throat swabs of contact persons in a homeless facility were analysed for Hib carriage.

Results: An increase in invasive Hib cases between November 2024 and January 2025 prompted the identification of three cases in Hamburg occurring within a two-week period. Investigation by local health authorities revealed that all affected individuals had a history of substance use in Hamburg, and most were utilizing homeless services. To date, nine cases – six in Hamburg, two in Mecklenburg-Western Pomerania, and one in Lower Saxony – have been confirmed to be part of to the outbreak, with three resulting in death. Additionally, for three suspected cases, two in Hamburg, one in Berlin, WGS is ongoing.

An ad hoc carriage investigation conducted in a homeless shelter found no additional Hib carriers among 47 residents. Genome sequencing showed that all nine confirmed outbreak-related isolates differed by fewer than six alleles in the PubMLST core genome MLST (cgMLST) scheme. Phylogenetic analysis showed that the outbreak strains are distinct from other circulating European strains. Further findings will be reported as the outbreak continues.

Conclusions: This outbreak underscores the potential for Hib transmission in a vulnerable population even in the post-vaccine era and highlights the critical importance of sustained surveillance. Spatio-temporal and genomic analyses are essential epidemiological tools for tracking disease spread and informing public health responses.

WS14.03
Increasing prevalence of KPC-3-producing *Klebsiella pneumoniae* in Germany, 2021-2024

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Question: Multidrug-resistant strains of *Klebsiella pneumoniae* are a major cause of both hospital- and community-acquired infections, including sepsis and pneumonia. The production of KPC-type carbapenemases is a key mechanism of carbapenem resistance in this species. While KPC-2 is currently the most frequently detected KPC-variant at the German National Reference Centre for Multidrug-resistant Bacteria (n=486 in 2024), recent data indicate a rapid increase of KPC-3-producing *K. pneumoniae* in Germany. Between 2021 and 2024, the annual detected numbers of KPC-3-producing *K. pneumoniae* in Germany increased from 56 to 203 including an increase of 34.2 % from 2023 to 2024. This rate of increase in *K. pneumoniae* is more than twice as high as observed for *bla*KPC-2 over the same period.

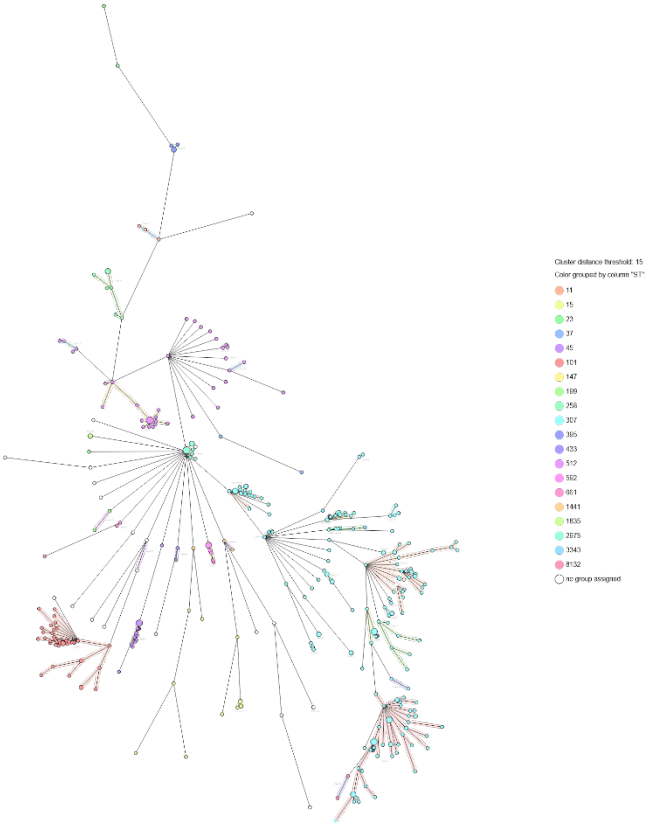
Methods: Within the integrated genomic surveillance, we subject all carbapenemase-positive *K. pneumoniae* isolates to whole genome sequencing (WGS). From this dataset we selected all KPC-3-producing isolates and employed phylogenetic analysis and virulence profiling to characterise the dissemination and molecular features of KPC-3-*producing K. pneumoniae* isolates in Germany.

Results: Our WGS dataset, compiled over a period of 4 years, comprised 410 *K. pneumoniae* isolates carrying blaKPC-3 representing 35 different sequence types (STs). The most prevalent STs were ST307 (n=204), ST512 (n=45), and ST101 (n=45). Sample type information was available for 398 isolates (97 %) with rectal swabs (n=124) and urine (n=95) being the most common specimen sources. Notably, approximately 7 % of isolates (n=29) harboured at least one additional carbapenemase gene, with *bla*NDM-1 being the most frequently detected carbapenemase alongside *bla*KPC-3 (n=13). Furthermore, 99 % of isolates carried at least one extended spectrum β-lactamase (ESBL)-encoding gene.

Core genome multilocus sequence typing (cgMLST) identified 39 clusters of two or more isolates using the consensus threshold of 15 allelic differences for *K. pneumoniae*. The largest cluster, comprising 61 isolates, belonged to ST307. This international high-risk clone, first described in Europe in 2008, exhibits genomic traits which contribute to its adaptation to the human host and healthcare environments. Several hypervirulence-associated genes including *yersiniabactin* and *colibactin* were identified in ST307 isolates from the two largest detected clusters.

Conclusions: The rapid dissemination of carbapenemase producing Enterobacterales including *K. pneumoniae* carrying *bla*KPC-3 poses a serious threat by limiting treatment options for severe infections. Consequently, early detection and surveillance with subsequent analyses are essential to monitor transmission chains, curb the spread of resistance, and reduce its impact on patient outcomes as well as healthcare systems in Germany.

Fig. 1



WS14.04
Genomic surveillance and resistance profiling of *Enterococcus faecium* in Germany: insights from the National Reference Center (NRC) for Enterococci, 2024

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Introduction: *Enterococcus faecium*, particularly vancomycin-resistant strains (VREfm), remains a significant driver of healthcare-associated infections. The NRC for Enterococci plays a central role in characterizing resistance mechanisms and supporting outbreak investigations. The implementation of intensified genomic surveillance, particularly of invasive blood culture isolates, including both VREfm and vancomycin-susceptible isolates (VSEfm), has enabled deeper insights into the distribution of clonal lineages at both national and institutional levels.

Objectives: We present genomic trends, resistance profiles, and molecular resistance characteristics of *E. faecium* isolates submitted to the NRC in 2024, with comparisons to previous years. Particular emphasis is placed on the distribution of sequence and complex types, as well as the proportion of vancomycin and linezolid resistance genes.

Materials & Methods: Between November 2023 and October 2024, a total of 1,367 clinical *Enterococcus* isolates were submitted by 104 diagnostic laboratories. Species identification was performed via PCR, *sodA* sequencing or Maldi-ToF MS. Antimicrobial susceptibility testing was conducted using broth microdilution and Etest®. Resistance genes (*vanA*, *vanB*, *cfr*, *optrA*, *poxtA*) were detected via multiplex PCR. Whole-genome sequencing (WGS) and core genome multilocus sequence typing (cgMLST) were applied to 824 isolates, including 689 from bloodstream infections and 150 from suspected outbreaks.

Results: Among VREfm isolates, 55% harbored *vanA* and 44% *vanB*, while previous years were dominated by *vanB* genotype. WGS-based analysis revealed substantial clonal diversity on a national scale Notably, sequence types such as ST80 and ST117 were dominant and exhibited broad geographic distribution, underscoring their epidemic potential, while patterns on regional scale often showed dominance of specific complex types. VREfm and VSEfm isolates from both, outbreak contexts and surveillance, showed clonal relatedness at local levels (e.g. from same submitting institutions). These genomic approaches are essential for distinguishing outbreak-related isolates from sporadic cases and for informing infection control strategies Linezolid resistance among VREfm remained prominent (23% of VREfm are LVREfm).

Conclusion: Continued integration of high-resolution genomics into national surveillance efforts is critical to maintaining the NRC"s role as an early warning system for emerging MDR clones, especially in cases of none-notifiable diseases or pathogens like VREfm. Voluntary submission of invasive *E. faecium* and VRE isolates allows the recognition of local, regional and country-wide dissemination of strain types, but also allows a valuable feedback for participating institutions of regional and local trends in the light of more general dynamics.

WS14.05

An update on Leptospirosis in Germany – latest outbreak linked to pet rats

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Leptospirosis is a globally important bacterial zoonosis caused by pathogenic *Leptospira* species. Transmission occurs through direct or indirect contact of small skin lesions or mucous membranes with the urine of infected animals. Small mammals, such as rodents, are an important reservoir for the pathogen. In Germany, leptospirosis is considered a rare disease. However, a high incidence of unreported cases is assumed, as a majority of cases remain undiagnosed due to a subclinical or mild course of disease and symptoms are typically non-specific. Recent outbreaks of leptospirosis in Germany occurred in 2006 among triathlon participants and in 2007 and 2014 among farm workers (Brockmann et al. 2010, Desai et al. 2009, Dreesman et al. 2016, Fiecek et al. 2017).

In 2023 an outbreak of leptospirosis was associated with the keeping of fancy rats as pets. The consultant laboratory for leptospirosis tested 33 serum samples of humans who had been in contact with potentially infected rats and 56 urine samples of these rats. Human serum samples were investigated using the microscopic agglutination test (MAT) and an in-house ELISA. Rat urine samples were analysed by a PCR targeting the *LipL32* gene to detect presence of pathogenic *Leptospira*. Where possible, samples were further typed using a PCR targeting *secY* and multilocus sequence typing (MLST).

Three serum samples showed positive results in MAT and ELISA (IgG and IgM) and serogroup Icterohaemorrhagiae was detected. One additional serum sample showed positive ELISA results for IgG and IgM and three serum samples for IgM only. In rat urine samples, nine positive samples were determined by PCR (*LipL32*) and five samples could be typed as *Leptospira interrogans*.

Overall, contact with rats should be given greater consideration as a source of infection in the future and owners of pet rats should be advised of the potential risk of infection.

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WS14.06

Toxigenic *Corynebacterium ulcerans* in raw milk of a cow with acute mastitis and the risk of milk-associated diphtheria in humans

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Question: Historically, a considerable number of diphtheria outbreaks, mainly in the UK and the USA, have been epidemiologically or bacteriologically linked to the consumption of milk and dairy products. However, with the introduction of milk pasteurization and hygienic improvements in livestock farming and animal welfare, no cases of milk-linked human diphtheria outbreaks or bovine mastitis due to toxigenic corynebacterial have been reported in the recent decades. Here we report the first isolation of a toxigenic *Corynebacterium ulcerans* strain from the milk of a cow with acute mastitis within nearly 40 years and outside of UK or Finland.

Materials & Methods*:* The isolated strain was analysed by state-of-the-art bacteriological methods including toxigenicity testing by a novel Lateral Flow Immunoassay and Elek test, molecular typing was done by whole genome sequencing and MLST/cgMLST analysis.

Results*:* The obtained sequence type ST-331 is also found in human isolates of cutaneous diphtheria. cgMLST analysis, however, found no close relationship to 43 human ST-331 isolates from our German strain collection or to two animal samples from a zoonic cluster of this ST.

Conclusion*:* The risk of milk-associated diphtheria due to *C. ulcerans*, although today extremely rare, should be avoided by milk pasteurization and the respective hygienic standards.

WS15.01

Sustainability in microbiology and IPC – ten key strategies for a greener laboratory and healthcare secto

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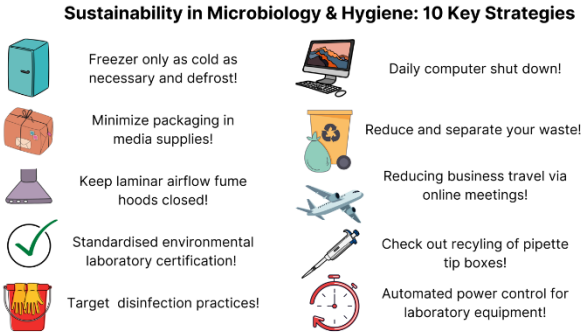
Question: The healthcare sector is responsible for approximately 5% of global greenhouse gas emissions, with microbiology laboratories and infection prevention and control (IPC) practices contributing significantly through energy-intensive processes and resource consumption. Although maintaining IPC standards is essential, there is an urgent need to reduce the ecological footprint of microbiology and IPC practices. This work aimed to identify practical strategies to make microbiology and IPC more sustainable without compromising infection prevention.

Methods: A structured approach was used to develop ten key strategies for ecological improvement in microbiology and IPC. This process included literature review, expert consultations, and synthesis of implementation experiences in microbiology and IPC settings. The focus was on identifying scalable, pragmatic, and effective measures that address waste management, energy consumption, material use, and behavioral change.

Results: Ten strategies were defined to target various aspects of laboratory and IPC operations. Waste reduction was emphasized through minimizing single-use plastics and packaging materials (1), separation (2), and recycling programs (e.g. pipette tip boxes) (3). Energy efficiency measures included optimized ultra-low-temperature freezer use (4), automated power control for laboratory equipment (5), shutting down IT equipment after working hours (6), and smarter management of fume hoods (7). Environmental certification systems (8), such as Eco-Management and Audit Scheme (EMAS), were identified as frameworks for systematic improvement, which can be included in existing quality management systems. Additional strategies focused on limiting disinfection to relevant areas (9), and reducing business travel through online and hybrid meetings (10). Together, these measures offer a comprehensive approach to reducing emissions, conserving resources, and promoting sustainability in microbiology and IPC practices.

Conclusions: Implementing sustainability strategies in microbiology and IPC is both feasible and necessary to align healthcare with global climate goals. The proposed measures demonstrate how laboratories and IPC departments can significantly reduce their ecological footprint while maintaining essential infection control standards. This framework offers practical guidance for other institutions aiming to contribute to a more sustainable healthcare system through environmentally responsible microbiological and IPC practices.

Fig. 1



WS15.02

AI-powered species identification with fluorescence images of bacterial smears

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Introduction: Accurate and rapid identification of bacterial species is crucial for clinical decision-making. Many bacterial species differ in shape and size in Gram-stained microscopic preparations. Based on this established observation, we wanted to optimise species identification using microscopy powered by AI. The aim was to develop a machine-learning based procedure for bacterial species identification using several fluorescent dyes on microscopic images of bacterial smears.

Materials & Methods: The seven most common bacterial species identified by MALDI-TOF were selected and stained with various fluorescence dyes (i.e. Acridine Orange, Auramine O, Calcofluor White, Congo Red, Rhodamine B, Rose Bengal and Trypan Blue). Data on relevant features (e.g. area, eccentricity, contrast, homogeneity, major/minor axis) were extracted from the segmented images and used to train and test a machine learning model for species classification. Model performance (CatBoost) was evaluated using the area under the receiver operating characteristic curve (AUROC).

Results: The prediction accuracy varied between the different species (AUROC): *Klebsiella pneumonia* (0.80), *Klebsiella oxytoca* (0.88), *Escherichia coli* (0.89), *Staphylococcus epidermidis* (0.94), *Enterococcus faecalis* (0.95), *Staphylococcus aureus* (0.97) and *Pseudomonas aeruginosa* (1.00). Additional species that were not part of the training dataset were reliably classified as 'unknown species'.

Conclusion: We provide a proof-of-concept that the ML-powered prediction of bacterial species based on microscopic images is promising. Due to low investment costs in infrastructure and low maintenance requirements, this procedure is well suited to be used in settings with limited resources.

WS15.03

In silico analyses of virulence genes in *K. gyiorum*

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Introduction: *Kerstersia gyiorum* is a rare human pathogenic bacterium that has been little studied to date. For this reason, little is known about its virulence properties. Herewith, genomic determinants of virulence based on *in silico* data are presented obtained from a defined collection of *K. gyiorum* strains.

Methods: The genomes of 14 isolates identified as *K. gyiorum* in routine microbiological diagnostics were assembled using PacBio sequencing technology. These genome data were then examined for the presence of virulence traits using the Virulence Factor Database (VFDB).

Results: Two genes (*epsS and lspG*) encoding type 2 secretion systems were identified. In addition, evidence was found for the presence of *barA/B and bauA/B/C/D/E*, which encode the siderophore acinetobactin, which has already been described in *Acinetobacter baumannii*. Furthermore, the genes *rmlA*, *rffG* and *kdsA*, which are involved in the synthesis of LPS, were detected. In addition, the genes *flgBCDEFGHIK, flhABCD, fliEFGIMPORS, motAB, fliAEFGIMNPQRS*, *cheARWY* were detected, which are responsible for motility (including the expression of flagella) and chemotaxis. Interestingly, *flaA* was not detected in one strain, namely DSM 109002. In addition, the genes *cheD (*encoding chemotaxis proteins) and *cheR* (encoding a methyltransferase) were detected. Not all genes could be detected in all strains; slight variants were found. Furthermore, the *plc* gene was detected, which encodes phospholipase C (PLC) and plays a central role in cellular signal transduction. Furthermore, the *katA* gene was identified, which encodes catalase A and mediates a protective function against oxidative stress. In addition, *clbS* was found, which encodes a cyclopropane hydrolase protein that provides protection against colibactin and thus cells from DNA damage.

Conclusion: This abstract presents initial findings on resistance genes in *K. gyiorum*. The virulence genes presented here also describe key characteristics of *K. gyiorum*, such as the secretion system, aerenesis, endotoxin formation and antiphagocytosis. These findings therefore provide initial insights for further research into *K. gyiorum*. However, due to the small number of isolates available, it is not yet possible to say whether the genes belong to the core or accessory genome of the whole species. Nonetheless, since the genes *flaA, cheD* and *cheR* were not detected in all strains, it can be assumed that they are part of the accessory genome.

WS15.04

Seroincidence of RSV, Influenza A/B, and SARS-CoV-2 among health care workers in the 2024/2025 winter season: insights from a longitudinal seroepidemiological cohort study

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Question: The incidence of acute respiratory infections (ARIs) requires particular attention in healthcare settings where vulnerable patient groups and highly exposed employees come into contact. The contribution to nosocomial transmission chains and workforce absenteeism poses a significant threat. Therefore, investigating the seroincidence of ARIs is fundamental to better understanding their spread and impact.

Methods: The prospective ARIPro cohort study assessed the incidence of RSV, Influenza A/B, and SARS-CoV-2 infections among healthcare workers before (October 2024) and after (April 2025) the winter season 2024/25. Per participation per timepoint a serum blood sample combinded with a study questionnaire on ARI infections and vaccinations was collected. SERION ELISA classic Respiratory Syncytial Virus IgG was used to quantify the antibody levels against RSV, using whole virus lysate as target. Individuals vaccinated against RSV were excluded from the analysis due to interference of vaccine-induced antibodies with the immunoassay. The Influenza antibody levels were quantified using SERION ELISA classic Influenza A and B IgG, targeting the nucleo/matrix proteins of Influenza A and B. Roche Elecsys Anti-SARS-CoV-2 antibody test, targeting the nucleocapsid was used to quantify antibody levels against SARS-CoV-2. Seroconversion was defined as a 2-fold increase of the IgG levels.

Results: The study cohort consisted of 426 healthcare workers (HCWs) who participated in the ARIPro study in both October 2024 and April 2025. After the 2024/2025 winter season, 7.8% (33/425, 95% CI: 5.6%–10.7%) of the study population showed seroconversion to RSV. 7.8% (33/426, 95% CI: 5.5%–10.7%) of HCWs seroconverted to Influenza A and 12.7% (54/426, 95% CI: 9.8%–16.2%) to Influenza B. Data on SARS-CoV-2 incidence will be presented at the conference.

Conclusions: The assessment of seroincidence is a suitable method to uncover the epidemiology of acute respiratory viruses, regardless of symptomatic manifestation and screening deficiencies. In the present study, Influenza B appeared to be the predominant pathogen during the 2024/2025 winter season, surpassing Influenza A and RSV. This preeminence contrasts with national surveillance data from the Robert Koch Institute (RKI), which reported higher incidences for Influenza A than for Influenza B and RSV. One possible explanation is that Influenza B and RSV are commonly associated with milder symptoms in adults, which may lead to a higher rate of unrecognized infections and, consequently, underreporting in routine surveillance. These findings underline the importance of using seroepidemiology in understanding the dynamics of ARI occurrence.

WS15.05

False-positive galactomannan values in bronchoalveolar lavage fluid are significantly more common in critically ill patients after aspiration

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Introduction: Invasive pulmonary aspergillosis (IPA) is increasingly diagnosed in non-neutropenic ICU patients. In this population, galactomannan (GM) in bronchoalveolar lavage fluid (BALF) has become the most important microbiological parameter. However, there is increasing evidence that a considerable number of false-positive BALF GM tests lead to misdiagnosis and antifungal overtreatment.

Objective: To examine if aspiration is causing false-positive BALF GM tests.

Methods: All adult, non-surgical ICU patients who underwent a BAL with testing for GM (Platelia Aspergillus Ag Assay, BioRad), mycological culture, and *Aspergillus* PCR (AsperGenious, PathoNostics) from 11/2020 until 03/2025 were retrospectively included. Additional data were collected to classify the patients for IPA following the FUNDICU and the EORTC/MSGERC 2020 criteria, respectively. The likelihood of previous aspiration was assessed on the basis of 18 risk factors for aspiration (e.g. resuscitation or dysphagia).

Results: 356 BALF specimens from 180 ICU patients were included in this study. A positive BALF GM (ODI≥0.5) was determined in 69 (19.4%) BALF specimens of 51 (28.3%) patients. In this group, 21 (30.4%) BALF specimens of 17 (33.3%) patients were also positive for *Aspergillus* PCR and/or *Aspergillus* culture (true-positive GM group). The remaining 48 (69.6%) BALF specimens of 34 (66.7%) patients were GM-positive only (GM-only group). The frequency of patients with previous aspiration was significantly higher in the GM-only group (35/48; 72.9%) compared to the true-positive GM group (5/20; 25.0%, p<0.001).

Conclusions: Our results show that the majority of patients have only positive BALF GM values without positive *Aspergillus* culture or PCR. These patients also have a greatly increased aspiration rate, suggesting a link between aspiration and false-positive GM levels. A weighted aspiration score could be used to identify patients with likely false-positive GM results and reduce misdiagnosis of IPA and antifungal overtreatment.

WS15.06

Microbial dynamics and biofilm formation on bilioenteric catheters after liver transplantation

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Introduction: Following liver transplantation, the biliary tract may be colonized by gut microbiota due to surgical interventions. This colonization, especially on bilioenteric catheters, can result in infections such as cholangitis, often exacerbated by immunosuppressive treatments. Cholangitis can develop to life-threatening conditions like sepsis or organ failure. In catheter-associated biofilms gram-negative *Enterobacteriaceae* and gram-positive *Enterococcaceae* are the most prevalent families. Despite being less abundant, *Enterococcaceae* are isolated at similar frequencies from cholangitis patients, indicating potential supportive interactions within polymicrobial biofilms. Diagnosing the catheter-associated microbiome may help identify patients at risk of infection, enabling earlier and more targeted interventions.

Objectives: This study establishes a newly *in vitro* model simulating catheter-associated biofilm formation. It was used to investigate inter- and intraspecies interaction and spatial and temporal biofilm dynamics on bilioenteric catheters. Furthermore, these investigations aimed to examine the influence of intrinsic (genetic resistance) and extrinsic (bile acids, culturing conditions) factors.

Materials and Methods: Representative clinical isolates from bilioenteric catheters were used in both planktonic and biofilm cultures in an established *in vitro* model. Microscopy, colony-forming unit (CFU) counts, viability staining, and 16S rRNA sequencing were employed to assess microbial composition and biofilm structure. Bioinformatics was applied to analyse genetic characteristics relevant to microbial interactions and antibiotic resistance.

Results: Biofilm formation was observed on the internal catheter surface, suggesting it offers a protective niche. Dual-species biofilms showed more complex extracellular matrices than single-species biofilms. *Enterococcaceae* were five times more prevalent in biofilms, although *Klebsiella pneumoniae* remained dominant in mixed communities. Bile acids and incubation conditions altered community dynamics, with contact-dependent interactions likely playing a major role.

Summary: This study reveals that catheter-associated polymicrobial biofilms are shaped by both microbial and environmental factors. *Enterococcaceae* benefit from interactions with *Enterobacteriaceae* in maintaining presence within biofilms. The *in vitro* model effectively replicates key features of clinical microbiomes, making it suitable for future research. Incorporating microbiome diagnostics into post-transplant care may improve infection control and patient outcomes by enabling more precise, timely treatments.

WS16.01
MCOLN1 is essential for Coxiella burnetii egress via lysosomal exocytosis

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Intracellular bacteria evolved mechanisms to invade host cells, establish an intracellular niche, which allows intracellular replication, and exit the host cell after completion of the replication cycle to infect new target cells. *Coxiella burnetii* (*C. burnetii*) is a Gram-negative, obligate intracellular pathogen and the causative agent of the zoonotic disease Q fever. Resident alveolar macrophages are the first target cells, but *C. burnetii* spreads to other organs and cell types. While we have information about *C. burnetii* uptake and the maturation process of the phagolysosomal-like *C. burnetii*-containing vacuole (CCV), it is not well studied how *C. burnetii* exits its host cell to spread to other target cells. In previous work, we showed that egress partially depend on induction of host cell apoptosis.

Here, we show that an infection with *C. burnetii* triggers the activation of the transcription factor EB (TFEB), a master regulator of the autophagy and lysosomal development. The activation occurs in a time-dependent manner and depends on the size of the CCV. Importantly, TFEB activation during *C. burnetii* infection did not depend on inhibition of mTORC1, but instead on MCOLN1, which channels Ca^{2+} across the lysosomal membrane into the cell cytosol. Knock-down of MCOLN1 resulted in reduced TFEB activation, smaller CCVs and most importantly, reduced bacterial egress. This suggest that Ca^{2+} release from the CCV might induces lysosomal exocytosis. Indeed, we could show that the pH of the CCVs in proximity to the plasma membrane is elevated, which is a prerequisite for lysosomal exocytosis. These peripheral CCVs are positive for LAMP1 and release viable and infective bacteria, without inducing host cell death. The released bacteria are not covered by a membrane. Together, these data support our hypothesis that lysosomal exocytosis is involved in *C. burnetii* egress. In line with this assumption, LAMP1 and *C. burnetii* was stainable in non-permeabilized cells at sides of bacterial release, demonstrating the fusion of the lysosome with the plasma membrane. Importantly, while replication of *C. burnetii* is not inhibited in cells lacking LAMP1/2, egress is impaired.

Taken together, our data demonstrate that with increasing CCV sizes, TFEB is activated by release of Ca^{2+} from lysosomes via the MCOLN1 channel, which in turn enables further development of CCVs and increased cytosolic Ca^{2+} level. This triggers lysosomal exocytosis and egress of *C. burnetii* without cell death induction. Thus, our data demonstrate that *C. burnetii* uses at least two pathways for egressing: induction of apoptosis and lysosomal exocytosis.

WS16.02
Host cell-dependent control of cellular egress in *Orientia tsutsugamushi*

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Background and Aims: *Orientia tsutsugamushi* (OT) buds from the plasma membrane (PM) via a virus-like, non-lytic egress. However, whether this process varies between cell types and which molecular factors regulate it has not yet been

investigated. Here, we employed microscopic and quantitative molecular tools to examine the dynamics of OT budding in different host cell types and explored the involvement of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery.

Materials and Methods: Murine L929 fibroblasts and human HuH7 hepatoma cells were infected with OT at low and high multiplicities of infection (MOI). Bacterial budding was visualized by transmission electron microscopy (TEM), budding bacteria were quantified by qPCR, and the ESCRT adaptor protein Alix was tracked by confocal microscopy.

Results: In both cell lines, exiting OT showed a close association with the PM and formed a rear-pole bud neck, confirming a conserved ultrastructure. Quantitatively, budding density measured from TEM images at 5 dpi was approximately 2-fold higher in L929 than in HuH7 cells. Genome counts in culture supernatants were similar up to 5 days post infection (dpi), then increased by ~1 log in L929 but decreased by ~1 log in HuH7.

Three distinct egress profiles emerged:

1. Fibroblasts, low MOI: OT replication increased by 2 logs, with a budding peak at day 11 (replication followed by egress).

2. Fibroblasts, high MOI: no replication, budding peak at day 7 (replication-static, replication-independent egress).

3. Hepatoma cells, any MOI: replication undetectable, budding reduced by 1 log after day 5 (host restriction of replication and bacterial egress).

Thus, egress can occur independently of active bacterial replication and can either be induced or suppressed during infection with OT.

To characterize the budding sites of OT, we stained infected cells with a probe for cholesterol-rich microdomains — the fluorescently labeled cholera toxin B (CTB) subunit — and observed a close association between the CTB signal and PM-associated OT. This finding aligns with previous reports suggesting that OT exploits cholesterol-rich microdomains during its egress (Kim et al., *Microbial Pathogenesis*, 2013). Despite a reduction in overall Alix protein levels, we observed that the ESCRT adaptor protein Alix was redistributed in infected HuH7 cells, shifting from a cytosolic distribution to focal clusters, while it remained unchanged in L929 cells.

Summary: OT was found in cholesterol-rich microdomains prior to its non-lytic exit but differed in its ability to functionally exploit this platform across host cell types: fibroblasts supported robust budding even in the absence of bacterial replication, whereas hepatoma cells restricted it. Redistribution of the ESCRT component Alix was identified as a cellular response to OT infection and emerges as a potential target to limit OT egress.

WS16.03

What stresses you most? Detecting stress responses of uropathogenic Escherichia coli in intracellular bacterial communities

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Introduction: Uropathogenic *Escherichia coli* (UPEC) account for the majority of urinary tract infections (UTI). Despite antibiotic treatment, recurrence of infection is frequently reported. Invasion of superficial bladder cells by UPEC and subsequent development of intracellular bacterial communities (IBCs) is one reason of recurrent UTI. 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 can contribute to recurrent UTI.

Goals: In this project our aim is to identify if and which stresses intracellular bacteria composed in IBCs have to cope with and may trigger or contribute to bacterial exit from the cells.

Materials & Methods: We use an *in vitro* bladder epithelial model employing RT-112 bladder epithelial cells. After infection of RT-112 cells with UPEC and subsequent IBC formation, the activation of bacterial stress response was followed using stress promoter gene fusions and confocal microscopy. The following stress promoter gene fusions coupled to YFP were constructed: (1) P*gadA* to detect low pH stress, (2) P*dps* to detect multiple stresses like oxidative stress, general starvation response and long-term stationary viability, (3) P*crp* as a global regulator was utilized to observe stress related to glucose starvation and (4) P*recA* to detect DNA damage response. The fluorescence intensity of the stress reporter fusions was normalized to constitutively expressed CFP by P*ctx*.

Results: We studied the changes in stress response gene expression during the intracellular cycle of two different UPEC model isolates. In previous studies we observed differences in intracellular replication and exit of these two isolates. After infection of RT-112 cells and development of IBCs by UPEC we analyzed the stress reporter gene expression at 10h, 24h and 48h post infection. Our results show that the two UPEC isolates react differently during their intracellular cycle. We could detect significant differences in YFP expression between both isolates in at least one timepoint for every gene fusion. In addition we also observed differences in YFP expression of the same isolate depending on the timepoint of analysis.

Discussion: Our results show that the investigated UPEC isolates encountered different stresses during their intracellular life cycle. These results underline and complement previous results giving rise to different intracellular trafficking of the two varying UPEC isolates. Interestingly, the two model UPEC strains also differ in their intracellular replication capacity depending on the extracellular glucose availability. Furthermore, the exit behavior of the two model UPEC isolates can be differentially influenced by glucose concentrations. Taken together, our results strongly suggest different strategies used by different UPEC isolates to survive intracellularly and subsequently escape host cells.

WS16.04

E3 ubiquitin ligase LRSAM1 restricts intracellular Staphylococcus aureus survival

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Introduction: Intracellular invasion and persistence of *Staphylococcus aureus* serves as an effective strategy for the pathogen to evade the host immune response and antibiotic therapy that can result in chronic and relapsing infections, which are often refractory to therapy. Ubiquitination of bacterial surfaces by specific E3 ubiquitin ligases is a host cell strategy to target intracellular bacteria for degradation via selective autophagy. However, knowledge of the E3 ligases involved in bacterial ubiquitination is very limited, especially for Gram-positive bacteria such as *S. aureus*.

Method: Lung epithelial cells represent the first line of defence against infections of the respiratory tract. Therefore, we analysed A549 lung epithelial cells during *S. aureus* infection, with a particular focus on the role of the E3 ligase LRSAM1. In LRSAM1-deficient A549 cells we evaluated the host cell response to *S. aureus* infection compared to the one of wild-type control cells. We investigated the intracellular survival of *S. aureus* as well as bacterial surface ubiquitination, induction of selective autophagy and host cell death processes.

Results: By analysing LRSAM1-deficient cells, we observed a significant increase of intracellular bacteria, which was accompanied by an elevated host cell death mainly due to induction of apoptosis and necroptosis. In addition, LRSAM1 knockout led to a reduction in the ubiquitination of the bacterial surface. As consequence, elimination of the intracellular bacteria by selective autophagy was prevented, although the process of autophagy itself was strongly induced.

Conclusion: In conclusion, our results indicate a prominent role for LRSAM1 during *S. aureus* infection. LRSAM1-mediated ubiquitination of intracellular *S. aureus* plays a crucial role for its elimination via selective autophagy. Moreover, our results provide an opportunity to further investigate the specific substrates that are ubiquitinated on the bacterial surface. Understanding the molecular mechanisms underlying the role of LRSAM1 will help to develop new therapeutic strategies against host cell persistence of *S. aureus* or other intracellular pathogens.

WS16.05

Investigating the role of the mTORC-related kinase KIN during translational regulation in the *Plasmodium falciparum* blood stages

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Malaria remains a critical global health challenge, with over 247 million cases and 619,000 deaths annually. The disease results from infection with *Plasmodium* parasites, with *P. falciparum* being responsible for the most severe manifestations. During its complex life cycle, the parasite alternates between human and mosquito hosts, necessitating adaptation to vastly different environmental conditions and multiple stage transitions that depend on tight post-transcriptional regulation. In mammals, the mTORC pathway acts as a central regulatory mechanism that controls protein synthesis, cell growth, and stress responses. Although *P. falciparum* lacks key mTORC components like the mTORC1 and mTORC2 kinase complexes, it retains downstream elements such as the S6 kinase homolog PKB, the RNA polymerase III inhibitor Maf1, and the translational repressor DOZI, a putative 4E-BP. Moreover, it encodes homologs of upstream regulators, including PI3K, LanCL2 (7-Helix-1), and AMPK (KIN). AMPK, a cellular energy sensor in eukaryotes, modulates translation by inhibiting mTORC1 and promoting stress granule formation. Similarly, the plasmodial counterpart KIN senses nutrient availability during blood-stage replication and regulates parasite growth and survival. Here, we investigate the role of KIN in the *P. falciparum* blood stages using chemical approaches. In particular, we target its function as a translational regulator during asexual blood stage proliferation and gametocyte maturation up to gametogenesis by employing the AMPK/KIN inhibitor Dorsomorphin and the activator A-769662. Chemical inhibition of KIN impairs parasite replication by causing an arrest at the schizont stage. By contrast, KIN activation reduces merozoite production per schizont without otherwise perturbing blood-stage development, ultimately leading to reduced parasite proliferation. Interestingly, A-769662 treatment reduces the gametocyte conversion rate, whereas Dorsomorphin has no effect on this process. However, KIN inhibition by Dorsomorphin blocks gametocyte maturation at stage IV, underscoring KIN"s critical role in reaching mature stage V gametocytes. In addition to inhibiting gametocytogenesis, Dorsomorphin also impairs gametogenesis by reducing the ability of male gametocytes to exflagellate. Moreover, KIN inhibition disrupts the eIF2α-mediated stress response, emphasizing its involvement in cellular stress regulation. Collectively, our findings reveal a pivotal function for KIN in the translational control during both asexual development and sexual differentiation of the malaria parasite *P. falciparum*.

WS16.06

Unveiling the SNARE machinery of vesicle trafficking during the egress of *Plasmodium falciparum* gametocytes from red blood cells

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Malaria remains a major global health burden, caused by the protozoan parasite *Plasmodium falciparum*, which is transmitted to humans through the bite of infected *Anopheles* mosquitoes. A critical step in the parasite"s life cycle is the egress of mature gametocytes from infected red blood cells (RBCs), enabling their uptake by a mosquito during a blood meal. This egress follows an inside-out mechanism, where the parasitophorous vacuole membrane (PVM) ruptures before the RBC membrane (RBCM), a process facilitated by specialized parasite-derived vesicles. These vesicles include osmiophilic bodies (OBs), which contribute to PVM disruption, and *P. falciparum* egress vesicles (P-EVs) containing the perforin-like protein PPLP2, which mediates RBCM lysis. In a previous proximity-dependent BioID study, we identified 143 candidate gametocyte egress vesicle proteins (GEVPs), among which were several members of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) family—a group of proteins important for membrane fusion events in eukaryotic cells. Expanding on these findings, *in-silico* analyses were performed and 25 putative SNARE proteins in *P. falciparum* were identified, with transcriptional profiling revealing that most exhibit peak expression in gametocytes. In this study, we investigate the expression

patterns and subcellular localization of selected SNARE proteins in gametocytes using tagged transgenic lines including targeted knockdown (KD) system. Future work will employ the inducible knockout (KO) approaches to functionally dissect the roles of these SNAREs in egress and transmission. By elucidating the molecular machinery underlying this essential biological process, our research may contribute to the identification of novel strategies to disrupt malaria transmission.

WS17.01

UV-C vs. plasmid power: disarming antibiotic resistance at the genetic level

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Background: Mobile genetic elements carrying antibiotic resistance genes (ARGs) contribute to the spread of antimicrobial resistance in clinical environments. Ultraviolet-C (UV-C) light-emitting diodes (LEDs) are increasingly applied for surface disinfection, but their potential to compromise the structural and functional integrity of plasmid-encoded ARGs remains insufficiently characterized. Furthermore, it is unclear whether subinhibitory UV-C doses may promote horizontal gene transfer via transformation in clinically relevant bacteria. This study addresses both aspects by analyzing plasmid stability and transformation efficiency following UV-C LED exposure.

Methods: Two plasmids (pCR™-Blunt II-TOPO and pUC19) encoding ARGs were exposed to defined doses of UV-C LED light. Plasmid DNA integrity and conformation were assessed by DNA concentration measurements and gel electrophoresis. Gene functionality was evaluated through transformation assays using *Escherichia coli* DH5α. To determine whether UV-C induces natural competence, sublethal doses were tested in *Escherichia coli* and *Citrobacter freundii*, a species that typically lacks natural competence.

Results: UV-C irradiation caused dose-dependent DNA degradation and conformational changes in plasmids, with the TOPO plasmid (4655 bp) exhibiting greater susceptibility than pUC19 (2686 bp). Complete functional inactivation of the TOPO plasmid was achieved at 1,500 J/m², while pUC19 required higher doses for comparable reduction in transformation efficiency. Importantly, subinhibitory doses did not enhance transformation frequency in either bacterial species, indicating no competence-inducing effect under the applied experimental conditions.

Conclusion: UV-C LED irradiation effectively reduces the functionality of plasmid-encoded ARGs at sufficient doses without promoting natural competence. These findings support its use as an additional disinfection strategy to limit the spread of mobile ARGs in clinical settings. However, the required doses for complete inactivation exceed those typically applied for microbial disinfection, which may limit its practical implementation.

WS17.02

Cleaning of intensive care units with probiotics and its effect on the acquisition of Pseudomonas aeruginosa in blood cultures and tracheal aspirates – an interrupted time-series analysis (CLINO)

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Introduction: The hospital environment can play a crucial role in the transmission of pathogens that are able to cause healthcare-associated infections (HAIs). Probiotic cleaning products are an innovative and sustainable option for environmental cleaning. They may have the potential to improve existing cleaning strategies especially for pathogens that are highly persistent in the environment such as Pseudomonas aeruginosa (PAE). Therefore, we have replaced routine surface disinfection with routine probiotic cleaning.

Objectives: To assess the effect of probiotic cleaning on the acquisition of PAE in blood cultures and tracheal aspirates in comparison to conventional disinfection.

Materials & Methods: We conducted an interrupted time series analysis in participating intensive care units (ICUs) of our hospital. Regularly surface disinfection (1% incidine by Ecolab) used during baseline period (01/01/2018 – 31/12/2019) was replaced by a probiotic cleaning product (1% by HEIQ / Chrisal) during the intervention period (01/12/2022 – 30/11/2024). The probiotic cleaning product contained Bacillus subtilis (ATCC6051), Bacillus megaterium (ATCC14581), Bacillus licheniformis (ATCC12713), Bacillus pumilus (ATCC14884) und Bacillus amyloliquefaciens (DSL13563-0). The primary outcome was incidence of PAE in blood cultures and tracheal aspirates. Poisson regression and Cox proportional hazard regression models were applied. The trial has been registered at the German registry for clinical trials (DRKS00036014).

Results: In total, 13 ICUs with 14.323 patients during baseline and 15.198 patients during intervention period were included. Incidences of PAE in blood cultures and tracheal aspirates were 2.63 per 100 patients (95% CI 2.37 – 2.91) in the baseline and 1.68 per 100 patients (95% CI 1.48 – 1.90) in the intervention period. For the intervention period, hazard ratio of PAE in blood cultures and tracheal aspirates was 0.74 (95% CI 0.63 – 0.86, $p < 0.001$) compared with standard disinfection after adjusting for age, gender and comorbidities.

Summary: Probiotic cleaning significantly reduced the incidence of Pseudomonas aeruginosa in blood cultures and tracheal aspirates compared to conventional disinfection in a large ICU-based study. The findings suggest that probiotic cleaning may be an effective and sustainable add-on to improve environmental cleaning and prevent pathogen transmission in the hospital. Further research with multi-center cluster randomized trials are needed to confirm effectiveness and safety.

WS17.03

Impact of biofilm formation by vancomycin-resistant Enterococcus faecium (VREfm) on susceptibility to disinfectants used in clinical routine

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Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) poses major challenges to healthcare systems. In Germany, most VRE infections have been linked to the Multilocus Sequence Types (MLST) sequence types (ST) ST80, ST117 and the emerging ST1299. Studies show that biofilm formation increases biocide tolerance, therefore presumably disrupting hygiene measures. However, current testing protocols for disinfectants primarily employ planktonic bacteria. While *Enterococcus faecalis* (Efs) is well known for strong biofilm formation, it remains unclear whether *E. faecium* (Efm), especially VREfm of infected patients, exhibit similar behavior. Hence, in this study we assess the biofilm producing capacity of different VREfm and the efficacy of clinically used disinfectants on biofilms and their planktonic counterparts.

Methods: A total of 90 strains were employed: 82 clinical VREfm isolates, comprising 34 different MLST ST and 44 different core genome (cg) MLST Complex Types (CT), and eight control strains such as Efs DSM2570 and Vancomycin-susceptible *E. faecium* (VSE) DSM20477. After characterization of isolates regarding growth behavior and genetic resistance profile, biofilm formation was assessed by two different assays: the crystal violet (CV) assay via photometric measurement and the bead assay using colony forming units. To improve strain differentiation, the bead assay was implemented. Disparities in efficacy of disinfectants on biofilms compared to their planktonic counterparts were tested with 70 % ethanol, 2 % Gigasept®AF, Sterillium®, and 0.5 % Incidin®, as stated by the manufacturers.

Results: Growth analysis showed variability among VREfm isolates across and within sequence types. Using the CV assay, all tested Efm strains of different ST and CT were found to be weak biofilm producers compared to Efs. Furthermore, homogeneous low biofilm production was observed, despite substantial differences in planktonic growth among Efm strains. Application of the bead assay resulted in more heterogeneous and higher biofilm production among the isolates that correspond closer to their natural growth. Out of 84, 83 Efm isolates, including the most common ST (ST80, ST117, ST1299), showed a comparatively lower level of biofilm formation, apart from one ST822 strain with a similarly high level as Efs. All disinfectants tested, with given incubation times and concentrations, proved highly effective in hindering the growth of Efm in both planktonic and biofilm-bead cultures.

Conclusion: The overrepresentation of specific MLST ST VREfm subtypes in nosocomial infections remains unclear. Regarding its growth, the biofilm production capacity and the resistance to disinfectants, clinical VREfm are susceptible to elimination. Advantages for specific VREfm in hospital settings may therefore lie in other virulence factors or its tenacity in the absence of proper hygiene measures.

WS17.04

Theoretical framework for environmentally sustainable infection prevention and control in hospitals – results from expert interviews

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Question: Hospitals are being increasingly encouraged to reduce their impact on the environment. Infection prevention and control (IPC) and hygiene measures can contribute to reducing the length of stay in hospitals and avoiding subsequent interventions and are therefore intrinsically sustainable. However, they are also associated with high energy consumption, substantial use of disposable products, and the deployment of antimicrobial agents with ecotoxicological implications.

The aim of the study was to identify current practices that are performed under the pretext of IPC but are not recommended by IPC experts as well as guideline-concordant practices that may be adaptable under specific conditions.

Methods: As part of the project HOspitals hygiene Preventing Emissions (HOPE), we conducted problem-centered interviews with experts in IPC/hygiene and sustainability in healthcare. The interviews were recorded, transcribed and pseudonymized. We used the Grounded Theory to develop a theoretical framework. The project is funded by the Gemeinsamer Bundesausschuss Innovationsfond (grant number: 01VSF23019). Data protection and ethics advice was obtained (UMG 9/7/24).

Results: We interviewed 10 IPC experts and 10 experts in sustainability in healthcare. Based on the results, we developed a framework that presents the conditions, interventions/modifications, paths of action and objectives of the integration of IPC and sustainability as well as the interactions between and within these (figure 1).

The outer ring of the framework illustrates nine conditions that form the frame for the effective implementation of interventions and modifications aimed at enhancing environmental sustainability in IPC. These conditions were derived from the challenges identified in the interviews. The subsequent ring presents the clustered interventions and modifications proposed by the experts. This is followed in the next ring by the mechanism of action of the interventions or modifications. At the core of the framework lie the overarching goals towards which these efforts are directed. E.g., nine IPC experts reported that staff frequently lack the knowledge required to perform tasks in accordance with guidelines and rarely question the necessity or effectiveness of existing practices – therefore basic knowledge is a condition. An intervention might include IPC training and educational campaigns as suggested by nine IPC and one sustainability experts. These interventions function by debunking prevalent hygiene myths and prompting critical reflection on existing practices. The aim of these mechanisms is a reduction in non-indicated activities as stated by all IPC experts.

Conclusion: The framework highlights the complexity of modifying guideline-concordant and purported IPC practice to be more environmentally sustainable within hospital environments. Subsequent work will involve augmenting the framework with quantitative data to enhance its analytical robustness.

Fig. 1

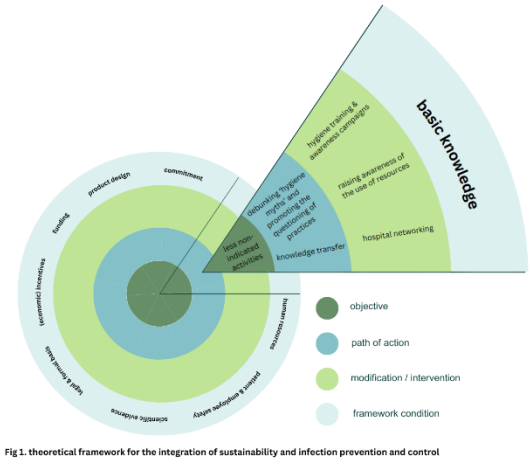

Fig 1. theoretical framework for the integration of sustainability and infection prevention and control

WS17.05
Resistenzen, Toleranzen oder was sonst? Verminderte Wirksamkeit von Desinfektionsmitteln bzw. ihren Wirkstoffen

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Die pandemische Ausbreitung von Antibiotikaresistenzen steht außer Frage. Wäre gegen Desinfektionsmittel und Antiseptika eine Toleranz- oder Resistenzentwicklung möglich, hätte das gravierende Folgen für die Infektionsprävention. Die Begriffe Toleranz und Resistenz sind in der Bewertung der klinischen Wirksamkeit von meist spezifisch wirksamen Antibiotika klar definiert. Auch wenn in letzter Zeit versucht wurde, neue Definitionen für eine verminderte Empfindlichkeit gegenüber Bioziden zu finden, empfiehlt es sich, die Begriffe Toleranz und Resistenz nicht im Kontext der Wirksamkeit von breit an mehreren Zielstrukturen aktiven Bioziden zu verwenden.

Da nach einer Desinfektionsmaßnahme möglichst keine relevanten replikationsfähigen Mikroorganismen wiedergefunden werden sollten, stellt die Replikationsfähigkeit nach einer korrekt erfolgten Desinfektion ein geeignetes Korrelat für die Wirksamkeitsbeurteilung dar. Wir schlagen deshalb als neuen Begriff die Replikationsfähigkeit nach Anwendungskonzentration (***r**eplication **c**apacity **a**fter **u**se **c**oncentration*, **RCAU**) als Beurteilungskriterium vor, wobei die Bestimmung einer RCAU jeweils nach der vom Hersteller eines Produkts definierten Einwirkzeit erfolgt. Die Bestimmung einer RCAU kann *in vitro* mit den bestehenden Verfahren der Überprüfung von Desinfektionsmitteln erfolgen. Insbesondere die Mikromethode eignet sich für erweiterte Bestimmungen der RCAU.

Wegen verschiedener intrinsischer Widerstandsfähigkeiten, physiologischer Zustände aber auch durch genetische Veränderungen können unterschiedliche Bewertungen einer RCAU bei Populationen von Mikroorganismen angenommen werden. Für die Versorgung von Patienten sind bei korrekter Anwendung nur jene Subpopulationen von Relevanz, welche auch nach der Desinfektionsmaßnahme eine Replikationsfähigkeit aufweisen.

Sofern Desinfektionsmittel auf Flächen und nicht im Kontext von Biofilmen angewendet werden, geht die Desinfektionsmittel-Kommission im VAH gegenwärtig davon aus, dass in der Desinfektionsmittel-Liste des VAH gelistete Produkte in den zertifizierten Anwendungskonzentrationen auch gegen antibiotikaresistente Mikroorganismen wirksam sind. Allerdings ist zu beachten, ob die intrinsische Widerstandsfähigkeit eines durch Desinfektion zu erfassenden vegetativen Erregers höher ist, als die der Prüforganismen zur Bestimmung der Bakterizidie/Levurozidie.

Wie bei der Ansiedlung von 4 MRGN in Abwasser-führenden Systemen in fortwährender Exposition gegenüber den unterschiedlichen Wirkstoffgruppen von Bioziden in verdünnter Konzentration, bieten Biolfilme Mikroorganismen gute Möglichkeiten zur Entwicklung einer erhöhten RCAU gegenüber Desinfektionsmitteln und Antiseptika. Das Auftreten mobiler genetischer Elemente, die mehrere Gene tragen, die für QAV- oder Antibiotikatoleranz kodieren und die Assoziation von QAV und 4 MRGN Clustern wirft die Frage auf, ob durch die weit verbreitete Verwendung von QAV das Entstehen von Antibiotikaresistenzen begünstigt wird.

WS18.01
Bacterial catabolism of phytate and inositol in the gut: activities and cross-feeding

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Phytate (or phytic acid, InsP6) is the main storage form of phosphorus and minerals in plants and is present in plant tissues such as bran and seeds of legumes and cereals, including oil seeds and nuts. Its dephosphorylated form, *myo*-inositol (*myo*-Ins), is a polyol that can be utilized by ~17% of all human gut species and 200 commensals as carbon and energy source. The specific phytase activities, the *myo*-Ins degradation capacities and the respective cross-feeding within the human gut microbiome, however, are largely unknown. To shed further light on the catabolism of phytate and *myo*-Ins by bacteria, we recently established a bioinformatic pipeline that allowed us to identify phytase genes and *myo*-Ins degradation genes in all validated bacterial genomes accessible by databases, indicating that 25% of all bacteria dephosphorylate phytate and 29% degrade *myo*-Ins, respectively. In a comparative One-Health culturomics study, we currently analyse swine feces as well as stool samples from participants of a nutrition study (NuEVA) that involves four diets (Western, flexitarian, vegetarian, and vegan diet) with varying but well-estimated phytate amounts. Indeed, a substantial amount of up to 70% of the gut bacteria, in particular aerobic commensals, appear to utilize *myo*-Ins, suggesting that a phytate-rich diet drives the microbiome towards bacteria that profit from phytate dephosphorylation and *myo*-Ins release. Phytase assays performed with gut isolates exhibited a high variance of activity, pointing to a pool of distinct phytases to be disclosed. Using supernatants of phytase-positive gut strains grown in the presence of phytate, we observed cross-feeding of *myo*-Ins from phytase producer to phytase-negative strains including the enteropathogen *Salmonella enterica*. To conclude, the data demonstrate that an unexpected broad spectrum of commensal bacteria potentially benefits from phytase activities by utilizing *myo*-Ins released by phytases as substrate for growth.

WS18.02

Glycolipid-protein and glycolipid-polysaccharide conjugates as potent targets for jump-start vaccination

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Introduction: The acronym *ESCAPE* (*Enterococcus faecium, Staphylococcus aureus, faecium*, *Clostridioides difficile, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacteriaceae*) summarizes the most frequently encountered pathogens in hospital-acquired bacterial infections. Since many of these nosocomial pathogens have already developed resistancies to commonly used front-line antibiotics, vaccination might be a promising tool to control the significant healthcare burden elicited by these bacterial pathogens. Since all of these bacteria colonize mucosal surfaces and can induce systemic infections, an immediate induction of protective immunity upon hospital admission is advantageous. Natural killer T (NKT) cells might be a promising cellular target in this context as they immediately release copious amounts of cytokines following engagement of their T cell receptor.

Goals: To "jump-start" immune responses independent of conventional T cell help, we combined protein or polysaccharide antigens of *ESCAPE* bacteria to alpha-GalactosylCeramide (a-GalCer), the prototypical glycolipid NKT cell agonist ligand.

Materials & Methods: We measured antibody responses to bacterial protein and polysaccharide antigens of ESCAPE bacteria. Moreover, we assessed the composition of the intestinal microbiota, *Clostridioides difficile* counts and intestinal inflammation using plating assays, qPCR, 16S rRNA sequencing and histopathological analyses.

Results: We detected IgA, IgM and IgG responses to protein or polysaccharide antigens of *ESCAPE* bacteria within a few days. Humoral antibody responses were long lasting, but not as persistent as observed with vaccines targeting conventional T cells. Polysaccharide-glycoconjugate vaccines induced glycan-specific antibodies in mice and substantially limited colitis and colonization with *Clostridioides difficile* after experimental infection. These anti-glycan antibodies selectively inhibited the colonization of the gut with *Clostridioides difficile* without disrupting the intestinal microbiota. The glycoconjugates induced long-term protection and were even superior to a toxin-targeting vaccine candidate in preventing *Clostridioides difficile*-mediated disease.

Summary: Glycolipid-protein conjugates "jump-start" mucosal and systemic humoral immune responses. Thus, these conjugates might provide immediately protective immunity, which is subject of current investigations in preclinical infection models. Glycoconjugate vaccines against *Clostridioides difficile* represent a complimentary approach to toxin-targeting strategies and are advancing through preclinical work. As anti-toxin immunoglobulins correlate with asymptomatic carriage, toxin-based vaccines may even expand the presence of *Clostridioides difficile* in the population. Vaccines targeting bacterial surface structures, in contrast, could limit the human reservoir due to the inhibition of intestinal colonization.

WS18.03

Competitive fitness of *Staphylococcus aureus* against nasal commensals depends on biosynthesis and acquisition of biotin

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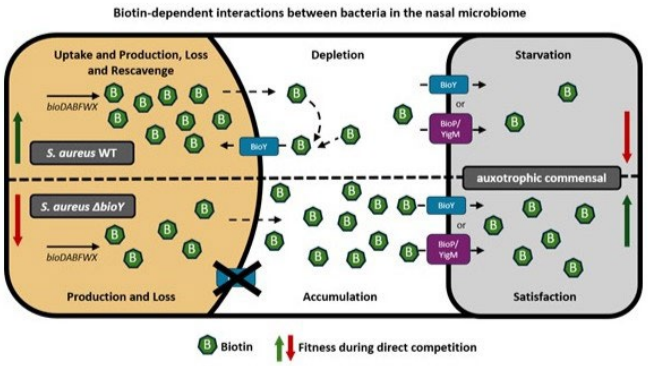
Question: *Staphylococcus aureus* is a major opportunistic pathogen and a frequent colonizer of the human nasal cavity, where it increases the risk of subsequent infections. Its presence is influenced by interactions with non-pathogenic members of the nasal microbiome, yet the molecular mechanisms underlying these interactions remain poorly understood. In this study, we investigate nutritional interactions between the pathogen and commensal species, with a specific focus on competition for the essential coenzyme biotin.

Methods: We conducted growth assays, co-culture experiments, biotin quantification, and genomic analysis of biotin biosynthesis and transport pathways across a range of nasal isolates, including *S. aureus*, coagulase-negative staphylococci (CoNS), and other representative commensals.

Results: We found that the nasal environment is biotin-limited and that *S. aureus* requires biotin for growth and membrane integrity. While *S. aureus* and some commensals are biotin prototrophs capable of releasing biotin into the environment, others—particularly CoNS—are auxotrophs and depend on external biotin sources. High-affinity biotin transport systems were present in both prototrophs and auxotrophs and conferred a competitive advantage in co-culture. In *S. aureus*, these systems serve a dual function: they support growth by minimizing biotin loss and simultaneously limit the availability of biotin to competing auxotrophic species.

Conclusions: Our findings indicate that biotin availability contributes to microbial interactions within the nasal microbiome. Nutrient-based competition and dependency may influence the colonization and persistence of *S. aureus*, highlighting a potential mechanism by which the microbiome modulates pathogen carriage.

Fig. 1



WS18.04

Suppression of multidrug-resistant *Escherichia coli* clinical isolates via cooperative niche exclusion

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The fight against multi-drug resistant Enterobacterales (MDR-E) has been designated a high priority by the World Health Organization (WHO). Colonization of the human gut by MDR-E, including MDR E. coli, is associated with an increased risk of infection and subsequent dissemination within the community. Various experimental interventions have been studied to promote the decolonization of the gut from MDR-E, such as antibiotic treatment or fecal microbiota transplantation (FMT). However, both approaches may have negative effects on the gut microbiota, including diarrhea and loss of colonization resistance. Conversely, the potential of probiotics to selectively decolonize the microbiota of carriers of MDR strains presents a promising alternative, particularly if these interventions achieve their goals without negatively impacting health-promoting commensals. Previous studies have shown that closely related commensal Enterobacterales can compete within the murine gut, leading to the displacement of less competitive species from the ecosystem. Based on individual differences in the capacity to spontaneously displace MDR-E from the gut, we hypothesize that the human microbiome serves as a significant reservoir for such probiotics, which have the potential to selectively decolonize MDR-E.

As a novel resource for identifying potentially probiotic bacteria, we generated a strain collection of Enterobacterales from 630 donors across four cohorts consisting of individuals from various age groups and nationalities. Because it is crucial to screen as many strains as possible due to the high genetic diversity of bacterial isolates, we established an ex vivo assay to identify strains with protective properties. In this study, we evaluated the strain-specific potential of 430 commensal Escherichia coli isolates to inhibit the growth of a multidrug-resistant (MDR) E. coli strain. Comparative analyses in in vitro, ex vivo, and mouse models revealed that only a subset of commensal strains could facilitate gut decolonization. Bioinformatic and experimental analyses of the antagonism for representative strains demonstrated how direct and indirect carbohydrate competition contributes to niche exclusion among E. coli strains. Finally, the combination of a protective E. coli strain with a Klebsiella oxytoca strain enhanced the gut decolonization potential against metabolically diverse MDR E. coli strains and additional MDR-E species, illustrating that rational, metabolically complementary design is essential for developing next-generation probiotics with broad-spectrum activity. Currently, we are evaluating the safety profile of the strains that have shown significant decolonization potential through bioinformatics, as well as phenotypic and functional analyses.

WS18.05
Spatial analysis of microbial communities
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The human body is a complex ecosystem harbouring billions of microbes. Sequencing-based approaches are commonly used to characterize microbiome composition at a global level. However, such approaches generally lack spatial resolution and cannot capture the fine-scale organization of microbial communities within host-associated microenvironments. To overcome this limitation, we adapted the CLASI-FISH (combinatorial labelling and spectral imaging fluorescence in situ hybridization) technique—originally developed for bacterial consortia—to enable simultaneous visualization of bacterial and fungal species within complex microbial communities.

Applying this method to the murine colon, we observed that bacteria were not randomly distributed throughout the lumen. Instead, they exhibited distinct spatial preferences, accumulating in proximity to the host epithelium and adhering to food particles. Probiotic *Lactobacillus* species frequently formed dense aggregates around digested food matter. Quantitative image analysis further revealed that *Lachnospira* spp. and *Bacteroides* spp. were preferentially found at short distances from the inner mucus layer and from food debris. These findings suggest that the gut microbiota in mice follows specific spatial organizational principles, rather than being randomly dispersed.

The combined use of multiplexed fluorescence imaging and spatial analysis offers a powerful approach to study host-associated microbial structure *in situ*. Building on these findings, we aim to apply this methodology to human duodenal biopsies from patients with celiac disease (CeD), in order to explore potential disease-associated patterns in microbial spatial organization and interkingdom interactions at the epithelial interface.

WS19.01
Influenza A Virus infection dynamics altered by TERC-mediated host signaling in lung tissue
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Alveolar type 2 (AT2) cells are essential progenitor cells responsible for maintaining pulmonary homeostasis and represent a key target for influenza A virus (IAV) replication. Dysregulation of AT2 cells contributes to inflammation and impaired tissue repair, particularly in the elderly, who experience increased severity and mortality from respiratory infections like IAV. However, the mechanisms driving age-related exacerbation of disease remain poorly understood. A hallmark of aging is the telomere shortening caused by the lack of telomerase activity. Telomerase consists of two compartments: the telomerase TERT and the RNA template TERC. Deletion of TERC results in dysfunctional telomerase activity leading to accelerated telomere shortening and premature aging in mice. Beyond its role as part of the telomerase complex, TERC, an lncRNA, also exerts regulatory functions in pathways such as inflammation and PI3K/Akt signaling.

This study aims to investigate the role of TERC, particularly its telomerase-independent function, in the disease manifestation during respiratory virus infection.

IAV infections were conducted *in vitro* using the human NCI-H441 cell line (an AT2-like cell line that produces surfactant) following transfection with TERC-specific siRNA, as well as in primary AT2 cells isolated from the lungs of TERC knockout

(TERC ko/ko) mice. Furthermore, a precision cut lung slice model (PCLS) was employed to study infection dynamics in lung tissue. IAV infection was also analyzed *in vivo* in TERC ko/ko and wild-type mice infected intranasally for 2 and 21 days. Viral loads were quantified via plaque assay, cytokine secretion was measured by flow cytometry, protein phosphorylation was assessed by western blot and phosphoproteomics. Global gene expression in infected AT2 cells was analyzed via mRNA sequencing.

Remarkably, TERC knockdown in NCI-H441 cells resulted in decreased viral replication and reduced inflammatory responses. This was associated with reduced activation of the PI3K/Akt signaling pathway, which is known to facilitate viral entry. Additionally, TERC depletion enhanced Hippo signaling and increased secretion of integrin beta-2 (ITGb2) and plasminogen activator inhibitor-1 (PAI-1), factors implicated in tissue repair mechanisms. These findings were corroborated in the PCLS model and *in vivo* experiments.

Our results reveal a novel role for TERC in modulating antiviral defense and lung repair during IAV infection, offering new insights into telomerase-independent functions of TERC in mice and human infection models.

WS19.02

How does *Coxiella burnetii* survive adverse intracellular conditions?

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Coxiella burnetii is an obligate intracellular zoonotic bacterium that causes Q fever. Infections can be either acute (mild flu-like illness, pneumonia or hepatitis) or chronic (endocarditis). Importantly, chronic Q fever develops months or years after primary infection without clinical symptoms, suggesting that *C. burnetii* persist within the host. Yet, information about the induction and/ or regulation of *C. burnetii* persistence is rare. We have shown that during infection of primary macrophages, citrate limitation results in inhibition of *C. burnetii* replication without affecting viability. Mechanistically, hypoxic conditions (0.5% O_2) lead to stabilization of HIF1α, which impairs STAT3 activity, resulting in reduction of the TCA cycle intermediate citrate. Of note, *C. burnetii* requires hypoxic conditions for axenic growth. Thus, lack of oxygen *per se* does not influence bacterial physiology, while hypoxia-induced limitation of citrate does. Here, we characterized *C. burnetii* under hypoxic conditions, to clarify how this bacterium survives this environmental stress condition. Thus, we infected primary murine macrophages with *C. burnetii* under normoxic (21% O_2) and hypoxic (0.5% O_2) conditions and analysed the gene expression profiles. Our data suggests that under hypoxic conditions *C. burnetii* does not undergo stringent response, but instead enters the SCV-like form, as the non-replicating persistent form. Further analysis by electron microscopy suggests that also morphological changes occur. Thus, the SCV-like persistent form is smaller in size and have dense chromatin material and a thicker cell wall. In addition, our data indicates that the SCV-like persistent form of *C. burnetii* is more infectious, more tolerant to antibiotics and less sensitive to clearance by IFNγ activated macrophages.

Taken together, hypoxic conditions induce citrate limitation. This nutritional stress induces a SCV-like persistent stage in *C. burnetii*, allowing the bacteria to survive this adverse condition. As the SCV-like persistent *C. burnetii* is more resistant against antibiotic treatment and clearance by activated macrophage, it hinders the elimination of the bacteria. Which in turn allows the pathogen to thrive once the conditions change in its favour.

WS19.03

Plasmid-specific phages inhibit the vertical and horizontal transmission of plasmids in *Escherichia coli* liquid cultures and biofilms

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Introduction: Bacterial conjugation plays a critical role in the dissemination of antimicrobial resistance (AMR), as resistance genes are frequently plasmid-encoded. Plasmid-specific phages exploit plasmid-encoded proteins—such as the conjugative transfer apparatus— to specifically recognize and destroy their bacterial hosts. Bacterial resistance to these phages often involves plasmid loss or downregulation of transfer apparatus expression, thereby limiting both vertical and horizontal plasmid transmission. Consequently, plasmid-specific phages may serve as effective tools to curb the spread of AMR genes via conjugation in key environments, including healthcare settings and livestock production systems.

Objectives: To develop and validate simple and robust methods for quantifying donor cells and transconjugants in complex bacterial populations under various culture conditions, enabling comparative evaluation of the capacity of different phage isolates to inhibit the transmission of conjugative plasmids.

Methods: We used recombineering to construct different sets of fluorescently labelled *Escherichia coli* strains, as well as conjugative plasmids and used flow-cytometry, fluorescence microscopy and image analysis to quantify donor and transconjugant populations in complex bacterial populations in liquid culture conditions and biofilms.

Results: Our findings demonstrate that the selected set of test strains and plasmids provides a reliable platform for assessing the efficacy of diverse plasmid-specific phages in inhibiting both vertical and horizontal transmission of conjugative plasmids within complex bacterial populations across varying culture conditions.

Summary: Fluorescently labelled donor and recipient bacterial populations, along with fluorescently tagged conjugative plasmids, provide powerful tools for analysing plasmid dissemination. These tools enable precise assessment of plasmid transfer dynamics in both the absence and presence of plasmid-specific phages, using flow cytometry and microscopic image analysis. Our findings underscore the utility of plasmid-specific phages as targeted interventions to limit the spread of antimicrobial resistance in diverse bacterial communities.

WS19.04

Detection and isolation of *Francisella tularensis* using recombinant reporter fusion proteins

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Introduction**:** The highly pathogenic bacterium *Francisella tularensis (F. tul)* is the causative agent of tularemia in animals

and humans. Three subspecies are recognized: *F. tul tularensis* (type A), *F. tul holarctica* (type B) and *F. tul mediasiatica.* The exceptional infectivity and pathogenicity – especially of the type A strains – and the potential misuse as an agent of bioterrorism or biological warfare (CDC Tier 1 "select agent") necessitate accurate and rapid identification of *F. tul.* Furthermore, the cultural isolation of these and other fastidious *Francisella* spp. from complex matrices such as ticks – one of primary transmission vectors of tularemia – remains a challenge. To address these challenges, we successfully developed a *F. tul* - specific single-chain variable fragment antibody (scFv)-reporter fusion protein (RFP) for PCR-independent, rapid and specific detection and enrichment of *F. tul*.

Methods: The RFP genetic fusion was assembled from synthetic DNA fragments encoding (i) a fluorescent protein and (ii) a *F. tul*-specific scFv. These fragments were fused by Gibson assembly and cloned into the expression plasmid pASG-IBA105 harboring a N-terminal Twin-Strep-Tag-epitope. The resulting RFP was heterologously produced in *Escherichia coli* and purified by affinity chromatography. To confirm specificity, binding of the RFP to a range of *Francisella* spp. and other bacteria was assessed by fluorescence microscopy. Direct detection of *F. tul* by fluorescence microscopy was also tested in blood, sputum and lymph node tissue. For the isolation of *F. tul* cells from complex matrices, the RFP was sequestered onto magnetic beads via the Twin-Strep-Tag epitope. These RFP-loaded magnetic beads were utilized to separate *F. tul* cells from buffered lysed tick material. These ticks were collected by dragging over low vegetation in the Nymphenburg Park in Munich. Pooled ticks were lysed by physical crushing and pre-tested for the presence of *F. tul* by PCR. PCR-positive tick pools were subjected to enrichment by magnetic separation. The enriched *F. tul* cells were plated onto agar plates and colonies could be obtained after incubation for three days at 37°C.

Results: All *F. tul* subspecies were successfully recognized by the recombinant RFP; more distantly related bacteria were not. The only non-*F. tul* bacteria bound by the RFP were *F. hispaniensis* and *Y. pestis*. Direct detection of *F. tul* cells by fluorescence microscopy in clinical samples from tularemia patients was successful. Overall, this microscopy-based detection method is sensitive, requires few steps and can be performed rapidly. Finally, utilizing RFP-loaded magnetic beads, we successfully isolated *F. tul* from collected wild ticks. The isolated strains were identified as *F. tul holarctica* by whole genome sequencing and a newly developed mismatch amplification assay based on specific single nucleotide polymorphisms (SNPs) to distinguish different *F. tul* subspecies isolates from one another.

WS19.05

CoxBase goes Wiki – how to create sustainability for genomic Q fever data

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Q fever is a zoonosis that occurs worldwide and is caused by the Gram-negative bacterium Coxiella burnetii. While it primarily affects livestock (like sheep, gotas and cattle) and other animals, it can also be transmitted to humans. Infections, and sometimes outbreaks, occur regularly in Germany. This is because the pathogen is found in large quantities in the faeces of infected animals and their ticks. In addition, a very low infectious dose can easily lead to human infection through inhaling aerosols from these materials. Furthermore, C. burnetii has high environmental stability, meaning that the animal materials and faeces of ticks can cause infection as pathogen-containing dust even after months of drying in the environment. In recent years, extensive genomic studies have made it possible to develop appropriate surveillance for the aforementioned animals. This makes it easier to identify and trace outbreaks. Following the creation of a central database by our working group (CoxBase; coxbase.q-gaps.de), an online platform for the epidemiological surveillance, visualisation, analysis and typing of Coxiella burnetii genomic sequences, as well as for tracking, further developments have been achieved that improve data availability for relevant users. As part of the "Wikipedia-Ressource", Wikibase is a versatile, open-source software platform developed by the Wikimedia Foundation. It provides the infrastructure and tools needed to create, manage, modify and query data. The best-known instance is Wikidata (https://www.wikidata.org/), which hosts more than one billion items and is maintained by a large global community of contributors. Wikibase offers a user-friendly web interface for entering primary structured data and can be efficiently queried via SPARQL (SPARQL Protocol and RDF Query Language) and connected to other knowledge graphs.. This new resource offers all the benefits of Wikibase, including an easy way to curate data collaboratively, query it using a powerful language, and connect it with other resources. We hope that this special open-access variant of genomic typing data on Coxiella burnetii will facilitate the work of interested research groups and users at the interface of genomic surveillance and public health, enabling them to conduct their own investigations, studies, analyses and interpretations of Q fever genomic surveillance data in future. We are confident that this application can also be transferred to data sets of other infectious agents, representing a new, simple, cost-effective and sustainable form of data availability for research, diagnostics and clinical use.

WS20.01

Machine learning for the prediction of antimicrobial resistance with MALDI-TOF mass spectrometry data

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Question: Rapid results from antimicrobial susceptibility testing (AST) are essential to guide the antimicrobial therapy of critically ill patients. However, AST results are usually only available 18-24 hours after species identification. Species

identification is carried out in most laboratories with MALDI-TOF mass spectrometry. These mass spectrometric data can also be used to predict antibiotic resistance (Weis et al. Nat Med. 2022). We wanted to know if we can reproduce results reported by Weis et al. and how the ML-algorithms perform when own data is used for training.

Methods: We used publicly available data (DRIAMS-A, Switzerland, 2015-2018) and own data from routine diagnostics (Germany, 2023-2024). After preprocessing mass spectrometry profiles (intensity transformation, smoothing, baseline removal, total ion count normalization, trimming) and binning into 3 m/z, we obtained 6000 features of continuous variables for each isolate. Isolates were binary labelled with AST results (resistant vs. susceptible/susceptible increased exposure, EUCAST V13 and V14).

Using nested cross-validation, we trained various classification models including regularized logistic regressions (glmnet), multilayer perceptrons (MLP), random forests, and gradient boosting machines (LightGBM, XGBoost). Grouped resampling was employed to address the clustered data structure caused by patients contributing several samples, while stratification and weighting were used to adjust for class imbalances. Antimicrobial resistance was predicted for *Escherichia coli* (3rd gen. cephalosporins), *Klebsiella pneumoniae* (3rd gen. cephalosporins) and *Staphylococcus aureus* (oxacillin). Predictive performance on the respective test data was calculated as the mean area under the receiver operating characteristic curve (AUROC) for each model.

Results: We were able to reproduce results of Weis et al. by training ML-models on their data, which resulted in similar AUROCs for the various species-antimicrobial agent-combinations. Predictive performance (AUROC) was best for ceftriaxone-resistance in *E. coli* using LightGBM (0.75), ceftriaxone-resistance in *K. pneumoniae* using XGBoost (0.75) and oxacillin-resistance in *S. aureus* using MLP (0.82)

When using our own mass spectra for model training (*E. coli* [n=7785], *K. pneumoniae* [n=2381], *S. aureus* [n=4564]) performance was even better. The predictive performance (AUROC) was best for cefotaxime-resistance in *E. coli* using LightGBM (0.80), cefotaxime-resistance in *K. pneumoniae* using XGBoost (0.75) and oxacillin-resistance in *S. aureus* using random forest (0.85).

Conclusion: The predictive performance of ML models for antimicrobial resistances based on MALDI-TOF data reported by Weis et al. is reproducible when trained on external datasets, suggesting a general use of the models. Whether the performance can be improved by adding further data (patient or environmental data) or by optimizing the processing of MALDI-TOF profiles still needs to be investigated.

WS20.02

The increase in 4MRGN *Escherichia coli* in a tertiary care hospital in northern Germany is caused by various clones with different OXA carbapenemases

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Objectives: The rapid global spread of carbapenemases poses a major public health threat due to limited treatment options and high mortality rates. In a tertiary care hospital in northern Germany a significant increase in carbapenemase-producing *E. coli* was observed. Understanding the local epidemiology is crucial for effective infection control and antimicrobial stewardship.

Methods: In total 74 clinical strains of *E. coli* harbouring a carbapenemase gene and have been isolated in 2018 to 2024 in a tertiary care hospital in northern Germany were characterized using whole genome sequencing. Clonality of the isolates (cgMLST and classical MLST) and their resistance genes were analyzed using seqsphere (Ridom, Münster, Germany). Additionally, the clinical isolates were compared isolates from several studies from the wastewater system of the same hospital.

Results: From 2018 to 2024 74 carbapenemase-producing *E. coli* were observed with 12, 14 and 26 isolates in 2022 to 2024, respectively. The isolates could be divided in 32 different MLST sequence types (STs) and six isolates with incomplete allele sets. The most frequent STs observed were ST38 (n=10), ST131 (n=8), ST167 (n=8), and ST69 (n=6). Close clonal relationship could only be observed in three pairs of isolates. Characterization of carbepenemase genes showed eleven different genes. Most prominent (n=42) were *bla*$_{OXA}$ genes (*bla*$_{OXA-244}$ [n=18], *bla*$_{OXA-48}$ [n=13], *bla*$_{OXA-181}$ [n=9 , *bla*$_{OXA-162}$ [n=1], *bla*$_{OXA-484}$ [n=1]) followed by *bla*$_{NDM}$ genes (*bla*$_{NDM-5}$ [n=15], *bla*$_{NDM-1}$ [n=5], *bla*$_{NDM-4}$ [n=1] , *bla*$_{NDM-19}$ [n=1]). Additionally, *bla*$_{KPC-2}$ (n=6) and *bla*$_{VIM-1}$ (n=6) were observed. Two isolates displayed two carbapenemase genes in parallel. The strong increase in 2024 was primarily caused by isolates carrying *bla*$_{OXA}$ genes (18/26; 69,2 %). The 18 *bla*$_{OXA}$ carrying isolates in 2024 could be separated in 11 different STs. Three STs observed more than once in 2024 could be further subdivided by the observation of different *bla*$_{OXA}$ genes. No genetic link to environmental isolates from the hospital wastewater system was observed.

Conclusions: The increase of carbapenemase-producing *E. coli* in a tertiary care hospital in northern Germany is primarily caused by highly divers clones and not by transmission events. The data show that different plasmids with *bla*$_{OXA}$ genes are spreading, and therefore a further continuous increase in carbapenemase-producing *E. coli* is to be anticipated. There was no evidence of the hospital wastewater system as a source for isolates detected in patients, suggesting that this transmission route, which is frequently described for other *Enterobacterales*, plays a minor role for *E. coli*.

WS20.03

Emergence of NDM-producing carbapenem-resistant Pseudomonas aeruginosa ST308 in Vietnam: results from an ICU admission screening study in Hanoi, Vietnam, 2023

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Objectives: Vietnam is among the countries most affected by antimicrobial resistance (AMR) in the Asia-Pacific. While multidrug-resistant (MDR) Enterobacterales have been extensively studied, genomic data on MDR *Pseudomonas aeruginosa* in Vietnam remains scarce. To address this, we characterized 20 carbapenem-resistant *P. aeruginosa* (CRPA) isolates from rectal colonization of ICU patients.

Methods: Screening for CRPA was conducted using a selective chromogenic medium (mSuperCARBA). Species identification was achieved through MALDI-TOF mass spectrometry, while antimicrobial susceptibility testing (AST) was performed using the Vitek®2 system and broth microdilution. Whole-genome sequencing (WGS) was performed using the Illumina NextSeq platform.

Results: Twenty CRPA isolates were collected from rectal swabs of 691 patients admitted to the ICUs of the 108 Military Central Hospital in Hanoi, Vietnam, between July 1, 2023, and October 31, 2023. The predominant multilocus sequence type (MLST) was ST308, accounting for 50% (10/20) of the isolates. Notably, 70% (14/20) of the CRPA isolates harboured genes encoding metallo-β-lactamases (MBL), with *bla*NDM-1 being the most prevalent (86%, 12/14), followed by *bla*IMP-26 (14%, 2/14). Comparison with published ST308 genomes suggest a distinct NDM+ ST308 clade emerging in Vietnam. Cefiderocol resistance was observed in 50% (10/20) of isolates. Colistin demonstrated the most favourable susceptibility profile, with 90% (18/20) of isolates remaining susceptible.

Conclusion: A significant proportion of CRPA isolates in our study were MBL producers, with high levels of resistance to novel β-lactams and β-lactam/β-lactamase inhibitor combinations. These findings underscore the urgent need for effective infection prevention and control strategies to mitigate the further spread of MBL-producing CRPA.

WS20.04

Patients, plasmids and the puzzle of ICU transmission – a genomic deep dive into Carbapenemase producing Enterobacterales

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Introduction: Whole Genome Sequencing (WGS) enables the detection of Carbapenemase producing Enterobacterales (CPE)-transmissions via temporally non-concurrent exposure[1]. These transmissions often go unnoticed by surveillance focusing on concurrent spatiotemporal connections. In our hospital, we noticed admission to the intensive care unit (ICU) was common among patients with hospital-acquired (ha) VIM-CPE. However, due to intensified screening in ICUs at our hospital, it was unclear if the ICUs were the sites of transmission or only reflect shared risk factors. Clarifying this association is critical for targeted infection prevention strategies.

Goal: We aimed to identify the most likely transmission route for patients with ha-VIM-CPE and ICU admission, focusing on potential transmissions on the ICU.

Methods: We included inpatient episodes from 2018 to 2021 with detection of *bla*$_{VIM}$ CPE. Ha-inpatient episodes were defined as VIM-CPE detection >2 days after admission and at least one prior negative screening. Using short- and long-read WGS, we determined genomic relationships on core-genome and plasmid level. The time at risk of acquisition (TARA) was defined as time between last negative and first positive CPE-screening. For ha-inpatient episodes with TARA overlapping any of our four ICUs, we analyzed patient movement data (wards and rooms) and procedures (e.g. surgery, endoscopy, indwelling devices) to identify the most likely transmission route.

Results: Among 43 inpatient episodes with *bla*$_{VIM}$ CPE, 22 were hospital-acquired. WGS revealed genomic relatedness among 17 ha-isolates: 16 were grouped into four clusters based on core-genome similarity, and one showed relatedness only at the plasmid level. Of the 17 WGS-related inpatient episodes, 14 patients had been treated in an ICU at some point, and 10 of these had an ICU stay during their TARA. Among these 10 patients, seven had a TARA overlapping with a stay on the surgical ICU. In five of these seven, the most likely transmission route was indirect exposure within this ICU via shared room or ward. Since the patients had no temporal overlap and no shared intervention or procedure was identified, we suspect transmission through environmental sources. For the three patients whose TARA overlapped with other ICUs, no likely transmission route could be identified.

Summary: While most patients with genomically related ha-VIM-CPEs had an ICU stay, the ICU was not necessarily the site of acquisition. However, we identified complex transmission clusters involving a surgical ICU, highlighting this ICU"s potential role in CPE transmission. Our findings demonstrate the value of combining WGS with detailed epidemiological and screening data to disentangle complex transmission dynamics. This integrated approach can help prioritize targeted infection control measures in high-risk areas.

1 Marimuthu K et al. Whole genome sequencing reveals hidden transmission of carbapenemase-producing Enterobacterales. Nat Commun. 2022.

WS20.05

Uncommon genomic rearrangements drive vancomycin resistance in *vanB*-positive *Enterococcus faecium*

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Introduction: The *vanB* operon is composed of the *vanY*, *vanW*, *vanH*, *vanB* and *vanX* resistance genes and regulated by the two-component regulatory system *vanR* and *vanS*. As part of routine diagnostic and surveillance studies, we have sequenced over 3,000 vancomycin-resistant *Enterococcus faecium* (VREfm) isolates on the MiSeq platform and noticed that some isolates lacked regulatory genes. We aimed to investigate the *vanB* expression of these isolates.

Material and Methods: Raw-reads were assembled using Velvet and the *vanB* cassette was detected using ARMFinderPLus (Ridom SeqSphere+). SPAdes and SKESA assemblies were used for confirmation. Vancomycin-

resistance was confirmed by disc diffusion and broth microdilution. Van-gene presence was tested by PCR. Long-read sequencing was performed on MinION followed by Flye or Unicycler assembly. Endogenous protein expression of VanB was detected with the aid of monoclonal antibody generated by hybridoma technology and used in dot blot ELISA.

Results: We investigated the *vanB* cassette of >1700 isolates collected between 2013-2023. 12 isolates were missing regulatory genes or the ligase; 11 lacked *vanR* and *vanS*, while one isolate lacked *vanB*, confirmed also by different assembly algorithms. Susceptibility testing revealed that 10 of the 12 isolates were vancomycin susceptible, linked with the absence of the regulatory genes or the ligase. However, two ST117/CT71 isolates were vancomycin-resistant, despite lacking *vanR* or *vanS*. Expression of the *vanB* cassette was confirmed by dot blot ELISA in the absence and presence of vancomycin. Long-read sequencing identified in one isolate up to 3 copies of the *vanB* gene cluster (*vanY*, *vanW*, *vanB* and *vanX*) bracketed by IS*Enfa3*. In the second isolate, phage genes were detected upstream of the *vanB* gene cluster (*vanY*, *vanW*, *vanH*, *vanB* and *vanX*). In both cases, alternative promoters could regulate the expression of the VanB cluster leading to the observed resistant phenotype.

Conclusion: Retrospective analysis identified genotypically susceptible isolates lacking the response-regulator and sensor-kinase, but were phenotypically vancomycin-resistant, mediated through alternative mechanisms of gene expression due to uncommon genomic rearrangements. Our findings highlight the diversity of vancomycin resistance mechanisms in *E. faecium* and underscore the need for further investigation.

WS20.06

Isolation of hospital-associated vancomycin resistant *Enterococcus faecium* clones from German rivers

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Introduction: Previous studies have revealed high clonality of clinical vancomycin resistant *Enterococcus faecium* (VREf) isolated from different German hospitals, with ST117/CT71 in particular showing a remarkable clonality between sites [1,2].

Objectives: The GAP project was initiated to investigate VREf isolated from German rivers and wastewater treatment plants in areas close to Hamburg, Lübeck, Freiburg, and Cologne/Bonn, and compare them to our previously sequenced clinical isolates.

Methods: Environmental isolates (n=203, 73 from rivers isolated 2016-2024, and 130 from wastewater influent/effluent isolated 2016-2018), were plated onto appropriate media. Confirmed VREf were sequenced (MiSeq) and compared by cgMLST (SeqSphere) to previously sequenced VREf [1,2]. The rivers were not connected to wastewater treatment plants that received hospital wastewater.

Results: The majority of environmental VREf were *vanB*-positive (n=168) and the remaining were *vanA*-positive (n=35). MLST analysis revealed 124 ST117, and 60 ST80. Nine isolates (collected 2023-2024) were ST1299, an ST recently described as dominant in Bavaria. Five isolates were ST323, two were ST2176, and one each ST192, ST375, and ST1478. cgMLST analysis using a threshold of 20 alleles reveals clustering of river and wastewater isolates from multiple sites, and some identical isolates. Coincidentally, 117 of the ST117 were the epidemic CT71.

Using the same 20 allele threshold, 1300 clinical VREf cluster with the environmental isolates. With a lower threshold of ≤3 alleles, 82 clinical VREf clustered with the environmental VREf. Interestingly some clinical isolates were identical to environmental isolates despite them being isolated in different regions (Figure).

Summary: These data show that clinically relevant VREf strains can be isolated not only from wastewater, but also from rivers which were not connected to a hospital upstream of the collection point, suggesting that the isolates are present in the community. Furthermore, the VREf appear to persist over the 8-year period that samples were collected, particularly the epidemic CT71 strain. Therefore, VREf can be considered a One-Health problem with a reach beyond the clinics. Further work is necessary to understand how the isolates are cycled back into the community and how to break this cycle.

[1] Biehl L. et al. Euro Surveill. 2022 Jan;27(2):2001876.

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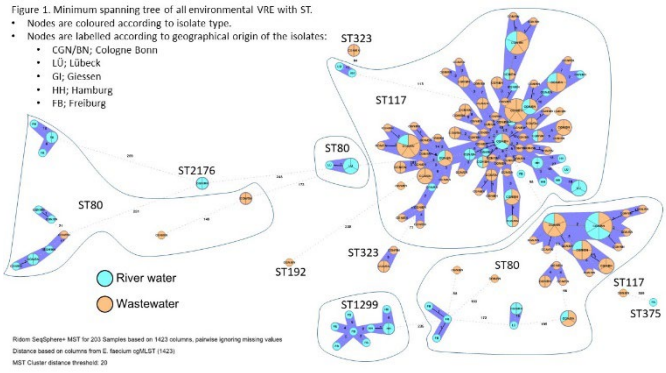
Fig. 1



WS21.01

Early diagnosis of Mucormycosis using PCR from serum and plasma

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Background: Infections caused by mucorales can be fatal in immunosuppressed patients. However, diagnosis is challenging and often delayed because no blood biomarkers exist and detection usually requires invasive samples. Recent studies describe PCR-based methods for detecting mucorales DNA in blood, which could enable early and non-invasive diagnosis (1). The aim of this retrospective study is to investigate the value of PCR detection from serum or plasma for diagnostic purposes.

Methods: Retrospectively, 42 haemato-oncological and immunosuppressed patients with proven or probable mucormycosis were identified over a period of ten years. Serum and plasma samples taken before and after the initial diagnosis (day 0) were analysed using a mucorales-specific multiplex real-time PCR (2). In addition, a comparison was drawn between the initial detection by culture, PCR from samples other than blood, and blankophor staining.

Results: At the time of submission, preliminary results from 20 patients are available. In 12 out of 20 patients, mucorales DNA was detected by PCR from serum or plasma between 1 and 39 days prior to the initial diagnosis (day 0). Only 8 out of 20 patients had positive culture results.

Conclusions: The preliminary data suggest that detection of mucorales DNA in peripheral blood by PCR allows earlier diagnosis of mucormycosis. This could support screening of high-risk patients and thus enable earlier therapy.

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WS21.02

Metagenomic pathogen detection as an adjunct multi-line diagnostic tool

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Question: As an early adopter of a standardized, metagenomic next-generation sequencing (mNGS) diagnostic tool, the University Medical-Center Mainz has analyzed over 2,000 patient samples using DISQVER® (CE-IVD, Noscendo GmbH, Germany). As the enhanced sensitivity of mNGS has previously been explored, we aimed to investigate the diagnostic performance and relevance of mNGS pathogen detection, as compared to standard of-care (SOC) blood culture (BC) across different medical departments. Specifically, we investigated a subset of more than 1,200 patient samples, which were analyzed by both, mNGS and SOC blood culture within a time frame of +/- 7 days.

Methods: Blood samples for mNGS diagnostics were collected as part of routine clinical protocols alongside SOC diagnostics. Diagnostic performance was compared on a pathogen-specific level for each sample. Pathogens were grouped into distinct tiers based on clinical relevance and microbiological features.

Results: A total of 2,030 blood samples, from five different clinical departments, were analyzed using the commercially available mNGS test DISQVER®. Of these, 1,249 samples were taken within a time frame of +/- 7 days of a corresponding blood culture, and qualified for further investigation. A notable development of mNGS usage towards a second-line diagnostics was observed throughout all clinical departments. Furthermore, in the hematology department, mNGS was also employed as a first-line diagnostic tool in well-defined clinical indications. Focusing on bacterial results, mNGS yielded a 34.4% positivity compared to 24. 6% in blood culture. While 130 findings of 23 different species were detected both by mNGS and BC, NGS provided 632 additional findings of 187 different species, of which 17% are usually not cultivable in blood culture. While 235 findings of 51 species were detected by BC only, more than 60% of those were attributed to possible contaminants.

The increased use of mNGS as a second-line diagnostic tool after SOC BC highlights its value in complex infectious disease cases where SOC diagnostics often fall short.

Conclusions: Our findings demonstrate a high diagnostic relevance of mNGS as an adjunct to standard-of-care infectious disease diagnostics. mNGS is a well-regarded and established method, particularly valuable for complex infection cases, serving as an effective mutliline-diagnostic tool in complementing the diagnostic framework.

Routine mNGS testing becomes both practical and scalable, by performing in-house sample preparation, fostering closer microbiological oversight and delivering timely, context-specific interpretations for patient treatment.

WS21.03

FISHseq for molecular diagnosis of *Bartonella* endocarditis

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Background: *Bartonella* are causative agents of blood culture-negative infective endocarditis (BCNIE), yet their diagnosis remains challenging, particularly with conventional diagnostic techniques. Fluorescence in situ hybridization combined with 16S rRNA gene sequencing (FISHseq) offers promising potential not only for identifying the *Bartonella* species but also for providing insights into its activity, in situ localization, and spatial organization within infected tissues. This diagnostic approach may overcome diagnostic challenges associated with BCNIE and improve detection of the fastidious, Gram-negative bacteria.

Methods: Between January 2017 and May 2025, native heart valve and cardiovascular prostheses samples from a total of 1.920 patients with suspected or confirmed infective endocarditis were analyzed using FISHseq. In addition to an already validated *B. quintana*-specific FISH probe, we optimized and validated a *Bartonella*-specific probe excluding *B. quintana* for use in FISH.

Results: By FISHseq, *Bartonella* endocarditis was diagnosed in 30 patients with BCNIE, with *B. quintana* identified in 23 cases and *B. henselae* in 7. In vivo-grown biofilms were observed in all 23 patients with *B. quintana* endocarditis and in 5 out of 7 patients with *B. henselae* endocarditis. Additionally, a *Bartonella*-specific FISH probe (excluding *B. quintana*) successfully visualized *B. henselae* in all 7 cases, enhancing diagnostic precision.

Conclusions: FISHseq proved crucial for detecting *Bartonella* in all IE cases. It is an effective tool for diagnosing *Bartonella* endocarditis. Increasing awareness of *Bartonella* as a potential cause of BCNIE and the implementation of appropriate diagnostic workflows are essential. Further research is needed to determine optimal treatment strategies, particularly given the therapeutic challenges posed by biofilm formation. *B. henselae* endocarditis should be considered in individuals at risk for IE and in regular contact with cats.

WS21.04
Diagnosis of severe fungal infections using next-generation sequencing of cell-free DNA – a single center experience

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Background and aims: Systemic fungal infections remain a clinical challenge with high rates of mortality especially in immunocompromised patients. Early treatment is crucial, but diagnosis is often delayed, due to low sensitivity rates and long turnaround times of established diagnostic tests. In microbiology, Next Generation Sequencing (NGS) could complement the conventional characterization of pathogens based on morphology, staining properties and metabolic criteria with a genomic definition of pathogens. In clinical practice NGS has enormous potential, but is currently mainly used in research. We describe our experience using this method for the diagnosis of fungal pathogens in patients with severe infections.

Methods: Clinical data of all fungal infections diagnosed by the commercially available DISQVER® test (Noscendo GmbH, Duisburg, Germany) were recorded from patients between 2021 and 2023. Briefly, for the test plasma is generated from whole blood and cfDNA is isolated using the QIAsymphony instrument. NGS libraries are prepared and subjected to 75 bp SR sequencing on an Illumina NextSeq instrument. The sequenced reads are uploaded into the cloud and after quality checks run through the DISQVER pipeline, which removes human DNA sequences, classifies non-human sequences to microbial taxonomies and assesses the relevance of the identified species by comparison to control cohorts and run controls.

Results: In 18 hospitalized patients with severe infections, relevant fungal pathogens were detected. Two patients were excluded from further analyses due to incomplete data. Median age was 65 years, 44% (7/16) were female and all patients were immunocompromised. Common causes for immunosuppression were hematological diseases, solid organ transplant, and liver cirrhosis. Detected fungal species were *Aspergillus fumigatus* (n=10), *Rhizopus/Rhizomucor* species (n=4), *Histoplasma capsulatum* (n=1), *Pneumocystis jirovecii* (n=1), *Trichosporon asahii* (n=1) and *Malassezia globosa* (n=1). In all cases, the diagnosis was consistent with the clinical presentations and the mortality was high with 56% (9/16). The focus was pulmonary in most cases (n=10, 63%). In three cases (19%), no focus was identified. In ten patients (63%), the diagnosis of fungal infection was first or only established by NGS.

Discussion: While this data represents no head-to-head comparison between conventional testing and NGS of cfDNA, this method provides a promising tool for challenging pathogens such as fungi, especially in severely or critically ill patients and those with immunosuppression. The high mortality rate underlines the need for improvement of diagnostic and treatment strategies, and future clinical trials are needed to assess the impact of such molecular diagnostic methods on clinical outcome.

Summary: In this case series, next-generation sequencing of cell-free DNA facilitated diagnosis of invasive fungal infections in immunocompromised patients.

WS21.05

Rapid and user-friendly discrimination of highly virulent *Streptococcus agalactiae* clonal complexes by MALDI-TOF MS

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Background: Streptococcus *agalactiae* (Group B streptococci; GBS) is the most common causative agent of neonatal bacterial meningitidis.These infections are burdened by high morbidity, mortality and post-infection severe sequelae. Most of the cases are associated with a highly virulent clone, defined as serotype III and clonal complex 17 (CC17), but in the 1990s another clone (CC1) emerged as significant cause of infection in newborns as well as in adult immunocompromised patients. In this study, we investigated MALDI-TOF MS, a well-established method for bacterial identification worldwide, for its potential to discriminate between the highly virulent GBS CC17 and CC1 clones, alongside its other known subtyping applications.

Methods: A total of N=143 previously well-characterized GBS strains were included in this study. The strains belong to 19 different sequence types and 6 clonal complexes, namely CC17 (n=80), CC23 (n=16), CC12 (n=15), CC19 (n=14), CC1 (n=11) and CC498 (n=4), and one isolate each belonging to ST26, ST41 and ST529.

MALDI-TOF MS spectra were acquired and analysed with the MALDI Biotyper® system (MBT - Bruker Daltonics, Germany), using the default setting and procedure for routine measurement. For each isolate, two spots were measured using the direct smear method, from an overnight culture on CBA plate.

Results: For both CC17 and CC1, a specific marker was found in the MALDI spectra. Most of CC17 (69/80 – 86.3%) isolates showed the presence of a specific peak at 7617 m/z, which was present in none of the other isolates (0/63), which all showed a peak at 7637 m/z, absent in CC17. These peaks were found to be corresponding to two protein variants, determined by an amino acid exchange (R25H) in the *S. agalactiae* 50S ribosomal protein L35. In all CC1 isolates (11/11), a specific peak at 6251 m/z was observed, and was absent in all the other strains. This peak was found to be related to a 6250-Da ST1 specific protein.

Conclusions: MALDI-TOF MS showed to be able to discriminate the highly virulent clones of *S. agalactiae*. The implementation of this potential subtyping application, after validation and regulatory approval, could allow prompt and extremely routine-friendly detection and screening of GBS isolates, which could represent a relevant aid in the prevention of the transmission from colonized mothers to newborns.

Fig. 1

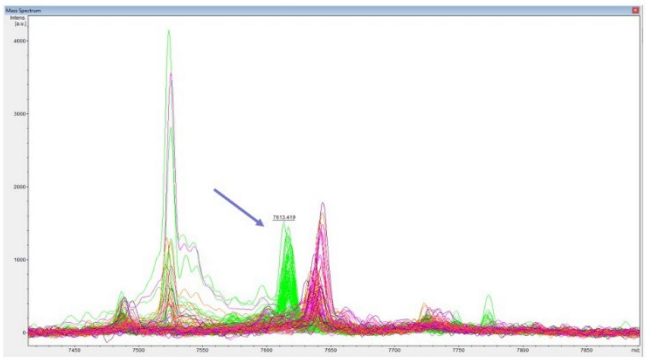

Figure 1. MBT spectra of all *S. agalactiae* strains included in the study in the mass range 7450-7850 Da. The different clonal complexes are shown in different colors, with CC17 in green. The specific CC17 peak 7617 m/z is clearly differentiable from the 7637 m/z peak (different colours) present in all the other strains but CC17 isolates.

Fig. 2

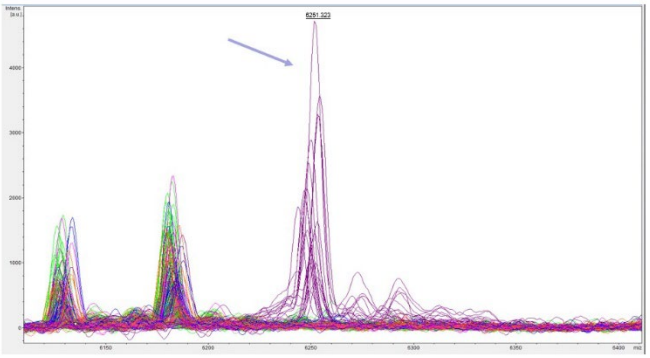

Figure 2. MBT spectra of all *S. agalactiae* strains included in the study in the mass range 6100-6400 Da. The different clonal complexes are shown in different colors, with CC1 in violet. The peak 6251 m/z is present only in CC1 isolates. None of the other strains shows a peak in the same mass range.

WS21.06

A theragnostic microbiological approach to chronic endometritis: combining culture and rapid molecular diagnostics

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Introduction: Chronic endometritis (CE) is an important differential diagnosis in cases of unexplained infertility. It is believed to be caused by bacterial inflammation, and the diagnosis is typically made using immunohistochemical detection of CD138+ plasma cells in endometrial tissue. Despite the absence of routine microbiological testing, empirical antibiotic treatment remains the standard of care for CE.

Objectives: The aim of this study was to perform microbiological and molecular analysis on endometrial biopsies taken for CE diagnostics in order to describe the microbial species identified depending on the diagnostic method, and to discuss these findings in the context of potential individualized therapy recommendations, following a theragnostic approach.

Materials and Methods: A total of 1,586 endometrial biopsies submitted for immunohistochemical diagnosis of CE were

additionally subjected to culture-based and loop-mediated isothermal amplification (LAMP)-based microbiological analysis. The LAMP assays targeted common urogenital pathogens, including *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Gardnerella vaginalis*, as well as Lactobacillus species. Descriptive statistical analysis was conducted to assess patient and sample characteristics, using two-sided Fisher"s exact tests.

Results: Microbial cultures were positive in 64% of samples from patients without prior antibiotic treatment, in 76.5% after one prior therapy, and increased to 83% after more than one previous treatment ($p < 0.001$). Notably, *Enterococcus faecalis* ($p < 0.001$) and coagulase-negative staphylococci ($p = 0.016$) were detected more frequently with increasing numbers of prior treatments.

LAMP-based analysis identified at least one uropathogen in 14% of samples from untreated patients and in 17% of samples following at least one prior treatment. While the overall detection rate did not increase with the number of previous therapies, detection of *Ureaplasma urealyticum* did ($p = 0.013$). *Gardnerella vaginalis* was identified in only 0.4% of culture-based tests, compared to 8.1% in LAMP-based assays ($p < 0.001$).

Lactobacillus species were detected in 50% of untreated cases, 57% after one, and 67% after more than one prior therapy. Although the overall *Lactobacillus* species distribution remained stable across groups, *L. iners* was detected more frequently in CE-positive cases after one prior antibiotic treatment compared to CE-negative samples (19% vs. 12%, $p = 0.044$).

Conclusion: Microbiological and molecular analyses of endometrial biopsies can be integrated into routine diagnostics. LAMP offers valuable complementary diagnostic information, particularly for detecting uropathogens. These findings support the potential for individualized, rational anti-infective therapy recommendations, which warrant further clinical validation.

WS22.01

Reference pangenomes improve 'omics analysis of fungi by capturing their genetic diversity

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Fungi harbor a tremendous amount of genomic diversity, including marked differences in gene content even within the same species. A prominent example is *Aspergillus fumigatus*, a ubiquitous environmental mold responsible for an estimated 1.5 million deaths annually. Only 69% of the total genes of the species are conserved in all isolates, with a large number showing presence-absence variation. Due to their absence in the reference strains, the role of these accessory genes in stress resistance, metabolism, and virulence remains unknown. To create a tool that captures species" diversity with the ultimate goal of understanding the function of these accessory genes, we used 26 near-chromosomal level genome assemblies to create a pangenome reference for *A. fumigatus*. This reference has a length of 38 Mbp, 30% longer than the current Af293 reference, and encodes 2,260 ORFs absent in Af293.

This novel tool can be used for the unbiased but computationally straightforward analysis of genomic and transcriptomic data from diverse strains. As a demonstration that the graph pangenome better captures *A. fumigatus*" diversity, alignment of 300 genomic and 166 transcriptomic data resulted in significantly more reads aligned than the linear reference. Additional work analyzing a set of serial isolates from the same patient, collected over a two-year period, demonstrated that reference pangenomes can quantify genomic adaptations during chronic infections that are usually overlooked by short-read sequencing. This analysis identified 101 structural variants (genomic events greater than 50 base pairs) that were not detected using traditional structural variant calling methods. Altogether this work highlights the value of reference pangenomes for improving our understanding of strain heterogeneity and how it contributes to diverse biological processes, including virulence.

WS22.02

A new reference genome for *Giardia duodenalis* assemblage B

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Introduction: Intestinal infections with the protozoan parasite *Giardia duodenalis* are widespread. Transmission of the environmentally resistant cysts occurs via fecal-oral routes and infections typically lead to diarrhea and other unspecific gastrointestinal complaints. *G. duodenalis* represents a species complex comprised of eight different assemblages (A-H). Each of these assemblages are distinguishable based on genetic information and show variable host specificities. Human giardiasis is almost exclusively mediated by the two assemblages A and B, whose genomes only share approximately 70% nucleic acid identity.

Objectives: While for assemblage A a representative and chromosome-scale reference genome is available, for assemblage B only a limited number of fragmented genomes exist. A noted reason is the higher allelic sequence heterogeneity (ASH) of assemblage B versus A in its tetraploid sets of five chromosomes. Here, we aimed at generating a chromosome-scale reference genome for assemblage B.

Materials & Methods: Axenic cultures of five assemblage B parasites from our own biobank were propagated, DNA was extracted and hybrid genomes were assembled from PacBio and Illumina sequencing reads [1]. An additional Hi-C dataset was generated on selected isolates to include genome-wide chromatin interactions in the analysis to finally generate a chromosome-scale genome for *G. duodenalis* assemblage B. Annotations were generated using standard bioinformatic workflows.

Results: Based on hybrid assembly of PacBio and Illumina sequence datasets, the generated reference genomes consisted of 62-124 scaffolds [1]. In this study, we identified one isolate with an unexpectedly low ASH. To further improve the genome assembly, we generated Hi-C datasets from this isolate and were able to merge the scaffolds finally to five fragments, representing the expected chromosome number for this organism. The completely assembled genome comprised 12.3 MB and annotations retrieved approximately 5.500 predicted proteins. Comparison of the assemblage B reference with assemblage A revealed large chromosome rearrangements between the two assemblages.

Conclusion: We were able to generate the first chromosome-scale reference genome for *G. duodenalis* assemblage B. Next, we will exploit this reference genome to map additional assemblage B genomes from patients in order to resolve population structure of this assemblage.

[1] Klotz C, Schmid MW, Winter K, Ignatius R, Weisz F, Saghaug CS, et al. Highly contiguous genomes of human clinical isolates of Giardia duodenalis reveal assemblage- and sub-assemblage-specific presence-absence variation in protein-coding genes. Microb Genom. 2023;9(3).

WS22.03

From environment to Gut: The evolutionary path of *Candida albicans*

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Objectives: *Candida albicans* is a common commensal yeast in the human gut microbiome of industrialized societies, but also a major cause of severe infections. In contrast, colonization rates are low among populations largely unaffected by industrialization. Environmental reservoirs of *C. albicans* are poorly understood, and the extent to which strains from these sources differ from human-associated ones remains unclear. This study aims to investigate the evolution and adaptation of *C. albicans* to the human host in the context of industrialization and modern medicine.

Methods: We examined over 40 *C. albicans* strains collected from isolated human populations, animals, and diverse environmental sources. These strains were subjected to metabolic profiling, genome sequencing, antifungal susceptibility testing, and virulence assays. Laboratory evolution experiments simulated adaptation to sugars typical for diets in industrialized societies. Additionally, ancient DNA recovered from paleofeces and dental calculus enabled partial reconstruction of historical *C. albicans* genomes from a time before wide-spread industrialization.

Results: Human-derived strains generally caused more damage to human epithelial cells in vitro compared to environmental strains, suggesting host-specific adaptations. Unexpectedly, some of the commensal strains were able to inflict more damage than the clinical isolates. Comparative genomics revealed significant variation in the sequence of *ECE1*, a key virulence gene, as a potential genetic basis. Our metabolic assays revealed that while most strains were able to metabolize common dietary sugars, human-derived strains showed more inter-strain variability in metabolic profiles and a narrower carbon utilization spectrum. Several environmental strains furthermore showed reduced antifungal susceptibilties, indicating environmental pre-adaptations to certain drugs. In evolution experiments, strains adapted to specific sugars showed trade-offs in forms of reduced growth under other conditions, reflecting the metabolic diversity of the isolated strains. Finally, a partially reconstructed genome from a century-old primate-derived *C. albicans* isolate provided insights into the evolutionary trajectory of *C. albicans* before widespread medical intervention.

Conclusions: Our findings suggest that environmental strains are largely metabolically pre-adapted to colonize the human gut, can show low antifungal susceptibility, and can rapidly adapt to available dietary carbon sources. These results support the view that *C. albicans* may have been a transient colonizer in the past, becoming a dominant member of the human mycobiome only in the wake of industrialization and modern lifestyle changes.

WS22.04

***Candida albicans* exploits host immune activation for increased stress resistance**

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During microbial infections, a dynamic interplay between the pathogen and the host takes place. Therefore, an appropriate adaptation to the changing host environments is crucial for microbial persistence within the host. *Candida albicans* is an opportunistic pathogenic yeast that commonly colonizes mucosal surfaces. Under certain predisposing conditions, involving microbiome destabilization, disruption of epithelial barriers, and immunosuppression, *C. albicans* can switch from the commensal to a pathogenic stage. Its coexistence with humans, has fostered the evolution of adaptation strategies to cope with the host immune response during early stages of infection. Understanding how the inflammatory responses impact fungal adaptation and immune evasion strategies is crucial to comprehend disease pathogenesis.

To identify host immune mediators promoting *C. albicans* adaptation, we developed an inflammation-adaptation model in which different immune cell types are stimulated with heat-killed *C. albicans* morphotypes. Culture supernatant from stimulated immune cells is then used to grow the fungus under different stress conditions, mimicking the host immune-related stresses. Exemplary, fungal growth was significantly increased under oxidative and fever-like stress conditions, when cultured in the supernatant obtained from *Candida* hyphae stimulated macrophages, compared to the unstimulated one.

Analysis of the exoproteome revealed that exposure to *Candida* hyphae induced the release of specific proteins from macrophages. When *C. albicans* subsequently encounters these proteins, they influence stress resistance and, consequently, the ability to escape immune cells. Several recombinant proteins, ranging from metabolic enzymes, to potential surface biding proteins and signaling molecules, were selected and their capacity to individually increase fungal stress tolerance was assessed *in vitro*. The impact of individual protein supplementation on fungal growth was dependent on the specific stress condition and culture environment, suggesting that the overall stress resistance conferred by the proteins released in response to hyphae relies on the combination of several factors, rather than one single molecule. Proteins like HMGB-1 or SHP-1, both proteins directly involved in immune signaling modulation, seemed to be more effective in alleviating oxidative stress, while the addition of metabolic enzymes like lactate dehydrogenase (LDH) rather acted beneficial under heat-stress.

In summary, we could show that immune responses to *C. albicans* determin the release of specific proteins in the inflammatory host environment. These subsequently, shape fungal adaptations to overcome immune-related stresses. How specific proteins affect fungal growth under immune-like stress conditions was assessed, while the mechanism underlying the observed increased stress tolerance induced by each particular protein remains an ongoing focus of our research.

WS22.05

Unravelling the impact of interferon-immunotherapy on epithelial resistance to *Candida albicans* translocation

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Invasive candidiasis is challenging to treat due to compromised immune system and the limited success of antifungal treatment alone. Immunotherapy has been posited as an approach to augment host defence of immunocompromised patients. Despite the beneficial role of the cytokine interferon gamma (IFN-γ), on augmenting myeloid antifungal activity, it remains unclear how an acute increase of IFN-γ levels influences intestinal epithelial cells that represent the first barrier against Candida albicans. Using an in vitro intestinal epithelial model, the association between fungal translocation, breakdown of epithelial barrier integrity and tight junction disassembly, upon acute treatment with IFN-γ was evaluated. We found that C. albicans translocation is increased upon IFN-γ treatment. Moreover, expression of junction proteins, are downregulated in the presence of IFN-γ. Accordingly, microscopy revealed disorganization of the tight-junction belt. We next exposed intestinal epithelial cells to the acute IFN-γ treatment during infection with *C. albicans* mutants lacking crucial virulence genes of. This included mutants deficient in the adhesin and invasin Als3 (*als3Δ/Δ*), the toxin candidalysin (*ece1Δ/Δ*), filamentation (*efg1Δ/Δ*/*cph1Δ/Δ*) as well as mutants with reduced proteolytic activity (*sap1/2/3Δ/Δ* & *sap4/5/6Δ/Δ*). Strikingly, fungal translocation of the *ece1Δ/Δ* and *als3Δ/Δ* mutants was also increased by acute exposure to IFN-γ. Though, *sap1/2/3*D/D and *sap4/5/6*D/D mutants showed no difference in translocation with IFN-γ exposure. Collectively, our data shows that reorganized barrier junctions following IFN-γ treatment make epithelial barrier more permissive to C. albicans translocation via damage-independent mechanisms, shedding a light on potential detrimental effects of interferon immunotherapy on barriers defence against C. albicans infection.

WS22.06

Host inflammation primes the opportunist *Candida albicans* for impact – A case of "trained pathogenicity"?

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As a common commensal fungus, *Candida albicans* is part of the healthy human microbiome. However, it is also responsible for an unacceptably high number of mucosal and, in immunocompromised patients, life-threatening infections. The commensal-to-pathogen switch during invasion of host tissues exposes the fungus suddenly to new host niches and promptly changing environments, driven by inflammation. During co-evolution with the human host, the fungus found ways to persist in us, which inherently requires fungal adaptations to outlast stress imposed by the immune system. To mechanistically unravel, how the opportunist *C. albicans* might use inflammation as a signal to anticipate and avoid its clearance, we established an *in vitro* inflammation adaptation model. By stimulating human immune cells and using their supernatants as surrogate for an inflammatory environment, in which we grow *C. albicans*, we assessed inflammation-triggered changes in fungal pathogenicity and stress resistance. We applied live-cell imaging to dynamically record the immune cell stimulation process and capture *Candida"s* competitive fitness against immune cells, when exposed to inflammation. To molecularly understand the underlying fungal adaptations, we characterized the composition of the inflammatory environments by fractionation and proteomics and the fungal transcriptional response to inflammation by RNAseq. Particularly supernatants of macrophages, which were stimulated with fungal hyphae, elevated *C. albicans* stress resistance. Although both, a pathogenic and a commensal isolate in this environment increased their stress resistance, their transcriptional response and different requirements of specific protein fractions, suggested strain-specific adaptations. Correspondingly, this environment could train the commensal strain to escape from macrophages, but not increase escape of the already pathogenic strain. Stress adaptation was associated with supernatants of macrophages undergoing cell death in response to fungal hyphae, although lysis alone was insufficient. The encounter of hyphae resulted in a rich macrophage exoproteome, based on which we selected recombinant proteins to screen for induction of fungal stress adaptations and already identified first adaptation factor candidates. Our findings demonstrate that inflammatory signals can induce fungal adaptations to overcome infection-relevant stresses, including immune cells. Identification of single host proteins and specific fungal transcriptional signatures that regulate adaptation to inflammation could aid establish new biomarkers and targets for host-directed or antivirulence-based therapies in candidiasis. To solidify clinical implications of our study, we are currently assessing, how inflammatory environments generated by macrophages of primary immunodeficiency patients with lifelong elevated risk of fungal infections, differentially prime fungal stress adaptations.

PS01.001

Salmonella SopB delays disease progression and tissue inflammation *in vivo*

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The enteric pathogen Salmonella enterica subsp. enterica serovar Typhimurium (S. Typhimurium) contributes significantly to childhood mortality particularly in developing countries. The Salmonella pathogenicity island (SPI)-1 encoded type 3 secretion system (T3SS) effector protein Salmonella outer protein B (SopB) has been shown to manipulate bacterial internalization, cell survival, cell trafficking and host cell signaling. Using a recently established murine neonatal infection model, we analyzed the functional role of SopB by comparing SopB-deficient (ΔsopB) with SopB proficient S. Typhimurium infected animals. Unexpectedly, ΔsopB S. Typhimurium infected animals showed an accelerated disease course with premature mortality. Although the intraepithelial bacterial burden and systemic spread were similar, a significantly increased early proinflammatory cytokine response with increased expression of Cxcl1, Cxcl2, Mcp1 were noted in the absence of SopB as early as day 1 p.i. In accordance, the recruitment of neutrophils and monocytes to the lamina propria was increased in ΔsopB S. Typhimurium infected mice leading to a more pronounced TNFa-mediated inflammatory response and necroptosis-dependent epithelial cell death. This effect in vivo was independent of the phosphatidylinositol phosphatase activity of SopB but required an intact N-terminal domain. A comparative phosphoproteome and transcriptome analysis of infected epithelial cells in vitro and in vivo revealed an early increase in ERK/MAPK but also mTOR signalling in the absence of SopB. Taken together, my results suggest that SopB manipulates early cell signaling and membrane trafficking in a phosphatidylinositol phosphatase-independent manner to reduce mucosal inflammation and prevent early mortality likely leading to enhanced host transmission.

PS01.003

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

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Introduction: *Legionella pneumophila,* the causative agent of a life-threatening pneumonia, intracellularly replicates in a specialized compartment in lung macrophages, the *Legionella*-containing vacuole (LCV). During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II (Lsp) and type IVB (Dot/Icm) secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins and the GDSL hydrolases PlaA, PlaC and PlaD, are encoded in the genome.

Goals: We here focus on the characterization of the phospholipase PlaD and shows various differences to the other GDSL hydrolases PlaA and PlaC. We aim to understand the importance, secretion path, and mode of action in infection and activation mechanism of PlaD.

Materials and methods: We investigated the mode of secretion of PlaD by means of Western blotting and protein translocation assay. Additionally, we determined its binding to various lipids and its interactions with eukaryotic proteins by means of lipid-protein-overlay assays, proximity ligation, and pull down assays. Further, we analyzed the localization of PlaD during infection via immunofluorescence microscopy.

Results: We showed that, during infection, PlaD is Dot/Icm-dependently injected into the host cell cytoplasm where it localizes to distinct organelles. Moreover, we demonstrated that PlaD binds to a subset of phosphoinositide species and interacts with a class of regulatory proteins of the host cell. Additionally, our data revealed that the C-terminal half of PlaD is essential for its secretion and phosphoinositide binding but dispensable for interaction with the regulatory proteins.

Summary: Based on its Dot/Icm dependent injection into the host cell cytoplasm, we classify PlaD as a novel type IVB secreted effector protein of *L. pneumophila*. We propose that PlaD is involved in the regulation of host cell signaling cascades during infection.

PS01.005

Coexistence of mucoid *Staphylococcus argenteus* and non-mucoid *Staphylococcus aureus* in the airways of a person with cystic fibrosis

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Introduction: *Staphylococcus argenteus* is a recently described, coagulase-positive species closely related to *Staphylococcus aureus*, both phenotypically and genetically, and part of the *S. aureus* complex along with *S. schweitzeri* [1]. Unlike *S. aureus*, persistent infections caused by *S. argenteus* in people with cystic fibrosis (pwCF) are rarely reported. In a recent prospective multicenter study on the prevalence and clinical significance of mucoid *S. aureus* in pwCF airways [2], both mucoid and non-mucoid *S. argenteus* were co-isolated with *S. aureus* from a single pwCF at the first outpatient visit. At the second visit, *S. aureus* was replaced by a mucoid *S. argenteus* strain. Following cefuroxime treatment, *S. argenteus* was undetectable in further visits.

Methods: Throat swabs from five quarterly visits were analyzed in the microbiological lab of the University Hospital Münster. Ten staphylococcal isolates per sample were identified by MALDI-TOF MS, *spa*-typing, and whole genome sequencing. Pigmentation and hemolysis were assessed on Columbia Blood agar; mucoidity via Congo Red agar, and biofilm formation was quantified in a microtiter plate assay. Antibiotic susceptibility was tested using Vitek2 and Etest. *In vitro* competition experiments were conducted by co-culturing both species at a 1:1 ratio to simulate airway colonization.

Results: Each species exhibited uniform *spa*-types: *S. aureus* (yellowish, non-hemolytic) as t4379 and *S. argenteus* (grey, strong hemolysis) as t5078. The mucoid phenotype of *S. argenteus* was linked to a mutation in *icaR*, coding for the repressor of the intercellular adhesion-operon, leading to pronounced polysaccharide-dependent biofilm production. *S. aureus* isolates were highly penicillin-resistant, whereas *S. argenteus* isolates were sensitive. *In vitro*, *S. argenteus* exhibited a higher growth rate and displaced *S. aureus*, with the mucoid variant becoming dominant. Subinhibitory cefuroxime levels had an impact on interspecies dynamics, leading to the replacement of *S. argenteus* by *S. aureus*.

Conclusion: This study presents the first molecular characterization of mucoid *S. argenteus* from the airways of a pwCF. Mucoid and non-mucoid *S. argenteus* coexisted with *S. aureus* in the CF lungs, suggesting clinical relevance. Phenotypic differentiation between both subspecies and both phenotypes is not always as evident as it was in this case. Accurate species identification is essential to guide effective therapy, as biofilm-producing *S. argenteus* may contribute to chronic airway infections. In case of *S. aureus,* mucoid variants may – but do not always – display enhanced resistance and persistence linked to biofilm formation [2,3]. Future studies should consider both species in pwCF.

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PS01.007

The clinical relevance of *Staphylococcus borealis* as an emerging potential uropathogen: a comprehensive analysis of patient isolates

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Introduction: *Staphylococcus borealis* (SB) is a newly identified species of coagulase-negative staphylococci. Until recently, routine microbiological labs were unable to distinguish SB from its closest relative, *Staphylococcus haemolyticus* (SH). A few studies describe SB as a commensal like SH; however, SB has been reported to produce urease, a trait typically associated with uropathogens. Additionally, SB colonies show yellow pigmentation, a phenotype usually restricted to *Staphylococcus aureus* (SA) [1]. The clinical relevance of SB in human disease remains largely unknown.

Methods: SB and SH from all clinical samples submitted to the microbiological lab of the University Hospital Münster over 16 months were identified by MALDI-TOF MS and confirmed by PacBio-based whole genome sequencing (WGS). Colony pigmentation was evaluated on P-agar; urease activity on urease agar plates. Biofilm formation was analyzed by the Congo Red agar method and a microtiter plate assay. Antibiotic susceptibility was tested using Vitek2 and agar diffusion. To assess clinical relevance, patients" clinical data were reviewed.

Results: A selection of SB isolates from our strain collection was analyzed, originating either from hospitalized patients (n=82) or andrology outpatients (n=54). SH isolates from hospitalized (n=85) and andrology patients (n=22) served as the control group. WGS showed that SB cannot be reliably distinguished from SH by MALDI-TOF MS. Among SB isolates with scores ≥ 2 (70%), WGS confirmed the correct species in 75%; for scores < 2 (30%), in only 20% of cases. Here, an *ad hoc*-cgMLST scheme was developed, enabling high-resolution intra-species analysis. Yellow pigmentation was observed in only 85% of SB isolates, and 20% lacked urease activity. Biofilm formation by SB was inducible and occurred only in some isolates in glucose-supplemented TSB. These biofilms were protein-based unlike the polysaccharide-based biofilms typically produced by SA. SB showed a typical CoNS antibiotic resistance profile but with lower phenotypic resistance to β-lactam antibiotics, correlating with a lower prevalence of *mecA* (9.6% in SB vs. 29.4% in SH). Notably, SB exhibited a high resistance to fosfomycin (97% vs. 14% in SH), despite absence of the *fosB* gene. SB was mainly isolated from urine (81.7%), less often from urogenital swabs (4.9%), other swabs (12.2%) and blood (1.2%). Among hospitalized patients (median age: 66y), 86.6% were male; comorbidities included immunosuppression (24.4%), malignancy (28%) or transplant history (6.1%). UTI symptoms were often unclear. Andrology patients (median age: 37y) mainly provided ejaculate isolates (94.4%) and few from urine (5.6%). In contrast, SH isolates were found uniformly across all patient samples and both genders.

Conclusion: SB may be an emerging uropathogen, particularly in men, warranting further evaluation in future prospective studies with detailed clinical case data.

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PS01.009

Isolation and characterization of mucoid, hyper-biofilm-producing *Staphylococcus aureus* from respiratory samples of cystic fibrosis patients using Congo Red agar

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Introduction: Biofilm formation is a key virulence factor in many bacterial species, including *Staphylococcus aureus* (SA). In SA, the primary component of the biofilm matrix, polysaccharide intercellular adhesin (PIA), is synthesized by the products of the *icaADBC* operon. Expression of this operon is tightly regulated by IcaR, a transcriptional repressor encoded by the *icaR* gene, located upstream and separated by an intergenic region (IGR).

Our recent findings demonstrated that mutations in *icaR* or the IGR led to enhanced biofilm production and a mucoid colony phenotype in SA [1,2]. In a prospective, multicenter study, we observed a mucoid SA prevalence of 9.1% for mucoid SA among SA-positive persons with cystic fibrosis (pwCF). Moreover, pwCF harboring only mucoid SA - without co-infection by *Pseudomonas aeruginosa* - showed reduced lung function compared to those colonized exclusively by non-mucoid SA [3]. However, visually identifying mucoid SA on routine culture media can be challenging and often requires tactile assessment of the sticky colonies.

Methods: To improve detection, we analyzed 1,360 SA isolates from respiratory samples of 35 pwCF using Congo Red (CR) agar alongside standard Columbia Blood (CB) agar. For each unique combination of clonal lineage and colony phenotype, further characterization included microtiter plate experiments to assess biofilm formation, as well as detachment assays to identify matrix components. Mutations within the *ica* operon were determined via Sanger sequencing.

Results: Mucoid strains mostly exhibited a pyramidal, wrinkled colony morphology on CR agar and were readily identified by their characteristic appearance on CR agar. Among these, 121 out of 177 isolates (68.4%) produced substantial biofilm. Genetic analysis revealed that 53.1% of mucoid isolates harboured mutations in either *icaR* or the IGR.

Detachment assays of *ica*-independent biofilms suggested the involvement of alternative matrix components.

Conclusion: CR agar proved effective for identifying mucoid SA based on its distinctive colony morphology, which often correlated with increased biofilm production and mutations within the *ica* locus. However, the molecular mechanisms underlying biofilm formation in some mucoid SA strains remain unresolved and warrant further investigation.

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PS01.011

Rapid phenotypic antibiotic susceptibility testing of *Klebsiella spp.* using the RamanBioAssay™

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Klebsiella pneumoniae is one of the most important pathogens of infections acquired both in the healthcare context and in the community. Of particular concern are carbapenem-resistant *K. pneumoniae* (CR Kp), which produce carbapenemases that render last-line antibiotics ineffective and are associated with frequent hospital outbreaks with high mortality. Some of these strains also exhibit hypervirulent properties that enable severe infections even in otherwise healthy individuals. Due to their resistance and clinical impact, CR Kp have been classified by the WHO as a critical priority pathogen [1], highlighting the urgent need for rapid and reliable diagnostics. In this study, we aimed to expand the RamanBioAssay™ platform beyond previously tested combinations [2] to detect meropenem susceptibility in *K. pneumoniae* isolates, enabling rapid, label-free phenotypic antibiotic susceptibility testing (AST). Phenotypic responses were detected using Raman spectroscopy, based on distinctive spectral signatures. The results were compared to gold standard AST methods to validate performance and diagnostic accuracy. The assay successfully discriminated between susceptible and resistant isolates within 90 minutes of antibiotic exposure. The spectral changes in response to meropenem were reproducible and showed a high correlation with conventional AST results. Thanks to its speed and accuracy, this approach could significantly improve diagnostic precision, making it a promising tool to support timely treatment decisions and improve antimicrobial stewardship for various bacterial pathogens.

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PS01.013

High seroprevalence but low frequency of intrathecal anti-Epstein-Barr virus IgG suggests a unique role of Epstein-Barr virus infection in multiple sclerosis

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Introduction: Multiple sclerosis (MS) is strongly associated with high anti-Epstein-Barr virus (EBV) IgG seroprevalence. Many patients with MS have an intrathecal production of antiviral antibodies, which can be detected by elevated antibody indices (AI) [1,2]. In fact, the detection of intrathecal antibodies against measles, rubella, or varicella zoster (MRZ) viruses helps in the differential diagnosis of MS. Here, we compared the frequency of elevated AIs for anti-EBV IgG with that of other microbes in patients with MS who were seropositive for the respective antimicrobial antibodies.

Methods: The presence of IgG antibodies against EBV and *Borrelia burgdorferi*, cytomegalovirus, herpes simplex virus 1/2, MRZ virus, parvovirus B19, tick-borne encephalitis virus, mumps virus and *Toxoplasma gondii* was analyzed in paired serum and CSF samples from 50 MS patients using ELISAs (all EUROIMMUN).

Results: The prevalence of anti-EBV IgG in serum was 100% (50/50) while the frequency of elevated EBV-AIs was 10% (5/50) resulting in a seroprevalence-corrected frequency of 0.1. The prevalence of IgG against the other microbes in serum varied from 6.0% (*Borrelia burgdorferi*) to 94% (varicella zoster virus) while elevated AIs ranged between 0.0% (tick-borne encephalitis virus) to 73% (parvovirus B19, MRZ around 40%). The resulting seroprevalence-corrected frequencies ranged from 0.14 (herpes simplex virus 1/2) to 1.4 (parvovirus B19, MRZ virus around 0.45).

Conclusion: These data demonstrate a universal seroprevalence but a paradoxically low frequency of elevated AIs for anti-EBV IgG in patients with MS, in contrast to all other microbial pathogens tested. Hypothetically, pre-existing antibody-producing B cells may be primed to enter the CNS during and due to acute EBV infection, that is, at a time point before anti-EBV antibody producing cells are formed. Furthermore, combining MRZ parameters with parvovirus B19 could improve the sensitivity of the MRZ reaction for MS.

PS01.015

Detection of elevated intrathecal production of anti-viral capsid antigen antibodies of Epstein-Barr virus supports diagnosis of primary central nervous system post-transplant lymphoproliferative disorder

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Introduction: Primary central nervous system (CNS) post-transplantation lymphoproliferative disorder (PCNS-PTLD) is a rare but serious complication of chronic immunosuppression, mostly characterized by Epstein-Barr virus (EBV)-driven uncontrolled proliferating B cells that enter the CNS for currently unclear reasons. PCNS-PTLD diagnosis is usually based on brain biopsy. However, the presence of viruses in the CNS typically results in intrathecal antibody production, which can be detected by an elevated antibody index (AI) [1,2]. This study investigates the frequency of EBV-specific elevated AI in PCNS-PTLD patients and the applicability of the determination of elevated AI to support PCNS-PTLD diagnosis.

Methods: In paired CSF and serum samples from 9 PCNS-PTLD and 20 non-inflammatory neurological diseases (NINDs) patients, anti-Epstein-Barr nuclear antigen-1 (EBNA-1) IgG, anti-viral capsid antigen (VCA) IgG and IgA, and IgG against 10 further microbial pathogens were detected using established ELISAs (all EUROIMMUN).

Results: EBV-specific serum IgG was found in 9/9 (100%) and elevated AI for anti-VCA IgG, anti-VCA IgA, and anti-EBNA-1 IgG in 7/9 (77.8%), 6/9 (66.7%), and 2/9 (22.2%) PCNS-PTLD patients, respectively. In 2/9 (22.2%) patients (one without histological evidence for EBV), no EBV-specific elevated AI was detected. 17/20 NIND patients showed EBV-specific serum IgG, indicating prior EBV infection. These patients had no elevated AI for anti-VCA or anti-EBNA-1 IgG. Elevated AI for anti-measles virus, anti-toxoplasma gondii, and anti-varicella zoster virus IgG were found in 1/9 (11.1%), 1/8 (12.5%), and 3/8 (37.5%) PCNS-PTLD patients, respectively.

Conclusion: The frequent intrathecal production of anti-VCA IgG and anti-VCA IgA in PCNS-PTLD and the absence of elevated AI for EBV-specific antibodies in NIND patients suggest that elevated EBV-specific AIs could support the diagnosis of

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PS01.017

Antibody detection in the context of vaccination against tick-borne encephalitis virus using two IVDR approved ELISAs

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Introduction: This study investigated seroconversion in human serum samples between vaccine doses and after complete vaccination against tick-borne encephalitis (TBE), which requires three vaccine doses for basic immunization.

Methods: Panel A contained single serum samples from 50 individuals obtained after ≥1 vaccine dose against TBE.

Panel B contained 54 serum samples from nine individuals (six per person) obtained before and after first and second vaccine dose and six sera from one individual (#51) with sampling after second and third vaccine dose. Samples were analyzed for anti-TBE virus IgG and IgM antibodies using the two newly designed and IVDR approved Anti-TBE Virus ELISA 2.0 (IgG) and Anti-TBE Virus ELISA 2.0 (IgM) (EUROIMMUN).

Results: Overall, the results showed that the positivity rate for anti-TBE virus IgG antibodies increased with continued vaccination. Eight individuals from panel B had negative results in the Anti-TBE Virus ELISA 2.0 (IgG) before the first vaccine dose and antibody titers above the cut-off after the second vaccine dose, which then decreased again (e.g. individual #53). Individual #51 had a high IgG antibody titer (>200 RU/ml) after the third vaccine dose, which corresponds to an antibody level to be expected after completion of basic immunization. Only individual #57 had positive results in the Anti-TBE Virus ELISA 2.0 (IgM) after vaccination. Individual #55 had specific IgG antibodies before the first vaccine dose, which might be due to prior infection with TBE virus or undocumented vaccination against TBE.

Conclusions: The observed antibody dynamics are plausible in view of the ongoing vaccination and indicate the necessity of the third vaccine dose for immunization. Overall, the data show that the Anti-TBE Virus ELISA 2.0 (IgG) is suitable for the determination of the immune status and that specific IgM antibodies can be detected in individual cases after vaccination.

PS01.019

Rapid detection and molecular multiparameter typing of PVL-positive *Staphylococcus aureus* isolates from clinical samples from Lithuania

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Introduction: *Staphylococcus aureus* (SA) is a major human commensal and pathogen, with the Panton-Valentine leukocidin (PVL) genes associated with increased virulence and severe clinical manifestations such as skin and soft tissue infections and necrotizing pneumonia (which can have a high fatality rate of up to 50%). PVL-positive strains, especially

community-associated methicillin-resistant SA (CA-MRSA), pose a significant public health threat due to their combination of heightened virulence and antibiotic resistance. Rapid detection and molecular typing of these isolates are essential for effective epidemiological surveillance and infection control, particularly in countries like Lithuania where comprehensive data remain scarce.

Material and methods: The collection of clinical SA isolates from two hospitals in 2018-2019 and 2024 and the collection from the community in 2012-2020 as well as screening for PVL genes using RT-PCR were performed at Vilnius University. Positive isolates were then characterized at Leibniz-IPHT Jena using DNA-microarray based assays that facilitated not only the detection of virulence genes including PVL and resistance markers, but also an assignment to CCs and strains. In addition, isolates harvested directly from the agar plate were tested for PVL production using an experimental lateral flow (LF) test.

Results: Out of 1296 SA isolates (1170 MSSA and 126 MRSA), 124 yielded PVL-positive PCR results. 100 of these isolates were available for genotyping. The MRSA rate among these PVL-positives was 60%. For two methicillin-susceptible isolates (MSSA), PVL could not be confirmed by array and LF. The most common PVL-MRSA strain was an ACME-negative CC8-MRSA-IV (n=43). Out of 47 SA isolates from "Hospital 2", 42 belonged to this epidemic strain. The other strains from this hospital belonged to CC8-MRSA-[IV+ACME] "USA300" (n=3), CC80-MRSA-IV (n=1) and CC22-MRSA-IV (n=1). Isolates from the other hospital were assigned to eleven different clonal complexes, the most common were CC30-MSSA (n=13), CC121-MSSA (n=12) and CC8-MRSA-[IV+ACME] "USA300" (n=11). A concordance of PVL detection by array and LF was observed in 100/100 isolates (100%).

Discussion: The high MRSA rate among PVL-positive SA isolates exceeds the national average and signals a concerning overlap of virulence and resistance. The concentration of CC8-MRSA-IV in one hospital might indicate a local outbreak, while the presence of diverse clonal complexes in other hospitals suggests polyclonal, community-borne transmission and a potential importation of international lineages, as seen elsewhere in Europe. Rapid PVL detection is essential to prevent outbreaks and improve clinical outcomes. In settings with limited data, such as Lithuania, molecular typing, rapid screening and strengthened infection control are critical. Given its clinical relevance, routine PVL screening —via PCR or lateral flow assays—in routine diagnostics should be seriously considered.

PS01.021
Investigation of antimicrobial susceptibility in *K. gyiorum*

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Introduction: *gyiorum* is a bacterium that rarely occurs in routine diagnostics but is capable to cause infections in humans. For this reason, only limited data on *K. gyiorum* from a medical perspective is available. This particularly applies information on antimicrobial susceptibility. Therefore, results from phenotypic testing in correlation with *in silico* data for this species are presented here for the first time.

Methods: In this study, we used isolates from a total of 14 strains that had previously been identified as *K. gyiorum*. Whole genome sequencing of the strains was conducted using PacBio sequencing technology. Afterwards, the species was confirmed as *K. gyiorum* using dDDH. The antimicrobial susceptibility was determined by gradient diffusion test against amoxicillin/clavulanic acid, ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, azithromycin, erythromycin, clarithromycin, cefepime, ceftazidime, cefuroxime, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, doripenem, ertapenem, imipenem, meropenem, aztreonam, colistin, doxycycline, fosfomycin, nitrofurantoin, rifampicin, tigecycline and trimethoprim/sulfamethoazole. In addition *in silico* analyses were performed using the "Comprehensive Antibiotic Resistance Database" (CARD). By comparing the phenotypic data with the genomic data, a gene was then sought that could explain the antimicrobial resistance.

Results: The results from antimicrobial susceptibility testing are presented as MIC values only (EUCAST v. 15.0 does not provide guidelines to determine the susceptibility to rare human pathogenic bacteria). Low MIC values were found for amoxicillin/clavulanic acid, for ampicillin, for piperacillin/tazobactam, ertapenem, imipenem and meropenem. In contrast, high (or higher) MIC values were observed for fosfomycin and nitrofurazone, for azithromycin, erythromycin, clarithromycin, ciprofloxacin, levofloxacin and moxifloxacin (summary table 1). When determining resistance genes using "CARD", it was noticeable that only "strict hits" and no "perfect hits" were given. This indicates the presence of variants of known resistance genes. There were indications of a variant of the *catB*3 gene, which is responsible for the development of resistance to phenicol antibiotics. Furthermore, a variant of the *adeF* gene (encoding an efflux pump) was identified, which is responsible for the development of resistance to fluoroquinolones and macrolides.

Conclusion: The increased MIC values for quinolones and macrolides could be explained by the proposed variant of the *adeF* gene. However, these questions will need to be clarified in the course of further research into this species. Due to the high MIC values for fosfomycin and nitrofurazone, it can be assumed that the isolates in this collection are resistant to these antibiotics. Unfortunately, the CARD analysis did not reveal any evidence of a resistance gene that could explain the resistance.

Fig. 1

Antibiotic	Range (in µg/ml)
amoxicillin/clavulanic acid	0.125 - and 0.25
ampicillin	0.125 - 0.5
piperacillin/tazobactam	0.25 - 0.5
ertapenem	0.004 - 0.016
imipenem	0.25 - 1
meropenem	0.032 - 0.125
fosfomycin	64 - 256
nitrofurazone	64 - 256
azithromycin	1 - 8
erythromycin	4 - 8
clarithromycin	4 -16
ciprofloxacin	1 -
levofloxacin	0.25 - 8
moxifloxacin	0.125 - 8

PS01.023

Human *Staphylococcus pseudintermedius* spondylodiscitis in a patient without known animal contact

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Introduction: Spondylodiscitis encompasses related pathological entities, such as vertebral osteomyelitis, spondylitis, and discitis. In humans, the most common causative agent is *Staphylococcus aureus*. *Staphylococcus pseudintermedius*, a common colonizer of dogs and notorious agent of canine pyoderma, is rarely reported in human infections and usually linked to animal contact. This case presents a rare instance of spondylodiscitis caused by *S. pseudintermedius* in a young male with no known animal exposure, highlighting its pathological potential in human infection.

Objectives: This case report aims to describe the clinical presentation, microbiological diagnosis, treatment, and genetic characterization of *S. pseudintermedius* in a human case of spondylodiscitis without animal contact, emphasizing the need for advanced diagnostic techniques and awareness of this pathogen within and beyond the One Health perspective.

Materials & Methods: A 24-year-old man with documented penicillin allergy presented following a traumatic fall with an unstable L4 vertebral fracture requiring surgical stabilization. Following surgery and after discharge against medical advice, the patient was readmitted with signs of infection. Diagnostic evaluation included blood cultures, tissue sampling, MRI imaging, and laboratory inflammatory markers. Bacterial identification was performed using MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing utilized the VITEK 2 system and disk diffusion method. Whole genome sequencing (WGS) was conducted on three patient isolates for genetic characterization.

Results: Findings indicating spondylodiscitis at the L4 level with MRI were confirmed by elevated inflammatory markers (CRP 282 mg/L; WBC 11.9 10^9/L). *S. pseudintermedius* was isolated from multiple tissue samples and blood cultures, with all isolates demonstrating identical susceptibility patterns and morphological features. Empiric clindamycin therapy was replaced by daptomycin treatment and, subsequently, by oral levofloxacin and rifampicin leading to a successful outcome. WGS revealed all three isolates have identical chromosomes and a small plasmid, confirming clonal infection. Genomic analysis identified multiple resistance genes and virulence factors. Multilocus sequence typing classified the isolates as ST2051, a rare sequence type previously reported only once in a feline isolate from Poland.

Conclusion: This rare case underscores *S. pseudintermedius* as a potential zoonotic pathogen, even without animal contact. The strain harboured significant antimicrobial resistance and virulence determinants, highlighting the pathogenic capability of this primarily veterinary pathogen. Furthermore, this report on atypical causative pathogens of musculoskeletal infections emphasizes the importance of accurate microbiological identification techniques and the value of WGS in characterizing unusual pathogens.

PS01.025

Improvement of the serodiagnosis of tick- and louse-borne relapsing fever

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Background: Louse- and Tick-Borne Relapsing Fever (RF), caused by various *Borrelia* species, comes along with recurrent episodes of fatal fever. Especially in low-resources countries, the diagnosis of this neglected infectious disease is challenging. Microscopy of blood smears is still considered the gold standard, whereas PCR-based methods are not implemented in most African countries. Recently, we succeeded in developing two immunoassays, ELISA and Lineblot, for the serodiagnosis of RF. This study deals with the amendment of both immunoassays.

Methods: The highly immunoreactive Variable Major Proteins (Vmps) originating from *Borrelia recurrentis* were assessed in different combinations with the already implemented antigens in these immunoassays (GlpQ and CihC-N). A wide serum panel including samples from RF patients, syphilis, leptospirosis, malaria, leishmaniasis, tuberculosis, HIV, CMV and EBV, as well as healthy blood donors, was utilised to determine the most discriminating combination of antigens resulting in a low cross-reactivity and a high specificity and sensitivity.

Results: Among different combinations investigated GlpQ and VlpD proves to be most promising antigens. An increase in sensitivity by 42.9 % (reaching 92.9 %) was obtained for the IgM Lineblot assay, with a minor reduction of the specificity of 3.9 % (resulting in 85.5 %). In terms of the IgG Lineblot assay, the specificity was elevated by 13.1 % (rising to 89.4 %) without an impairment of sensitivity (92.9 %).

For the IgM ELISA, an improvement to 96.6 % (increase of 15.4 %) for specificity, while the sensitivity remains at 100.0 %. In case of the IgG ELISA, a slight increase in specificity to 1.1 % (to 100.0 %), alongside a decrease in sensitivity of 7.1 % (to 92.9 %) was observed.

Discussion: Including the combination of GlpQ and VlpD as the most immunoreactive antigens, we were able to improve both immunoassays, which are now suitable for the serodiagnosis of Louse- and Tick-borne RF. Field studies to assess the feasibility of the improved assays are ongoing. Nevertheless, the development of two reliable *in vitro* immunoassays will improve the diagnostic and may enhance the awareness of this neglected tropical disease in certain African countries.

PS01.027

The role of viridans streptococci and anaerobic bacteria of the oral cavity in children with parapneumonic pleural effusions/empyema – a nationwide hospital-based surveillance study (Germany, 2010/11-2022/23)

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Background: Parapneumonic pleural effusions/empyema (PPE/PE) are serious complications of community-acquired pneumonia in children. While *Streptococcus pneumoniae* and *Streptococcus pyogenes* are established major pathogens, the clinical relevance of oral cavity bacteria (viridans streptococci and anaerobic bacteria; VS/AA) in paediatric PPE/PE is largely unclear.

Methods: A prospective nationwide hospital-based surveillance study in Germany recorded children aged S. pneumoniae- or *S. pyogenes*-associated PPE/PE (reference group) using multivariable regression analysis.

Results: Among 1,242 children with any identified PPE/PE-associated pathogens, 115 (9.3%) presented with VS/AA and 818 (65.9%) with *S. pneumoniae* or *S. pyogenes*. Compared to the reference group, children with VS/AA-associated PPE/PE were older (median 11.4 vs. 3.6 years, p<0.001) and had more underlying diseases (46.1% vs. 22.2%, p<0.001), mainly complex neurological comorbidities (25.2%). In multivariable analyses, VS/AA vs. reference patients showed a similar duration of hospital stay (median 20 days vs. 18 days, p=0.467), and a similar proportion required PICU care (80.0% vs. 81.5%, p=0.992). VS/AA patients had a longer time from symptom onset to hospital admission or to discharge (by 4.1 days, 95%CI 2.5-5.7, p<0.001, and by 5.2 days, 95%CI 2.1-8.3, p=0.001, respectively), and needed longer PICU treatment (by 3.1 days, 95%CI 0.4-5.9, p=0.025). They showed more frequently pulmonary complications, especially atelectasis (OR 1.9, 95%CI 1.2-3.0, p=0.006) and pulmonary abscess (OR 1.7, 95%CI 1.0-2.8, p=0.040). They were more likely to develop sequelae (OR 2.3, 95%CI 1.5-3.7, p<0.001) but less likely to develop sepsis/SIRS (OR 0.4, 95%CI 0.2-0.7, p=0.004) than the reference group.

Conclusion: VS/AA-associated PPE/PE particularly affected older children and those with complex comorbidities. In patients at risk, empirical antibiotic treatment should include anaerobic coverage, and distinct clinical features should be considered in therapeutic management.

PS01.029

Detection of colistin resistance in *Acinetobacter baumannii* using a rapid MALDI-TOF MS-based assay

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Introduction: Polymyxins exert their antibacterial effect by directly targeting the bacterial cell membrane. Modification of Lipid A leads to colistin resistance by hindering membrane access through electrostatic repulsion. Although current guidelines recommend polymyxins only as an alternative treatment for carbapenem-resistant Enterobacterales and *Acinetobacter baumannii* due to its poor pharmacokinetic and pharmacodynamic profile, it remains a last-resort antibiotic particularly in regions where novel beta-lactam/beta-lactamase inhibitors are not available.

Its use is hampered by the time-consuming and labor-intensive broth microdilution method (BMD), which is the accepted reference method for testing colistin susceptibility. Additionally, there is no reliable diffusion-based method, and validated rapid AST methods are rare. With the increasing incidence of carbapenem-resistant Gram-negative bacterial infections, there is an urgent need to develop rapid and accurate methods for testing colistin resistance.

Aims: In this study, we evaluated the outcome of a novel rapid matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS)-based assay for testing colistin resistance in *Acinetobacter baumannii* through Lipid A characterization, using BMD as the reference method.

Materials and Methods: The colistin resistance of 60 clinical isolates of *A. baumannii* were tested using a rapid MALDI-TOF MS-based RUO assay. Lipid A molecules from the bacterial cell membrane were extracted using the MBT Lipid Xtract Kit (RUO) (Bruker, Germany). Spectra acquisition and automated evaluation were conducted with the prototype LipidART Module (RUO) of MBT Compass HT (RUO) on a MALDI Biotyper® siriusTM System (Bruker) in negative ion mode. The results were then compared to BMD MICs obtained using the MICRONAUT MIC-Strip Colistin (Bruker).

Results: Based on BMD results, 30 strains were identified as colistin-susceptible and 30 strains as colistin-resistant. The prototype MBT LipidART Module (RUO) revealed a sensitivity of 96.7% (29/30) and a specificity of 93.3% (28/30) for detecting of colistin resistance in *A. baumannii*. The overall validity of the assay was 100% (60/60).

Conclusions: The research-use-only Lipid workflow works as an effective and rapid screening tool for detecting colistin resistance in clinical *A. baumannii* strains.

This method may offer the potential for speeding up diagnostics, provided that it may be made available as an IVD device in the future.

PS01.031

Sore throat with septic complications – a case report

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We report on a 25-year-old patient who was referred to us with a suspected bronchopulmonary infection. He complained of progressive exertional dyspnea and left-sided chest pain that was depending on breathing. In his medical history, he had had a pharyngitis a few weeks earlier. Laboratory tests indicated an inflammation. The chest X-ray was unremarkable; however, computer tomography revealed multiple abscess-forming pulmonary lesions with left-sided pleural effusion and suspected gas infiltrations in the twelfth thoracic vertebra. An additional magnetic resonance imaging of the spine showed a spondylitis. With no microbiological pathogen detected in blood, urine, sputum, effusion punctate, and bronchoalveolar lavage – blood cultures had been taken only after established antibiotic therapy – a DNA analysis using Next Generation Sequencing (NGS) was performed to detect bacterial DNA in the patient"s blood. Here, small DNA amounts of Fusobacterium necrophorum were detected, a typical pathogen for abscess-forming oropharyngeal or dental infections. Further specific diagnostics revealed thrombosis of the left internal jugular vein as well as two infected molars in

the right lower jaw and left upper jaw. The findings were consistent with Lemierre"s syndrome, a rare disease primarily affecting young adults, characterized by septic thrombophlebitis following acute infections in the oropharyngeal area and septic-embolic foci, especially in the lungs. The anaerobic gram-negative pathogen Fusobacterium necrophorum can usually be detected. Getting back to our patient, antibiotic therapy, video-assisted thoracoscopy with empyema evacuation, dental restoration, and full anticoagulation for jugular vein thrombosis were performed therapeutically. He was discharged after three weeks of inpatient treatment. In our case, the infection focus of the already at admission present septic condition initially remained unclear. Complicating matters, we couldn"t detect a pathogen by means of traditional microbiological diagnostics. Ultimately, only molecular pathogen diagnostics, in our case by sequencing bacterial DNA from the serum, revealed a bacterium that provided crucial information about the sepsis focus. This allowed for further diagnostic and therapeutic steps to be initiated in a timely manner potentially averting a severe course of the disease. The described case illustrates the importance of careful microbiological sampling and the potential of molecular pathogen diagnostics.

We published this case in the journal "Die Innere Medizin" on September 24th in 2024 (volume 66, pages 129-131).

PS01.033
Towards a pragmatic diagnostic workflow for the detection of hypervirulent *Klebsiella pneumoniae* in routine diagnostics

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Introduction: Hypervirulent *Klebsiella pneumoniae* (hvKp) are associated with pyogenic, community-acquired infections at multiple sites in otherwise immunocompetent patients. An increasing dissemination of hvKp with and without carbapenem resistance is observed worldwide, but effective surveillance in the hospital setting are mostly lacking. Therefore, screening strategies are becoming increasingly important to implement effective infection prevention and control (IPC) measures in hospitals, as well as to guide the clinical management of patients with hvKp.

Aims: Our main goal of this pilot study is to establish a pragmatic diagnostic workflow in our routine microbiology laboratory for the detection and surveillance of hvKp.

Methods: We introduced a step-by-step diagnostic procedure into the routine microbiological diagnostics at Muenster University Hospital in October 2024. First, all *K. pneumoniae*, excluding those isolated from multidrug resistance screening, are screened using the string test. A positive result is indicated by the formation of a viscous string stretching from the bacterial colony to the loop measuring ≥5 mm. String-positive *K. pneumoniae* are subjected to whole genome sequencing (WGS) to detect five genotypic virulence markers (*peg-344*, *iroB*, *iucA*, *rmpA* and *rmpA2*) that are strongly associated with the hvKp phenotype (AUC 0.962) (*1*). HvKp classification was based on all five biomarkers being present. The remaining isolates were classified as classical *K. pneumoniae* (cKp).

Results: In total, 232 patients (55% male, 10/2024-4/2025) with a *K. pneumoniae* infection were screened with the string test. Of these, 24 isolates (10.3%) were string-positive, of which ten isolates (42%) were classified as hvKp by WGS. The proportion of hvKp compared to cKp was highest when isolated from deep-seated abscesses (5 (100%) vs. 0 (0%)), and blood culture (1 (100%) vs 0 (0%)), followed by respiratory specimen (3 (50%) vs. 3 (50%)) and urine (1 (8%) vs. 11 (92%)). All hvKp showed the expected phenotype for *K. pneumoniae* (ampicillin resistant), but none of the isolates acquired carbapenem resistance.

Conclusion: The predictive significance of the string test depends strongly on the source of the *K. pneumoniae* isolation. A positive string test in *K. pneumoniae* isolates from invasive specimen is highly suspicious for hvKp. Hyperviscous phenotypes (string-positive) from urine are rarely associated with hvKp. As part of the diagnostic workflow, WGS can make a diagnostic contribution to the discrimination of hvKp from cKp.

1. T. A. Russo *et al.*, Differentiation of hypervirulent and classical Klebsiella pneumoniae with acquired drug resistance. *mBio* **15**, e0286723 (2024).

PS01.035
Pseudo-outbreak with *Mycobacterium chelonae* in a microbiological laboratory: lessons learned

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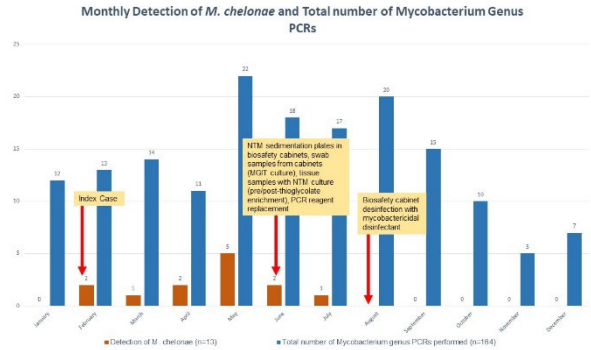
Background: *Mycobacterium chelonae* is a rapidly growing non-tuberculous mycobacterium that belongs to the *Mycobacterium chelonae-abscessus* complex. *M. chelonae* is associated with skin and soft tissue infections, surgical site infections, cosmetic interventions and tattooing. (Pseudo-) outbreaks with *M. chelonae* were associated with contaminated cardiac valve bioprotheses or bronchoscopes. In 2024, we sporadically detected *M. chelonae* by PCR in various clinical specimen without clinical correlation or cultural growth. Here, we report on a pseudo-outbreak of *M. chelonae* most likely arising from contaminated surfaces in our microbiology laboratory.

Methods: We conducted a descriptive epidemiological study to investigate a pseudo-outbreak of *M. chelonae* from February to August of 2024. A positive case was defined as the detection of *M. chelonae* from tissues samples and aspirates using an in-house mycobacterium genus PCR targeting the 16S rRNA gene with subsequent Sanger sequencing for species identification (BLAST database). In the absence of any epidemiological links between the cases, we suspected an environmental source of *M. chelonae*. An interdisciplinary outbreak team was formed to coordinate the following investigations: Non-tuberculous mycobacteria sedimentation plates (NTM) were placed under the biosafety cabinets, swabs were taken from biosafety cabinets and screened for *M. chelonae* by culture (MGIT), additional NTM plates were used for *M. chelonae* culture of routine tissue samples before and after enrichment in Thioglycolate broth and PCR reagents were replaced to assess possible reagent-related contamination. As an intervention, we changed the weekly routine cleaning of biosafety cabinets to a mycobactericidal disinfectant (ULTRASOL Oxy Wipes) once a week instead of a nucleic acid disinfectant.

Results: The suspected index case presented in February 2024 with a skin and soft tissue infection. Here, *M. chelonae* was detected by PCR and culture. Between February and August 2024, 115 mycobacterial genus PCRs were performed on clinical samples. A total of 13 *M. chelonae*-positive samples (11.3%) were considered part of the pseudo-outbreak. None of the environmental investigations detected *M. chelonae*. After implementing routine cleaning of the biosafety cabinets using a mycobactericidal disinfectant in August 2024, no further *M. chelonae* was detected.

Conclusion: After changing the biosafety cabinet disinfection protocol — from a nucleic acid disinfectant to a mycobactericidal agent — our mycobacterial species PCR no longer detected any *M. chelonae* contamination in tissue samples and aspirates. *Mycobacterium chelonae-abscessus* complex species are common in the environment and persist well on surfaces. Using a surface disinfectant with mycobactericidal activity after i.e. spills with NTM samples or periodically, is an easy measure to prevent laboratory contaminations.

Fig. 1



PS01.037
Microfluidic RT-PCR platform for rapid detection of uropathogens in urinary tract infections
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Urinary tract infections (UTIs) are among the most frequent bacterial infections worldwide, with an estimated 50–60% of women affected at least once in their lifetime (1). Microoorganisms responsible for UTIs, e.g. *E. coli*, can travel from the bladder to the kidneys via the ureters, potentially leading to pyelonephritis. This ascending infection represents a significant clinical concern, as it may result in severe complications and ultimately impair kidney function if not promptly diagnosed and appropriately treated (2).

In light of rising antimicrobial resistance, the need for rapid, reliable, and targeted diagnostic tools has become increasingly urgent. Conventional methods such as urinalysis and urine cultures are considered gold standard but are often time-consuming and not suitable for immediate, point-of-care diagnostic. To address these limitations, we developed a lab-on-a-chip system for the direct detection of UTI pathogens using probe-based real-time PCR. This microfluidic platform enables the identification of Gram-positive and Gram-negative bacteria, as well as *Candida albicans*, directly from urine samples without the need for enrichment or culture steps.

The chip integrates several key functions: sample filtration, ultrasonic lysis of microorganisms, automated fluid handling, and real-time PCR detection. After the pathogens are captured and lysed on the chip, the extracted nucleic acids are mixed with a RT-PCR master mix and transferred into a reaction chamber containing lyophilized, pathogen-specific primer-probe mixes. Fluorescent signal generation is enabled through a FAM-quencher system, allowing for specific and sensitive detection of target DNA sequences.

Primer-probe sets were designed based on the genomic sequences published by Fukumoto et al. (3) and Enderle et al. (4), and validated using genomic DNA from Gram-positive and Gram-negative bacteria as well as fungi. The assay achieved high specificity, detecting all target organisms except *Staphylococcus saprophyticus*. No significant cross-reactivity was detected under the tested conditions. Primer efficiency ranged between 91.2% and 114.1%, with reliable detection limits down to 4×10^{-5} ng DNA per reaction for *E. coli*, *P. mirabilis*, and *P. aeruginosa*.

This diagnostic platform offers rapid turnaround times and high analytical sensitivity, making it particularly suitable for point-of-care applications in clinical and outpatient settings.

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PS01.039
Investigation of different methods for identification of *Escherichia hermannii*
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Introduction: The species *Escherichia hermannii* (*Atlantibacter hermannii)* was first described in 1982. The main reservoir of *E. hermannii* is the environment (e.g., in drinking water). However, there are isolated reports of infections caused by *E. hermannii*, suggesting that it is a rare opportunistic pathogen. In order to clarify the pathogenicity and clinical significance of a rare human pathogenic bacterium, it is always necessary to first create a clearly defined collection of clinical strains of the respective species. Since these isolates originate from microbiological routine diagnostics, it is therefore important to determine whether

there are routine diagnostic procedures that can be used for reliable identification of *E. hermannii*.

Methods: In this study, a total of eight isolates were examined, which were primarily identified as *E. hermannii* in routine diagnostics at the Institute of Medical Microbiology and Virology, University Hospital Carl Gustav Carus Dresden. First, the genomes of the isolates were obtained using Illumina sequencing technology, and the identity of the species was confirmed using the Type Strain Genome Server (TYGS). The identity of all isolates was then re-examined using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and sequencing of the 16S rRNA gene.

Results: The species *E. hermannii* was confirmed in all isolates using the (TYGS), which uses digital DNA-DNA hybridization (dDDH) for species identification. The results of MALDI-TOF MS also showed identification with a score value of over 2.0, confirming the species. Sequencing of the 16S rRNA gene also showed an identity of 99.87 to 100% when compared to sequence of the type strain at the National Center for Biotechnology Information (NCBI) database. This also indicates a secure species identification.

Conclusion: The results presented here demonstrate that MALDI-TOF MS and sequencing of the 16S rRNA gene are suitable for identification of the species *E. hermannii*. However, MALDI TOF MS is significantly easier to use, easier to integrate into the workflow, and cheaper for conducting analyses than sequencing of the 16S rRNA gene. Therefore, this method is preferable. However, it should be noted that the results presented here are preliminary in nature due to the small number of strains. For this reason, our results must be supplemented by further investigations in the future.

PS01.041

In silico investigations into the virulence of _Hafnia paralvei_

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Introduction: The bacterial species *Hafnia paralvei* belongs to the *Hafniaceae* family within the *Enterobacterales* order. It is closely related to *Hafnia alvei*. Since it is challenging to clearly distinguish between the two species in routine diagnostics, it is difficult to make statements about the prevalence or differences in virulence compared to *Hafnia alvei*. This abstract therefore presents *in silico* results on *Hafnia paralvei* that provide valuable insights into the virulence properties of this species.

Methods: In the present study, isolates were examined that were initially identified as *Hafnia alvei* in routine diagnostics. The genomes of the isolates were obtained using Illumina sequencing technology and a total of 26 isolates were then clearly confirmed as *Hafnia paralvei* via the Type Strain Genome Server (TYGS). Afterwards, the Virulence Factor Database (VFDB) was used to search for the presence of virulence genes.

Results: The analysis identified genes responsible for motility (*flgB)* and chemotaxis (including *cheB*, *cheD*, *flgB*), stress resistance (including *clpP),* modulation of the immune system (including *galE, rafE),* magnesium transport (*mgtB*) and adherence (including *pilW*). *Hafnia alvei* and *Hafnia paralvei* employ different mechanisms for iron uptake: *H. alvei* utilizes the *chu* and *shuS* genes, whereas *H. paralvei* carries the *shuA* gene in certain cases. *H. paralvei* has a type VI secretion system (T6SS) with the *hcp2* and *vipB-mglB* genes. The *hcp2* gene has also been found in *H. alvei*. However, the *vipB-mglB* gene is exclusively present in *H. paralvei*.

Conclusion: *In silico* analyses of virulence genes can provide valuable initial information about the pathogenicity of a species that has hardly been studied before. For this reason, results such as those presented here are valuable and can serve as an important basis for further investigation and research into rare or little-studied human pathogenic species.

PS01.043

Development of a rapid test for the detection of antibiotic resistant _Neisseria gonorrhoeae_

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The WHO estimates that *Neisseria gonorrhoeae* (NG) is the third most common sexually transmitted infection in the world, with approximately 106 million new infections annually. Detection of NG is performed by time-consuming and expensive real-time-PCR-analysis, requiring specialised equipment and skilled personnel. Hence, there is an unmet medical need for the development of a rapid and easy-to-use antibody-based Lateral Flow Assay (LFA), performed directly by the attending physician. Tests of this type already exist for NG, but they usually have low specificity or sensitivity, cannot detect antibiotic resistance, and are not approved for routine diagnostics.

To identify better target antigens for NG detection by LFA, whole-genome-sequences of 70 NG strains were analyzed and screened for conserved genes. Out of 1567 alleles, 88 ORFs with 100% identity were determined. Of those, the most highly expressed ORFs were identified by qRT-PCR against a collection of unrelated NG strains with distant relationships (based on core-genome MLST analysis). The genes were ranked and five were selected, cloned and expressed in *E. coli* pET29b-expression system. Mice were immunized with these recombinant antigens and monoclonal antibodies (moabs) against each of these target proteins were generated by hybridoma technology. These moabs will be screened for binding to the endogenous NG protein and ultimately one antibody pair has to be selected to bind to one NG antigen in the NG-LFA.

Antibiotic resistance in NG against penicillin, quinolones, cephalosporins, tetracyclines, and aminoglycosides is based on single amino acid mutations in the *gyrA, penA, mtr, norM*, or *penB* genes. Based on Wang et al. (2020), a novel LFA format will be developed to detect these mutations in the corresponding mRNAs by using single-stranded DNA sequences immobilized in a line on the LFA strip. The mRNAs of the lysed bacterial isolate to be tested form a heteroduplex strand with the complementary DNA, which is then detected

by a gold-conjugated monoclonal antibody (mAb S9.6, Bou-Nader et al. 2022) and thus displays a visible line when the target mRNA is present. The DNA-RNA hybridization conditions must be combined with the antibody-based detection of the selected NG antigen so that only the mRNAs of mutated target genes bind, thus indicating "resistance." Due to the simple and rapid adaptation of the DNA sequence to be immobilized, new mutations or variants can be quickly included in the heteroduplex LFA test, thus offering a high degree of flexibility.

Our approach will allow a fast and specific detection of NG combined with its antibiotic resistance determinants by using an affordable LFA. The implementation of antimicrobial resistance (AMR) in the NG-LFA will ensure appropriate treatment of patients with effective antibiotics and prevent AMR development against last-resort antibiotics.

PS01.045

Pilot study for the differentiation of *Clostridium botulinum Group I and Clostridium sporogenes* using *MALDI-TOF MS* and unsupervised machine learning

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Clostridium botulinum Group I and *Clostridium sporogenes* are phylogenetically closely related anaerobic spore-forming bacteria. Accurate differentiation between these species is essential for clinical diagnostics and food safety.

We analyzed a collection of *C. botulinum* Group I and *C. sporogenes* strains using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Proteomic data were processed and analyzed using unsupervised machine learning techniques, using a consensus feature selection strategy as described by Dematheis *et. al*. (2022) for dimensionality reduction and peptide marker identification.

The analysis revealed distinct clustering patterns, allowing for successful differentiation between the two species based on their proteomic profiles. However, attempts to classify strains based on botulinum neurotoxin (BoNT) type were unsuccessful, likely due to the absence of toxin expression under standard culture conditions. To address this limitation, we tested various liquid and biphasic media optimized for BoNT production, aiming to induce toxin expression and identify associated peaks in the MALDI-TOF spectra.

These preliminary findings demonstrate the potential of combining MALDI-TOF MS with unsupervised machine learning for the differentiation of closely related *Clostridium* species. Further optimization of culture conditions is necessary to enable the detection of BoNT associated peaks at the proteomic level.

PS01.047

Towards routine implementation: standardizing agar-based phage susceptibility testing

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Introduction: Bacteriophages are promising to target multidrug-resistant infections, with a personalized approach preferred due to their high specificity. Therefore, reliable phage susceptibility testing is essential. Recognized reference methods such as spot test and double agar overlay assay lack standardization. To address this, we developed a standardized agar-based phage susceptibility test (PST) for use in routine diagnostic microbiology laboratories [1, 2].

Aims: This study evaluates a standardized agar-based phage susceptibility test in routine diagnostics. A nationwide blinded inter-laboratory comparison of PST assesses the method"s reproducibility, reliability, and applicability across labs.

Methods: The evaluation of standardized phage susceptibility testing was carried out successively. In the first stage, an in-house approach was employed to assess diagnostic applicability and reproducibility. Intra- and inter-assay variability was evaluated by 28 phage-testing-inexperienced staff members within our routine diagnostic setting. Following protocol optimization, the second stage involved a blinded, nationwide inter-laboratory comparison. The standardized protocol was distributed to 12 diagnostic laboratories across Germany, which conducted both intra- and inter-assay testing.

Results: So far, the in-house approach from the first stage demonstrated excellent inter- and intra-assay consistency, indicating that the test is highly feasible and can be executed reliably by all participating staff. False-positive results (2.46%) were more frequent than inconclusive results (1.26%) and false negatives (0.30%). Notably, inconclusive and false-negative results occurred predominantly on the second day of testing, potentially due to contamination of the phage suspension by individual handling errors. Structural methodological issues were addressed and resolved through protocol adjustments to prevent such occurrences in the second evaluation phase. **Conclusion**

Our proposed standardized PST shows good applicability and reproducibility in routine diagnostics, even when performed by phage-testing inexperienced academic or technical staff. Initial results highlight the importance of protocol compliance and contamination risk in high-titer phage lysates. Ongoing inter-laboratory test data of the second stage will further validate the robustness and standardization potential of the method.

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PS01.049

Investigation of different methods for identification of *Kerstersia gyiorum*

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Introduction: The bacterial species *Kerstersia gyiorum* was first described in 2003. *K. gyiorum* is a gram-negative proteobacterium of the class *Betaproteobacteria*, which grows under aerobic conditions singly, in pairs or in short chains. *K. gyiorum* have been isolated in humans from stool, sputum and leg wounds. However, today there are only few case reports available that underscore the pathogenic potential of *K. gyiorum*. For this reason, it needs to be regarded as a rare pathogen. In order to determine the pathogenicity of a rare pathogenic species, a collection of clearly defined isolates must first be compiled. Since the strains originate from routine clinical diagnostics, it is therefore important to know whether there is a suitable routine method for reliably identifying the species that can be applied to build up a large strain collection.

Methods: For this study, a collection of 15 isolates was used, which were primarily identified as *K. gyiorum* at the Institute for Medical Microbiology and Virology (Carl Gustav Carus University Hospital, Dresden). The complete genomes of these strains were assembled using Pacbio sequencing technology. The species was then confirmed using the Type Strain Genome Server (TYGS), which uses dDDH for strain identification. Afterwards the strains were analysed by MALDI TOF MS, VITEK 2 and sequencing of the 16S rRNA gene. Finally, the results obtained by routine methods were compared to the TYGS results.

Results: A total of 14 isolates were securely identified with dDDH values between 80.7% and 91.5% and thus confirmed as *K. gyiorum*. One isolate (DSM 105837), however, was determined to have a value of 29.4, suggesting that this is a species that has not yet been described. Using MALDI TOF MS analysis, all isolates except DSM 105837 showed score values above 2.0 and thus were identified as *K. gyiorum*. DSM 105837 however was identified with a score of 1.94 on genus level. Sequencing of the 16S rRNA gene revealed that it is not possible to reliably distinguish between *K. gyiorum* and *Bordetella* spp. When looking at the VITEK 2 results, it became obvious that identification was not possible in five cases. Furthermore, *Pandoraea* spp. was identified as excellent in four cases and as *Pseudomonas fluorescens* in four cases. In one case, *Pseudomonas alcaligenes* was given as the result.

Conclusion: An identification strategy based on the evaluation of whole genomes, such as dDDH, is currently the best option for reliable identification of a bacterial species and should therefore be used for as a reference for method comparisons in bacteriological diagnostics. MALDI TOF MS appears to be the most suitable method for identifying *K. gyiorum* in routine diagnostics. This is particularly evident in the fact that the species, which has not yet been described, could not be reliably identified at the species level, but only at the genus level. In contrast, the sequencing of the 16S rRNA gene and VITEK 2 must be considered unreliable.

PS01.051
Enabling rapid treatment of urinary tract infections using a Raman-spectroscopy-based assay

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In a world where antibiotic resistance has already reached a threatening level for global health, it is of great importance to develop and implement user-friendly and reliable point-of-care tests (POCT) that doctors can use in their daily work. Our team at the Leibniz-IPHT is working on a chip-based testing method that not only allows the identification of bacterial species causing urinary tract infections but also enables the assessment of the effectiveness of various antibiotics on these bacteria.

The current development phase focuses on simplifying the preprocessing steps required in a laboratory for such an analysis to the point where highly specialized personnel are no longer necessary. The ultimate goal is to enable on-site antibiotic susceptibility testing to allow timely and targeted therapy for each individual patient.

The identification of bacterial species and information about resistances is achieved through the use of Raman spectroscopy, a promising phenotypic method that offers both high sensitivity and specificity, making it a powerful tool for medical diagnostics. An important aspect of developing innovative Raman applications is the choice of materials for the sample preparation platform. To obtain high-quality spectra, we have chosen metal-coated filter substrates to minimize background interference. This enables Raman spectroscopic analysis to be carried out directly on the chip, avoiding additional handling time.

Figure 1. Schematic representation of the workflow for the proposed antibiotic susceptibility test with Raman spectroscopic readout and example spectra of *E. coli* measured on the newly developed Raman substrate.

Acknowledgement:

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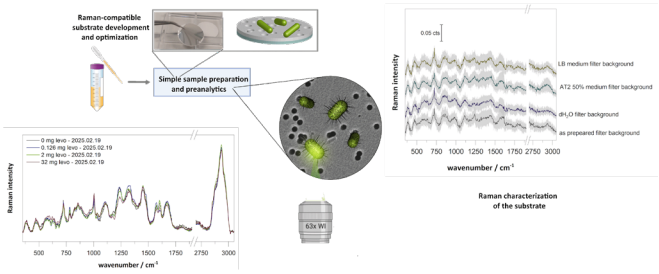
Fig. 1



PS01.053
From biomaterials to biofilms: unlocking new applications for the "ClicKit-Well" In Vitro test platform

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Introduction: Reliable, standardized in vitro testing is essential for evaluating biomaterials and surface-functionalized (implant) materials and for understanding their interaction with biological tissues. The "ClicKit-Well", a patented test system [1] that creates standardized, fluid-tight wells directly on solid biomaterial surfaces, has proven its

value in cell-based assays. However, an equally pressing need, highlighted by the COVID-19 pandemic, exists in the field of microbiology: the standardized testing of antimicrobial surfaces. Here, we present our strategy to expand the "ClicKit-Well" platform into this domain, develop a licensing model, and scale production to enable market access.

Materials and Methods: Originally developed within the VIP+ project "UniBioface" (Aug 2022 – Jul 2025), the "ClicKit-Well" allows for fluid-tight well formation on various materials, mimicking conventional multiwell plates. Initial validation focused on ISO 10993-5 compatibility, material stability, and reproducibility of assay results.

In the current "TRACe-M" project (2025–2026), supported by the Leistungszentrum Smart Production and Materials, the platform is being adapted for microbiological applications:

- Identification of testing requirements via industry and lab surveys
- Usability testing for microbial assays
- Establishment of biofilmmass assay (Crystal violet staining) combined with a biofilm viability test within the "ClicKit-Well" and comparative evaluation with standardized tests conducted by accredited testing laboratories
- Design-for-manufacturing studies for large-scale production

Results: The "ClicKit-Well" system has successfully fulfilled all functional requirements for standard cytocompatibility and cell-based assays. Additionally, a biofilmmass assay was conducted within the "ClicKit-Well" to evaluate its suitability and reproducibility for microbiological applications. Building on this foundation, ongoing usability evaluations with microbiology partners are assessing the system"s performance in microbial assay contexts. Initial findings confirm:

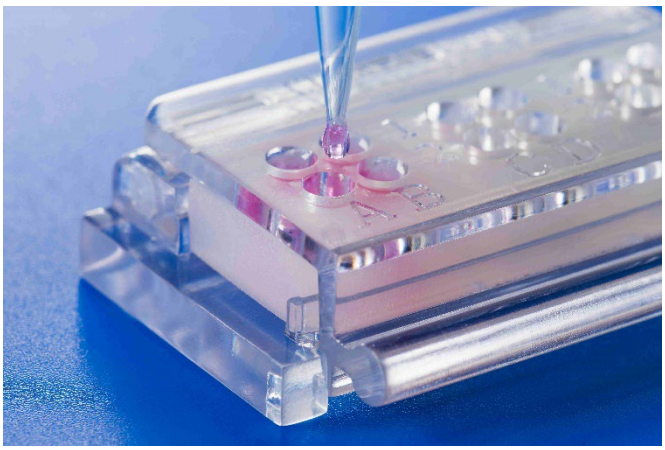
- Suitability of geometry and sealing for bacterial suspensions
- High potential for inter-laboratory comparability
- Clear demand for a commercially available testing solution

Conclusions: Originally developed and validated for biomaterials testing, the "ClicKit-Well" platform is now being strategically extended to address the growing demand for reproducible in vitro testing of antimicrobial surfaces. Supported by IP protection and a high TRL, current efforts focus on targeted market communication, usability validation, and licensing in combination with injection-molded mass production. This approach bridges the gap between academic innovation and industrial implementation – transforming a validated laboratory tool into a scalable, standardized testing solution.

References

[1] Issleib C. et al., 2020, Patent: "Arrangement For Performing In Vitro Biocompatibility Tests", DE102018221415

Fig. 1



PS01.055

Digital droplet-based reporter assays: approaches for photonic characterization of infection

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Droplet-based microfluidics [1, 2] offers an innovative approach for detecting pathogenic bacteria. It enables the quantification and differentiation of infection-relevant microorganisms within complex samples, aiming at improved diagnostic sensitivity and specificity.

Within the framework of the Leibniz Center for Photonics in Infection Research (LPI), a microfluidic chip, that provides droplet generation, incubation and analysis of digital microbial single-cell reporter assays, was developed. Parallel generation and monitoring of droplets inoculated with single cells or small populations of pathogens is realized. The assays exploit chromogenic and fluorogenic reporter substrates to detect enzymatic and metabolic activities. In addition, pathogen-drug interaction assays are performed to investigate the interactions between microorganisms and therapeutic substances.

Independent growth of pathogenic strains in individual droplets (~35 pL) is enabled as well as quantitative evaluation by optical image processing. In addition, the selectivity and specificity can be variably adjusted by the choice of reporter substrate. The microbiological digital droplet reporter assay, in combination with a robust optical setup, demonstrates the potential for rapid, on-chip bacterial cultivation and screening, while increasing multiplexing capability.

Figure 1: Digital droplet–based reporter assay for viable bacteria detection. General workflow: droplet generation, incubation, storage and detection inside of one microscope slide sized microfluidic-chip.

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[2] Watterson W et al., eLife, 2020, 9:e56998

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Acknowledgements:

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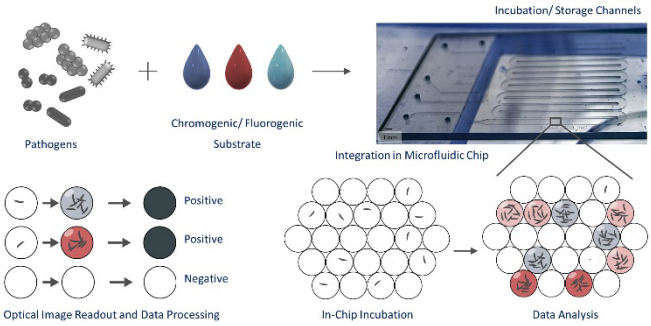
Fig. 1



PS01.057
Epidemiology and antimicrobial susceptibility of Corynebacterium aurimucosum in outpatient clinical samples (2022–2024)

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Background: *Corynebacterium aurimucosum* is a non-diphtheric species of Corynebacterium and part of the human skin and mucosal flora. In routine microbiological diagnostics, it is often considered a contaminant, and its clinical relevance remains unclear. However, case reports suggest potential pathogenicity, particularly in immunocompromised patients and in opportunistic or nosocomial infections. This study aimed to describe epidemiological characteristics and antimicrobial resistance profiles of *C. aurimucosum* isolates from outpatient clinical samples.

Methods: We conducted a retrospective descriptive analysis of *C. aurimucosum* isolates collected from outpatients in the Rhine-Ruhr metropolitan region between 2022 and 2024. Specimens included urine, urogenital samples, wound swabs, blood cultures, and dialysates. Specimens were cultured using standard microbiology methods. Identification was performed via MALDI-TOF MS, and susceptibility testing followed EUCAST guidelines using standard disk diffusion on BD Mueller Hinton Fastidious Agar. Demographic data were extracted from the LIS MOLIS via HyBASE™. To assess differences in proportions, Pearson's chi-square test was used. A p-value of <0.05 was considered statistically significant.

Results: A total of 1,253 *C. aurimucosum* isolates were identified, with 84% from female patients and a median patient age of 55 years (IQR 32–71). Most isolates originated from urine (73%), followed by urogenital (15%) and wound samples (11%). Among 35,351 urine samples tested in 2024, 30,793 showed bacterial growth. *C. aurimucosum* was detected in 369 cases (1.2%). Among women with a positive urine test in 2024 (90%), the proportion of *C. aurimucosum* was 1.4%, compared to 0.6% among men with a positive urine test (79%) ($p<0.05$). In 2024, 29.5% of the *C. aurimucosum*-positive urine isolates were detected in pure culture or with mucosal flora only, whereas 70.5% showed co-detection with potential (13.6%) or typical (56.9%) uropathogens.

Susceptibility testing (n=281) showed high susceptibility to vancomycin (99.6%), tetracycline (91.5%), and linezolid (100%). High resistance was observed for clindamycin (96.8%) and penicillin (98.6%). Resistance to ciprofloxacin (40.6%) and moxifloxacin (44.6%) was also observed, with significantly higher rates in male compared to female patients for ciprofloxacin in urine (56% vs. 31.6%, $p<0.05$) and moxifloxacin in wound swabs (63.3% vs. 46.2%, $p<0.05$).

Conclusion: *C. aurimucosum* was regularly detected in outpatient samples indicating that it should not be routinely dismissed as a contaminant, particularly when isolated in pure culture. However, its frequent co-detection with established uropathogens highlights the importance of cautious interpretation within the clinical context. The fluoroquinolone resistance pattern and respective gender-related differences suggest potential selective pressure or strain variability and highlight the importance of targeted susceptibility testing.

PS01.059
Evaluation of Fourier-Transform Infrared Spectroscopy (FTIR) for monitoring of Carbapenemase-producing Bacteria

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Introduction: Data of the German National Reference Center (NRZ) for multidrug-resistant gram-negative bacteria show a steady increase in the number of carbapenemase-producing bacteria [1]. As carbapenemases can drastically limit therapeutic options, continuous monitoring and typing of these strains would be favorable to unveil transmission events in hospitals.

Fourier transform infrared spectroscopy (FTIR) has been emerging as a reliable, fast and routine-friendly method for strain typing, proving to work for different microbial species.

In this study, we typed all carbapenemase-producing bacteria collected over a 15-months period using FTIR.

Methods: A total of N=70 carbapenemase-producing strains from regular microbiology routine samples between January 2024 and March 2025 were analyzed: 34 *E. coli*, 25 *K. pneumoniae* and 11 *A. baumannii* isolates. In-house PCR assays comprising KPC, NDM, VIM, OXA-48 group, OXA-23 and GIM were used for the determination of the carbapenemase.

For FTIR analysis, the strains were grown on Columbia sheep blood agar for 24 h at 36 °C, in three independent biological replicates. Spectra were acquired, processed and analyzed by the IR Biotyper® system (IRBT - Bruker Daltonics, Germany), accordingly to the manufacturer"s recommendation. Similarity analysis was performed by Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA). Results of IR Biotyper were compared to epidemiological data.

Results: For the n = 34 *E. coli*, FTIR analysis showed altogether 4 clusters: Two clusters comprising samples of 2 different patients each being treated on different wards of the

same hospital. The carbapenemase matched in each case (OXA-48). In addition, there were 2 clusters each consisting of 2 patients from different hospitals of a common hospital group (Fig. 1).

For the *K. pneumoniae* FTIR also detected 1 cluster with 3 isolates – all NDM and OXA-48 positive - from different hospitals of the same hospital group. Moreover, there was 1 cluster with 2 isolates originating from the same ward.

For the *A. baumannii,* 1 cluster with 3 isolates from different wards of the same hospital was detected.

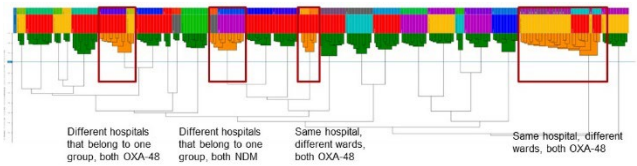
Conclusions: In this study, FTIR was used to type carbapenemase-producing strains of a 15-months period of a microbiology routine laboratory. Several small clusters within wards, hospitals or hospital groups were found, possibly indications of transmission events. Together with epidemiological data, these FTIR-based information can be used for further investigation.

The implementation of FTIR as near-time monitoring tool into routine workflows could significantly contribute to detection of transmission events and subsequent adaptation of hygiene measures to prevent further spreading of potentially life-threatening microbes.

References:

[1] Pfennigwerth et al., Epid Bull 2025;20/21:3-11, doi: 10.25646/13143

Fig. 1



PS01.061
A 3D bone model to study the pathogenesis of osteomyelitis

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Bone is a complex, highly specialized tissue that constantly undergoes remodelling throughout lifetime. Those remodelling processes are mediated by the osteogenic activity of osteoblasts on the one and osteoclastic bone resorption on the other side. At the same time, bone is very prone to injuries, mainly fractures, for which the life-time prevalence is 44 % for people above the age of 55. Additionally, the risk for fractures significantly rises in old age due to osteoporosis, so bone health plays an increasingly important role in an aging society. It is therefore important to deepen the understanding of cellular mechanisms underlying bone maintenance and the influence of external, lifestyle-associated factors like diet to be able to develop efficient therapy and prevention strategies for bone injuries.

Here, a 3D cell culture model of bone including the major bone cell types, osteocytes, osteoblasts and osteoclasts, is to be established. The model allows the cells to interact with each other under physiological conditions in a standardized system and can thereby provide an experimental platform for a diverse number of projects concerned with bone health. Starting from an experimental set-up that includes the murine cell lines MLO-Y4, MC3T3-E1 and RAW 264.7 cocultured in and on a sandwich-like collagen scaffold, the model will be advanced towards a realistic bone model for human primary cells. Methods like immunofluorescence staining, flow cytometry and cytotoxicity testing are currently implemented and optimized for combined use with the 3D model.

The model will then be integrated into already ongoing research investigating how bacterial infections affect bone cells as well as for testing and optimization of newly developed bioimplant materials that combine both antimicrobial and bone-cell supporting properties.

PS01.063
Clinical epidemiology and antimicrobial susceptibility of carbapenemase-producing enterobacterales from a German university hospital during a 5-year period

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Background: Infections caused by carbapenemase-producing Enterobacterales (CPE) represent a threat for health care systems and patients due to the limited therapeutic options and increased mortality.

Objective: The primary purpose of this study was to assess the prevalence and distribution of carbapenemase genes among screening- and clinical isolates of Enterobacterales from a German University Hospital during a 5-year observational period and to determine the antimicrobial resistance rates of CPE.

Methods: From January 2020 to December 2024, all Enterobacterales isolated from screening- and clinical samples were molecularly investigated for carbapenemases in every case of elevated minimum inhibitory concentration (MIC) of ertapenem (> 0.125 mg/l), meropenem (> 0.125 mg/l) or imipenem (> 1 mg/ml), depending on the Enterobacterales species. After detection of carbapenemases, antimicrobial susceptibility testing by broth microdilution was executed.

Results: Overall, 101 CPE were identified. The annual number of CPE detected increased from 2020 (n=5) to 2024 (n=38), as did the diversity of carbapenemase genes detected (2020, n=2; 2024, n=8). Most of the CPE strains harbored only one carbapenemase gene, such as *blaOXA-48* (n=32, 31.7%), *blaNDM* (n=27, 26.7%), *blaVIM* (n=14, 13.9%), *blaKPC* (n=8, 7.9%), and *blaOXA-181* (n=5, 4.9%). Moreover, 15 CPE carried two different carbapenemase genes. Regarding the antimicrobial resistance, only one of 30 CPE isolates tested showed resistance to aztreonam/avibactam (3.3%). In contrast, the resistance rates for cefiderocol and ceftazidime/avibactam amounted respectively to 12.8% and 53.9%.

Conclusions: The increasing incidence of CPE at our hospital is in line with the rising prevalence of CPE in Germany, which partly resulted from the admission and treatment of wounded soldiers from Ukraine. The newly available reserve antibiotic aztreonam/avibactam showed promising *in vitro* activity against CPE.

Figure 1: Distribution of different carbapenemase-patterns (one or more carbapenemase-types per isolate) among Enterobacterales isolates of this study (n=101)

Figure 2: Annual frequency (%) of carbapenemase-patterns (one or more carbapenemase-types per isolate) among Enterobacterales isolates of this study (n=101) during the observational period 2020-2024

Fig. 1

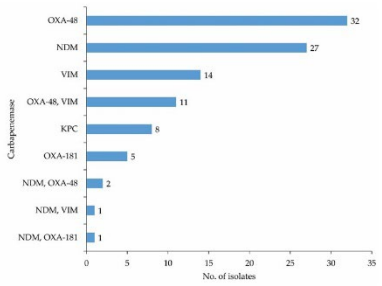
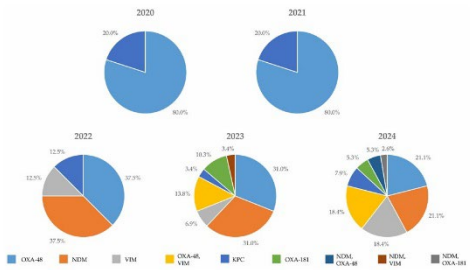


Fig. 2



PS01.065
Surveillance of bloodstream infections in a German pediatric cancer unit during a 5-year period: microbial spectrum, antimicrobial susceptibility and effectiveness of preventive taurolidine lock

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Background: Bloodstream infections (BSIs) are a major cause of morbidity and mortality in pediatric patients with cancer. A significant proportion of BSIs are associated with the use of a central venous catheter (CVC). The aim of the present study was to evaluate the spectrum and the antimicrobial susceptibility of microorganisms causing BSI in a uniformly monitored and treated cohort of patients of a pediatric cancer unit. In addition, we evaluated the effectiveness of preventive antimicrobial lock therapy with taurolidine-citrate solution in reducing BSI-rates.

Methods: We retrospectively assessed demographic, clinical and microbiological data of BSIs during a 5-year period from January 2019 to December 2023. Preventive lock treatment with instillation of taurolidine-citrate solution into the CVC was performed from January 2019 to April 2022, interrupted from May to December 2022 (only instillation of saline solution) and, after a suspected increase of the BSI episodes, reintroduced from January 2023. We also calculated the incidence rates with 95% confidence intervals (CI) for BSIs per 100 patient months comparing CVC locks with taurolidine-citrate solution vs. saline solution.

Results: In 45 patients, a total of 57 BSI episodes were recorded and 60 microorganisms (36 Gram-positive bacteria, 23 Gram-negative bacteria and 1 yeast) were cultured. The most frequently isolated bacteria were the *Staphylococcus* spp. (n = 20, 33.3 %) and *Enterobacterales* (n = 17, 28.3 %). Regarding the antimicrobial susceptibility, all Gram-positive bacteria were susceptible to vancomycin. Among the Gram-negative bacteria, only four isolates (17.4 %) showed a resistance against piperacillin/tazobactam and only one isolate a resistance against meropenem (4.3 %). Incidence of BSIs was highest from May to December 2022 (19.6 per 100 patient months; 95% CI: 11.0 – 32.4 per 100 patient months) when CVCs were locked with saline instead of taurolidine-citrate solution.

Conclusions: In this study, *Staphylococcus* spp. and *Enterobacterales* were the most frequently isolated microorganisms among pediatric cancer patients with BSI. None of the isolated Gram-positive bacteria was resistant to vancomycin, and resistance to piperacillin/tazobactam and meropenem was rare among the Gram-negative bacteria. The lock of CVCs with taurolidine apparently contributed to the prevention of BSIs in the above-mentioned patients.

PS01.067
Typhoid, paratyphoid and enteric infections between the years 1872 and 2023 in Bremen, Germany

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In the 19th century and 20th century many hygienic and public health measures were planned and implemented in Germany. Statistical and public health reporting became also mandatory. As a federal state Bremen introduced a reporting of several infectious diseases since the year 1872. This is about 30 years precedent to the German national state. Therefore, Bremen is a favourable model to study the effects of early health measures on the distribution of infectious diseases. In Bremen city the introduction of a central drinking water supply using a sand filtration was accompanied by sharply falling numbers of reported typhoid cases. Other measures were the continuous upgrade of the sewage system, food monitoring, the construction of a central slaughterhouse, the opening of public bathes and the establishment of a hygienic institute with a disinfection institution. Because the cities of Bremen and Bremerhaven were very important ports for emigration, additional measures were taken in related fields. Typhoid outbreaks were associated with flooding and contaminated milk supply. Possibly ascending case numbers were also the result of improved contact tracing. A large outbreak of paratyphoid happened in 1959/60 supposed to be related to food. Large outbreaks of dysentery happened in 1904 and 1918 at the end of World War I in coincidence with returning soldiers. The last outbreak of cholera was reported for the year 1866. Only a few cholera cases were detected during the huge Hamburg outbreak in 1892. A profound negative effect of World War I and II on the infection situation can clearly be seen, too. In the last decades only a few cases of typhoid, paratyphoid or dysentery were reported per year in maximum.

PS01.069

Deciphering the impact of gut microbiota-derived short chain fatty acids of varying chain lengths on carbapenem-resistant *E. coli*

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Multidrug-resistant (MDR) *Enterobacteriaceae* represent a significant global health threat, with intestinal members of this family frequently causing infections. In the gut, short-chain fatty acids (SCFAs)—predominantly acetate (C2), propionate (C3), and butyrate (C4)—are key microbial metabolites produced through the fermentation of dietary fibers. Less abundant SCFAs, such as valerate (C5) and caproate (C6), are generated via chain elongation pathways from precursors mentioned above.

While SCFAs are known to influence bacterial growth and physiology, the molecular mechanisms underlying the effect of alkyl chain length of SCFAs on the fitness of *Enterobacteriaceae* remain insufficiently explored. Moreover, the impact of the antibiotic resistance mechanism, particularly the loss of porins OmpC and OmpF, on responses of the respective bacteria is hardly investigated.

This research aims to explore how various length of SCFAs influence the growth of both wild-type (WT) and double knockout (DKO; ΔompC/ΔompF) gut derived *E. coli* ATCC 8739 strain and the model strain K12 MG1655 under batch culture conditions. Comparative analysis for single deletion mutant strains (either ΔompC or ΔompF) were performed as well. The molecular mechanisms underlying responses will be explored as well.

Preliminary results demonstrate that valerate and caproate exert much stronger inhibitory effects on *E. coli* growth compared with acetate, propionate, and butyrate, suggesting a chain length-dependent antibacterial activity. Transcriptomic (RNA-Seq) and proteomic profiling will be employed to uncover the gene and protein expression signatures underlying these differential responses.

The outcomes of this research are expected to advance our understanding of how SCFA structure and bacterial resistance traits interact to control survival of multidrug resistant *E. coli* in the gut. Results may assist the development of microbiota-based strategies for preventing or reversing colonization by carbapenem-resistant *Enterobacteriaceae.*

Keywords: Antibiotic resistance, gut microbiota, carbon structures, short chain fatty acids.

PS01.071

High-throughput protein microarrays as a comprehensive screening tool enabling Carbapenemase-specific POC diagnostics

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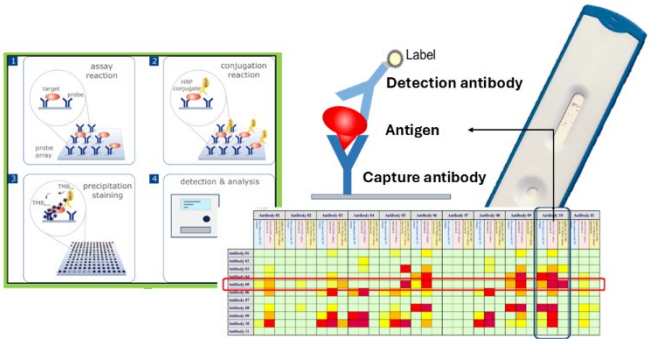
Carbapenemase-producing bacteria undermine the efficacy of carbapenems, a class of last-resort antibiotics used primarily to treat infections caused by multidrug-resistant Gram-negative pathogens. Carbapenemases are among the most alarming antimicrobial resistance mechanisms because they inactivate all β-lactam antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems—leaving clinicians with few or no therapeutic options. The genes encoding these enzymes are typically located on mobile genetic elements, which facilitate rapid horizontal gene transfer among different bacterial species. These plasmids often carry toxin-antitoxin systems that promote long-term persistence in bacterial populations, even in the absence of antibiotic pressure. Carbapenem-resistant *Enterobacteriaceae* (CRE) often colonize the gastrointestinal tract without symptoms, serving as silent reservoirs for further dissemination. Infections caused by CRE are associated with high morbidity and mortality and are frequently resistant to multiple drug classes.

Given the urgent clinical need for rapid diagnostics, immunochromatographic assays represent a promising approach for point-of-care detection. However, the development of such assays is often hindered by the time-consuming process of identifying high-affinity antibody pairs. To accelerate this process, we evaluated a protein microarray platform as a high-throughput screening tool to identify optimal monoclonal antibody (mAb) pairs targeting the most clinically relevant carbapenemases. Monoclonal antibodies derived from hybridoma libraries and commercial sources were spotted in triplicate at three serial dilutions each and tested in a single experiment against lysates from reference strains expressing KPC, NDM, IMP, VIM, OXA-23/48/58, and MCR-1 enzymes.

Signal intensities were quantified and normalised, and diagnostic performance was assessed across four thresholds. A cut-off >0.2 yielded the best balance, with approximately 61% balanced accuracy and ≥99% specificity. Around 5% of tested antibodies showed strong, reproducible reactivity. For several targets—such as KPC, IMP, VIM, OXA-58, and mcr-1—100% sensitivity was achieved. Notably, a bivalent formulation comprising the mAbs blaNDM-22_CT and blaNDM-24_CT—used together both as capture and as detection antibodies—yielded the highest sensitivity and specificity for detecting diverse NDM allelic variants. In addition, the array allowed simultaneous mapping of cross-reactivity, a key advantage over conventional ELISA workflows.

Our findings confirm that protein-based microarrays offer a robust, efficient platform for antibody pair selection, reducing reagent use while accelerating assay development. The validated antibody pairs are directly applicable to ELISA or lateral flow test formats and provide a strong foundation for next-generation diagnostics capable of detecting an evolving panel of carbapenemases in clinical settings.

Fig. 1



PS01.073
The risk to spread bacteria by shooting disposable gloves
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Question: Using medical gloves is a routine infection prevention and control measure that protects both patients and medical staff from the transmission of infectious agents. It is important to properly remove and dispose medical gloves after use to prevent contamination of the hands and surrounding surfaces. However, the practice of shooting gloves into the bin during removal can occasionally be observed, either playfully or for convenience. The objectives were to determine how often gloves are shot by medical staff and to assess, if shooting gloves contaminate nearby surfaces.

Methods: We conducted an international online survey on glove shooting to find out whether this practice is popular. In a laboratory experiment, we contaminated gloves with defined concentration (10^7 colony forming units [CFU]) of *Escherichia coli* and *Staphylococcus aureus* from the outside (to mimick a contamination with patient material) and in a separate experiment from the inside (to mimick contamination with the personal bacterial flora). Chromogenic agar plates selective for *E. coli* and *S. aureus* were placed on the ground between the shooter and the bin (150 cm distance) in a grid of 13 x 13 cm^2 sub-squares. After shooting, plates were cultured to quantify CFU.

Results: Of the 1584 respondents, 65.1% (n=1031) have seen somebody "shooting gloves" while the majority (69.8%, n= 1106) has never tried it. The majority had some concerns regarding environmental contamination by shooting gloves (arithmetic mean: 2.7 of a Likert scale from "no concerns" [1] to high concerns [5]).

In the laboratory simulation, bacterial contamination of the plates on the floor was highest where the gloves were removed. The contamination of plates (mean number of CFU/plate) was higher for *E. coli* compared to *S. aureus* when gloves were contaminated from inside (7.0 vs 2.6) or outside (14.2 vs. 4.4). A contamination of the floor near the bin was not detected.

Conclusion: Some healthcare workers know or practice glove shooting. Contrary to the reported concerns of respondents, the contamination of the floor after shooting gloves is low.

PS01.075
***Candidozyma auris*: Is it time for an admission screening in German hospitals?**
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Introduction: *Candidozyma* (formerly *Candida*) *auris* is an emerging pathogen with multiple antifungal resistances. It is linked to severe invasive infections in critical ill patients. In addition, *C. auris* is easily transmitted from patient to patient and is associated with long-lasting difficult-to-control hospital outbreaks. Screening on admission for the early detection of unknown carriers has not yet been established at our hospital (Charité – Universitätsmedizin Berlin, Germany).

Objectives: Decide on admission screening based on own data from previous *C. auris* cases at Charité and on a non-systematic literature search.

Materials & Methods: We collected data on *C. auris* epidemiology from medical records, microbiological results and infection control measures at Charité as well as from the literature. Furthermore, we conducted a pilot study on the cultural detection of *C. auris* to test our in-house diagnostic procedures. We used samples from patients sent to our laboratory for the screening of multidrug-resistant gram-negative bacteria. The samples were inoculated onto CHROMagar™ Candida Plus plates (Mast) and incubated at a temperature of 37 °C for 48 hours.

Results: Since 2021, when the first patient with *C. auris* was admitted to Charité, a further nine *C. auris* cases have been identified in our hospital. Of these 10 patients with *C. auris*, seven had an infection, while three were colonised. Seven patients were already infected or colonised by *C. auris* at the time of admission. Prior to admission, all of these seven patients had received antimicrobial therapy, five of whom (71.4%) had been treated at a healthcare facility abroad. A total of three patients acquired *C. auris* as a result of transmission events at Charité. These transmissions occurred before *C. auris* in the index cases had been diagnosed. *C. auris* was frequently detected in skin swabs, regardless of whether the patient was infected or colonised. Between September 2024 and January 2025, 3,079 samples from 1,351 patients were tested for *C. auris* in the context of our pilot study. Of these, 60% were rectal swabs, 19% were wound swabs, and 17% were swabs of the nose and throat. No *C. auris* cases were diagnosed.

Conclusion: Even though no cases of *C. auris* were identified, the pilot study demonstrated that the procedures in our laboratory are straightforward. Based on our findings and data from the literature, we will now start screening patients, who have previously received treatment at a healthcare facility abroad, for *C. auris* upon their admission to Charité. The aim of this screening is to detect unknown carriers as soon as possible and avoid transmission. For screening purposes, skin swabs (including from the axilla, groin and navel) and wound swabs are tested for *C. auris* at our laboratory.

PS01.077
Emergence of antibiotic-resistant bacterial strains: insights from quantum mechanics
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Microbial resistance to antibiotics has reached crisis stage; the effectiveness of current strategies for preventing or managing the emergence and spread of infections caused by antibiotic resistant bacteria is limited. It has been theorized that quantum mechanics may shed light on the origin and mechanism of microbial drug resistance. In this presentation, the advances and challenges in the field of quantum biology—especially in the context of pathogenic microbes are discussed. A new approach is proposed to determine if, indeed, quantum phenomena are relevant in microbiology and useful in the ongoing work to address microbial resistance. Information plays a key role in biology, microbiology, and quantum mechanics. The microbial genome encodes information about the surrounding, which allows bacteria to exploit environmental resources and protect against external

adverse effects, such as antibiotics. Quantum systems, e.g., molecules in a quantum state, show matter-wave behavior, superposition, and environment-related information flow; as emphasized by Anton Zeilinger, the quantum system is nothing other than the consistently constructed referent of the information represented in the quantum state. Quantum mechanical phenomena, such superpositions (each of the components of a quantum system is simultaneously present), may be relevant in the flow of information between bacterial components and antibiotic exposure, resulting in changes in the bacterial genome. However, there is as yet no direct experimental evidence of molecular matter-wave behavior of bacterial components. One reason for this may be that quantum systems entangle rapidly with environmental components, resulting in "decoherence" (transition from quantum to classical state). Only the observed outcome of an intracellular quantum event could serve as evidence of quantum mechanical processes at the cellular level. The extremely short time period, i.e., femtoseconds, in which coherence is maintained at the cellular or molecular level, is the challenge for researchers attempting to discover quantum states in microbial populations. Although there are as yet no published evidence of quantum processes in the development of bacterial resistance, this does not rule out the possibility, once the problem of decoherence and extremely brief time frames can be resolved. Solving the "arms race" of drugs and microbes by shifting to a more predictive approach may lead to breakthroughs in the problem of microbial resistance. Reference Bertlmann R A, Zeilinger A (eds.) Quantum [Un]speakables. Springer 2002, p. 252 Keywords: microbial resistance; quantum microbiology, quantum coherence, quantum-mechanical superposition

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PS01.079

Mind the Gap(N): GapN as a potential novel antimicrobial target in *Streptococcus pyogenes*

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In 2024, macrolide-resistant *S. pyogenes* were added to the WHO list of priority pathogens, highlighting the urgent need for new antimicrobial strategies against this important pathogen [1]. The non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase GapN is a promising antimicrobial target in *S. pyogenes* [2]. As these bacteria lack the oxidative branch of the pentose phosphate pathway, GapN serves as the main NADPH-producing enzyme [3].

In this study, potential competitive inhibitors were identified by *in silico* docking of compound libraries against the substrate-binding pocket of GapN using a homology model of the tetrameric holoenzyme as the structural basis.

Among the screened compounds, glyoxal bisulfite exhibited the strongest inhibition of GapN activity *in vitro*. It also showed dose-dependent impairment of GapN and markedly increased the Km for its natural substrate glyceraldehyde 3-phosphate, indicating a competitive inhibition.

Complementary to this, fragment-based crystallographic soaking experiments revealed that the low molecular weight compounds pyrimidine-5-amine and 4-hydroxypyridazine bind in the NADP(H)-binding pocket of GapN. When applied to the purified GapN enzyme *in vitro*, both fragments caused an impairment and revealed additive or slight synergistic effects when combined with glyoxal bisulfite. When assessed in *S. pyogenes* survival assays, the combination of glyoxal bisulfite and 4-hydroxypyridazine led to accelerated bacterial killing, suggesting a potentiated antimicrobial effect.

Taken together, our results demonstrate glyoxal bisulfite - alone or in combination with cofactor-binding fragments - as a promising starting point for the development of specific GapN inhibitors with antimicrobial potential.

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PS01.081

Comparison of broth microdilution and agar diffusion for antimicrobial susceptibility testing of *Granulicatella adiacens*

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Questions: *Granulicatella adiacens* is gram-positive coccus formerly classified as nutritionally variant streptococcus. It is part of the normal oropharyngeal flora, but can also cause infections (e.g. bacteremia, endocarditis). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) does not provide clinical breakpoints neither for minimal inhibitory concentration (MIC) nor for inhibition zone diameters for *G. adiacens*. The objectives of this study were to estimate susceptibility rates using Clinical and Laboratory Standards Institute (CLSI, M45) MIC breakpoints for clinical *G. adiacens* isolates and to correlate MIC and inhibition zone diameter.

Methods: We tested 34 clinical isolates from three laboratories in Germany. For broth microdilution, we used commercial 96-well plates following the manufacturer"s instruction (Merlin, Bruker, Bremen, Germany). The MIC was interpreted with clinical breakpoints from CLSI M45 as EUCAST does not provide clinical breakpoints for *G. adiacens* yet. For agar diffusion, we followed modified EUCAST methodology using chocolate agar (e.g. instead of Mueller-Hinton Agar ± defibrinated horse blood) but used antibiotic disks with concentrations recommended by EUCAST.

Results: All isolates were susceptible to ceftriaxone, imipenem, meropenem, and vancomycin when using CLSI breakpoints (100%, n=34/34). Most of the isolates were susceptible to ampicillin (94%; n=32/34), ciprofloxacin (85%; n=29/34), penicillin, and clindamycin (both 74%; n=25/34). *G. adiacens* frequently exhibited resistance against erythromycin (56% resistant, (n=19/34)). MICs and inhibition zone diameters were correlated for benzylpenicillin and vancomycin.

Conclusions: *G. adiacens* have promising susceptibility rates to ceftriaxone carbapenems and vancomycin. Good correlation between MIC and inhibition zone diameter was observed for benzylpenicillin and vancomycin suggesting potential breakpoints for disk diffusion.

PS01.083

Prevalence and antibiotic sensitivity of bacteria on door handles in selected hospitals in Benin City, Nigeria

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Microorganisms are everywhere including the surfaces of inanimate objects and contacts with these objects transfer microbes to humans, e.g. door handles and door frames. *Staphylococcus aureus* has been evolving and developing resistance to antibiotics for years. Methicillin resistance *Staphylococcus aureus* (MRSA) has become prime nosocomial pathogens for patients in hospitals, homes and workplaces. The aim of this study was to determine the prevalence and survival of methicillin resistance *Staphylococcus aureus* and other bacteria on door handles in selected hospitals in Benin City, Nigeria. Three (3) hospitals were used for the study and the study was carried out for a period of 12 months. Swab samples were collected from the door handles of thirteen (13) sampling points of the hospitals. The samples were studied using standard bacteriological techniques and molecular biology techniques were used for further characterization of the bacterial isolates, and virulence factors of the bacterial isolates recovered from the door handles were analysed. *Staphylococcus aureus* was subjected to methicillin sensitivity test and the survival rate of the isolates on the door handles for a period of twenty – five (25) days was investigated and the transfer of isolates from door handles to humans using five (5) panellists through contact was determined. The results of the bacterial population ranged from 2.13 ± 0.08 x 105 to 6.93 ± 1.26 x 105 cfu/m2. The bacterial isolates were characterized and identified to include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus epidermidis, Streptococcus faecalis and Salmonella enterica.* The virulent factors of *Staphylococcus aureus* showed 30% formation for slime, 60% formation for haemagglutination, 60% formation haemolysis and 70% formation for capsule respectively. *Staphylococcus aureus* isolates isolated, 75 % were found to harbor methicillin resistant gene (*MecA* gene). Survival rate of *Staphylococcus aureus* and *Escherichia coli* showed counts of 6.33 ± 0.88 x 105cfu/cm2 and 11.00 ± 0.58x 105cfu/cm2 respectively on door handle after 25 days. Transfer of isolates from inoculated door handle to the fifth panelist showed that *S. aureus* and *E. coli* were 3.41 and 11.22 % viable respectively. Door handles from hospital setting were found to harbor some bacterial isolates and were resistant to some antibiotics after curing. *Staphylococcus aureus* and *Escherichia coli* were found to be viable on door handles for 25 days. Therefore, cleaning of door handles with disinfectant should be recommended, in order to preventing transmission of infections in the hospital environment and possible outbreak of diseases.

PS01.085

Awareness of antibiotic resistance across medical specialties: results from AntibioResDE, Germany 2024

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Introduction: Recognizing antibiotic resistance as a growing problem is essential to promoting good prescribing practice. Most people self-evaluate their performance as above average compared to their peers in many aspects of daily life. This estimation is important for self-reflection and to further improve practices.

Materials: We invited physicians and dentists via their specialty associations and state medical boards to participate in an anonymous online survey on Voxco© on antibiotic resistance information. The AntibioResDE survey was open from March to September 2024. Prescribers were asked to assess the following statement: “The successful treatment of bacterial infections with antibiotics will become significantly more difficult in the next 10 years” in their own specialty, in other specialties, in Germany, in Europe and worldwide. The options were likely, possible, unlikely and no opinion for each of these five settings. Using Stata™ 17, we performed univariate analysis of this statement in relation to medical specialty, years in practice and gender.

Results: A total of 2 945 physicians and dentists completed our survey; 1 387 (47%) worked in private medical practice, 1 055 (35%) worked in hospitals and 518 (18%) were dentists. Eight distinct specialties had between 55 and 518 respondents each. The majority of respondents deemed the statement that the successful treatment of bacterial infections will become more difficult in the next 10 years to be likely in all five settings, ranging from 51% within their own specialty to 67% in other specialties and from 63% in Germany to 77% worldwide. Within medical specialties, urologists were most likely to affirm this statement (68%) followed by general practitioners (62%) and internists (60%) for their own specialty and paediatricians (79%), gynaecologists (77%) and general practitioners (75%) for other specialties. Within Germany, gynaecologists (69%), paediatricians (68%) and general practitioners (68%) were the most concerned while ear-nose-throat specalists (59%) and anaesthesiologists (60%) were least concerned. Anaesthesiologists (90%) and paediatricians (88%) predicted the most difficulties within 10 years worldwide. Prescribers with fewer years of professional experience more frequently predicted issues than their colleagues with longer experience across all five settings (78% with

Fig. 1

Table 1: Percentage of respondents stating that it was likely that the "successful treatment of bacterial infections with antibiotics will become significantly more difficult in the next 10 years" by medical specialty, setting, years in professional practice and gender, AntibioResDE, Germany 2024

	own specialty (%)	other specialty (%)	in Germany (%)	in Europe (%)	worldwide (%)
SPECIALTY					
urology (n=333)	68,2	70,0	67,3	78,6	82,5
general medicine (n=377)	62,1	74,8	67,7	76,7	81,8
internal medicine (n=309)	59,9	66,0	62,9	75,2	84,3
anaesthesiology (n=74)	56,8	56,8	59,5	86,3	90,4
surgery (n=218)	53,2	65,7	60,5	72,9	79,6
gynaecology (n=55)	52,7	76,9	68,5	67,9	84,6
ear-nose-throat (n=484)	51,2	63,5	59,1	66,8	76,2
paediatrics (n=331)	49,6	79,3	67,7	82,2	88,0
dentistry (n=505)	32,3	73,8	63,2	63,0	67,3
SETTING					
hospital (n=1 025)	54,2	66,8	62,4	76,4	84,1
private practice (n=1 380)	56,5	71,4	65,0	73,7	80,2
dentistry (n=505)	32,3	73,8	63,2	63,0	67,3
YEARS IN PRACTICE					
0-4 years (n=194)	60,8	80,5	75,3	83,9	88,5
5-9 years (n=333)	51,4	71,8	62,1	74,5	81,7
10-14 years (n=432)	49,8	73,9	65,3	78,2	83,3
15-19 years (n=451)	53,2	70,3	65,1	70,6	77,6
20+ years (n=1 496)	50,1	67,3	61,8	70,1	77,1
GENDER					
female (n=1 440)	54,3	74,2	68,0	75,1	80,1
male (n=1 425)	48,8	66,2	59,6	70,8	78,6

PS01.087
Nanoscale surface modification of Titanium alloys reduces bacterial adherence without compromising osseointegration

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Biomaterials are widely used in clinical practice, but as foreign bodies, they can promote microbial colonization and biofilm formation, leading to biomaterial-associated infections (BAIs). Though relatively rare, BAIs are difficult to treat due to biofilms that shield bacteria from the immune system and antibiotics, often requiring implant removal [1,2]. With rising antibiotic resistance, there is an urgent need for strategies that both prevent and treat infections.

This project aims to reduce bacterial adherence during early stages of implant infection by modifying titanium alloy surfaces. While microscale roughness supports bone integration, it can also provide a niche for bacteria [3]. We propose using nanoscale surface roughness to discourage bacterial colonization while enhancing osseointegration.

Ti-6Al-4V samples were polished, ultrasonically cleaned, and etched in 10 mol/L NaOH at 50 °C for 5 minutes to 24 hours to produce varying nanoscale roughness. Unetched samples served as controls. After etching, all samples were rinsed, cleaned, and dried. Surface wettability was assessed via static contact angle measurements. AFM and SEM confirmed increasing surface roughness with longer etching durations.

Based on roughness (Rq), we selected three etched groups (approx. 10 nm, 20 nm, and 40 nm) plus the control. To assess bacterial adhesion, we exposed the samples to *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *and Escherichia coli* (*E. coli*), followed by Cyto9 staining and fluorescence imaging. Bacterial attachment was significantly reduced on rougher surfaces after both 1 h and 24 h incubation. We used GFP-expressing strains as a confirmation method for bacterial viabilty. We observed lower bacterial colonization on nanorough samples compared to unetched control after 1 h. We also performed SEM imaging to examine bacterial morphology and surface interaction. Bacteria strongly adhered to smoother surfaces, while adhesion was reduced on rougher ones. To evaluate cytocompatibility, we cultured human osteosarcoma cells (SaOs-2) on both etched and control samples for 24 h. IF staining was used for visualization, which confirmed robust cell attachment and spreading on nanorough surfaces.

In conclusion, NaOH-induced nanoscale roughness reduced bacterial adhesion without compromising host cell compatibility. This surface modification strategy may support the development of infection-resistant, cell-friendly implant material.

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PS01.089
Advancing targeted protein degradation strategies against bacterial pathogens

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Question: New strategies in antibiotic drug discovery are essential due to rising antimicrobial resistances and challenges in conventional drug development. Among the most critical pathogens, the ESKAPE pathogens and *Mycobacterium tuberculosis* contribute to more than five million lethal infections worldwide each year. Recently, core components of the mycobacterial protein quality control system, namely the ClpC1:ClpP1P2 system, emerged as attractive drug targets for *M. tuberculosis*. BacPROTAC antibiotics (Bacterial Proteolysis Targeting Chimeras) offer unique opportunities for drug discovery exploiting the concept of targeted protein degradation: Those bivalent small molecules hijack the bacterial proteolytic machinery and induce proximity to a drug target of interest. As a consequence, the bacterial drug target is degraded, forcing bacteria into growth arrest or cell death. We aimed to develop BacPROTACs which can target endogenous proteins of *Mycobacterium tuberculosis* and to pioneer the technology towards a broader range of bacteria including ESKAPE pathogens. In parallel, we aimed to understand the BacPROTAC"s mode of action in order to decipher bacterial self defense mechanisms and to expand the concept towards formerly undruggable drug targets.

Methods: With a combination of rational design and experimental validation, we developed Homo-BacPROTACs against the essential mycobacterial protein ClpC1. Iterations between SAR studies and activity assays *in vitro* and *in vivo* were combined with quantitative proteomics and resistance screens to optimize molecules against *M. tuberculosis* H37Rv.

In parallel, alternative ligands and activity screens against non-essential proteins paved the way towards first BacPROTACs against *Staphylococcus aureus*.

Results: Homo-BacPROTACs enable the simultaneous degradation of the essential mycobacterial protein ClpC1 together with its defence system ClpC2. The dual Clp degrader, built from linked cyclomarin A heads, was highly efficient in killing pathogenic *M. tuberculosis*, being >100-fold more potent than the parent antibiotic cyclomarin A. The next generation of binders showed an even higher potency in mycobacteria, successfully overcame resistance mutations of the first BacPROTACs and enables the development of the BacPROTAC technology towards Gram positive pathogens like *S. aureus*.

Conclusion: Overall, our data reveal Clp scavenger proteins as an important defense for protein quality control in mycobacteria and highlight the potential of BacPROTACs as antitubercular drugs. Our data indicate that the concept of small-molecule degraders can be maintained by BacPROTACs. Their mechanistic advantages over canonical occupancy-driven agents suggest this technology as a promising future antibacterial strategy.

PS01.091
Malaria in returning travelers: a risk for personal and public health in Germany? A Middle-Franconian case study

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Background: Among others, globalization is characterized by increased human travel activities due to work, vacation or visiting family and friends. Following bites by *Anopheles* (*An.*) mosquitoes in many tropical regions of the world, travelers may acquire infections with *Plasmodium* (*P.*) species, the causative agents of malaria, which can lead to severe disease and (re-)introduction of the parasites into malaria-free countries. Until the 1940s, endemic malaria occurred in Middle Franconia, Germany. In the present study, we related the prevalence of *Anopheles* vectors in the region of Erlangen and the number of *Plasmodium* infections diagnosed at the University Hospital of Erlangen (UKER), which is relevant for estimating the risk of future autochthonous transmission.

Methods: Mosquito surveillance data were provided by the citizen science project "Mückenatlas" (mosquito atlas), the German mosquito database (CULBASE) and Biogents AG. Travelers, who consulted the vaccination outpatient clinic at UKER, were questioned for their destinations. The cases of malaria diagnosed at UKER from January 2010 to March 2025 were retrieved from the laboratory and patient information systems at UKER.

Results: Three known vectors of *Plasmodium* species are prevalent in Erlangen: *An. messeae, An. maculipennis* sensu stricto and *An. plumbeus*. In 2024, travelers (n=614) were mostly planning to visit the WHO African Region (n=239, 38.9%), followed by the Region of the Americas (n=152, 24.8%) and the South-East Asia Region (n=140, 22.8%). From 2010 until March 2025, confirmed malaria cases (n=40; number of patients with initially suspected malaria: n=317) primarily resulted from travelling to Africa, especially Nigeria (n=10) and Cameroon (n=5), and were mainly due to *P. falciparum* (n=28, 70%). In 2024, two patients with *P. falciparum* infection required intensive-care treatment, with one fatal outcome. *P. vivax* and *P. ovale* accounted for only a small proportion of the malaria cases at UKER (n=7, 17.5%). One of these patients did not receive primaquine therapy due to pregnancy, leading to a recrudescence after several months. Another patient sought medical help only five months after the presumed date of infection due to an initial lack of symptoms. None of the malaria patients diagnosed took proper anti-malarial chemoprophylaxis.

Conclusions: Prior counselling as well as adequate chemo- and exposure prophylaxis are critical for preventing *Plasmodium* infections during travelling to high-risk regions. As *Anopheles* species occurring in Middle Franconia and other non-endemic areas are capable of autochthonous malaria parasite transmission, patients with subclinical, untreated or incompletely treated *P. vivax* or *P. ovale* infections may pose a risk for public health. To avoid unexpected autochthonous malaria, mosquito surveillance measures are advisable. *Plasmodium* infections need to be excluded in patients with fever after return from malaria-endemic areas.

PS01.093
Host adaption of Phage K for enhanced targeting of *S. aureus* USA300 in lung infection models

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Staphylococcus aureus (*S. aureus*) USA300 is a prominent community-associated MRSA (methicillin-resistant *Staphylococcus aureus)* strain, posing a serious public health concern due to its aggressive pathogenicity and association with necrotizing pneumonia. Bacteriophages, owing to their high host specificity and potent lytic activity, offer a promising alternative therapeutic strategy.

In this study, phage K was adapted to infect *S. aureus* USA300 through serial propagation. The resulting mutant, phage KJ25, exhibited significantly larger plaque diameters ($p < 0.001$) and greater suppression of bacterial growth over 24 hours at several multiplicities of infection (MOI 1, 0.1, 0.01) compared to the wild-type phage. While analysis of early metabolic activity indicated stronger initial lysis by phage K, KJ25 achieved superior long-term control of bacterial proliferation, suggesting altered phage tropism following adaptation.

To investigate underlying mechanisms, whole-genome sequencing of both wild-type and adapted phage, along with transcriptomic analysis of infected bacteria, was performed to identify genetic changes associated with altered host specificity. Furthermore, the efficacy of KJ25 was validated *in vitro* using A549 lung epithelial cells and *ex vivo* in a human lung tissue slice model, both showing reduced bacterial burden upon phage treatment.

In conclusion, host adaptation of phage K to USA300 yielded a novel variant, KJ25, with enhanced lytic activity and

therapeutic potential against *S. aureus* in pulmonary infection models. This work highlights the potential of phage adaptation as a strategy for developing targeted treatments for antibiotic-resistant bacterial infections.

PS01.095

Efflux activity influenced by antibiotic concentration and bacterial growth conditions: towards a quantitative link between efflux and bacterial fitness

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Efflux pumps are key contributors to antibiotic resistance, yet the relationship between efflux activity, antibiotic concentration, and bacterial fitness under different growth conditions remains to be fully understood (Vareschi, Jaut et al, 2025). In this study, we investigate how efflux activity of *Escherichia coli* cells responds to changes in antibiotic concentration, under different growth conditions, using wild-type strains and efflux overexpressor strains. Our experimental approach combines Nile red efflux assays (Bohnert et al, 2010) with growth curve measurements, with the aim of correlating efflux activity with fitness.

Our results reveal that efflux activity is modulated both by antibiotic concentration and by the physiological growth state of the bacteria. Notably, we find that efflux overexpression increases fitness in the presence of antibiotic only above a threshold antibiotic concentration. We further investigate how nutrient limitation alters the fitness benefits of efflux for different degrees of antibiotic challenge. Our findings highlight the complex interplay between efflux pump activity, nutrient availability, antibiotic challenge and bacterial fitness. A better understanding of this relationship is essential for improving antibiotic treatment strategies and advancing our ability to predict and control antimicrobial resistance evolution.

Vareschi, Jaut et al. 2025 Trends in Microbiology, https://doi.org/10.1016/j.tim.2025.04.012 .

Bohnert et al. 2010 Antimicrobial agents and chemotherapy 54: https://doi.org/10.1128/aac.00620-10

PS01.097

To test or not to test: a qualitative study on how individuals decided to (not) self-sample for SARS-CoV-2 mail-in gargle tests in Baden-Württemberg, Germany

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Background and aim: Throughout the COVID-19 pandemic, testing for SARS-CoV-2 has been implemented worldwide on an unprecedented scale as an important measure against the pandemic. Despite the success of testing strategies largely depending on individuals" willingness to test, user perspectives on SARS-CoV-2 tests remained scarce. We aim to contribute to an in-depth understanding of SARS-CoV-2 testing behavior and to inform the design of testing interventions for future pandemics.

Methods: Conducted amid the second pandemic wave (late 2020 – early 2021) during and after a multi-arm trial evaluating SARS-CoV-2 surveillance strategies in the federal state Baden-Württemberg, Germany, this qualitative sub-study aimed to assess factors that influenced the willingness to test for SARS-CoV-2. Interviews were held remotely via telephone or online, were transcribed verbatim and analyzed using thematic analysis informed by health behavior theories.

Results: Individuals" testing decisions must be contextualized within their broader socio-ecological context and experiences gained over the course of a pandemic. Various factors influenced decisions to (not) get tested, including: attitudes towards testing and pandemic measures in general, trade-offs between perceived benefits and potential social and psychological costs of testing, the perceived threat of COVID-19, health literacy, health status, self-efficacy and implementation aspects.

Conclusions: To ensure effective testing strategies with a high willingness to test, our findings highlight the need for public health interventions to propagate the rationale and (prosocial) benefits of testing, promote health literacy and self-efficacy, tailor testing approaches to individuals" abilities, build trust, and address potential ethical dilemmas as a potential burden of testing.

PS01.099

Developement of sample preparation from clinical swabs using ionic liquids for downstream molecular applications

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Efficient and rapid sample preparation is essential for the molecular analysis of clinical samples such as nasopharyngeal, wound and/or rectal swab. A major challenge lies in the effective lysis of Gram-positive bacteria, which possess a thick peptidoglycan layer that confers increased structural stability and resistance to chemical and physical lysis methods.

Our study aimed to optimize bacterial DNA extraction from clinical swabs through comparative lysis protocols, with a focus on maximizing yield and compatibility with downstream molecular methods such as qPCR and digital PCR. Classical (polyester) and FLOQ® (flocked nylon) swabs (Copan Italia, Brescia, Italy) were evaluated using *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative), as well as *Bacillus atrophaeus* and *Staphylococcus aureus* (Gram-positive) as model organisms. Lysis protocols included treatment with phosphate-buffered saline (PBS) as control, LyseMax® buffer (biotechrabbit, Berlin, Germany), and ionic liquids (ILs). DNA recovery and PCR compatibility were assessed both directly and after magnetic bead-based purification using Nanoreactor-Beads® (BLINK AG, Jena, Germany).

Lysis performance varied by both bacterial species and swab type. Gram-negative bacteria yielded high DNA recovery rates

with IL-based lysis conditions (around 100%). In contrast, Gram-positive species showed lower lysis efficiency (60 – around 100%). In comparison to PBS and LyseMax buffer, ILs improved lysis efficiency, particularly for Gram-positive strains, but showed strong inhibitory effects on downstream PCR applications unless DNA was first diluted. FLOQ® swabs demonstrated a slightly better bacterial cell transfer than Classical swabs. However, DNA release worked better with Classical swabs. DNA-bound ILs could be efficiently removed using Nanoreactor-Beads, enabling direct use in digital PCR with only minor losses in performance.

The study highlights the importance of both chemical lysis and swab handling parameters in maximizing DNA recovery from clinical swab samples. While ILs show promising results in terms of lysis efficiency, their inhibitory effects require mitigation via DNA purification before downstream use. In addition, manual handling—particularly agitation speed and resuspension time—proved to be critical factors influencing reproducibility and DNA yield, underscoring the need for standardized workflows. Future work will include testing these protocols on complex clinical matrices, such as spiked saliva and rectal swab samples, to evaluate real-world applicability and robustness.

PS01.101
Rapid identification of difficult-to-culture pathogens in bacteremia by nanopore sequencing
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The current gold standard for diagnosing bacteremia remains the incubation of conventional blood culture bottles followed by subcultivation on agar plates. However, several bacteria are challenging to cultivate due to different relevant conditions such as prolonged generation time, nutritional requirements, prior antiinfective therapy and preclinical factors. Molecular diagnostic methods offer a promising alternative by combining high sensitivity, rapid testing and low turn-around times as well as detection of unculturable pathogens. Indeed, PCR-based approaches are limited by their reliance on probes that have been specifically designed for each pathogen, thereby restricting the number of bacteria that can be detected. In contrast, Next Generation Sequencing (NGS) aided by nanopore technology allows a rapid analysis of both culturable and difficult-to-culture bacteria.

This study aimed to establish a robust sequencing workflow comprising high-yield nucleic acid extraction, user-friendly library preparation and sequencing procedures as well as a reproducible bioinformatic pipeline. Our results show that certain nucleic acid extraction methods are reliable and provide highly differential quantities of prokaryotic reads. Therefore, we determined the diagnostic sensitivity of relevant extraction methods. Afterwards, a proof-of-concept was performed by spiking blood cultures with various difficult-to-culture bacteria. During several time points after sample incubation, the detection of culturable challenging bacteria was successful achieved via the Shotgun-NGS approach with a following verification by 16s-rDNA-PCR.

In conclusion, we propose a rapid, standardized and easily reproducible protocol for molecular pathogen detection from blood culture material using nanopore sequencing. Key advantages of this approach include sample multiplexing and real-time analysis which contributes to its high efficiency in the diagnostic workflow for bacteremia. The main advantage of this protocol is highlighted by the ability to detect culturable as well as fastidious bacteria. We propose that sequencing of blood culture samples may emerge as a valuable method for uncovering difficult-to-culture pathogens in clinical microbiology.

PS01.103
From raw reads to real impact: making metagenomics clinically useful
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Introduction: Clinical metagenomics is increasingly entering routine medical practice across a range of disciplines. Here we share our experiences implementing the IVDR compliant software platform DISQVER® with a focus on usability, stability and standardisation. Additionally we provide insight into how DISQVER® works in daily diagnostic use.

Objectives: We aim to demonstrate how standardised, CE-IVD-compliant metagenomic diagnostics developed in close collaboration with microbiologists and clinicians can be implemented across laboratories to enable reliable, comparable, interpretable pathogen detection in routine clinical care.

Materials & Methods: Using next-generation sequencing of cell-free DNA to determine relevant pathogens in blood and other bodily fluids becomes more and more available through offerings of various manufacturers. With rising adoption, the need for robust, standardised processes grows. We present regulatory and technical strategies for enabling clinical metagenomics in diverse laboratory environments. In addition, we describe the underlying workflow of DISQVER®, including how analytical results are generated, filtered and presented for diagnostic interpretation.

Results: DISQVER® is developed under CE-IVD (IVDR) regulations as Software-as-Medical-Device (SaMD) and ISO 13485 accreditation. This includes controls for software development, database quality, technical documentation and risk management. The test has been clinically validated in more than eight studies across intensive care, hematology and COVID-19 coinfection, with further studies ongoing. Over the past years, over 10,000 patient samples have been processed, contributing significantly to the continuous refinement of the underlying pathogen database and improvement of the evaluation algorithm. This iterative development, guided by close collaboration with clinical experts, supports improved sensitivity, specificity, and clinical relevance of reported results. Ongoing innovation addresses emerging challenges in preanalytics, contamination control, commensal differentiation, automation and data protection.

Summary: Translating clinical metagenomics into routine care requires navigating regulatory, technical, and data protection challenges. Our standardised workflows and validated platform form the basis for reproducible results and allow other laboratories to adopt comparable diagnostics. This standardisation enables cross-laboratory consistency, while the medical validation of results must be performed based on the clinical context.

PS01.105

Nasal colonisation and prosthetic joint infection by *S. aureus*: smoking gun evidence in a clinical case

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Introduction: *Staphylococcus aureus* is a common colonizer but it can also cause systemic, life-threatening disease. Nasal carriage of *S. aureus* might be regarded as a risk factor for endogenous infections of surgical sites and, especially, of prosthetic joints, due to the bacterium´s ability to form biofilms. Thus, decolonisation treatment is discussed as simple intervention to reduce the risk of infection after procedures such as total joint arthroplasty.

Material and methods: An elderly male patient was admitted in Jan. 2025 to the emergency room of the Bautzen hospital after knee arthroplasty in Nov. 2024 by another hospital. The patient showed signs of inflammation of the knee. A revision of the joint was performed immediately. However, he developed septic shock, multiorgan failure and necrotising myositis around the surgical site, showing a CRP of 507 mg/L, procalcitonin of 34.9 µg/L and myoglobin of 6706 µg/L. Thus, the implant was removed two days after admission. Initially, ampicillin+sulbactam and, later, meropenem and gentamicin were given. The patient was admitted to the ICU.

Blood cultures, an aspirate from the knee and nasal swabs yielded methicillin-susceptible *S. aureus*, so that the therapy was continued with flucloxacillin and clindamycin.

The *S. aureus* isolates from knee and nasal swab were genotyped by microarray and finally sequenced applying nanopore technology.

Results: The knee aspirate and nasal isolates proved to be identical by microarray hybridization, being identified as enterotoxin P (*sep*) positive CC7-MSSA. Sequencing showed them to belong to *spa* type t091 and MLST sequence type 7. The chromosomes of the two isolates differed in only 258 gaps/insertions/deletions and eight nucleotide substitutions (SNPs). For comparison, a sequence of an epidemiologically independent *sep*-positive CC7-MSSA (GenBank CP015646) was analysed. It differed from either isolate in 1670 or 1676 SNPs and in 44,142 or 44,198 gaps/insertions/deletions, respectively (with a chromosome measuring around 2.77 megabases). The gene content of the plasmids of the two isolates were identical, but one isolate showed a partial duplication which might, or might not, be a sequencing artifact.

The patient recovered and was, six weeks later, transferred to a rehabilitation centre. He was weaned and dialysis was ceased. By late May, he was discharged back home.

Discussion: Nasal and the aspirate isolates proved to be near-identical, to an extend where the error rate of the sequencing technology might be the limiting factor. Thus, an endogenous infection of the prosthetic joint by a nasal coloniser is the most likely scenario. This means that the entire episode might easily have been prevented by timely decolonisation with, e.g., mupirocin. Considering the life-threatening risk of an (endogenous) orthopedic surgical site infection with *S. aureus*, the safety and the low cost of decolonisation, there is no reason to forego this intervention.

PS01.107

Molecular microarray-based method for characterizing clinical strains of vancomycin-resistant *Enterococcus* (VRE) from Romania

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Introduction: *Enterococcus* species, especially *E. faecium,* and *E. faecalis*, have emerged as significant opportunistic pathogens in healthcare settings. The emergence of vancomycin-resistant *Enterococcus* (VRE), driven by multidrug resistance, and the ability to acquire resistance genes through horizontal gene transfer, threatens patient safety by limiting treatment options and increasing healthcare costs. Conventional phenotypic and PCR-based methods often lack resolution and require multiple tests. In contrast, DNA microarray-based assays enable rapid, comprehensive profiling of clinical isolates, transmission tracking, and enhancing outbreak detection.

Objectives: This study aimed to apply a validated DNA microarray-based assay to characterize clinical VRE isolates from Romania, focusing on species identification, resistance gene profiling, virulence factor detection, and comparison with whole-genome sequencing data to evaluate assay accuracy and epidemiological studies.

Materials & Methods: In this study, eighty non-duplicate clinical isolates of *E.faecium* from Romania were comprehensively characterized. Species identification, resistance profiling, and virulence gene detection were performed using a high-throughput DNA microarray previously developed for VRE [1]. The procedure involved genomic DNA isolation, parallel labeling, and stringent hybridization. Microarray results were compared to theoretical predictions based on genome sequences of the same strains to validate the findings.

Results: All isolates were identified as *E. faecium*. A high prevalence of multidrug resistance isolates was observed, with the aminoglycoside-modifying enzyme gene (*aac(6')*) detected in all isolates, and the macrolide resistance gene *(erm(B))* was present in 99% of isolates. The vancomycin resistance gene *vanA* was found in 66% of isolates. Among the virulence factors, the *esp* gene was present in 85% of isolates. The microarray provided enhanced typing resolution compared to conventional methods and showed high concordance with genome sequencing. confirming its robustness for detailed molecular epidemiology.

Conclusion: This study highlights the urgent need for advanced molecular typing methods to monitor VRE in healthcare settings, especially in low-resource regions. DNA microarray-based assays provide rapid, ultrasensitive, and cost-effective for simultaneously identifying species, resistance genes, and virulence factors. Their high-resolution

typing enables accurate outbreak detection and transmission tracking key to effective infection control.

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PS01.109

Development of an Integrated Genomic Surveillance (IGS) of public health relevant pathogens in Germany for infectious disease control

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Introduction: Integrated Genomic Surveillance (IGS) is used for surveillance of pathogens, infectious diseases and transmission events and is becoming increasingly important worldwide. It complements existing infectious disease surveillance by linking results from whole genome sequencing (WGS) and bioinformatic genome analysis of pathogens with other relevant pathogen data and selected epidemiological information from the affected individuals.

Objectives: Linked data can be used to pursue various objectives. One of the IGS's primary objectives is to rapidly identify significant pathogen dissemination and to ascertain infection links, even across disparate time periods and locations. This capability facilitates timelier outbreak detection and transmission tracking, and enables the early implementation of targeted measures to effectively combat outbreaks by health authorities.

Moreover, the IGS enables the continuous detection and monitoring of pathogen variants with evolving characteristics, such as transmissibility and virulence. In doing so, the IGS makes a significant contribution to the decision-making processes.

Methods: Building on the preliminary work of the IGS in recent years, an internationally comparable IGS has been under development for Germany since 2021 as part of two projects funded by the Federal Ministry of Health. Essential processes and infrastructure are being developed in collaboration with National Reference Centers (NRZ), Consultant Laboratories (KL), state laboratories, private labs, the University Medicine Network (NUM), and public health authorities.

Results: The necessary framework for the systematic sequencing of reportable pathogens in the respective NRZ/KL is currently being developed. This includes expanding the German Electronic Reporting and Information system (DEMIS) for transmitting pathogen genome sequencing data to the RKI, establishing a robust IT infrastructure for genome analysis, linking WGS data with epidemiological data, as well as visualizing and providing insights gained from the IGS to various stakeholders, such as public health authorities.

Conclusion: The currently developed German wide IGS infrastructure enables rapid and sensitive detection of infectious disease outbreaks and comprehensive monitoring of relevant trends regarding pathogen variants. The integration of all relevant stakeholders is crucial for a sustainable IGS system.

PS01.111

Genetic characterization of clinical carbapenem-resistant *Klebsiella pneumoniae* isolates from Bavaria, Germany, 2021 – 2025

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Background: *Klebsiella pneumoniae* has emerged as a global public health threat due to the high mortality associated with carbapenem-resistant strains. Effective surveillance strategies are therefore essential to monitor the regional spread of high-risk clonal lineages and guide treatment options.

Aims: This study aimed to identify the predominant genetic lineages, including major resistance and virulence determinants, of clinical carbapenem-resistant *K. pneumoniae* in Bavaria, Germany.

Methods: Between May 2021 and May 2025, a total of 544 putative carbapenem-resistant *K. pneumoniae* isolates were collected from patients admitted to 118 different healthcare sites across Bavaria, Germany. Subsequent whole genome sequencing was performed using Illumina technology. De novo assemblies were analyzed for multilocus sequence typing (MLST) using SeqSphere+, resistance genes were identified via the Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca), and virulence factors were detected using the Kleborate genotyping pipeline (https://github.com/katholt/Kleborate).

Results: A total of 535 isolates (98.3%) were classified as belonging to the *K. pneumoniae* species complex (KpSC), which included *K. pneumoniae* (n=521, 97.4%), *K. quasipneumoniae* (n=7, 1.3%), and *K. variicola* (n=7, 1.3%). The majority of KpSC isolates were obtained from rectal swabs (n=178, 33.3%), urinary tract infections (n=104, 19.4%), and bloodstream infections (n=46, 8.6%). Our collection of KpSC isolates exhibited high diversity, encompassing 121 different sequence types (ST). Most KpSC isolates belonged to international high-risk clonal lineages, specifically ST147 (n=101, 18.9%), ST395 (n=65, 12.1%), and ST307 (n=46, 8.6%).

A total of 398 KpSC isolates (74.4%) expressed at least one carbapenemase-encoding gene, with KPC-2 (n=74, 13.8%), NDM-1 (n=69, 12.9%), and OXA-48 (n=59, 11%) being the most common carbapenemases. Moreover, 68 isolates (12.7%) produced two carbapenemases, with co-expression of NDM-1/OXA-48 detected in 32 isolates (6.0%) and NDM-5/OXA-48 in 15 isolates (2.8%) being the most frequent combinations.

Additionally, many KpSC isolates tested positive for known hypervirulence markers, including yersiniabactin (n=328, 61.3%), aerobactin (n=148, 27.7%), rmpA/A2 (n=43, 8.0%), salmochelin (n=17, 3.2%), and colibactin (n=14, 2.6%).

Conclusion: To our knowledge, this is the first genomic study specifically investigating carbapenem-resistant *K. pneumoniae* in Bavaria, Germany. The emergence and

spread of global high-risk lineages, primarily KpSC ST147, along with the high prevalence of KPC-2 and NDM-1 carbapenemases is particularly concerning.

PS01.113
High-resolution microbial identification using 16S-rRNA and 5.8S/ITS-rRNA gene region sequencing: comparative applications and insights from a small-scale diagnostic laboratory
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Introduction: Microbial identification underpins many disciplines, including clinical diagnostics, food safety, environmental monitoring, and industrial biotechnology. Traditional phenotypic methods often fall short in resolving microorganisms to the species level—particularly in the case of fastidious, slow-growing, or unculturable organisms. Molecular methods such as ribosomal RNA (rRNA) gene sequencing now serve as gold standards due to their reproducibility, sensitivity, and taxonomic resolution. This study explores the practical use of 16S-rRNA sequencing for bacterial identification and 5.8S/ITS-rRNA region sequencing for fungi and yeasts within a resource-limited diagnostic laboratory setting.

Objectives: Our primary goal is to demonstrate the practical advantages, limitations, and comparative value of 16S and ITS sequencing as applied to microbial identification across diverse domains. We also aim to share operational strategies for implementing these technologies effectively in a small laboratory setting.

Materials & Methods: We process samples from a variety of sources, including clinical isolates, environmental swabs, food products, and culture collections.

- **Amplification:** For bacteria, we amplify the full 16S rRNA gene using universal primers. For fungi and yeasts, we target the ITS1–5.8S–ITS2 region.
- **Sequencing Approaches:** Sanger sequencing is used for single isolates or low-throughput samples due to its clarity and ease of analysis.
- **Bioinformatics:** Sequences are compared against curated databases using BLAST for taxonomic assignment.

Results:

- **Environmental Applications:** Identification of clinically relevant from air plates and swabs is routine.
- **Food Microbiology:** ITS sequencing enabled accurate identification of spoilage yeasts in dairy and bakery products, while 16S helped trace pathogens.
- **Industrial Relevance:** Identification of microbial strains involved in fermentation and biodegradation supported quality control and strain tracking for clients.
- **Operational Efficiency:** Despite lacking in-house NGS capabilities, streamlined intake processes, hybrid sequencing strategies, and curated databases enabled timely, accurate identifications.

Summary: 16S and 5.8S/ITS-rRNA sequencing offer small-scale laboratories a cost-effective, high-resolution approach to microbial identification. These tools provide accuracy, culture-independence, and cross-domain applicability, though they are limited by database quality, taxonomic resolution, and resource constraints. By adopting strategic workflows—using Sanger sequencing as a frontline tool our lab effectively overcomes infrastructural limitations. Looking ahead, we aim to incorporate shotgun metagenomics for deeper insights and maintain curated taxonomic clusters to address unresolved species-level distinctions. This work highlights how small labs can remain vital contributors to microbial surveillance and diagnostics through thoughtful integration of molecular tools and bioinformatics.

PS01.115
Enhanced culture-independent diagnosis of heart valve and lymph node infections using full-length 16S rDNA Nanopore sequencing
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Introduction: Culture-negative infections -often resulting from prior antibiotic use, slow-growing pathogens, or delayed sample transport- present significant diagnostic challenges. While 16S rRNA gene Sanger sequencing is commonly used to identify culture-negative samples, it has certain limitations, including poor detection of polymicrobial infections and delayed turnaround times, as it is typically outsourced. Full-length 16S rRNA gene nanopore sequencing offers a rapid, in-house alternative with high-resolution microbial profiling that may overcome these limitations.

Objectives: This study evaluated the diagnostic performance of full-length 16S rRNA gene nanopore sequencing compared to conventional Sanger sequencing, with a focus on detection accuracy, species resolution, and utility in polymicrobial infections.

Methods: Eighty clinical samples (49 lymph node tissues and 31 heart valves) submitted for routine microbiological diagnostics underwent standard culture, Sanger sequencing, and nanopore-based full-length 16S rRNA gene sequencing.

Results: Nanopore sequencing demonstrated improved sensitivity of 93% in detecting pathogenic bacteria, particularly in culture-negative samples that produced mixed or ambiguous sequences by Sanger sequencing. It identified additional bacterial species missed by both culture and Sanger sequencing, offering enhanced resolution and faster turnaround for clinically relevant diagnoses. However, to ensure cost-efficiency, samples typically need to be batched for a full sequencing run, which may delay processing in low-throughput settings.

Conclusion: Full-length 16S rRNA nanopore sequencing is a promising diagnostic tool for complex or culture-negative infections, especially in time-sensitive clinical scenarios. Further validation in larger patient cohorts is warranted to support its integration into routine diagnostics.

PS01.117
Evaluation of Fourier transform infrared spectroscopy for typing of *Klebsiella pneumoniae* strains involved in hospital outbreaks
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Introduction: *Klebsiella pneumoniae* (*Kp*) is frequently associated with nosocomial infections, particularly due to the emergence of multidrug-resistant strains. Therefore, rapid and reliable typing methods are crucial for the prompt identification and tracking of strain transmission. In recent years, Fourier Transform Infrared Spectroscopy (FTIR) has emerged as a fast, effective, and user-friendly method for bacterial typing.

Question: This study aimed to evaluate the possibility of applying FTIR to characterize a dataset of *Kp* strains involved in hospital outbreaks and to assess its potential for enhancing infection control and tracking transmission dynamics.

Methods: A total of 37 well-characterized (whole genome sequencing) carbapenemase-producing *Kp* isolates were included in this study. The strains were primarily collected during three outbreak events at the University Medical Center Göttingen. Also, several unrelated and reference strains were analyzed. The isolates belong to different sequence types, namely ST11, ST15 (including outbreak 1), ST20, ST23, ST101 (including outbreak 2), ST147 (including outbreak 3), ST258, ST395, ST1031. Different capsule types are present.

For FTIR spectra acquisition, the strains were cultivated at 35 °C for 24±2 h on three different media: Columbia blood agar (Becton Dickinson, Germany), Müller-Hinton agar (VWR Chemicals, Belgium), and MacConkey agar (Thermofisher, Germany). Three biological replicates in three independent days were included. Spectra acquisition, processing and analysis were performed using the IR Biotyper® system (IRBT; Bruker Daltonics, Germany) Data analysis was performed by hierarchical cluster analysis and principal component analysis.

Results: The IRBT clustering enabled differentiation of *Kp* strains at different genetic levels. The STs were clearly distinguishable one from another. Furthermore, within the same ST, a further discrimination between clonally related and unrelated strains was observed. Specifically, sub-clusters of indistinguishable strains were identified within ST15, ST101, and ST147, corresponding to three distinct outbreaks. The remaining isolates, which were not part of specific clonal groups, clustered as singletons. No significant differences in discriminatory power were observed between the three culture media.

Conclusions: In this study, FTIR allowed the clear differentiation of *Kp* strains in relation to the combination of ST and capsule type. Outbreaks strains were successfully distinguished from unrelated strains, showing a high concordance with molecular analysis. Therefore, FTIR could be an effective method for the detection of epidemiologically relevant strains and could represent a reliable alternative for microbiological surveillance in healthcare settings.

Fig. 1

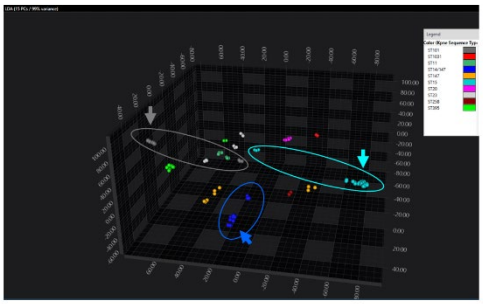

Figure 1. 3D PCA/LDA scatterplot showing the clustering of the isolates. Each geometric form represents a spectrum, and the color indicates the STs. The ellipses highlight the 3 STs which included clonal (indicated by the arrow) and not related isolates.

PS01.119
Evaluation of carbapenemase/ESBL chromogenic media for Fourier-transform infrared spectroscopy

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Introduction: Chromogenic media are widely used in microbiology for a fast detection of numerous defined target microorganisms. In the field of hospital hygiene, they represent a great aid for a prompt detection and preliminary identification of antibiotic-resistant strains. In the last years, Fourier-transform infrared spectroscopy (FT-IR) has been emerging and spreading worldwide as a promising methodology for a reliable, fast and user-friendly strain typing method. To date, the possibility to use chromogenic media was not recommended, because of the lack of performance data and theoretical possible interferences of the chromophore molecules with the absorption of IR radiation.

In this study, we evaluated the performance of FT-IR with different chromogenic media for four among the most clinically relevant *Enterobacteriaceae* species.

Methods: A total of 90 well-characterized carbapenemase-producing strains (n=30 *K. pneumoniae*, n=20 *E. coli*, n=20 *E. cloacae* complex and n=20 *C. freundii*), were tested. The datasets included clonally related strains and non-related strains. The strains were cultivated overnight at 35±2 °C on Columbia blood agar (CBA - Becton Dickinson), Brilliance™ CRE and Brilliance™ ESBL (Thermofisher), chromID® ESBL and chromID® CARBA (bioMérieux) and CHROMagar™ KPC and CHROMagar™ ESBL (CHROMagar), in three independent biological replicates. FT-IR analysis was performed by the IR Biotyper® system (Bruker Daltonics). The IR clustering using spectra measured from cultures on chromogenic media was compared with the results of the reference method.

Results: All chromogenic media allowed a similar discriminatory power, with high concordance with the reference method. Regarding *K. pneumoniae* and *C. freundii*, for all media, the concordance was 100%. Regarding *E. coli*, on Brilliance™ ESBL and CHROMagar™ ESBL two not-related isolates clustered together (concordance 95%). For *E. cloacae* complex, on Brilliance™ CRE and ChromID® Carba, two not-related isolates clustered together (concordance 95%). No interference of the chromophores was observed in the spectra quality.

Conclusions: This study proves in principle the suitability of carbapenemase/ESBL chromogenic media for FT-IR analysis for the most clinically relevant *Enterobacteriaceae* species

involved in hospital outbreaks. No differences in terms of discriminatory power and spectra quality was observed in comparison with Columbia blood agar. The use of chromogenic media could potentially allow a shortening of the turn-around-time. Further studies including other media, other manufacturers, other microbial species are necessary.

PS01.121
Plasmid analysis of KPC-2-producing *Enterobacterales* from hospital wastewaters

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Introduction: Carbapenem-resistant *Enterobacterales* represent a major public health concern due limited treatment options and the potential of horizontal spread of carbapenemases. Among these, KPC-2-producing *Klebsiella pneumoniae* play a critical role in nosocomial outbreaks worldwide [1].

Studies have already demonstrated the importance of wastewater from hospitals, wastewater treatment plants (WWTP) and surface waters as a source of carbapenem resistance determinants [2]. Although no infections have yet been reported from swimming lakes or other open waters, a better understanding of carbapenemase gene exchange between bacteria in the environment is needed to minimize the risk of emergence and further spread of resistant pathogens.

Goals and Methods: We performed comprehensive genome analyses of KPC-2-producing *Enterobacterales* isolates obtained from wastewater of 7 hospitals, samples from 6 WWTPs (without UV treatment) and river water collected in 2020 [2]. Short-read and long-read genome sequencing was performed for 104 and 11 *bla*KPC-2-carrying isolates, respectively. By MOB-suit analysis, we were able to assign the *bla*KPC-2 plasmids to an Incompatibility (Inc) type. For further analysis we focused on the frequently detected IncX3 plasmids (n=17) and compared them to a clinical KPC-2-producing *K. pneumoniae* outbreak strain (2010-13) from one of the hospitals.

Results: Analysis of the IncX3 plasmids showed that almost all plasmids carried ESBL gene *bla*SHV-12 in addition to *bla*KPC-2. Two further plasmids also carried beta-lactamase genes *bla*OXA-10 and *bla*TEM, respectively. The 17 plasmids were found in seven different species, mainly in *Escherichia coli* (n=6) and *Citrobacter famerii* (n=4). These were detected in wastewater of 2 hospitals and the connected WWTP (see Table). We were able to close (circularize) five plasmids from two *C. famerii*, two *E. coli* and the clinical *K. pneumoniae*. BLAST Ring Image Generator analysis of the plasmids showed that the environmental plasmids share very similar backbone and Tn4401-like transposon structures as the clinical isolate, regardless of species.

Conclusions: Our analyses showed a high diversity in plasmids carrying *bla*KPC-2 in hospital wastewater. The similarity of IncX3 backbones and *bla*KPC-2 transposon structures in different *Enterobacterales* species indicates the extent of horizontal gene transfer. A deeper understanding of horizontal gene transfer is essential to unravel the mechanisms driving the spread of *bla*KPC carbapenemase genes between bacterial species in this region, and to implement strategies for monitoring and limiting the spread of these resistance determinants in the environment.

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Fig. 1

KPC-2-producing *Enterobacterales* with IncX3 plasmids

Strain ID	Species	Num. contigs	Plasmid size [bp]	Inc. type	Source	Period 2020	Beta-lactamases		
182-21	*Escherichia coli*	4	57734	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	OXA-10
201-21	*Escherichia coli*	3	55208	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	TEM-1
221-21	*Citrobacter farmeri*	1	52270	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV	
222-21	*Escherichia coli*	1	52472	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
227-21	*Escherichia coli*	1	53292	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
238-21	*Escherichia coli*	2	57061	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
249-21	*Klebsiella ornithinolytica*	2	53729	IncX3	Hospital wastewater (1)	summer	KPC-2	SHV-2	
268-21	*Klebsiella ornithinolytica*	2	54401	IncX3	Hospital wastewater (1)	summer	KPC-2	SHV-2	
272-21	*Klebsiella pneumoniae*	2	68456	IncX3	Hospital wastewater (1)	summer	KPC-2		
374-20-1	*Serratia marcescens*	6	52355	IncX3	Hospital wastewater (1)	summer	KPC-2	SHV-2	
419-20	*Citrobacter farmeri*	1	62930	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
435-20	*Citrobacter farmeri*	2	52272	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
438-20	*Citrobacter farmeri*	2	52397	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
456-20	*Serratia marcescens*	4	51093	IncX3	Hospital wastewater (1)	summer	KPC-2	SHV-2	
498-20	*Citrobacter portucalensis*	3	55818	IncX3	Hospital wastewater (4)	winter	KPC-2	SHV-2	
513-20	*Escherichia coli*	4	53334	IncX3	Hospital wastewater (1)	winter	KPC-2	SHV-2	
518-20	*Serratia marcescens*	3	53729	IncX3	WWTP (4)	winter	KPC-2	SHV-2	
237-22	*Klebsiella pneumoniae*	1	54492	IncX3	Human patient		KPC-2	SHV-2	

PS01.123
Identification of a novel pathogenic island in a hybrid uropathogenic/enteroaggregative *E. coli* (UPEC/EAEC) of O3:H2 serotype

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Introduction: Pathogenic *Escherichia coli* is responsible for a variety of human diseases. Strains that infect the gastrointestinal tract are classified as diarrheagenic *E. coli* (DEC), while those associated with urinary tract infections (UTIs) are collectively termed uropathogenic *E. coli* (UPEC) and are part of a larger group called extraintestinal pathogenic *E. coli* (ExPEC). These strains exhibit high genomic plasticity, enabling the acquisition of mobile genetic elements that may enhance their pathogenic potential across different anatomical sites.

Objective: This study aimed to genetically characterize nine *E. coli* isolates of serotype O3:H2 obtained from stool or urine samples.

Methods: Whole genome sequencing was performed on nine *E. coli* of serotype O3:H2 isolates - three from urine and six from feces - using short-read (Illumina) and long-read (Oxford Nanopore Technologies MinION) platforms.

Findings: All isolates belonged to serotype O3:H2, phylogroup A, and sequence type 10, and exhibited a close phylogenetic relationship. Notably, all nine isolates carried hallmark genes of both EAEC and UPEC pathotypes, designating them as UPEC/EAEC hybrid strains. Among them, three isolates obtained from UTI cases contained a novel chromosomal pathogenicity island (PAI), approximately 34.0 kb in size and composed of 37 coding sequences (CDSs), including an operon with potential to encode the AfaE-V adhesin. This PAI was inserted downstream of tRNA-*pheV*, contributing to a mosaic PAI-like structure. UPEC-associated genes (*kpsMT*, *iutA*, *papA/C*, and *afaA*) were

embedded within these chromosomal PAI-like regions, forming modular genomic mosaics of variable size (86.4 kb to 165.3 kb) and content (88 to 162 CDSs), consistently inserted downstream of tRNA-*pheV*.

Conclusion: Our findings reveal a novel chromosomal PAI in hybrid UPEC/EAEC isolates of serotype O3:H2, highlighting their remarkable genomic flexibility. This adaptability may play a critical role in their evolutionary process and ability to cause infections at both intestinal and extraintestinal sites in the human host.

Keywords: hybrid UPEC/EAEC; pathogenic island; urinary tract infections.

PS01.125
Molecular characterization of a hybrid EnteroPathogenic/EnteroAggregative (EPEC/EAEC) *Escherichia coli* of Serotype O3:H2

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Introduction: The acquisition of mobile genetic elements carrying virulence factor-encoding genes has allowed *Escherichia coli* to evolve into isolates that can cause a range of diseases in humans. Enteropathogenic (EPEC) and enteroaggregative (EAEC) *E. coli* are two distinct pathotypes that use specific virulence strategies to cause diarrhea. EPEC is characterized by the presence of a chromosomal pathogenicity island known as the locus of enterocyte effacement (LEE region), while EAEC is defined by the presence of a high-molecular-weight plasmid (pAA) that encodes aggregative adherence fimbriae (AAF/I-AAF/V). During the investigation of a diarrheal outbreak in Brazil, one EPEC, four EAEC, and three hybrid EPEC/EAEC isolates of the O3:H2 serotype were obtained.

Methods: To identify the genetic elements responsible for the emergence of the hybrid EPEC/EAEC, all eight isolates were subjected to whole genome sequencing using Illumina technology. Additionally, four representative isolates (one EPEC, two EAEC, and one hybrid EPEC/EAEC) were subjected to long-read sequencing, hybrid assembly (combining short and long reads), and comparative genomic analysis.

Findings: All eight isolates belonged to phylogroup A and were assigned to sequence types (ST) 8087 (one EPEC, two EAEC, and three hybrids) or ST10 (two EAEC). Single-nucleotide polymorphism (SNP)-based phylogenomic analysis revealed that all O3:H2 isolates clustered together, suggesting a closer relationship among them. Comparative analysis of the chromosomes from the four representative isolates revealed that the hybrid EPEC/EAEC isolate harbored a prophage region containing EPEC-associated virulence genes (*nleB*, *nleF*, and *nleH2*), as well as genes encoding a putative type VI secretion system. The LEE region of both the EPEC and hybrid EPEC/EAEC isolates was identified as subtype 8, with 100% identity and coverage. The pAA plasmids of the EAEC and the hybrid isolates shared approximately 100% identity over at least 87.9% of their sequences and carried the *aggDCBA* operon, which encodes AAF/I.

Conclusion: Our findings indicate that all O3:H2 *E. coli* isolates analyzed in this study likely originated from a common ancestor. Furthermore, the data suggest that the EPEC isolate acquired the pAA plasmid, leading to the emergence of the hybrid EPEC/EAEC.

PS01.127
Innovative method for rapid, automated strain typing and source tracking using Fourier-transform infrared spectroscopy

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Introduction: Fourier-transform infrared spectroscopy (FT-IR) offers a modern, rapid, and cost-efficient approach for identifying various microbial species. A growing number of successful applications have recently emerged across diverse fields such as medicine, veterinary science, food safety, and probiotics. However, determining an appropriate clustering cut-off remains unresolved. The thresholds produced by hierarchical clustering algorithms are highly dependent on the dataset"s diversity and quality, often varying significantly between datasets. As a result, a cut-off suitable for one dataset may not be applicable to another.

This study introduces a novel, automated, and operator-independent method for defining cut-offs and reporting results.

Methods: This study examined the most clinically relevant bacterial species of the ESKAPEE group (*Enterobacter cloacae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Escherichia coli*) as well as other less common but always more frequently isolated in outbreak contexts like *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *Serratia marcescens*, for which strain-level typing may be necessary.

FT-IR spectra were acquired, processed, and analysed using the IR Biotyper® system (Bruker Daltonics, Germany). For each species and culture medium combination a spectral distance model was developed using training strains. Two clustering thresholds were defined, creating three similarity categories (high, moderate, low). The model was then used to classify the remaining test isolates (12 to 140 test strains per combination), with results presented as a nearest-neighbour list. Classification accuracy was assessed by comparing outcomes with those from the corresponding reference method.

Results: For all the species included in the study, the novel approach showed an average accuracy of 97% (median 98%). Most errors were found in *P. aeruginosa* cultivated on Mueller-Hinton (88%), while *A. baumannii* achieved 100% accuracy. Interestingly, *P. aeruginosa* from tryptic soy was identified with 96% accuracy, highlighting the importance of the growth conditions. The whole workflow is shown in **Figure 1.**

Conclusions: The approach introduced in this study holds the potential to mark a significant breakthrough in FT-IR-based strain typing. The newly developed algorithms aim to provide a fully automated, universal, and ready-to-implement typing solution for microbiology laboratories. Nevertheless, further validation is required to confirm the method"s applicability to a

broader range of microbial species across different global settings.

Fig. 1

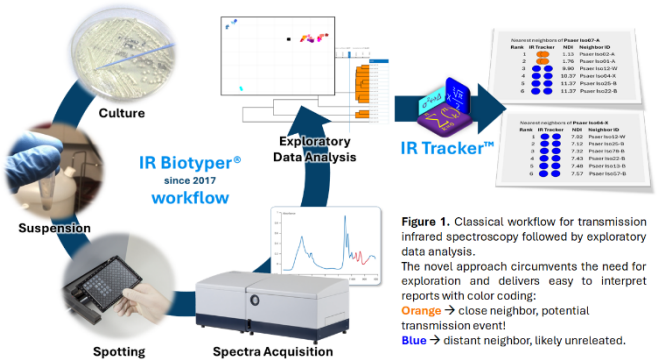

Figure 1. Classical workflow for transmission infrared spectroscopy followed by exploratory data analysis.
The novel approach circumvents the need for exploration and delivers easy to interpret reports with color coding:
Orange → close neighbor, potential transmission event!
Blue → distant neighbor, likely unreleated.

PS01.129
Towards rapid pathogen identification on Agar plates using deep learning

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Introduction: The widespread use of broad-spectrum antibiotics to promote growth and prevent disease in healthy animals and prior to pathogen confirmation in infections of humans contributes to the emergence of antimicrobial resistance. In microbiological diagnostics of infections and bacterial colonization, pathogen identification is typically performed by culturing clinical specimens on semisolid agar media. The growth characteristics including the colony morphologies of bacteria and fungi are used to identify specific pathogens. These are essential for subsequent laboratory analyses and therapeutic decisions. Accelerating microbiological diagnostics using Artificial Intelligence (AI) methods has the potential to shorten the duration of pathogen identification improving diagnostic accuracy and thus diminishing empirical antibiotic therapies.

Objectives: The aim of this study is to investigate the potential of Machine Learning (ML) methods for classifying bacterial and fungal species on culture plates using photographic image data. By creating a significantly larger training dataset than in current literature, this study seeks to develop a robust and generalizable ML model that can support or enhance current diagnostic procedures.

Materials & Methods: The dataset comprises over 100,000 microbiological examinations collected at the University Hospital Düsseldorf. It includes images of macroscopic bacterial cultures grown on semisolid agar media, representing clinically relevant categories such as MRSA (methicillin-resistant *Staphylococcus aureus*), VRE (vancomycin-resistant enterococci), MRGN (multidrug-resistant Gram-negative bacteria) and various urinary tract pathogens. Culture and incubation procedures are fully automated using the WASPLab system (COPAN). All images are labelled by an experienced medical microbiologist. We compared different machine learning architectures for classifying pathogen species.

Results: Preliminary results suggest that image-based ML models can reliably classify bacterial species with high accuracy. Convolutional Neural Networks (CNN)-based architectures trained from scratch demonstrate promising performance, with accuracies up to 97% (ROC-AUC 98%). Furthermore, the classification of bacterial species from culture plate images used in resistance screenings appears to be more accurate and consistent than classifications based on images from urinary tract pathogen screenings.

Summary: This study demonstrates the potential of machine learning to assist in classifying bacterial species on culture media. A significantly larger dataset combined with modern ML techniques will enable the creation of powerful diagnostic models. These models could shorten diagnostic turnaround times and support doctors in managing microbiology and infections. The final model is currently being developed and the dataset is being prepared. Both should contribute to the development and validation of image-based microbiological diagnostic tools.

PS01.131
A comprehensive, hospital-wide compliance monitoring and feedback system to support hand hygiene at a maximum-care hospital

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Introduction: Hand hygiene with alcohol-based disinfectants is the most important infection prevention and control (IPC) measure in hospitals to reduce the risk of nosocomial infections and the spread of potentially problematic pathogens such as multidrug-resistant bacteria. The main challenge is to reach and maintain hand hygiene compliance at an appropriate level over the long term and to generate sustainable, institution-wide acceptance of good hand hygiene.

Methods: We present the structure and design of a hospital-wide, comprehensive hand hygiene compliance monitoring and feedback system that was implemented at AGAPLESION Diakonieklinikum Rotenburg, a maximum-care hospital in Northern Germany. Moreover, we report initial results and experiences from the years 2019–2023.

Results: Starting in 2018/19, the surveillance system was gradually rolled out across all 18 somatic regular wards, two intensive care units and one selected functional area. The central component of this system are repeated direct hand hygiene compliance observations (following "Aktion Saubere Hände" standards) by trained IPC nurses. The observation frequency depends on the achieved compliance result:

- Overall compliance rate > 75%: Re-observation in the following year (when the 75% threshold is reached for the first time, a follow-up observation after six months is planned to verify the result.)
- Overall compliance rate between 50% and 75%: Re-observation in less than 6 months
- Overall Compliance rate < 50%: Re-observation in less than 4 months

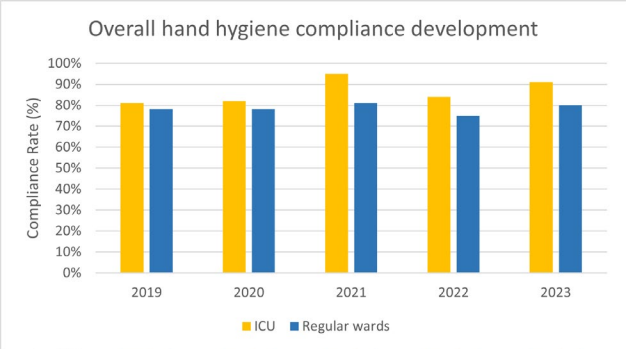
The results are shared—both written and verbally—with staff across all levels of hierarchy, including hospital management.

Furthermore, based on the specific observations, tailored training sessions are provided.

Since implementation, we observed a stable or slightly increasing compliance rate at an overall acceptable/good level (see Figure 1). Comparatively high compliance rates were noted in regular pediatric wards (mean 2019-2023: 89%). In most cases, nursing staff outperformed physicians (e.g., regular internal medicine wards 2023: nursing staff 86% vs. physicians 69% compliance rate). In the functional area, compliance results were lower (mean 2020-2023: 72%) to those found on the wards.

Discussion: The comprehensive approach described here has supported both practical hand hygiene behavior and institutional acceptance at our hospital, especially during the COVID-19 pandemic. In addition, further observations (e.g., usage of personal protective equipment, handling of vascular catheters,) could be conducted during hand hygiene compliance monitoring. Noteworthy is the very high personnel time requirement for providing and evaluating compliance observation including structured feedback.A more detailed evaluation of the concept and results is planned. In particular, the further developments in the aftermath of the COVID-19 pandemic are of interest.

Fig. 1



PS01.133

Immunogenicity and safety of a second COVID-19 booster and Influenza vaccination coadministration

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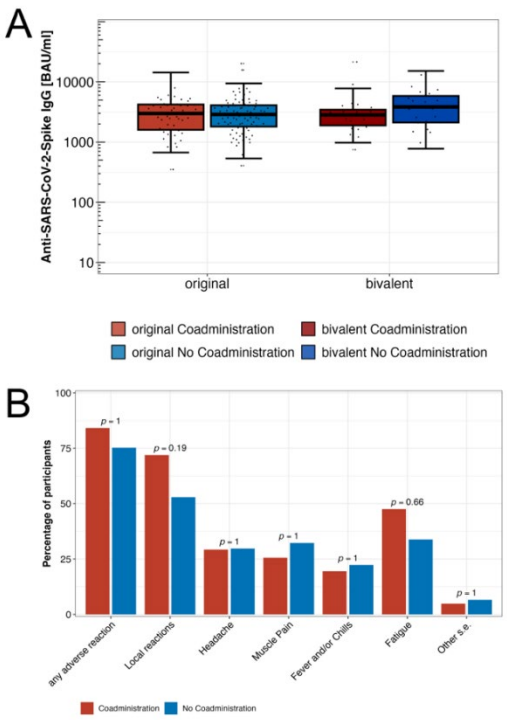
Question: In the post-COVID-19 pandemic era, the coadministration of seasonal COVID-19 booster and Influenza vaccines continues to provide effective immunization across broad sections of the population, particularly being established and examined with the third dose of monovalent COVID-19 vaccines. However, data on bivalent COVID-19 vaccines and further booster doses remain limited. To date, only a few studies have investigated coadministration for booster vaccinations, often yielding heterogeneous results based on small sample sizes and excluding Omicron XBB.1-adapted vaccines. This study aims to evaluate the immunogenicity and safety of coadministered versus separately administered COVID-19 and influenza vaccines for the fourth COVID-19 dose, using both bivalent and monovalent vaccines.

Methods: Within the CoVacSer trial, differences in Anti-SARS-CoV-2-Spike IgG antibody titers and occurring adverse events were assessed after the fourth mono- or bivalent COVID-19 vaccination with a BNT162mRNA vaccine with (intervention group) or without (control group) coadministration of a seasonal quadrivalent Influenza vaccine (Influvac Tetra vaccine 2022/2023). Anti-SARS-CoV-2-Spike IgG levels were determined 14 to 59 days after vaccination by SERION ELISA agile SARS-CoV-2 IgG.

Results: Of the 203 individuals included, 40.4% (82/203) received a fourth COVID-19 vaccination coadministered with a seasonal Influenza vaccination. The remaining 59.6% (121/203) received a fourth COVID-19 vaccination only. Comparison between intervention and control group revealed no significant differences in post-vaccination Anti-SARS-CoV-2-Spike IgG titers overall, even when stratified by COVID-19 vaccines (all p-adj.>0.99). No significant differences were found in queried adverse event categories (all p-adj.>0.19).

Conclusions: Coadministration of COVID-19 and Influenza vaccines provides a streamlined approach to seasonal immunization with minimal infrastructure requirements. The findings regarding immunogenicity and adverse events indicates that this strategy does not compromise the COVID-19 vaccine immunogenicity and is well tolerated - including both monovalent and bivalent formulations.

Fig. 1



PS01.135

Bacteriophages as alternative therapeutic for nasal eradication of *Staphylococcus aureus*

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Question: *Staphylococcus aureus* is a major human pathogen which causes a wide variety of infections. *S. aureus* colonizes approximately the nares of 20% of the human population. Decolonization treatments prior to invasive medical inventions lead to a reduced risk in subsequent infections. The proven method for decolonization of *S. aureus* is a treatment with the antibiotic mupirocin, which leads to problems on the long run. Firstly *S. aureus* recolonizes usually after the treatment has stopped. Secondly antibiotic resistance to mupirocin in *S. aureus* is emerging. In addition, S*. aureus* is part of a complex microbiome in the human nares. Other bacterial species have promoting or suppressive effects on *S. aureus* nasal colonization. The effect of mupirocin treatment on the nasal microbiome is poorly understood on the species and strain level. In this study we determine the global effect of nasal decolonization strategies on the nasal microbiome. Furthermore, we propose bacteriophages as decolonization agents which feature increased precision and selectivity.

Methods: We used a combination of *in vitro* techniques and *in silico* prediction to determine antimicrobial susceptibility and colonization dynamics upon mupirocin or phage treatment of a comprehensive collection of nasal commensals. Furthermore, we validated our results with *in vivo* colonization data.

Results: We show that mupirocin, the state-of-the art decolonization agent, inhibits constituents of the nasal microbiota selectively due to the presence of naturally occurring resistance alleles. Observed composition changes in co-culture experiments caused by mupirocin treatment correlate well with *in vivo* colonization data. In addition, we demonstrate that bacteriophages targeting the pathogen *S. aureus* show improved selectivity by sparing beneficial staphylococcal commensals.

Conclusion: We show that mupirocin treatment has notable collateral side effects on nasal commensals perturbing the composition of nasal microbiomes. Side effects are especially pronounced on other- often harmless - commensal staphylococci, which are frequently antagonizing *S. aureus* colonization. Hence, we propose alternative antimicrobial approaches targeting molecular structures which are unique to pathogenic commensals. Our results emphasize that bacteriophages could be potent selective delocalization agents. Furthermore, we demonstrate that personalized metagenomic data can be utilized to inform the selection of patient-specific phage cocktails allowing potentially selective and controlled eradication of *S. aureus* from the human nose.

PS01.137

Impact of pathogen-related genetic factors for long-term colonization of vancomycin-resistant enterococci (VRE)

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Question: Vancomycin-resistant enterococci (VRE) are multidrug-resistant pathogens of great importance in the hospital setting. As infections are often preceded by VRE colonization, the duration of VRE-carrier status has an important impact on the development of VRE infections. The impact of patient-associated risk regarding VRE-colonization is already well studied. However, little is known about pathogen-related factors that might contribute to a long-term VRE colonization. The primary aim of this study is to identify virulence genes that are associated with a VRE long-term carrier status. In addition, we want to analyse the degree of genetic relatedness between VRE isolates obtained sequentially from patients over time to investigate possible recolonization during long-term VRE colonization.

Methods: From 01/2021 to 12/2023, all patients of the University Hospital Münster with first detection of VRE colonization and at least three follow-up VRE tests were included in the study. The patients were stratified into short-term carriers (STc, VRE clearance <10 weeks) and long-term carriers (LTc, no clearance or clearance ≥10 weeks), respectively. A patient was considered cleared of VRE if three VRE-negative samples were available without subsequent positive samples. All first isolates of those patients were subjected to whole-genome sequencing (WGS). Based on these data, multilocus sequence typing (MLST), core genome (cg)MLST and screening for the presence of 38 known or putative virulence genes was performed.

Within the LTc group, if available, WGS data of the most recent VRE follow-up isolate was compared with WGS data of the first isolate using cgMLST. Isolates that differed in ≤ 5 alleles were considered related.

Results: From 134 patients, 51 fulfilled our definition for STc and 76 for LTc. Among the first colonization isolates of these patients, the distribution of MLST sequence types (ST117= 49%; ST80= 37%, other ST= 13%) and virulence genes showed no significant difference between the two groups. Of the analysed virulence markers, only *lwpB* (cell surface component in bacterial adherence) reached statistical significance in the univariable analysis (p=0.038) which did not persist in the following multivariable logistic regression model.

The cgMLST comparison of first and most recent isolates from LTc (n= 37) revealed two groups: Up to a colonization period of 100 weeks, both isolates were rather identical in cgMLST (difference in ≤ 5 alleles, 86%). Above 100 weeks, the proportion of non-related isolates in comparison to the first colonization isolate (cgMLST allele distances 17 to 427; 66%) increased.

Conclusion: In this study, we could not show an association between the analysed virulence genes and VRE long-term carrier status. In some cases, long-term colonization appears to occur through several successive colonization with different VRE isolates. Further analyses are needed to detect potential risk factors that promote VRE long-term carrier status.

PS01.139

COVID-19 and influenza: how healthcare workers´ vaccination intention turns into realization

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Question: Vaccination among healthcare workers (HCWs) enhances their protection, ensuring improved workability and better patient care. The German Standing Committee on Vaccination (STIKO) recommends annual autumn vaccination of HCWs with patient contact against COVID-19 and Influenza. This study examines HCWs´ adherence to their own prior vaccination intention.

Methods: In October 2024 and April 2025, HCWs aged ≥18 years working with and without patient contact and participating in the ARIPro study completed a questionnaire via RedCap. Logistic regression analysis was employed to identify factors influencing vaccination intention and realization. Variables included age, sex, occupation, contact with children, quality of life and smoking.

Results: Among the 542 (80.4% female) HCWs enrolled in October 2024, 41.2% intended to receive the seasonal Influenza vaccine only, 1.1% intended only the COVID-19 vaccination, and 16.3% intended to receive both. 41.4% opted for neither vaccine.

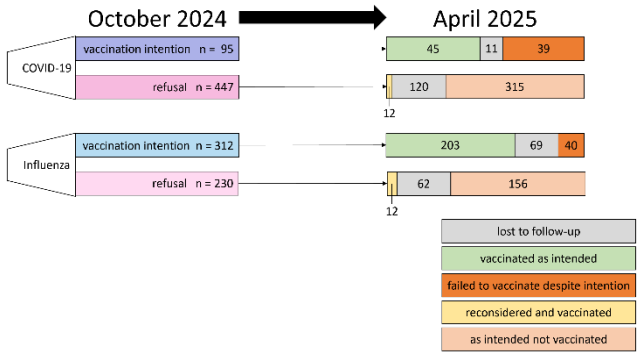
Regarding HCW with stated Influenza vaccination intention, 203 of 312 (65.1%) successfully adhered to their intention, while 40 (12.8%) did not receive the vaccination, despite initially expressing intent to do so. Among the 230 HCWs without Influenza vaccination intention, 156 (67.8%) were not vaccinated, 62 (27.0%) were lost to follow-up and 12 (5.2%) were vaccinated without having expressed prior vaccination intention.

Multiple logistic regression showed two significant factors: Physicians (compared to HCW without patient contact) were significantly more likely to realize their COVID-19 vaccination intention (odds ratio (OR): 5.6; 95% CI 1.2-30.9). Older age was the only factor significantly associated with realizing an existing Influenza vaccination intention (OR 1.053 (95% CI 1.024-1.086) per life year). Among re-participants, the COVID-19 vaccination rate was 13.9%, the Influenza vaccination rate was 52.3%.

Conclusions: While the majority of HCWs intended to receive the seasonal Influenza vaccination, less than a fifth planned to vaccinate against COVID-19. Among these, physicians exhibited a markedly higher realization of COVID-19 vaccination. While older age already was seen as a factor boosting Influenza vaccination intention, it now proved to be significant in realizing prior intention. Both factors may reflect awareness of those infections.

41.4% of all HCWs intend to forgo both vaccines, in contrast to official recommendations. With every eighth person failing to realize their existing Influenza vaccination – and almost every second HCW not realizing the already low COVID-19 vaccination intention, this underscores the necessity for further discourse and the implementation of targeted interventions.

Fig. 1



PS01.141
Methicillin-resistant *Staphylococcus aureus* (MRSA) in Germany: demographic developments and outbreak patterns (2015–2024)

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a key cause of healthcare-associated infections. Following the stabilisation of invasive MRSA infections in Germany and Europe in recent years, further analysis is needed to assess demographic developments and outbreak patterns.

Methods: We analysed MRSA notification data transmitted to the Robert Koch Institute (RKI) from 2015 to 2024 under the MRSA notification category. For demographic analysis (time, federal state, age, sex), we only included notified cases fulfilling the reference definition (invasive MRSA infections confirmed in blood or cerebrospinal fluid). For outbreak analysis, the dataset was extended to include all notified MRSA cases linked to outbreaks, including cases that did not meet the reference definition (e.g., colonisations or non-invasive infections). Outbreaks were identified using an outbreak identifier within the case information. For the demographic analysis, we calculated incidences per 100,000 inhabitants and applied chi-squared tests for comparisons.

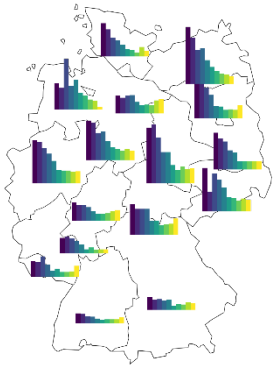
Results: Between 2015 and 2024, a total of 19,486 invasive MRSA cases were reported to the RKI. The distribution of cases across federal states changed significantly over time (p<0.005). Incidence declined nationally, from 4.4 to 1.4 per 100,000 inhabitants, with pronounced decreases in Berlin (8.7 to 1.9), Mecklenburg-Western Pomerania (8.4 to 1.1), and Saxony-Anhalt (8.2 to 2.2) (Figure). Children and young adults (1–29 years) had the lowest incidence (0.1–0.3 per 100,000). Incidence increased steadily from age 50 onward, with men consistently showing higher rates than women. Among individuals ≥80 years, incidence reached 14.3 in men and 5.6 in women. For outbreak analysis, additional 1,022 MRSA cases not fulfilling the reference definition were included, resulting in a total number of 20,508 cases. In total, 200 outbreaks comprising 696 cases were identified. Of the outbreak cases, 37% (242/696) were classified as infections, including 23% (157/696) as invasive infections.

Conclusion: MRSA incidence declined between 2015 and 2024, yet clear age- and sex-related risk patterns persist, particularly affecting older men. Outbreak numbers remain relatively low and notification of invasive cases only captures less than ¼ of outbreak cases. However, this analysis included only MRSA cases reported under the dedicated

MRSA notification category and may therefore be subject to underreporting. To enhance future efforts, integrated genomic surveillance (IGS)—linking routine epidemiologic data with genomic information on isolates—could provide additional critical insights.

Figure: Incidence of notified invasive methicillin-resistant Staphylococcus aureus (MRSA) infections per 100,000 inhabitants by German federal state, 2015–2024 (n=19,486). Colour code: 2015 (dark blue) to 2024 (yellow), with intermediate years represented by progressively lighter shades of blue to yellow.

Fig. 1



PS01.143
Beyond the outbreak: what hospital sinks reveal about everyday transmission risks
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Introduction: Hospital wastewater systems, particularly in intensive care units (ICUs), are increasingly recognized as hidden reservoirs for bacterial contamination and potential sources of healthcare-associated infections. While various components of the sink infrastructure may harbor pathogens, their relative significance and contribution to microbial diversity and resistance remain poorly understood.

Objectives: This study aimed to systematically assess bacterial contamination at multiple structural points within hospital sinks, characterize microbial diversity and resistance gene profiles, and evaluate the role of faucet use in bacterial dispersion.

Materials & Methods: Environmental samples were collected from four components of ICU sink units: splash area, wash basin, sink strainer, and siphon. Prevalence and species diversity were determined via culture-based methods and MALDI-TOF MS for species identification. Selected isolates were analyzed for resistance genes using the CarbDetect AS-2 Kit (Alere Technologies). The influence of faucet use on microbial dispersion was simulated to assess surface contamination in the surrounding area.

Results: The sink strainer and siphon, as components in direct contact with wastewater, exhibited the highest prevalence and greatest species diversity. These sites predominantly harbored gram-negative organisms such as *Citrobacter freundii, Enterobacter cloacae, Klebsiella pneumoniae, and Pseudomonas aeruginosa*, many of which carried resistance genes encoding carbapenemases, extended-spectrum β-lactamases, aminoglycoside-modifying enzymes, quinolone resistance determinants, and virulence factors. The sink strainer revealed the most diverse microbial spectrum and is thus considered the most representative sampling site. Faucet operation increased bacterial dispersion into the splash area, reinforcing its relevance as a high-risk transmission interface.

Conclusion: Internal sink components, such as strainers and siphons, serve as key reservoirs for both resistant and susceptible gram-negative bacteria. These findings underscore the need for targeted investigations to quantify the true burden of waterborne colonization and infection in routine clinical care, beyond outbreak scenarios. Broader analyses including non-resistant strains are essential to accurately evaluate wastewater-associated transmission risks and to inform evidence-based strategies for infection prevention, infrastructure design, and hygiene control in healthcare settings.

PS01.145
Multicenter prevalence of gram-negative rods with reduced susceptibility to carbapenems and analysis of infection control measures
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Question: Multidrug-resistant gram-negative rods (MRGN) present a significant challenge for hospitals in terms of infection prevention and control (IPC) measures and antibiotic therapy. Patients colonized with "4MRGN" isolates are routinely subjected to contact precautions as recommended by German guidelines. Isolates that do not meet the "4MRGN" criteria, but phenotypically show a reduced susceptibility to carbapenems (redCS), must be reported to the health authorities as recommended in RKI case definitions 2023. However, no national recommendations for IPC measures exist.

The aim of this study was (i) to determine the prevalence of isolates with redCS in four German third-level hospitals in 2024 and (ii) to evaluate related IPC strategies. Whole genome sequencing (WGS) was used in a subset of isolates to further characterize redCS.

Methods: We included Enterobacterales and *Acinetobacter baumanii* complex (ABC) isolates with redCS detected at four German hospitals in 2024.

At University Hospital Münster (UHM) the collection of futher isolates continued until March 2025. Here resistance phenotypes were confirmed using Etest and interpreted according to EUCAST clinical breakpoints. In Enterobacterales carbapenemases were excluded by first-line screening tests. All UHM isolates were subjected to WGS and subsequent core genome multilocus sequence typing (cgMLST) to analyse genetic relationships. Presence of resistance genes was analysed with AMRFinderPlus.

Results: Isolates with redCS are rare in all participating hospitals (UHM in 2024 (1300 beds, n=3), Cologne Merheim Medical Centre (700 beds, n=13), University Hospital Regensburg (890 beds, n=12) and Würzburg (1452 beds, n=7)). No hospital applied IPC measures recommended for "4MRGN" in case of redCS.

At the UHM, ten redCS isolates underwent WGS: eight *Klebsiella* spp., one *E. coli* and one ABC. cgMLST showed, that three *K. oxytoca* isolates form a closely related genetic cluster. Here epidemiological information confirmed hospital associated transmission.

Two redCS isolates had to be reclassified as "4MRGN" during the study. First, a *K. oxytoca* with an increased ertapenem MIC (1.5 mg/l), but no carbapenemase detected. Here the isolate showed meropenem resistance in subsequent samples. The corresponding patient received prolonged carbapenem therapy.

Second, an ABC with an increased MIC for meropenem (3 mg/l) showed the presence of $bla_{\text{OXA-66}}$ in WGS. The respective patient came from a high-prevalence country for carbapenemases.

Conclusions: Our study indicates that redCS isolates are rare. Currently they are classified by phenotype. Our WGS analysis suggest "red flags" in redCS isolates for IPC. Carbapenemases that are not detected by first-line screening tests and isolates that progress to "4MRGN" under prolonged antibiotic therapy. This suggests that species- and patient-dependent factors should be considered when applying IPC measures. Further data analysis will be needed to recommend a targeted IPC strategy.

PS01.147

Identification of potential clusters of multidrug-resistant gram-negative bacteria in acute-on-chronic liver failure patients

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Introduction: Acute-on-chronic liver failure (ACLF) is a life-threatening condition characterized by acute hepatic decompensation, frequently progressing to multi-organ failure. Infections with multidrug-resistant Gram-negative (MDRGN) bacteria significantly worsen prognosis and limit therapeutic options. Liver transplantation (LT) remains the only curative treatment, but MDRGN infection or colonization contraindicate LT.

Objectives: To assess the prevalence of carbapenem-susceptible MDRGN in pre-ACLF LT candidates and to characterize the underlying genetic determinants of resistance, with emphasis on molecular epidemiology and potential transmission events.

Materials & Methods: Between 2021 and 2023, 48 LT candidates at University Hospital Frankfurt were screened for MDRGN. Species identification, antimicrobial susceptibility testing (EUCAST), and PCR-based detection of carbapenemase genes were followed by whole-genome sequencing (Illumina NovaSeq) and bioinformatic analysis. Patient records were reviewed to identify potential transmission links.

Results: MDRGN colonization was detected in 46% of patients (22/48), yielding 45 isolates (predominantly *E. coli*; n=42). Most isolates were resistant to both cephalosporins and fluoroquinolones (n=41), while two harbored the carbapenemase gene *blaOXA-244*. Sequence types ST744, ST131, and ST38 were most prevalent. Core genome MLST and pangenome analyses revealed high genetic variability, including within identical STs. One likely transmission cluster involving ST38 was identified, though no direct hospital overlap was documented.

Conclusions: MDRGN colonization is frequent among ACLF patients evaluated for LT, with high genetic heterogeneity suggesting independent and primarily community or prior healthcare acquisition. Genomic surveillance enables the detection of cryptic transmission events and supports infection control efforts in transplant settings.

PS01.149

Evaluation of an algorithm for the digital surveillance of urinary tract infections (UTI) on the intensive care unit (ICU)

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Introduction: Surveillance of hospital-acquired infections in Germany is routinely conducted via manual chart review; this approach is resource intensive and prone to subjectivity. Analyzing routine electronic data via algorithm to conduct surveillance may present a worthwhile alternative to this approach; we expanded upon a previously validated algorithm to include urinary tract infections. We compared data derived from manual chart review to that which was extracted from hospital and laboratory information systems (HIS/LIS) to evaluate nosocomial infections and MDRO acquisition.

Materials/Methods: Data was obtained from one university medical center ICU over a period of 9 months. The infection prevention and control team collected clinical data in accordance with the Protection against Infection Act (IfSG). Manual chart review was then conducted according to the ICU-KISS module"s protocol provided by the National Reference Center for the Surveillance of Nosocomial Infections (NRZ). Routine data was extracted from HIS/LIS and analyzed via an algorithm that was originally developed for the EFFECT study; ward-movement data was linked with microbiological test results, generating a data set that allows for evaluation as to whether or not a urinary tract infection was nosocomial/ICU-acquired.

Results: Within a time period of nine months, manual chart review conducted according to ICU-KISS detected nine cases of ICU-acquired urinary tract infection whereas the algorithm identified twenty cases. The case discrepancy of eleven cases can be explained as follows: human error in manual chart review allowed for one case to be missed, seven cases were found within two calendar days of ICU step-down (and therefore still considered ICU-acquired) and three cases were identified without the fever prerequisite required to identify nosocomial UTIs in the KISS protocol.

Discussion: The data regarding nosocomial infections generated by algorithm identifies more cases of ICU-acquired

UTIs than manual chart review. This study not only suggests but also shows that hospital infection surveillance based on electronically generated routine data is an effective and sustainable alternative to the subjective, resource-intensive and time-consuming method based on manual chart review.

PS01.151
Development and construction of a washbasin according to hospital hygiene aspects
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Introduction: Among other factors, the recommendation of the Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) "Anforderungen der Hygiene an abwasserführende Systeme in medizinischen Einrichtungen" identifies washbasins as a proven source of nosocomial infections, particularly through aerosol and droplet formation at the drain or when the water drains. In conventional sinks, the drain is usually positioned centrally in front of the back wall in the sink well, and a drain set inserted into it connects the sink to the siphon's immersion pipe. The drain set is more or less sharp-edged at various points, with internal struts and undercuts for mounting a perforated plate or plug that covers the drain from above. The flow breaks off at all edges of this device as the water drains away, forming aerosols that are contaminated by the biofilm on the struts and covers, which are difficult to clean without tools. The use of cleaning and disinfection chemicals is largely ineffective, as the biofilm responsible for the aerosol contamination cannot be reached (little or no contact and exposure time).

Objective and implementation: As an alternative to conventional washbasins, a sink for all applications with increased hygienic relevance was developed. The objective was to design the shape and positioning of the drain in such a manner that it (1) drains the water without releasing splashes and aerosols and (2) can be cleaned completely and without any tools other than hands and wipes. The drain is moved from the sink well into the rear wall in a flow-optimized shape. Fittings inserted into the drain with sharp edges, undercuts and mechanisms are avoided (hair, soap and other dirt residues cannot get stuck or accumulate to feed nearby biofilm). The transition piece for the siphon is fitted directly under the sink. The resulting cavern is shaped as a confuser, which guides the draining water film into the drain with reduced turbulence. All transition radii are generously rounded (r≥5mm), thus stalling at sharp edges and thus release of splashes and aerosols are avoided. In addition, large radii enable complete cleanability, including the first few centimetres of the drain itself. A further advantage of this arrangement is that water does not overflow the drain when the tap is opened, preventing splashing water from the siphon.

Recent measurements (particle counting, particle image velocimetry, visualizations) confirm that under normal conditions water drains without aerosol release, which is never the case in conventional sinks.

The drain is published as a national (DE 10 2023 130 203 A1) and international (WO 2025/093528 A1) patent.

PS01.153
Innovative air filtration: cold plasma technology shows efficacy against viral contaminants using bacteriophage surrogates
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Introduction: The COVID-19 pandemic has highlighted the urgent need for advanced air disinfection to reduce airborne virus transmission in high-risk indoor environments. Combining air filtration with robust viral inactivation could mitigate risks in healthcare, public, and transport settings. Bacteriophages offer safe surrogates for such studies.

Objective: We have assessed the efficacy of cold atmospheric plasma (CAP) generated by a volume dielectric barrier discharge (DBD) device for virus inactivation on air filters.

Materials & Methods: To simulate a room air purifier, the CAP source and the particle filters were arranged consecutively at a distance of 100 mm in a ventilation duct. A fan provided constant airflow in the ventilation duct, directing reactive gas species from the plasma source onto the air filter. Three bacteriophage strains—MS2, PhiX174, and Phi6—were tested as surrogates. Filter segments were inoculated with eight 2-µl droplets of phage suspension (each ~10^6 Plaque Forming Unit (PFU)). For the control condition, identically inoculated filter segments were processed in the same manner but without exposure to CAP treatment. Post-exposure, residual infectious phages were quantified by plaque assay, and inactivation efficiency (% PFU reduction compared to controls) was calculated.

Results: CAP treatment produced significant, time-dependent inactivation of all three bacteriophages on air filters, as determined by comparison to untreated control filters that were processed in parallel without CAP exposure. Specifically, MS2 inactivation efficiencies relative to these controls were 70%, 78%, and 84% after 10, 20, and 30 minutes, respectively ($p < 0.001–0.0001$). PhiX174 inactivation was 74%, 84%, and 89% ($p < 0.01–0.0001$) at the corresponding time points. For Phi6, complete inactivation (100%) was observed at all tested time points ($p < 0.0001–0.05$). These data demonstrate that all reported reductions refer directly to the untreated control condition.

Discussion: These results confirm that CAP generated by a volume DBD device achieves robust viral inactivation across different phage models and exposure times, including complete elimination of Phi6. The use of MS2 (single-stranded RNA), PhiX174 (single-stranded DNA), and Phi6 (enveloped double-stranded RNA) as surrogates constitutes a scientifically robust model for clinically relevant, potentially pathogenic human viruses, such as noroviruses, enteroviruses, Parvovirus B19, adenoviruses, coronaviruses (e.g., SARS-CoV-2), respiratory syncytial virus (RSV), and influenzaviruses A and B. These findings indicate the potential of CAP for air decontamination across a broad spectrum of clinically important human viruses. The clinical impact, however, requires further investigation in future studies.

PS01.155
Evaluation of universal screening for MRSA in a metropolitan hospital trust

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Introduction: Although invasive infections by methicillin-resistant *Staphylococcus aureus* (MRSA) have decreased in Germany, MRSA remains a formidable threat. While the drop has likely been due to risk-based screening and improvements in infection control, analyses of long-term effects of specific interventions on the occurrence of MRSA in hospitals are sparse.

Aims: We present a survey of the occurrence of MRSA and methicillin-susceptible *S. aureus* (MSSA) in clinically relevant specimens (CRS) over 11 years (January 2012 to May 2023) and document changes associated with the introduction of universal nasopharyngeal MRSA screening among admissions of a metropolitan hospital trust (1,400 beds) in October 2017 (Figure, vertical dashed line). Before October 2017 MRSA admission screening was risk-based. Screening for MSSA carriers was not performed.

Materials and Methods: Data regarding detection of MRSA and MSSA were extracted from the laboratory information system. Patient admission and discharge dates were provided by the controlling department of the hospital trust. CRS included normally sterile specimens (e.g. blood cultures), respiratory samples, wound swabs, and urine. The observation period was arbitrarily sliced into 23 "seasons" lasting 6 months each (except the first and last with 3 and 8 months, respectively). Densities of MSSA/MRSA in CRS were calculated by dividing deduplicated laboratory confirmed occurrences by patient days adjusted for specimen densities. Only specimens and patient days after a minimum inpatient stay of 48 hours were included. Modeling was performed using negative binomial regression with interrupted time series analysis to estimate percent changes before and after October 2017.

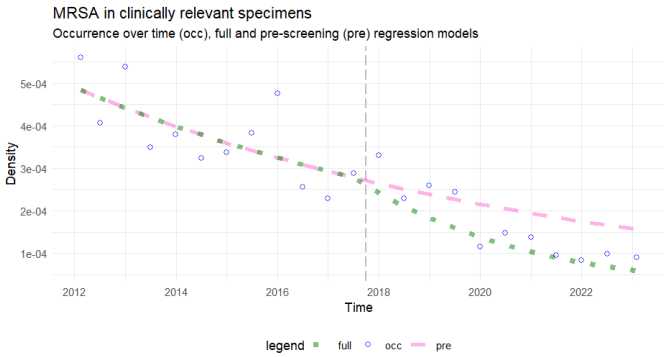
Results: Data pertaining to 648,838 hospital admissions were intersected with 433,137 CRS. Specimens from 167,972 admissions were included. Densities of CRS microbiology requests rose over the observation period, with increases for samples taken within the first 48 hours of inpatient stay (x 3.3) surpassing later stages (x 1.4). The proportion of admissions screened increased from 15% pre- to over 90% from November 2017 onwards. Density of MRSA in CRS decreased even before introduction of universal screening by -10,4% per annum (Figure, dashed pink line). After October 2017, an additional significant negative term per "season" (-8.5%, p=0.002) was found (Figure, dotted green line). Nevertheless, the occurrence of MSSA, which increased by 3.7% p.a. pre intervention, also followed a negative trend of -5.1% per "season" after universal screening.

Conclusions: We document the accelerated waning of MRSA in CRS following the introduction of universal screening. As a decrease was also seen for MSSA, we assume an unspecific effect appearing concurrently with screening efforts. The drop of MRSA in CRS can thus not solely be attributed to universal screening.

Figure

MRSA density in clinically relevant specimens. Please refer to abstract text.

Fig. 1



PS01.157

Prospective, patient-reported surveillance of post-Caesarean surgical site infections using mobile-accessible online surveys: preliminary data from the SECURE sectio study

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Background: Post-caesarean surgical site infections (SSIs) are a common and costly burden in maternal healthcare. In Germany, these infections are currently recorded retrospectively in a nationwide hospital infection surveillance system ("KISS"). However, given short postoperative hospital stays and shortcomings in post-discharge surveillance methodology, we presume that the current system significantly underestimates the true infection rate. To improve monitoring of obstetric SSI, we introduced a prospective, patient-reported surveillance system using a mobile-accessible online survey at the University Hospital Bonn (UKB).

Method: This is a preliminary analysis of the SECURE Sectio study (DRKS00033988). During their hospital stay, postpartum patients were invited to participate in the study via scanning a QR Code, which directed them to an automated online survey system. Follow-up questionnaires were distributed via email and/or mobile phone message on postpartum days 7,14, and 30. The surveys collected information on symptoms suggestive of SSIs and any healthcare consultations or treatments received. If an SSI was suspected based on survey responses, participants were asked to be contacted by phone to confirm the diagnosis and gather additional clinical details. Data were collected from October 2024 to April 2025 at UKB. SSIs were defined according to the criteria for postoperative superficial wound infections by the National Reference Center for Surveillance of Nosocomial Infections (NRZ).

Results: Out of 52 recruited patients, 7 (13.5%) self-reported clinical signs of a superficial postoperative SSI. Two patients (3.8%) were re-admitted to UKB with an SSI. Antibiotics were administered in 57.1% of the SSI cases. We found no significant difference for the frequencies of gestational diabetes, diabetes mellitus and secondary cesarean sections between patients with SSI and no SSI.

Discussion: Preliminary data from the SECURE Sectio study suggest that the rate of superficial post-caesarean SSIs may currently be underestimated by the NRZ KISS surveillance system. Surveillance based on patient-reported infection events may be more appropriate to capture the true SSI burden in postpartum patients. Recruitment of patients is currently ongoing to determine the infection rate, main risk factors, and to design targeted interventions to reduce infection rates and antibiotic use.

Fig. 1

	SSI (n = 7)	No SSI (n = 45)	P-value
Antibiotic use, n (%)	4 (57.1)	—	<0.001
Age, mean (SD), years	32.1 (4.3)	33.2 (5.0)	0.57
BMI, mean (SD), kg/m²	23.2 (4.3)	27.3 (6.5)	0.11
Hospital stay, mean (SD), days	3.6 (1.1)	4.2 (2.0)	0.54
Smoking, n (%)	—	4 (8.9)	0.99
Gestational diabetes, n (%)	1 (14.3)	12 (26.7)	0.66
Diabetes mellitus, n (%)	1 (14.3)	—	0.13
Multiparity, mean (SD)	1.7 (0.8)	1.6 (0.8)	0.71
Prior cesarean section, n (%)	3 (42.9)	17 (37.8)	0.99
Secondary cesarean section, n (%)	2 (28.6)	24 (53.3)	0.42

PS01.159
Effects of sub-MIC antibiotic exposure on Staphylococcal biofilm formation
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Question: Biofilm-forming and multidrug-resistant bacteria pose a major challenge, particularly in implant-associated infections. In addition to developing new antibiotics, optimizing the use of existing agents is increasingly relevant. Dalbavancin, a lipoglycopeptide with strong activity against gram-positive bacteria, including MRSA, and a long half-life, is promising for systemic and local use. We have previously shown that local release of dalbavancin reduces inflammation and bacterial spread after biomaterial implantation *in vivo*. Here, we mimicked declining antibiotic levels during local release and investigated the effect of subinhibitory concentrations on bacterial adherence and biofilm formation compared to rifampicin and minocycline.

Methods: *Staphylococcus aureus* ATCC 35556 was grown in antibiotic concentrations ranging from 2x MIC to 1/32x MIC in 96-well polystyrene plates using broth microdilution. In accordance with EUCAST recommendations, susceptibility testing for dalbavancin was performed in final concentrations of 0.002% Tween 80. After 24 h of incubation in tryptic soy broth, wells were washed three times with phosphate-buffered saline. Then, biofilm biomass was quantified using crystal violet staining (0.06%) referenced to an untreated control, or serial dilutions were plated to determine colony-forming units (CFU/ml).

Results: While bacterial cell counts remained stable across all tested antibiotic concentrations, noticeable differences in biofilm formation were observed. Rifampicin slightly increased relative biofilm mass in all tested concentrations. Minocycline reduced adherent biofilm biomass at lower sub-MIC concentrations (1/8x and 1/4x MIC), while higher selective pressure (1/2x MIC) resulted in biofilm mass comparable to the untreated control. Although the combination of rifampicin and minocycline increased bacterial susceptibility, no additional inhibition of biofilm formation was observed compared to minocycline exposure alone. Finally, dalbavancin demonstrated a strong, concentration-dependent reduction in biofilm mass, particularly at 1/4x and 1/2x MIC.

Conclusions: Our data underscore the importance of determining both CFU/ml and biofilm mass to gain a comprehensive understanding of antibiotic induced changes in biofilm formation. The results suggest a specific antibiofilm effect for dalbavancin at subinhibitory concentrations. While rifampicin is often used in combination with minocycline to reduce the development of resistance, our data indicate that its presence may attenuate antibiofilm effects. Ongoing qPCR and SEM analyses will help to elucidate the mechanistic and structural basis for these observations and will guide the rational design of future anti-infective drug-eluting biomaterials.

PS01.161
Assessment of the prevalence of pediculosis capitis and related effective features among primary schoolchildren in Ahvaz County, Southwest of Iran
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The present research was carried out to investigate the prevalence of head lice and related effective features among students in primary schools of Ahvaz County; Iran; to suitable training programs via improve the health promotion of the students in this county during winter 2016 and spring 2017. The sample size was 5930 students that was determined by using a single population proportion formula. In this analytical–descriptive study, samples were selected by a multistep, classification random sampling strategy. The data was collected in two parts, a carefully visual examination of the hair and scalp with head lice comb, and an interview to complete a questionnaire about epidemiologic factors. The collected data was evaluated using SPSS software, version 22. These data were compared using a Chi-square analysis, and a p value of less than 0.05 was considered to be significant. The overall head lice infestation rate in the studied population was 26.3%. The revalence rate was 15.2% in the boys and 37.9%in the girls, while the rate of infestation was 25.1%in the urban areas and 36.9%in the villages. Also, a statistical significant relationship was observed between Pediculosis capitis and gender, geographical area, type of home, access to tap water, hygiene bath at home, bath times per week, the number of family individuals, sleeping in the common bedroom, use common bedding, daily comb, use common comb, use common towel, use common scarf, history of infestation, presence health educator in school, access to primary health services, knowledge about head lice, infestation in the family, performing personal hygiene, itching on the head and hair style, length, density, and color. The high prevalence of head lice among students in the majority of primary schools in Ahvaz County is attributable to inadequate access to health educators, sanitation facilities, inattention to personal health, and other related factors. The controlling program must be done more efficiently to prevent the prevalence of head lice infestation. It is suggested that the people"s knowledge and attitude about biology, clinical presentation, prevention, and treatment strategies of pediculosis capitis should be improved.

Keywords Epidemiology . Pediculus capitis . Morbidity . Risk factors . Iran

PS01.163

Comparative efficacy of permethrin 1%, lindane 1%, and dimeticone 4% for the treatment of head louse infestation in Iran

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Pediculosis capitis occurs worldwide. Children in the age group of 5–13 years are the ordinary victims. Population-based studies show that the prevalence of pediculosis is 1.6–13.4% in various regions of Iran. In this observer-blinded trial, we conducted a study to evaluate the comparative efficacy and safety of permethrin 1% and lindane 1% shampoos and dimeticone 4% lotion to cure head lice infestation in children aged 7–13 years in female primary schools in Ahvaz City, Iran. Children with head lice were randomized to receive each treatment. Two applications of permethrin 1% or dimeticone 4% were done, 1 week apart. However, lindane 1% was used just once for treatment cases. Data analysis was done using SPSS software. Recovery differences between the groups were tested using the chi-squared test. Four hundred forty-four louse-infested schoolgirls were randomly treated with them. Permethrin, lindane, dimeticone, and placebo produced the recovery rates 56.8, 31.5, 51.4, and 10.8% on day 2 posttreatment,respectively. Cure rates were in the permethrin group on days 8 and 15, respectively, 69.4 and 90.1%. The success rates were in the lindane group on days 8 and 15, respectively, 73 and 86.5%. The success rates were in the dimeticone group on days 8 and 15, respectively, 60.4 and 94.6%. It is recommended dimeticone lotion (4%) as a very effective and safe pediculicide for pediculosis control. With proper application, dimethicone can rapidly cure head lice infestation with minimal reinfestation.

Keywords: Pediculosis . Children . Primary schools . Lindane . Permethrin . Dimeticone . Head lice . Iran

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PS01.165

A six-year descriptive-analytical study of *Pediculosis Capitis* in the Southwestern Iran

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Head lice infection is one of the most common diseases of all ages and has a global spread. The disease is more common in areas with high population density and lack of public health. Population growth and poor health are factors that exacerbate this infestation. This study aimed to determine the prevalence of head pediculosis among school children in urban and rural areas of Eastern Ahvaz, southwestern Iran. In this descriptive-analytical study, 5730 pupils were examined. Pupils were selected by multistep method. The hair on the head, back of the neck, and around the students' ears was examined to find out nits or live lice. Analysis was done by Chi-square l tests using SPSS software, version 18. A P- value of less than 0.05 was considered to be significant. Totally 5730 pupils from elementary, middle and high schools were randomly selected . Data was collected through school screening programs by trained persons using a questionnaire that included the information on the diagnostic result of head lice. The screening method was by inspection. The results and demographic data were analyzed by SPSS software. Main outcome measure: We considered the following demographic and epidemiological parameters: age, gender, educational level, history of infestation, season and geographical area. The infestation of head lice was 5730 cases. Of those affected with pediculosis, 75.6% lived in villages and 24.4% were rural residents. Most of the patients were found in the autumn (60%). Overall, 79.6% of students with pediculosis studied in primary schools, and 17.9% of those infested with pediculosis had a previous history of this infestation. The prevalence of pediculosis was higher in females than in males (97.2% vs. 2.8%, respectively). Our results on the higher prevalence of head pediculosis in females than in males are in line with many previous researches. Meanwhile, the rate of infested children was different according to the age groups. These differences might be associated with behavioral variations in different genders and age groups.

Key words: Head lice, Epidemiology, Iran

PS01.167

A study on the efficacy of cold plasma disinfection on various relevant human pathogenic bacterial species

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Introduction: The global rise in antibiotic-resistant bacteria presents a growing challenge to public health, as many bacterial infections are becoming increasingly difficult to treat with conventional antibiotics. Consequently, there is an urgent need to explore alternative mechanisms of bacterial eradication. One promising approach is the use of cold plasma, a non-thermal ionized gas that has been shown to effectively inactivate microorganisms. The aim of this study was to evaluate the bactericidal efficacy of cold atmospheric plasma (CAP) on a broad panel of clinically relevant Gram-positive and Gram-negative bacterial pathogens, with a focus on potential applications in infection control.

Material and methods: The bactericidal efficacy of CAP was tested using different plasma applicators on a variety of bacterial isolates. The plasma devices used are a modified ViroMed medical system operating with Indoor air. Suspensions of twenty-one human-pathogenic bacterial species, including 10 Gram-positive and 11 Gram-negative strains, were plated in different dilutions on blood agar, followed by exposure to CAP for defined durations (60–180 seconds) at different working distances. Experiments were done at room temperature to exclude thermally induced effects. After a 24-hour incubation at 37°C, colony counts were assessed to determine the impact of CAP on bacterial survival.

Results: The study demonstrated a statistically significant bactericidal effect across all tested Gram-positive and Gram-negative species. Quantitative analysis revealed a marked reduction in colony-forming units (CFUs) in all treated samples, indicating a consistent antimicrobial efficacy. Some clinical isolates, including *Staphylococcus aureus*, *Escherichia coli*, and *Citrobacter freundii*, exhibited complete growth inhibition following the longest exposure duration. This suggest a time-dependent antimicrobial response. Control plates, which were not exposed to cold plasma, showed consistent bacterial growth.

Discussion: These findings highlight the potential of CAP as a novel antimicrobial tool in medicine, particularly in the management of chronic wounds, skin and soft tissue

infections and for treating multi-drug-resistant bacteria. While early experimental concepts suggest applicability to respiratory infections, such as ventilator-associated pneumonia, clinical implementation in this area is actual exploratory. CAP offers several advantages, including a multifactorial antimicrobial mechanism that reduces the likelihood of classical resistance development and toxicological studies indicate good tolerance under controlled conditions and no adverse effects on human tissue. The results of this study contribute to the growing body of evidence supporting cold plasma as a viable alternative to traditional antibiotic treatments, with significant implications for the treatment of bacterial infections that are increasingly resistant to conventional therapies.

PS01.169

Investigation of the relationship between *Legionella* concentrations and total cell counts in drinking water plumbing systems and flat water meters

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Introduction: According to the German Drinking Water Ordinance, *Legionella* spp. have to be tested in buildings under certain conditions. The presence of bacteria and *Legionella* in individual components of the drinking water plumbing system (DWPS), such as branch pipes, fittings or angle valves, can cause local contamination that does not originate from the circulating hot water system. The significance of flat water meters for the detection of *Legionella* in water samples is not yet known.

Aims: The aim of the study was to investigate the potential occurrence of *Legionella* and water-associated bacteria in flat water meters and to assess their impact on water samples from the tap.

Material and methods: 136 water samples were taken from the central and peripheral areas of the DWPS in both hot (PWH) and cold water (PWC) from 22 flats in 11 apartment blocks in Hamburg and Schleswig-Holstein. Samples were taken from washbasins in the peripheral area in accordance with purpose C of DIN EN ISO 19458, with the Drinking Water Ordinance after 1 litre of drainage volume and after 3 litres in order to record the riser. Between the 1 and 3 litre samples, 38 flat water meters were removed and taken to the laboratory for analysis. The water meters were filled with sterile NaCl solution and analysed after one hour. Analysis of *Legionella* was carried out in accordance with DIN EN ISO 11731 and in parallel by flow cytometry using an accredited method to determine the total cell count (TCC)/ml.

Results: In all 62 samples from the central DWPS, *Legionella* concentrations were below the limit of quantification. In water meters from PWC, *Legionella* up to > 60,000 CFU/100 ml were detectable in 17 out of 20 samples, in water meters from PWH in 3 out of 17 up to 1,100 CFU/100 ml. The TCC in the water meters were between 112,000 and 1.5 x 107 cells/ml.

In drinking water samples from the flats, the high proportion of cold water samples with evidence of legionella (80 %) in the samples after 3 litres of drainage volume and replacement of the water meter was striking. This is not the case for hot water.

In cold water, a positive monotone relationship between the *Legionella* concentration in the meter and the concentrations in the water samples were seen (p=0.047). For TCC, this correlation is strongly positive (p<0.001). Since there were only 3 hot water meters with *Legionella* detected, no definite conclusions regarding a relationship between concentrations in the meter and in the water samples can be drawn.

Conclusions: Cold water plays a relevant role in the occurrence of *Legionella* in DWPS. Flat water meters can be contaminated with *Legionella* in high concentrations. In cold water, there is a positive monotone relationship between concentrations in meter samples and samples from the flats for both microbiological parameters.

This correlation was not recognisable in hot water, but cannot be ruled out due to the low number of *Legionella*-positive meter samples.

PS01.171

Neuorganisation des praktischen Studentenkurses Medizinische Mikrobiologie in der Humanmedizin

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Einleitung: Im Zuge der zunehmenden Verzahnung der medizinischen Fächer und der Einführung von innovativen Lehrformaten, haben wir unseren praktischen Studentenkurs im Fach "Medizinische Mikrobiologie" neu organisiert. In diesem Kurs sollen die Studierenden im 6. Fachsemester das Arbeiten mit Mikroorganismen erlernen, wichtige Grundkenntnisse erlangen und abschließend ihr Wissen in einer kurzen mündlichen Prüfung präsentieren.

Durchführung und Ergebnisse: Unsere Veranstaltung besteht aus 5 Nachmittagen (jeweils 3,5 h; bestehend aus 2,5 h Kurs und 1 h POL-Besprechung), an denen die Themen (i) Einführung und multiresistente Erreger, (ii) Haut-, Wund-, Knocheninfektionen & Blutstrominfektionen, (iii) Infektionen des ZNS und des Respirationstraktes, (iv) sexuell übertragbare Infektionen, Harnwegsinfektionen und Infektionen in der Schwangerschaft und (v) Infektionen des Gastrointestinaltraktes und bei Reiserückkehrern gelehrt werden. In dem Kurs lernen die Studierenden in Kleingruppen anhand von Fallbeispielen, bei denen Krankheitsbilder beschrieben werden und selbständig die Patientenproben (vorgelegtes Material) bearbeiten werden. Dies beinhaltet die Identifizierung von Keimen, die Testung von Resistenzen, das Anfärben von Präparaten und Mikroskopie, sowie die Analyse von PCR-Messungen und Serologie. Die Ergebnisse werden in einem Kursheft zusammengefasst, das neben weiteren Vorbereitungsmaterialen zum Lernen dient. Der praktische Kursteil wird durch zwei begleitende POL-Fälle ergänzt, die wichtige Symptomkomplexe bei Infektionen, nämlich Fieber unklarer Genese und unklarer persistierender Husten, beinhalten. Zur Erfolgskontrolle wird abschließend ein mündliches Testat durchgeführt. Dabei wird jeder Studierende nach einem standardisierten Schema in ein wissenschaftliches Gespräch eingebunden, bei dem neben Faktenwissen auch fachliche Zusammenhänge und logisches Denken überprüft werden.

Bewertung des Kurses: Die Kurstage, die POL-Fälle und das Abschlussprüfung werden von den Studierenden sehr gut bewertet. Besonders wird das praktische Arbeiten und die enge und intensive Betreuung im Kurs und in den POL-Fällen gelobt. Die überwiegend guten Lernerfolge werden auch in der Benotung der mündlichen Prüfungen sichtbar.

PS01.173

Hippo signaling-driven lung fibrosis by influenza a virus infection in lung fibroblast

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Respiratory viral infections are increasingly recognized as contributors to pulmonary fibrosis, primarily through the induction of epithelial cell dysfunction and alveolar macrophage-mediated inflammatory responses. The cellular crosstalk between alveolar macrophages and lung fibroblasts is a key driver of fibrotic remodeling. However, the precise molecular mechanisms underlying fibroblast activation in response to viral infection remain poorly defined. Importantly, the involvement of the Hippo signaling pathway, a critical regulator of tissue homeostasis, has yet to be comprehensively investigated in this context. To address this gap, the present study examines both direct and indirect effects of Influenza A virus (IAV) infection on fibrotic activation of lung fibroblasts.

For this purpose, patient-derived alveolar macrophages and a state-of-the-art primary human monocyte-derived alveolar macrophage-like (AML) cell model were infected with IAV. Following infection, viral load, cytokine release, and transcriptomic changes were assessed. To evaluate indirect effects on fibrotic processes, human primary lung fibroblasts (IMR-90) were treated with conditioned media (CM) from IAV-infected AML cells. In addition, IMR-90 cells were directly infected with IAV to examine modulation of the Hippo signaling pathway and the expression of fibrotic markers using proteomic and transcriptomic approaches.

Our findings reveal two distinct mechanisms by which IAV promotes fibrogenesis in lung fibroblasts: (I) indirectly, through pro-inflammatory and pro-fibrotic factors released by infected alveolar macrophages, and (II) directly, via IAV-induced modulation of the Hippo signaling pathway, leading to increased expression of fibrotic markers such as fibronectin and collagen I.

These results highlight IAV infection as a key driver of fibrosis in pulmonary fibroblasts and identify Hippo pathway dysregulation as a critical mediator of virus-induced fibrotic remodeling.

PS01.175

Gut microbiota in decompensated liver cirrhosis before and after TIPS

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Intestinal dysbiosis is a common accompanying symptom to decompensated liver cirrhosis (dLC), and is linked to impaired intestinal barrier function, systemic inflammation, and poor clinical outcomes. Underlying causes and clinical implications are, however, still not well explored. In a recent study, we demonstrated that gut microbiota in dLC patients can be stratified into three main groups that greatly differ in taxonomic composition and functional characteristics. In particular, one group (41% of patients) stood out that was characterized by *Enterococcus* spp. dominance, low bacterial cell counts and low capacities to produce short chain fatty acids and secondary bile acids. Patients assigned to this group frequently received antibiotics and showed an impaired intestinal barrier along with an elevated proinflammatory cytokine profile and an increased incidence of infections. The other groups were dominated by either *Bifidobacteria* spp. or diverse communities and had better clinical characteristics.

An immediate consequence of the progressive fibrotic scarring of hepatic tissue is hypertension in the portal vein, promoting inflammation and further complications, such as ascites and hepatorenal syndrome; it is also likely contributing to dysbiosis. Treatment involves placing a transjugular intrahepatic portosystemic shunt (TIPS) that lowers the portal pressure and reduces systemic inflammation. Currently, we investigate the impact of TIPS insertion on gut microbiota and inflammation in dLC patients and its relation to clinical outcomes applying quantitative genome-resolved metagenomics, cytokine measurements and various clinical assessments. Samples (n= 182; 69 patients) were collected before and up to four follow-ups until one year post TIPS insertion. In concordance with our previous work, we detected all three taxonomic groups mentioned above before TIPS. Longitudinal analyses suggested that *Enterococcus* spp. dominance was, however, transient and particularly centred around peri TIPS samples, whereas diversity significantly increased a few months post-insertion. Results suggest that TIPS placement has a positive influence on gut microbiota composition and function, potentially contributing to improved clinical outcomes. *Bifidobacteria* remained enriched throughout the complete observational period. We are currently integrating clinical data and inflammation parameters with functional metagenomics and metatranscriptomics in order to investigate the role of TIPS insertion on gut microbiota and clinical consequences in detail.

PS01.177

Linking *Candida albicans* protein kinases to cytotoxity – characterisation of Crk1 functions during epithelial invasion

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Microbial signal transduction pathways regulate adaptation to changing environmental conditions and facilitate the success of both commensal and pathogenic during interactions with the host. Most of these pathways are regulated by protein kinases. The opportunistic fungal pathogen *Candida albicans* exists as a harmless commensal on mucosal surfaces of most humans, but can also cause superficial to invasive infections upon certain circumstances. Both life styles require a complex network of signalling pathways.

The *C. albicans* genome was predicted to encode 108 protein kinases, yet nearly 50% remain uncharacterised. We aim to dissect the role of *C. albicans* protein kinases during the transition from commensal to pathogen.

We used *in vitro* intestinal epithelial cell (IEC) models to investigate the role of fungal protein kinases and to study their impact on the pathogenicity of *C. albicans*. First, we screened a library containing individual *C. albicans* mutants lacking each of the identified protein kinase genes for their ability to damage IEC. Mutants displaying altered IEC cytotoxicity were further tested for their growth and morphology phenotypes. Surprisingly, a mutant in which *CRK1* was deleted caused increased IEC cytotoxicity, despite slower growth and reduced hyphal length as compared to the parental wild type. To dissect the role of *CRK1* in pathogenicity, we monitored metabolic fitness by using Biolog Phenotypic Microarrays, and stress resistance by spot dilution assays, quantified the damage capacities towards other cell types, and quantified the adhesion, invasion and translocation potential.

Infection of various epithelial cell lines in different cell culture media revealed that the increased damage potential of *crk1*Δ/Δ is cell type- and media-dependent. Accordingly, metabolic profiling and spot dilution assays showed that *CRK1* is important for metabolic adaptations and resistance to cell wall stress. When investigating the stages of IEC infection, we found that adhesion of *crk1*Δ/Δ to IEC was slightly reduced, whereas invasion was significantly increased. In contrast, the ability to translocate through an IEC barrier in a transwell assay was reduced.

Collectively, we used *in vitro* models to investigate the role of protein kinases for *C. albicans* pathogenicity and identified the protein kinase gene *CRK1* as being critical for the regulation of processes linked to medium- and host cell type-specific cytotoxicity.

PS01.179

Suitability of metagenomic sequencing for small intestinal bacterial overgrowth diagnostics

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Pediatric intestinal failure (pIF) is frequently complicated by small intestinal bacterial overgrowth (SIBO), which can contribute to systemic infections, inflammation, and impaired nutrient absorption. Conventional SIBO diagnostics rely on culture of jejunal aspirates, which, although widely used, may underrepresent the diversity and complexity of the intestinal microbiota. In this study, we performed a direct comparison of culture-based microbiological assessments and untargeted metagenomic sequencing in different endoscopic sample types, aiming to characterize small intestinal microbial communities in children with pIF and to evaluate alternative sample types for SIBO diagnosis.

Jejunal samples (aspirates, brush swabs, biopsies) were collected from 44 children undergoing endoscopic evaluation for pIF. Standard culture methods were used to identify viable bacteria and determine total colony counts. In parallel, metagenomic sequencing was performed on DNA extracted from the same samples, enabling comprehensive, unbiased taxonomic profiling.

Culture-based methods detected SIBO (defined as >103 CFU/mL) in 66% of patients, primarily identifying *Enterobacteriaceae* such as *E. coli* and *Klebsiella spp.*. Metagenomic analysis detected both uncultured anaerobic bacteria and DNA signatures of oral commensals, likely inactivated by gastric passage, which were not represented in cultures. SIBO-positive samples were characterized by high abundance of bacterial reads belonging to one or few *Enterobacteriaceae* species. Anaerobic bacteria were in some instances found as concomitant flora in SIBO-positive samples by metagenomic sequencing, while being underrepresented in culture, but were never found by themselves in culture-negative samples. Overall, the metagenomic findings correlated well with culture-based SIBO diagnoses, supporting the reliability of both methods when samples are obtained endoscopically.

In contrast, stool or aspirates from small bowel stomas showed frequent false-positive SIBO results and did not reflect the microbiota of the proximal small intestine accurately, indicating that stoma samples are not suitable for SIBO diagnosis. Similarly, the utility of antibiotic therapy for eradication of SIBO in children with IF is not supported by our findings; the frequency of previous antibiotic therapy was not different between the SIBO / non-SIBO groups.

In conclusion, our findings underscore that SIBO is common among children with IF. Cultural diagnosis of endoscopic samples alone is sufficient for diagnosis, and mucosal biopsies and brush swabs are reliable alternatives to the gold standard of small bowel aspirates. Metagenomic sequencing reproduced and corroborated the cultural findings and revealed presence of additional uncultured or non-viable bacteria, but did not provide additional diagnostic insight.

PS01.181

Intimate association of *Campylobacter* with multidrug-resistant *Enterococcus* confers protection against diverse classes of antimicrobials

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Question: *Campylobacter jejuni* a major food-borne pathogen worldwide. Resistance to first-line fluorochinolone antibiotics (one of two recommended treatment options) is high. Surveillance of antimicrobial resistance is therefore of major importance. Here we report on two FQR *C. jejuni* isolates displaying reduced susceptibility towards multiple antimicrobials through close association with multi-drug resistant Enterococcus.

Methods: From 2010 to 2022, around 8500 clinical *Campylobacter* strains were analysed for their antibiotic resistance profiles within the framework of *Campylobacter* surveillance in Germany. For a subset of 2912 strains, genomic data were screened for antibiotic resistance genes and contamination with other bacterial species. Two strains with unusual antimicrobial resistance phenotypes were analysed in more detail, for example by means of scanning electron microscopy (SEM).

Results: Two *C. jejuni* clinical samples displayed a multi-drug resistance phenotype including resistance to fluorochinolones and macrolides. Genome analysis revealed that the samples, although derived from single colonies, also contained 10% of Enterococcus DNA reads. SEM confirmed the presence of coccoid bacteria in addition to spiral-shaped Campylobacter. *C. jejuni* benefited from intimate association with *E. faecium* or *E. faecalis* showing reduced susceptibility towards additional antimicrobial classes including macrolides.

Conclusions: Multi-drug resistance patterns in *Campylobacter* isolates may be due to intimate association with other intestinal bacteria. Specifically, association of FQR *C. jejuni* with MDR *Enterococcus* was observed and showed that *C. jejuni* can benefit by reduced susceptibility towards antimicrobials. Detection is possible by genome analysis.

PS01.183
Establishing an epidemiological platform for Carbapenem resistance in Egypt: using data visualization tool microreact and microarray for real-time surveillance and data integration

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The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) in Egypt, particularly in hospital settings, poses a serious public health threat. Carbapenemases are the most critical antimicrobial resistance mechanism globally, as they inactivate all β-lactam antibiotics—including penicillins, cephalosporins, and carbapenems—thus eliminating key therapeutic options for treating Gram-negative infections. The genes encoding these enzymes are often located on mobile genetic elements, which promote horizontal gene transfer and facilitate rapid global dissemination. Plasmids are further stabilized by toxin-antitoxin systems that ensure persistence even in the absence of antibiotic pressure. Infections caused by CRE are frequently associated with high morbidity and mortality, and resistance mechanisms often occur in combination, leading to multidrug resistance and very limited treatment options.

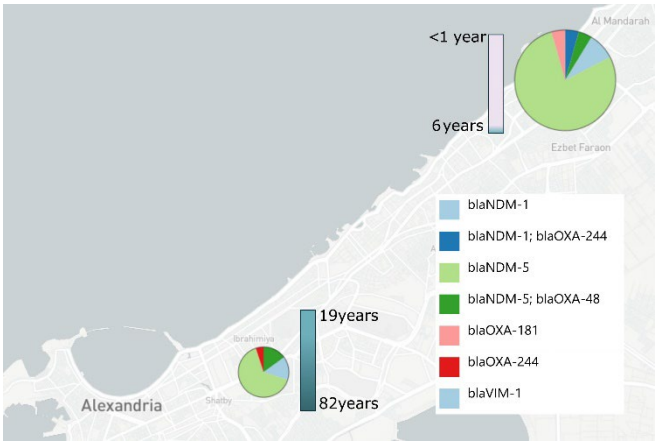
Our study aimed to establish an integrated surveillance approach for carbapenem-resistant *Escherichia coli* in Egypt by combining molecular microarray diagnostics, nanopore sequencing, and epidemiological data visualization. The goal was to enable timely detection, spatial-temporal tracking, and genotypic–phenotypic correlation to better understand resistance dynamics and inform infection control strategies.

A total of 43 *E. coli* isolates were collected from Egyptian hospitals between 2020 and 2023. Carbapenemase genes and resistance markers were identified using a combination of nanopore whole-genome sequencing and DNA-based microarray. Phenotypic antimicrobial susceptibility testing was performed using the VITEK2 system. The Microreact software platform was used to visualize and map the geographic and temporal distribution of resistant strains, allowing real-time epidemiological analysis.

Among the isolates, 72% carried the NDM-5 gene. Additional carbapenemase genes such as OXA-48 and VIM were also detected, predominantly in isolates from pediatric patients. The microarray achieved a diagnostic sensitivity of 92.9% and a specificity of 87.7% when compared with whole-genome sequencing. Phenotypic resistance profiles showed high concordance with genotypic data, with sensitivity and specificity rates of 95.6% and 95.2%, respectively. Microreact enabled intuitive visualization of strain distribution over time and geography, supporting outbreak tracing and resistance trend analysis.

Our study demonstrates the utility of combining molecular diagnostics, next-generation sequencing, and real-time epidemiological visualization for effective CRE surveillance. The strong correlation between genotypic and phenotypic resistance supports the use of DNA microarrays as a cost-effective alternative to whole-genome sequencing, particularly in resource-limited settings. The integrated approach offers a scalable strategy to monitor and respond to the evolving threat of multidrug-resistant *E. coli*, enhancing infection control and public health decision-making in Egypt.

Fig. 1



PS01.185
Insights into the genome of *Lactococcus lactis* and *Lactococcus cremoris* starter cultures

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Lactococcus lactis and *L. cremoris* strains are used as starter cultures in the manufacture of high-protein products. High-protein products often contain bitter peptides when they are obtained by first concentration and subsequent fermentation. The genes of the proteolytic system, especially *prtp*, are of particular interest here, as these may be associated with the presence of bitter peptides. In this project, two isolates from a starter culture were sequenced and compared.

The complete genome was sequenced using Illumina and Oxford Nanopore Technology, to obtain a zero-gap genome. Afterwards, the reads were assembled. The results show insights in the genome and plasmidome of each strain. Genomes of strains used as starter cultures contain plasmids with different sizes and gene clusters. The genomes were compared with known *L. lactis* and *L. cremoris* reference genomes, followed by bioinformatic analysis.

Analysis with Mauve software shows that almost 90 percent of the starter cultures genomes match the reference genomes. It is interesting to note that some genes are chromosomally encoded instead of plasmid-encoded or vary in their direction. For example, a Locally Collinear Block (LCB) of 34,579 base pairs of the chromosome of the *L. cremoris* strain is 66,700 bp downstream than in the reference strain *L. cremoris* MG1363. Furthermore, genes encoding a response regulator, which are plasmid encoded on the reference strain *L. lactis* 275, are part of the chromosomal DNA of the starter culture strain of *L. lactis*. Interestingly, the two *prtp* genes of the starter culture strains share 98% similarity according to ORthoANIu software. These findings can be used to answer further research questions about the genes encoding important enzymes and proteins of the proteolytic system and how they are responsible for the maintenance of bitter peptide levels in high protein products.

PS01.187

Functional analysis of a repetitive collagen-like GXY (CL-GXY) motif in *Bartonella* adhesin A of *B. henselae*

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Introduction: Adhesion to host tissues constitutes the initial and critical step in the establishment of infections. *Bartonella henselae* expresses the trimeric autotransporter adhesin (TAA) *Bartonella* adhesin A (BadA), which facilitates bacterial binding to the extracellular matrix (ECM) and promotes subsequent adhesion to endothelial cells. We identified a 310 amino acid long collagen-like 18-base pair GXY repeat motif (CL-GXY) within the BadA protein of *B. henselae* Houston-I Variant 2. Similar motifs have been described, e.g., in *Streptococcus pyogenes*. The biological function of the CL-GXY repeat in *B. henselae* remains unknown.

Material & Methods: Precise deletions of the *badA* gene and the 18-bp GXY repeat motif were generated via PCR-based cloning, Gibson Assembly, and homologous recombination. The resulting mutants were verified by PCR and Western blot analysis. Adhesion to ECM proteins will be assessed using ELISA and to endothelial cells via immunofluorescence microscopy and quantitative PCR.

Results: Targeted deletion of the entire *badA* gene and the specific 18-bp GXY repeat motif was successfully achieved. PCR and Western blot analyses confirmed correct integration of the constructs. Comparative analyses of adhesion to ECM proteins and endothelial cells are currently ongoing.

Conclusion: The GXY repeat motif appears to represent a unique structural feature of BadA in the *B. henselae* Houston-I Variant 2 strain, potentially contributing to strain-specific adhesion characteristics. Functional characterization of the CL-GXY motif will provide deeper insights into the modular architecture of TAAs and may guide the development of future anti-adhesion approaches.

PS01.189

In silico safety assessment of Exopolysaccharide-forming lactic acid bacterial strains for use as food starter cultures

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Lactic acid bacteria (LAB) are widely used as starter cultures in food fermentations due to their ability to improve sensory properties and enhance food safety by inhibiting the growth of undesirable microorganisms. Exopolysaccharide-forming LAB additionally contribute to the consistency and texture of various food products such as yogurt or sourdough. However, their application as starter cultures requires a detailed safety evaluation.

To investigate the suitability of selected LAB strains for use in food, an *in-silico* safety assessment was conducted in accordance with the European Food Safety Authority (EFSA) guidelines for whole-genome analysis of food-associated microorganisms. The complete genomes of five LAB strains were sequenced and bioinformatically analyzed.

A search for antimicrobial resistance (AMR) genes was conducted using the CARD database and the AMRFinderPlus tool. The presence of virulence genes, toxin genes, gelatinases, hyaluronidase and hemolysins, was assessed by the use of the VFDB and KEGG databases. Moreover, genes encoding amino acid decarboxylases which may be involved in biogenic amine production, were identified using KEGG annotations.

No AMR genes with ≥50% sequence identity were detected in any of the five LAB strains, indicating a low risk of horizontal transfer of resistance genes to pathogenic bacteria. Hemolysin-encoding genes were present in all strains. These are commonly found in LAB and are not considered a safety risk in the absence of phenotypic hemolytic activity, which was ruled out through blood agar testing. No other virulence genes could be identified. Furthermore, no genes encoding histidine or tyrosine decarboxylases—enzymes associated with the production of the most toxic biogenic amines such as histamine and tyramine—were detected.

In conclusion, this comprehensive genomic safety assessment indicates a favorable safety profile for all five LAB strains. The absence of detectable AMR genes, minimal presence of virulence factors, and lack of biogenic amine-associated genes support their suitability for use as food-grade starters cultures. Further evaluations in specific food matrices are recommended to confirm expression and functionality of relevant metabolic traits.

PS01.193

Challenges in laboratory diagnosis and antibiotic treatment options for a newly described *Pseudomonas aeruginosa* metallo-*β*-lactamase type GES-62 strain

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Antibiotic resistance is a major challenge in modern healthcare, as it severely limits the choice of treatment options. In particular, carbapenemase mediated carbapenem resistance in Pseudomonas aeruginosa poses an emerging health risk worldwide. Here, we discovered a hitherto unknown variant of the metallo-β-lactamase type GES in a P. aeruginosa strain by whole genome sequencing. This multidrug-resistant strain was isolated from bronchoalveolar lavage samples of a 61-year-old man, who suffered from respiratory insufficiency resulting from pneumonia. Ultimately, the patient succumbed to his condition, as there were no further treatment strategies. Given the high drug resistance of P. aeruginosa and its increasing role in severe infections, the implementation of methods for the rapid detection of carbapenemases is essential for optimizing therapeutic strategies and preventing nosocomial outbreaks.

PS02.002
The lower respiratory tract microbiome in patients with non-small cell lung cancer

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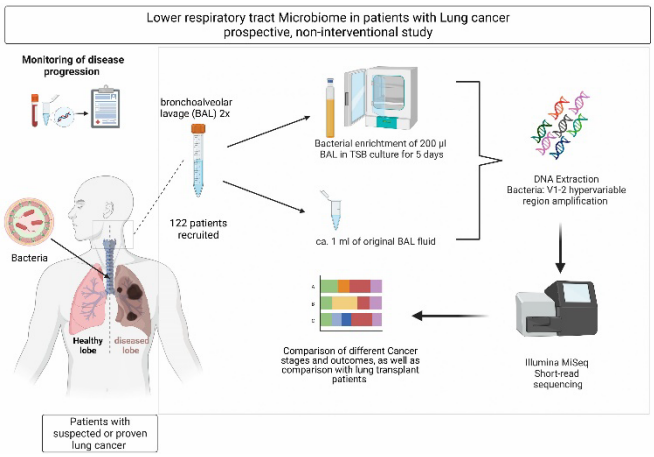
Introduction: The human microbiome plays a critical role in health and disease, with increasing evidence linking microbiome alterations to malignancies, particularly in the gastrointestinal tract. However, the role of the lower respiratory tract microbiome in non-small cell lung cancer (NSCLC) remains largely unexplored. This study aims to characterize microbiome diversity in NSCLC patients and assess its variations across histological subtypes and disease stages.

Patients and Methods: In this prospective study, patients undergoing diagnostic or preoperative bronchoscopy for suspected lung cancer were enrolled. Bronchial lavage samples were collected from both the affected and contralateral lobes. Microbial DNA was extracted and sequenced using Next-Generation Sequencing (NGS). Alpha diversity was assessed using Shannon and inverse Simpson indices, while statistical analyses (Wilcoxon, Mann-Whitney U, and Kruskal-Wallis tests) were performed to compare microbiome diversity based on gender, tumor location, histology, smoking status, and TNM staging. Bioinformatics tools, including LEfSe and MaAsLin2, were used to identify associations between bacterial features and clinical phenotypes.

Results: A total of 122 patients were included, of whom 87 (71.3%) had NSCLC, 16 (13.1%) had benign lesions, 9 (7.4%) had small cell lung cancer, and 10 (8.2%) had other malignancies. Among NSCLC patients, 57 (65.5%) had adenocarcinoma, 26 (29.9%) had squamous cell carcinoma, and 4 (4.6%) had other subtypes. Smoking history was reported in 76 NSCLC patients (87.4%), with 29 (38.6%) being current smokers. No substantial differences were detected between the affected and contralateral lobes. Alpha diversity was significantly lower in smokers compared to never-smokers (p=0.003) but did not differ between former and current smokers. Significant microbial diversity differences were observed across NSCLC TNM stages, with distinct bacterial taxa associated with NSCLC in non-smokers.

Conclusion: Our findings highlight significant variations in the lower respiratory tract microbiome of NSCLC patients based on smoking status and TNM staging. The differential abundance of bacterial species between smokers and non-smokers suggests a potential role in disease progression. These insights contribute to a better understanding of microbiome dysbiosis in lung cancer and its clinical implications.

Fig. 1



PS02.004
TED-Kasuistik – Biliäre Pseudolithiasis mit Obstruktion bei Hochdosis-Ceftriaxontherapie

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Hintergrund: Die Ceftriaxon-induzierte Gallenblasenpseudolithiasis ist eine Therapiekomplikation, die häufig bei Kindern, aber nur selten bei erwachsenen Patienten auftritt. Der hier vorgestellte edukative Fallbericht für klinisch-beratende Mikrobiologinnen und Mikrobiologen soll zur Ergänzung der spärlich verfügbaren klinischen Erfahrungen mit diesem Krankheitsbild beim erwachsenen Patienten beitragen. Fallbericht. Ein 41-jähriger männlicher Patient mit kulturnegativer Endokarditis erhielt 4 g Ceftriaxon pro Tag als Bestandteil seiner Antibiotikatherapie. Am 4. Behandlungstag zeigten sich in der Sonografie erstmals Anzeichen von Gallenblasen-Sludge und biliärer Pseudolithiasis. Am 17. Tag wiesen Bauchschmerzen über 30 Minuten sowie erhöhte Leber- und Pankreasenzyme auf eine temporäre Obstruktion hin und die Ceftriaxon-Therapie musste aufgrund dieser Komplikation vorzeitig abgebrochen werden.

Diskussion: Der Fall demonstriert eine oft übersehene, reversible Nebenwirkung einer Hochdosis-Ceftriaxon-Therapie sowie assoziierte Komplikationen. Obwohl Ceftriaxon-Therapien als normalerweise gut verträglich gelten, sollte die biliäre Pseudolithiasis als seltene, aber nicht

zu vernachlässigende Therapie-Nebenwirkung im Hinterkopf behalten werden.

PS02.006
Die Frankfurter Murmelklausur: Ein innovatives Prüfungskonzept
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Hintergrund: An der Universitätsmedizin Frankfurt werden im Praktikum der Medizinischen Mikrobiologie und Krankenhaushygiene für Studierende der Humanmedizin die Lehrinhalte anhand klinischer Fallbeispiele praxisorientiert gelehrt. Zur Vorbereitung auf die spätere teamorientierte Tätigkeit wird am Semesterende jeweils von zwei Studierenden in einem gemeinsamen Entscheidungsprozess ("murmeln") eine auf vier klinischen Fällen basierte Digitalprüfung mit jeweils fünf Key Feature-Fragen bearbeitet und (nicht) bestanden (sog. "Murmelklausur"). Die Akzeptanz dieses Prüfungsformates sollte in der Studierendenkohorte bestimmt werden.

Methoden: In einer im Anschluss an die Klausur mit der Webapplikation *soSci Survey* generierten Umfrage aus acht Fragen mit einer vierstufigen Likertskala wurden die Prüfungsteilnehmenden zum Einfluss des Prüfungsformats auf die Prüfungsvorbereitung, Wahrnehmung der Teamarbeit und zur subjektiven Leistungseinschätzung befragt. Darüber hinaus bestand die Möglichkeit, freie Kommentare abzugeben.

Ergebnisse: Von den 349 Klausurteilnehmenden (Bestehensquote: 93%, mittlere Prüfungsleistung 14,6 Fragen) haben 190 Studierende alle Fragen der Umfrage beantwortet. 78% der Studierenden gaben an, dass ihre Anspannung vor der Murmelklausur geringer bzw. deutlich geringer als vor bisherigen schriftlichen Einzelprüfungen war. 65% der Studierenden verneinten jedoch, dass sich ihr Prüfungsaufwand durch das Prüfungsformat reduziert habe. 99% der Studierenden haben die Teamarbeit als konstruktiv bis sehr konstruktiv wahrgenommen und 86% hat das gemeinsame Erarbeiten der Lösungen Spaß oder sogar sehr viel Spaß bereitet. 96% der Studierenden gaben an, die jeweiligen Lösungen einvernehmlich gefunden zu haben. 65% der Studierenden waren der Meinung, dass ihnen eine gute und 22% sogar sehr gute Prüfungsleistung gelungen sei. Von den 24 Studierenden (6,9%), die die Prüfung nicht bestanden haben, haben nur 3 Studierende (12,5%) an der Evaluation teilgenommen und ihre Prüfungsleistung realistisch als "nicht gut" eingeschätzt.

Fazit: Die teilnehmenden Studierenden bewerten das Prüfungsformat als weniger anspannend als eine vergleichbare Einzelprüfung, aber eindeutig als konstruktiv und unterhaltsam bei vergleichbarem Vorbereitungsaufwand. Das Format der sog. "Murmelklausur" trägt der Realität eines teambasierten Entscheidungsprozess in klinischen Fragestellungen Rechnung.

PS02.008
The deubiquitinating enzyme CYLD impairs NF-kB- and STAT1-dependent macrophage intrinsic immunity to *Staphylococcus aureus*
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In atopic dermatitis (AD), the lesional skin is frequently colonized by *Staphylococcus* (*S.*) *aureus*, contributing to the severity and clinical symptoms of the disease. The inflammatory milieu in the skin is characterized by a type 2 inflammatory signature, including M2 macrophages, which cannot eradicate *S. aureus*. Since *S. aureus* is effectively controlled in macrophages activated by pattern recognition receptor (PRR)-induced NF-κB and interferon (IFN)-γ-induced STAT1 stimulation, we hypothesized that pre-treatment with LPS as a PRR/TLR4-activating agent and IFN-g would induce effective control of *S. aureus* in monocyte-derived macrophages (MDMs) of AD patients. Our data show that the deubiquitinating enzyme CYLD is strongly expressed in skin macrophages and MDMs of AD patients compared to healthy controls and impairs the anti-staphylococcal activity of PRR-activated and IFN-treated MDMs. Functionally, CYLD impaired M1 macrophage polarization, as evidenced by reduced expression of CD80, TNF, and IL-6 upon LPS- and IFN-γ treatment in CYLD-deficient as compared to wild-type (WT) MDM/THP1 macrophages. Mechanistically, CYLD inhibited IFN-γ-induced STAT1 phosphorylation and activation by binding to STAT1 and inducing its K63 deubiquitination. CYLD also interacted with TRAF6 and NEMO/IKKγ in the MYD88 signaling pathway and with RIPK2 in the NOD2 pathway, leading to impaired activation of NF-kB. Inhibition of both STAT1 by siRNA and NF-κB by IKK inhibitor treatment, respectively, independently abolished the control of *S. aureus* in both CYLD-deficient and WT THP1 macrophages, which harbored identical high numbers of the pathogen. The in vivo inhibitory function of CYLD on the control of *S. aureus* was also observed upon infection of *Cyld*-deficient and WT mice. Collectively, these data illustrate that the increased expression of CYLD in macrophages of AD patients is a factor contributing to the ineffective control of *S. aureus*, diminishes M1 macrophage polarization, and that CYLD deletion unleashes the break on effective STAT1 and NF-kB-dependent control of *S. aureus*.

PS02.010
Bacterial aggregation in the intestinal microbiota
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Bacterial aggregation, involving single or multiple species, has been observed across a wide range of environmental and pathogenic contexts, indicating a potentially important ecological role also within human-associated microbiomes. In highly competitive ecosystems like the human gut, aggregation may offer protective benefits and confer a selective advantage. However, the specific physiological and ecological functions of bacterial aggregation in the intestinal environment remain to be elucidated.

To investigate the relevance of aggregation among gut bacteria, we isolated Enterobacteriaceae from fecal samples of patients in intensive care units (ICUs), collected within the MS-ICU study. These patients had received treatment with either meropenem, piperacillin/tazobactam, or no antibiotics. A total of 75 isolates were evaluated for their aggregation capacity. In parallel, we assessed their growth rates, biofilm formation abilities, and their resistance or tolerance to the administered antibiotics. We further explored correlations between these traits and aggregation behavior. Given the

recognized importance of bile acids in shaping intestinal microbiota composition and influencing disease progression during sepsis, we also examined the impact of bile acids on these phenotypes. Additionally, we tested whether aggregation influences evasion from phagocytosis or alters the host immune response.

Our findings reveal a high variability in aggregate-forming capabilities among the isolates, strongly influenced by the presence of bile acids. Interestingly, contrary to previous assumptions, aggregation was negatively correlated with biofilm formation and did not correlate with increased antibiotic tolerance or resistance. Furthermore, bacterial aggregates did not confer protection from the host immune system. Instead, they showed the capability of strongly triggering immune responses and were not uniformly protected from phagocytosis.

Overall, our study suggests that aggregation by Enterobacteriaceae in the human gut may not primarily serve to enhance survival via biofilm formation, antibiotic tolerance, or immune evasion. These results challenge commonly held assumptions about the benefits of bacterial aggregation and underscore the need for further investigation into its physiological significance. Understanding the role of aggregation could provide new insights into the maintenance of a healthy intestinal microbiota and into mechanisms underlying dysbiosis and infection, particularly in vulnerable patient populations such as those in intensive care units.

PS02.012
Human infections by the novel zoonotic species *Corynebacterium silvaticum* in Germany

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Question: The recently described *Corynebacterium (C.) silvaticum* is a unique group of diphtheria toxin (*tox*-) gene bearing species so far mainly found in wild boar and deers. Due to its close relationship to *C. ulcerans* a zoonotic potential has been suspected. Human infections due to *C. silvaticum* have not yet been described.

Materials & Methods: Strain identification was performed by biochemical differentiation, MALDI-TOF analysis, next generation sequencing (NGS)-based cgMLST and Average nucleotide identity (ANI) value comparison. Susceptibility testing was performed according to CLSI and EUCAST guidelines. Toxigenicity was analysed by real-time PCR, an optimized Elek test and Lateral Flow Immuno Assay (LFIA).

Results: We report the first three cases of human *C. silvaticum* infections with axillary lymphadenitis and abscess formation presumably, in two case probably originating from previously hunted and slaughtered infected wild boars.

Conclusion: Our three cases indicate that *C. silvaticum* may be a new zoonotic pathogen 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 animal processing.

PS02.014
An outbreak of foodborne botulism caused by commercially canned mushrooms from Russia

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Due to overall high safety standards in the producing industry foodborne botulism outbreaks induced by commercial food sources have been rarely seen within the EU for more than a decade. Here we report on an outbreak among two persons in Germany from unrelated households in Mai/June 2024 caused by commercially canned mushrooms from the Altai region in Russia. Both persons were of Russian/Eastern European ethnicity and had been severely affected requiring intensive care with ventilation.

In both cases type A foodborne botulism could be confirmed based on the presence of an BoNT/A2-producing *C. botulinum* strain in the fecal samples and BoNT/A in the serum of one patient. In collaboration with the local health authorities food items and food leftovers were collected from the households and supermarkets based on reports on the previous consumption. Glass jars of canned white mushrooms (*Russula delica*) in brine from the Altai Krai (Russia) from a local (Altai) producer were identified as the source of the outbreak. An analysis of recalled glass jars revealed presence of a BoNT/A2-producing *C. botulinum* strain in a number of jars. Furthermore, BoNT/A2 could be identified by ELISA, Endopep-suspension immunoassay (Endopep-SIA [1]), and LC-MS/MS in the affected glass jars thereby confirming the canned mushrooms as outbreak source. Other canned mushroom products distributed by the same company were unaffected. Interestingly, a search across Russian (social) media revealed that a similar product (*Russula delica* from the Altai) of the very same producer had been recalled in Russia earlier the year after a botulism outbreak in Irkutsk.

A comparison of the BoNT/A2 sequences identified in the current outbreak with the A2 sequences found in the NCBI databases revealed that the amino acid sequence contained 1 or more substitutions compared to other A2 sequences across the globe. Noteworthy, a single identical A2 sequence deposited by a Russian Institution was found.

In summary, mushrooms can be naturally contaminated with spores of BoNT-producing clostridia and canned mushrooms have been incriminated in botulism outbreaks in the past. Nowadays, commercial products are usually not involved in foodborne botulism outbreaks within the EU due to the high safety standards applied. However, foreign (non-EU) products from countries with less stringent safety or surveillance systems may enter the market, particular in niche areas. With respect to the Ukraine war, the current security situation prevents an efficient communication and early product

warning between the EU and Russia potentially affecting food safety and public health.

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PS02.016

Role of unusual nucleotides in Staphylococcus aureus

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Bacteria need to sense and response to their external environments in order to survive and grow under diverse circumstances. The signaling transductions for this purpose are often performed via molecular second messengers including cyclic nucleotides. In bacteria, cyclic adenosine 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) have been identified as important molecular messengers in diverse biological processes, such as glucose response, catabolite repression and bacterial cyst development. However, little is known about the function of 3',5'-cyclic cytosine monophosphate (cCMP) and 3',5'-cyclic uridine monophosphate (cUMP). Of note, earlier work demonstrated that cCMP and cUMP contribute to bacterial immunity against bacteriophage infection in *Escherichia coli* but it is still unclear whether these unusual cyclic nucleotides may have similar functions in other bacteria. Therefore, this project aimed to study what impact cUMP/cCMP signaling may have on *Staphylococcus aureus*, a medically important pathogen. Our data suggest that the cUMP/cCMP signaling machinery plays a crucial role in the adaptation of *S. aureus* and further regulates the interaction with non-staphylococcal organisms. Consequently, modulation of the signaling cascade involved may also be of therapeutic relevance and could help to develop new strategies for combating infections caused by drug-resistant *S. aureus*.

PS02.018

Immunological and microbiological properties of an uprising Gram-negative pathogen *Chryseobacterium indologenes*

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Introduction: Newly identified and sparsely characterized bacterial species represent an underappreciated but potentially significant threat to healthcare systems worldwide. Their pathogenic potential often goes unnoticed until infections arise in vulnerable patient populations. Among these, *Chryseobacterium indologenes* (*C indologenes*) - an environmental Gram-negative rod - has recently emerged as an opportunistic multi-resistant pathogen. In this study, we investigate for the first time the immunological and microbiological properties of *C. indologenes*, highlighting its relevance in the context of emerging infectious threats.

Materials and methods: We conducted a systematic literature research of all scientific publications available up to year 2024 to establish its epidemiological properties. Furthermore, *in silico* genomic analysis of eighteen clinical isolates was conducted to identify virulence factors genes, antibiotic resistance genes (ARGs) and mobile genomic elements. Moreover, early immune response to five clinical isolates was examined including alarmin signaling of A549-cells, cytokine signaling of THP1-cells and ROS-production assay in neutrophil infections. To assess possible intracellular survival, gentamycin protection assay was conducted. In addition to that, phenotypic antibiotic resistance was confirmed by gradient diffusion tests.

Results: *C. indologenes* infections showed a worldwide distribution, predominantly involving epithelial tissues. Genomic analysis identified capsule-biosynthesis clusters, oxidative-stress defense, magnesium-uptake systems, acid-resistance and proteases-encoding loci as core virulence modules. Isolates DSM 103855 and 103847 harbored the largest virulence-gene repertoire and triggered stronger host responses than other isolates including alarmin and cytokine gene upregulation, and the neutrophil ROS burst. Gentamycin protection assay revealed relevant CFU-count only for DSM 103847 suggesting intracellular persistence. Genotype–phenotype correlation showed uniform high resistance to β-lactams, variable susceptibility to trimethoprim–sulfamethoxazole, and full susceptibility to fluoroquinolones, matching ARG content.

Conclusion: In our study, most *C. indologenes* strains examined appeared to have a low-virulence yet clinically problematic because of extensive β-lactam resistance. A subset, exemplified by DSM 103855 and 103847, carries additional virulence determinants that enhance host-cell activation and intracellular survival, underscoring the need for strain-level surveillance and tailored therapy.

PS02.020

How stable are *Clostridioides difficile* toxins in stool samples?

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Question: TcdA and/or TcdB toxins can cause Clostridioides difficile infection (CDI). Detecting these toxins with enzyme immunoassays (EIAs) in stool samples is an integral part of the diagnostic work-up. As toxin stability can affect the reliability of EIAs, this study aimed to test the stability of TcdA and TcdB in stool samples.

Methods: First, the stability of TcdA and TcdB was assessed in stool samples spiked at two concentrations (20 ng/ml and 60 ng/ml) over 72 hours at 25 °C under ambient air conditions (mimicking transport conditions). Toxin concentration were measured by EIA hourly. To account for real-life conditions in CDI, toxin concentrations in stool samples from CDI patients were tested under identical conditions.

Results: In spiked stool samples, the concentration of TcdB decreased faster than that of TcdA. After 72 hours, the toxin concentrations were 94% (20 ng/ml TcdA), 93% (60 ng/ml TcdA), 68% (20 ng/ml TcdB) and 57% (60 ng/ml TcdB) of the initial concentrations. In contrast, toxin levels in stool samples from CDI patients increased to 206% of baseline levels within 24 hours, while median C. difficile cell counts remained almost unchanged (baseline: 2.5 x 10^4 CFU/ml; 24 hours: 3.9 x 10^4 CFU/ml).

Conclusion: TcdA and TcdB concentrations decreased in vitro, but this was offset by toxin production from viable C.

difficile, resulting in a net toxin increase. Thus, toxin instability is unlikely to interfere with toxin detection by EIA.

PS02.022
Strain-dependent internalisation of *S. aureus* in podocytes and renal epithelial cells
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Question: *Staphylococcus aureus* bacteremia (SAB) is associated with high mortality rates of 28–34%. *S. aureus* bacteriuria (SABU) secondary to SAB is frequently observed (7.8–39%), but its impact on outcomes is controversial. The pathomechanisms of *S. aureus* translocating from the blood to the urine have not been sufficiently characterised. The objective of our study was to investigate the interactions between *S. aureus* from SAB patients with or without SABU and distinct kidney cells.

Methods: We included *S. aureus* blood culture isolates from patients with SAB if a urine culture was taken within ±3 days of the blood culture collection. SABU was detected using standard culture and/or nucleic acid amplification tests on urine samples. Patients with risk factors for urinary tract infection (e.g. indwelling urinary catheter, hydronephrosis and/or kidney stones), missing urinalysis, and/or pregnant women were excluded.

We assessed the internalisation of *S. aureus* isolates into renal epithelial cells (A498) and human podocytes (AB8) by infecting the cells with *S. aureus* at an MOI of 50 for 1 or 3 hours. After removing the extracellular *S. aureus* with lysostaphin, we determined the number of intracellular bacteria by plating serial dilutions of the cell lysates.

Results: A total of 20 *S. aureus* isolates from patients (female:male ratio 1:1; median age 68.8 years) with (n = 11) and without (n = 9) SABU were included.

In AB8 podocytes, the internalisation of isolates from patients with SABU was significantly higher after three hours of incubation (median: 2.09 CFU/cell vs. 1.13 CFU/cell; p = 0.013) than from patients without SABU.

In the A498 cell line, the internalisation of the isolates from the SABU patients was significantly higher after one hour (median: 0.047 cfu/cell (n = 11) vs. 0.017 cfu/cell (n = 9); p = 0.012), and after three hours of incubation (median: 0.785 CFU/cell (n = 14) vs. 0.408 CFU/cell (n = 14); p = 0.009) compared to patients without SABU.

Conclusions: *S. aureus* strains known to induce SABU show high internalisation in the first renal cell lines of the blood-urine barrier compared to SABU-negative strains. Therefore, they may play a role in translocation from blood to urine. Direct interactions between *S. aureus* and kidney cells could also represent a new cornerstone in acute kidney injury during SAB.

PS02.024
More than a superantigen – streptococcal pyrogenic exotoxin J (SpeJ) shapes dendritic cell responses
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Background: *Streptococcus pyogenes* (GAS) is an exclusively human pathogen that can cause both mild and life-threatening infections, such as necrotizing soft tissue infections (NSTIs). During invasive infections, the genes encoding for control of virulence (Cov)R/S two-component system (TCS) are prone to mutations. These mutations can lead to a dysfunctional TCS and, consequently, to a hypervirulent bacterial phenotype. GAS strains harboring CovR/S mutations display increased superantigenic activity. This study aimed to investigate the impact of the CovR/S system, as well as superantigens, on the immune response of dendritic cells (DCs).

Methods: Human primary monocyte-derived (mo)DCs were infected with *emm1* GAS strains harboring either functional or dysfunctional CovR/S. Additionally, the strain MGAS5005 and its isogenic mutants lacking one or two superantigens were used for infections. DC maturation and the release of pro-inflammatory cytokines in response to infections were assessed via flow cytometry.

Results: Irrespective of CovR/S functionality, moDCs matured equally in response to all infections. However, impaired maturation was observed in moDCs infected with the CovR/S-dysfunctional MGAS5005 mutant lacking SpeJ. Consistent with the maturation data, the secretion of pro-inflammatory cytokines was also reduced.

Conclusion: In addition to its superantigenic activity, our study suggests a possible link between SpeJ and moDC maturation. Further studies are needed to elucidate the underlying mechanisms.

PS02.026
Identification of linear B-cell epitopes for improved Q fever serodiagnostics in sheep
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Introduction: Q fever is a globally distributed zoonotic disease caused by *Coxiella burnetii*. Infected ruminants, especially sheep and goats, often remain asymptomatic while shedding large quantities of bacteria during parturition, complicating detection and control. Commercially available ELISAs based on whole-cell antigens show a high variability in sensitivity and specificity. Identifying defined B-cell epitopes offers a promising strategy for developing more specific and reliable serological assays.

Objectives: This study aimed to identify linear B-cell epitopes from immunogenic proteins of *C. burnetii* and to evaluate their diagnostic potential in a peptide-based fluorescent ELISA.

Materials and methods: Immunoreactive proteins of *C. burnetii* field isolate 23QC0408 were identified by 2D gel electrophoresis and western blotting using sera from Q fever-positive sheep. Based on these findings and literature data, 30 linear B-cell epitopes were predicted using the IUPred3

algorithm, and synthesized as 20 to 30 aa peptides with N-terminal biotin tag. For seroreactivity screening, nine serum pools (comprising a total of 91 individual sheep sera) of known Q fever status (positive, negative, vaccinated) were tested in triplicate. Fluorescence signals were measured, and background correction was applied by subtracting 150 % of the signal from uncoated wells.

Results: Sixteen peptides demonstrated specific reactivity with Q fever-positive serum pools but not with Q fever negative serum pools. The most reactive peptides were those derived from GroEL, Mip, YbgF, and Com1. Peptide 22 (Mip) showed stable signals with all positive pools, indicating broad diagnostic potential. Peptide 4 (GroEL) demonstrated strong reactivity in sera obtained from a confirmed Q fever outbreak. Peptides 4, 21, 22 and 25 also showed reactivity with sera from vaccinated animals, suggesting antibody recognition following both infection and vaccination. No cross-reactivity was observed with Q fever negative serum pools.

Summary: A peptide-based fluorescent ELISA using selected B-cell epitopes of *C. burnetii* was established. The 15 of 30 peptides represent promising diagnostic targets for the serological surveillance of Q fever in sheep. Studies are currently being conducted with individual sheep sera, and validation on other host species such as goats and cattle is planned for the future.

PS02.028

Traditional herbal urologicals reduce infection with uropathogenic *Escherichia coli* by *i*nducing surface stress in bacteria and affecting host defenses

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Introduction: Urinary tract infection (UTI) is one of the most common infections worldwide with uropathogenic *E. coli* (UPEC) responsible for causing more than 80% of uncomplicated UTI cases. However, the classical treatments are becoming less effective due to the spread of antibiotic resistance. Several herbal extracts have been used since a long time in traditional medicine to treat UTI but their mode of action has not been investigated in detail [1]. We therefore investigate aqueous extracts of both individual plant species and mixtures of different plants.

Goal: To study the effect of plant extracts on bacterial growth and stress response as well as their impact on the host-UPEC interaction

Methods: Fluorescence-based reporter gene assays, yeast agglutination, adhesion & invasion assays, qRT-PCR

Results: At first, we checked the effect of the plant extracts on bacterial stress response using a stress reporter system in *E. coli* MG1655, consisting of transcriptional fusions of the promoterless reporter gene *yfp* to a set of promoters of stress-related genes. Most of the plant extracts caused envelope stress, which was specifically sensed by the BaeS-BaeR two-component system. Further, we analyzed the effect of the extracts on the functionality of type 1 fimbriae of UPEC strain CFT073. We observed that only some herbal extracts reduced yeast agglutination. To understand the effect of the plant extracts on host-pathogen interaction, we conducted invasion and adhesion assays using the urinary bladder epithelial cell line T-24 and UPEC strain CFT073. Most of the extracts significantly reduced bacterial adhesion and intracellular survival in a concentration-dependent manner**.** Further, we investigated whether the herbal extracts activate the innate immune response in the host. For this, we conducted qRT-PCR assays after incubation of different urinary bladder cell lines with the plant extracts. We observed that even without UPEC infection, most extracts induced significant expression of IL-6 and CXCL-3.

Conclusion: The tested plant extracts had no antibiotic effect on the bacteria and rarely inhibited the functionality of type 1 fimbriae, but often affected properties of the host cells, which led to a significant reduction of bacterial adherence and even more so intracellular bacterial survival. Most of the extracts elicit a significant induction of immune genes (IL-6, IL-8, CXCL-3) in both the T-24 and 5637 cells without UPEC infection suggesting a strong immunostimulatory effect.

References: [1] Tache, AM, Dinu, LD, and Vamanu E. (2022) *Applied Sciences* 12, 2635.

PS02.030

Region-specific spatial transcriptomics reveal distinct immunological functions in human Tuberculosis Granulomas

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A detailed understanding of host-pathogen interactions is essential to develop innovative strategies against multi-drug-resistant tuberculosis (MDR-TB). To investigate pathogenic mechanisms at the site of infection, we assembled a cohort of partial lung resections from patients with drug-susceptible, MDR, and XDR tuberculosis. Lung tissue was stratified based on pathological patterns—such as unaffected areas, diffuse inflammation, cellular granulomas, and necrotic granulomas—and tissue microarrays were constructed to integrate multiple patient samples and lesion types within single paraffin blocks. Using multispectral imaging, we identified key immunological regions at the protein level, including multinucleated giant cell areas, T cell- and macrophage-rich zones, early and late-stage granulomas, and tertiary lymphoid structures. Spatial transcriptomics was then employed to obtain region-specific gene expression profiles, which were correlated with distinct immunological functions. For example, macrophage-rich regions displayed both type I and type II interferon responses, while giant cell areas showed gene signatures related to pH regulation and acidification. The outer rim of granulomas—enriched in T and B cells—were characterized by growth factor signaling and complement activation pathways. These findings reveal distinct molecular signatures within defined morphological niches that are associated with either protective or pathological outcomes. Our results shed light on the immunometabolic landscape of pulmonary TB lesions and may inform the development of targeted, host-directed therapies.

PS02.032

Bupivacaine hydrochloride has antiviral and antimycotic properties during co-infection with influenza A viruses and *Aspergillus fumigatus in vitro*

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Respiratory infections are among the most common and consequential diseases worldwide that are associated with significant mortality. Infections with influenza A virus (IAV) and secondary infections caused by bacteria such as *Staphylococcus aureus* or opportunistic pathogens like the saprotrophic mold *Aspergillus fumigatus* often result in severe clinical outcomes, especially in the case of co-infections. The availability of suitable treatments is limited. Drug repurposing, which involves the use of already approved compounds for new therapeutic targets, enables the rapid and cost-effective development of alternative treatment options and represents a promising strategy. Previous studies have shown that local anaesthetics such as lidocaine and procaine act against viral, fungal and bacterial infections. Based on these findings, bupivacaine hydrochloride (bupivacaine), a structurally related local anaesthetic, was investigated in this study for its antimicrobial properties.

To explore these effects, *in vitro* experiments were performed using IAV-infected and IAV/*A-fumigatus* co-infected Madin-Darby Canine Kidney II (MDCK II) cells and Calu-3 human lung adenocarcinoma cells. First cytotoxicity assays were performed on the host cells to confirm tolerated concentrations. Possible antifungal effects of bupivacaine against *A. fumigatus* were analysed in a human cell-free environment. To investigate antiviral effects, MDCK II or Calu-3 cells were infected with IAV for 24 h with or without bupivacaine treatment at various non-toxic concentrations. In addition, Calu-3 cells were co-infected with IAV and *A. fumigatus* and treated with bupivacaine for 9 h. The antimicrobial effects of bupivacaine were analysed using various methods, including plaque assays, Western blotting, absorbance measurements, and immunofluorescence microscopy. The results indicate that bupivacaine inhibits the growth of *A. fumigatus* in a dose-dependent manner. Moreover, treatment with this local anaesthetic led to a significant reduction in IAV titers and to a reduced expression of viral proteins. Preliminary results from co-infection experiments indicate an inhibitory effect of bupivacaine against both tested pathogens.

In summary, bupivacaine showed both antiviral and antifungal effects *in vitro* at concentrations below the cytotoxic threshold. These results indicate a potentially expanded range of treatments for viral and fungal infections. Further investigations are required to clarify the underlying mechanisms and to validate these effects in more complex infection models.

PS02.034

Evaluation of DNA extraction methods for detecting antimicrobial resistance genes in wastewater

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The growing global concern regarding antimicrobial resistance (AMR) underscores the imperative for precise and reproducible methodologies for its detection and surveillance across diverse environmental matrices. Wastewater has been identified as a significant reservoir for the dissemination of AMR determinants. Accurate monitoring of antibiotic resistance genes (ARGs) and resistant microbial populations within wastewater systems necessitates the use of optimized DNA extraction protocols capable of yielding high-quality nucleic acids amenable to advanced molecular analyses.

In this study, we evaluated systematically the performance of five commercial DNA extraction kits: PowerFecal Pro (Qiagen), QIAamp DNA Microbiome (Qiagen), Wizard Enviro Total Nucleic Acid Kit (Promega), NucleoMag DNA Water Kit (Macherey-Nagel), and Quick-DNA/RNA Water Kit (Zymo Research) - using both the supernatant and pellet fractions of six wastewater samples collected from distinct sites. The evaluation focused on total DNA yield and the sensitivity of digital polymerase chain reaction (dPCR) for quantifying selected ARGs (*bla*KPC-2, *bla*NDM-1, *bla*GES-5, *bla*OXA-48, and *bla*VIM-1) using the QIAcuity system (Qiagen GmbH). Furthermore, the impact of each DNA extraction method on microbial community composition was assessed by high-throughput full-length 16S rRNA gene amplicon sequencing - based microbiome profiling.

Our results indicated that the Promega kit yielded significantly higher DNA concentrations from the supernatant compared to all other kits. For the pellet fraction, the Promega kit also produced significantly higher DNA yields compared to both Qiagen kits, though not significantly different from the Macherey-Nagel and Zymo Research kits. For the Promega kit, no significant differences were observed in the quantification of six ARGs between the supernatant and pellet fractions, as measured by dPCR. However, for the *bla*GES gene, a significant difference was observed between the supernatant and pellet when using the Zymo Research kit, both with and without the PCR inhibitor removal step.

At the microbiome level, the choice of DNA extraction kit had a notable effect on the resulting microbial community profiles. For instance, the family *Prevotellaceae* and the genus *Bacteroides* were underrepresented in samples processed using the QIAamp DNA Microbiome and NucleoMag DNA Water kits. Additionally, sequencing depth showed a significant positive correlation with the detection of specific taxa, such as members of the order *Enterobacterales*.

In conclusion, this study demonstrates that the choice of DNA extraction method is a critical factor that can introduce systematic biases in the assessment of microbial community composition. These findings provide a robust framework for selecting appropriate DNA extraction protocols to improve the accuracy and comparability of AMR surveillance efforts in wastewater.

PS02.036

Sub-lethal signals in the mitochondrial apoptosis pathway are essential for cytokine production and DNA damage in cells infected by *H. pylori*

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Introduction: *Helicobacter pylori* (*Hp*) colonizes the gastric mucus layer of around half of the world"s population. Although the infection is mostly asymptomatic, it is a major risk factor for gastritis, gastric and duodenal ulcers and gastric adenocarcinoma. *Hp* is recognized by epithelial cells and activates various signaling pathways through for instance pattern recognition receptors, resulting in inflammation. *Hp* can also induce DNA damage including double-strand breaks. The apoptosis system can be activated by infection, and

pathogens have pro- or antiapoptotic effects. We have recently shown that various intracellular pathogens (bacteria and viruses) trigger sub-lethal signals in the mitochondrial apoptotic pathway.

Methods: We infected human gastric carcinoma cells (AGS) with *Hp* and tested for sub-lethal signals in the mitochondrial apoptosis pathway. We determined cytokine/chemokine release and DNA damage as a readout. We further investigated the release of mitochondrial cytochrome *c* and SMAC by Western Blot and confocal microscopy. Additionally we analyzed morphological and ultrastructural changes of the mitochondrial network.

Results: Conditions could be established where infection with *Hp* induced sub-lethal mitochondrial apoptosis signaling in the absence of cell death which resulted in DNA-ds-breaks and secretion of cytokines. The pathway required the pattern recognition receptor NOD1 and specifically the BCL-2-family member BAX. Analysis of the mitochondrial network by confocal microscopy indicates that the release of intermembrane space proteins was not limited to individual mitochondria. Rather, large parts of the mitochondrial network released some SMAC and cytochrome c, apparently without compromising mitochondrial function. The release of SMAC appeared to be predominant over the release of cytochrome c.

Summary: The results identify sub-lethal signals in the mitochondrial apoptosis pathway during *Hp*-infection. Mitochondria can release intermembrane space proteins without losing their functional integrity, which is likely relevant to downstream inflammatory signaling. The evidence suggests that these signals contribute to inflammation and genome stress including mutations during chronic *Hp*-infection.

PS02.038

Wohlfahrtiimonas chitiniclastica: a potential disruptor of wound healing and glucose metabolism in diabetic foot ulcers

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Introduction: The worldwide increase in multidrug-resistant bacteria is drawing attention to previously underestimated pathogens and their impact on known infections. *Wohlfahrtiimonas chitiniclastica* has been lately identified in chronic, non-healing diabetic foot ulcers (DFUs), highlighting its emerging role in these infections.

Question: The aim of this study was to investigate the cellular and metabolic interactions of *W. chitiniclastica* in a diabetic wound environment.

Methods: To explore host-pathogen interactions, an epithelial cell line and monocyte cell line THP-1 cells were infected with *W. chitiniclastica.* Gene expression profiling was conducted. Furthermore, human neutrophils were isolated then infected with *W. chitiniclastica* to assess reactive oxygen species (ROS) production. To mimic diabetic conditions, infections were performed under varying glucose levels, followed by PCR for cytokine analysis. To assess potential effects on wound healing, a scratch assay was performed on epithelial cells infected with *W. chitiniclastica* for 24 hours, using a Keyence BZ-X800 microscope for analysis.The glucose metabolism of *W. chitiniclastica* was assessed by culturing the bacterium in media containing varying concentrations of glucose, followed by monitoring of its growth over time.

Results: Our results demonstrated that *W. chitiniclastica* had no significant effect on epithelial cells; however, it elicited a pronounced activation of THP-1 monocytes, characterized by elevated cytokine production. Notably, cytokine expression in THP-1 cells was attenuated under high-glucose conditions. Moreover, ROS production was generally reduced across all *W. chitiniclastica* strains, with the exception of one isolate. Surprisingly, the scratch assay revealed a significant delay in cell line closure in the presence of all *W. chitiniclastica* isolates or their supernatants. Additionally, all strains demonstrated robust growth in different glucose concentration environments, with no significant differences observed. This growth behaviour was comparable to that of "non-fermenting" bacterial species.

Conclusion: These findings suggest that *W. chitiniclastica* modulates immune responses in a glucose-dependent manner, potentially enabling immune evasion in the inflammatory environment of DFUs. *W. chitiniclastica* isolates significantly delayed wound closure, indicating a potential role in impaired chronic wound healing. Furthermore, *W. chitiniclastica´*s ability to grow in hyperglycaemic conditions without classical fermentation indicates oxidative glucose metabolism and a survival advantage.

PS02.040

Density-dependent suppression of the type III secretion system in *Yersinia* promotes replication and dissemination

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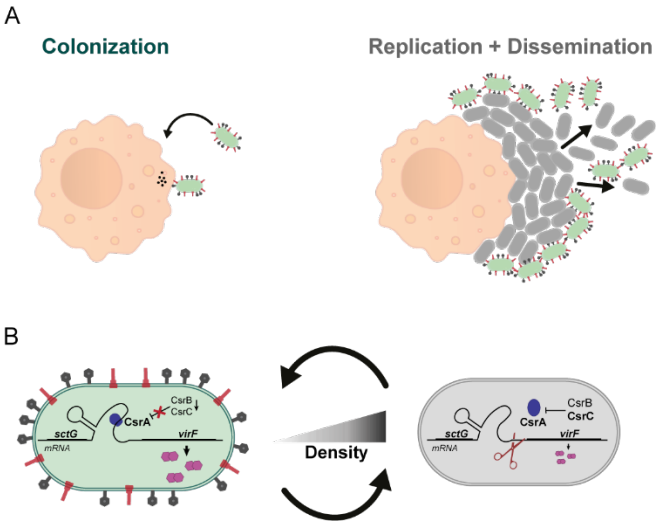
The type III secretion system (T3SS) is used by Gram-negative bacteria, including important pathogens, to manipulate host cells by injecting effector proteins. T3SS activity is tightly regulated and accompanied by a phenomenon known as secretion-associated growth inhibition (SAGI), where actively secreting bacteria cannot grow or divide. Therefore, a non-uniform expression of the T3SS may be advantageous during infection. *Yersinia enterocolitica*, a model for T3SS studies and immune evasion, was previously thought to uniformly express and assemble T3SSs, which are activated upon host cell contact. In this study, however, we found that *Yersinia* actively suppresses T3SS expression, assembly and activity at higher cell densities, such as those found inside bacterial microcolonies. This suppression is highly specific to the T3SS, reversible, and distinct from stationary phase adaptation. It is conferred by downregulation of the T3SS transcriptional activator VirF, whose expression *in trans* restores T3SS assembly and activity. Transcript analysis revealed that the phenotype is mediated by increased levels of the regulatory RNAs CsrBC, which destabilize the *virF* transcript by sequestration of the regulatory protein CsrA. In parallel, we observed a drastic reduction in bacterial cell adhesion caused by downregulation of the VirF-dependent adhesin YadA. We propose that this active suppression of T3SS secretion and cell attachment at higher local bacterial densities promotes a switch during *Yersinia* infection from a T3SS-active colonization stage to a bacterial replication and dissemination phase.

Fig. 1



PS02.042

External quality assessment of the detection of the rare zoonosis Q fever by molecular methods and serology

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Introduction: Querry fever (QF) is a zoonotic disease caused by the gram-negative bacteria *Coxiella (C.) burnetii* which can infect humans by inhaling airborne particles shed from infected small ruminants like sheep and goats. Germany has ranked the diseases as the second most important zoonosis for surveillance after avian influenza.

Objectives: As surveillance needs reliable diagnostic tools, the status of the quality of detection of QF in Germany will be assessed based on data from external quality assessment (EQA) schemes conducted by INSTAND e.V.

Materials & Methods: Qualitative data from twelve EQA surveys for the molecular detection of C.*burnetii* DNA (four samples each) was stratified by the usage of either in house (IH) or commercial assays (CA) and analyzed. For the serological analysis, qualitative and semi-quantitative data for IgM as well as IgG against phase I and phase II antigens was assessed in a method-dependent manner based on six EQA surveys (two serum samples each). Additionally, the interpretation of the serological results as provided by the participants indicating an acute, a past or a chronic QF infection was analyzed and compared to the donor"s history. Both EQA schemes were conducted worldwide between 2019 and 2024.

Results: While PCR-analysis was mostly done by in house assays (IH), most of the serology analysis was performed with commercial assays (CA). The PCR-analysis showed very good sensitivity (0.968-1.000) and specificity (0.891-1.000) throughout the observed period. There was no significant difference between IH and CA. The serology was more complex and stratified into an ELISA and an IFA cohort. Specificity was comparable between both methods (0.970-0.974). IFA was the more sensitive method for IgG phase I and phase II but not for IgM, as normally more laboratories using these methods were able to identify antibodies in the sample. This led to differences in the diagnostic interpretation of the serological results, as ~60% of ELISA-laboratories would have assessed a patient with a previous confirmed QF infection as having an acute infection, while ~60% of IFA-laboratories would suggest the patient had a chronic infection. Additionally, three donors with a suspected unrecognized previous infection would have been not identified as previously infected by 7% to 10% of laboratories the IFA cohort and by 14% to 28% of laboratories in the ELISA cohort. Semi-quantitative results show a missing harmonization for both cohorts.

Summary: The high quality of the laboratories shown here for direct and indirect *C. burnetii* detection methods provides a good basis for a reliable diagnostic management of Q fever.

PS02.044

News from the national reference centre for multidrug-resistant gram-negative bacteria

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Question: Multidrug-resistance in *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since only few 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 detailed analysis of the molecular epidemiology of carbapenemases is required.

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 10.203 isolates were investigated for carbapenemases at the National Reference Centre in 2024. Carbapenemases were found in 4.186 *Enterobacterales* strains, 583 of *P. aeruginosa* and 384 of *A. baumannii*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 999), NDM-1 (n = 667), NDM-5 (n = 598), KPC-2 (n = 534), OXA-244 (n = 526), VIM-1 (n = 484), KPC-3 (n = 255) and OXA-181 (n = 225), which were also found in various combinations, e.g. NDM-1/OXA-48 (n = 149). In total, 87 different carbapenemases or carbapenemase combinations were identified in Enterobacterales in 2024, with especially OXA-244 (+ 34.5 %), KPC-3 (+ 34.2 %), NDM-5 (+19.8 %), OXA-48 (+19.5 %) and KPC-2 (+ 17.6 %) showing significant increases compared to 2023. In *P. aeruginosa,* VIM-2 was again the most frequent carbapenemase (n = 326), followed by NDM-1 (n = 74), GIM-1 (n = 51) and IMP-1 (n = 32). OXA-23 was again the most frequent carbapenemase in *A. baumannii* (n = 247), followed by OXA-72 (n = 101) and NDM-1 (n = 37).

In 2024 the NRC has also introduced significant changes to the entry criteria for isolates sent to the NRC, which now

require a positive result in an unspecific phenotypic carbapenemase test or confirmation of a carbapenemase family by PCR or immunochromatography and first conclusions from this change will be presented.

Conclusions: A large variety of different carbapenemases is detected in Germany, especially in *Enterobacterales*. Compared to 2023, the increase of OXA-244, KPC-3 and NDM-5 in *Enterobacterales* is remarkable, while the number of detections of NDM-1 did not change much. In *P. aeruginosa*, VIM-2 remains the main carbapenemase and was again detected with rising frequency. In contrast, the number of detections of OXA-23 in *A. baumannii* has been stable.

PS02.046

Evaluation of FT-IR for the differentiation of Campylobacter jejuni strains regarding their potential to induce Guillain-Barré Syndrome

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Background: *Campylobacter jejuni*, the leading cause of bacterial gastroenteritis worldwide, can trigger post-infectious Guillain-Barré syndrome (GBS) due to molecular mimicry with gangliosides at the Ranvier nodes. The structurally analogous substance involved is a sialylated lipooligosaccharide (LOS) on the bacterial surface, for the synthesis of which the two alleles of the alpha-2,3 sialyltransferase (*cstII* or *cstIII*) serve as key enzymes. Strains that produce a sialylated LOS exhibit increased epithelial invasiveness and are more likely to be associated with bloody diarrhea. In contrast, there are also C. jejuni isolates that produce a non-sialylated LOS. Differentiating between these isolates is clinically significant, yet current methods require cost-intensive or labour-intensive genetic techniques. In this study, we evaluated the performance of FT-IRS spectroscopy for the discrimination of *C. jejuni* strains producing a sialylated LOS from those with a non-sialylated LOS.

Material/Methods: A total of 28 whole genome-sequenced *Campylobacter jejuni* isolates were investigated, representing various phylogenetic groups and carrying the *cst*II or *cst*III genes, or lacking any alpha-2,3 sialyltransferase, along with other genetic markers. These strains were isolated from both human and veterinary sources. Fourier-transform infrared (FT-IR) analysis was conducted using the IR Biotyper® system (IRBT - Bruker Daltonics, Bremen, Germany). Spectra were acquired from strains cultured on Columbia blood agar (Becton Dickinson, Germany) for 24 ± 1 hours at 42 °C in a microaerophilic environment. For each sample, three technical replicates from three independent biological replicates were measured. Exploratory data analysis was performed using hierarchical cluster analysis (HCA), principal component analysis (PCA), and linear discriminant analysis (LDA).

Results: Exploratory data analysis revealed that the *C. jejuni* isolates cluster significantly in relation to the presence or absence of one of the *cst* alleles. Furthermore, within the *cstIII*-positive cluster, a distinct subgrouping was observed that correlated entirely with the presence or absence of the two genes encoding a heterodimeric Tlp7, which functions as a formic acid chemotaxis receptor (**Figures 1 and 2**).

Conclusion: FT-IR methodology has demonstrated the potential to distinguish between *C. jejuni* strains that produce a sialylated LOS, which can trigger GBS, and those with non-sialylated LOS. Consequently, this approach could serve as a rapid and user-friendly solution for routine workflows. Further studies involving a larger number of strains are essential to confirm and reinforce these promising preliminary findings.

Fig. 1

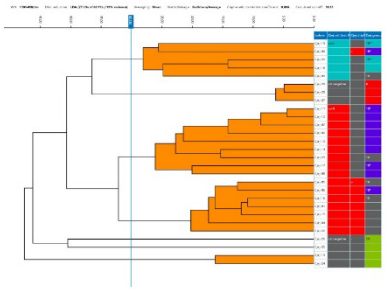

Figure 1. HCA results. On the left side, the IR Biotyper clustering is shown. On the right side, the isolate names, the *cst* gene (*cstII* in cyan, *cstIII* in red, absence of *cst* in grey), the presence of Tlp7 (positives in red, negatives in grey), *C. jejuni* group (1B** in cyan, 1B* in violet, 6 in re, 1A in grey and 2B in green) are shown, from left to right.

Fig. 2

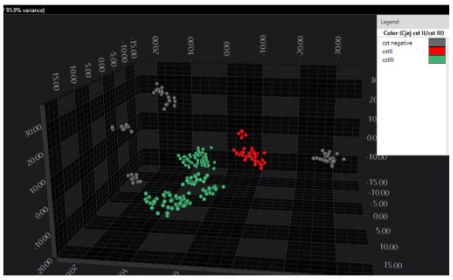

Figure 2. PCA/LDA 3D scatter plot results. Each geometric form represents a spectrum. *cstII*-positive strains are shown in red, *cstIII*-positive in green, and cst-negative in grey.

PS02.048

Influenza A Virus induces inflammatory responses in human Alveolar Macrophage-like cells

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Alveolar macrophages (AMs) are key immune sentinels in the lung, playing a central role in recognizing and responding to respiratory viruses. To better understand their involvement in Influenza A virus (IAV) pathogenesis, we established human alveolar macrophage-like (AML) cells by differentiating monocytes from healthy donors using GM-CSF, TGF-β, IL-10, and surfactant.

AML cells exhibited distinct morphology and marker expression compared to monocyte-derived macrophages (MDMs), including increased PPAR-γ, MARCO, and MRC1, and reduced MMP9 and CD36. Following IAV exposure, AML cells mounted a robust inflammatory response with elevated secretion of IL-6, IFN-λ2, TNF, IP-10, and IFN-β. Transcriptomic analysis revealed activation of key immune pathways, including type I interferon responses, pattern recognition receptor signalling, influenza A-related pathways, and JAK-STAT signalling, with strong enrichment in cytokine–cytokine receptor interactions.

These findings support the use of AML cells as a tractable *in vitro* model for studying alveolar macrophage responses to viral infection and provide insights into the mechanisms driving IAV-induced lung inflammation.

PS02.050

Borrelia tillae, a relapsing fever species from South Africa with human pathogenic potential

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Borrelia (B.) tillae was first described in 1961 by Zumpt and Organ as a novel relapsing fever species isolated from the soft tick *Ornithodoros zumpti* Heisch and Guggisberg 1953 in South Africa. The data collected in that study revealed significant differences between *B. tillae* and *B. duttonii* by using complement-fixation tests. These *Borrelia* species distributed in overlapping geographical regions but transmitted by different vectors. Rats appears to be the main reservoir hosts and a neurotropism has been proposed in infection studies with rats and mice but no data are available regarding the pathogenicity of *B. tillae* to humans.

In the present study, we sought to collect experimental evidence for intrinsic factors determining *B. tillae* competence to survive in human serum and overcome complement-mediated killing. Furthermore, we identified and characterized a host-interacting protein of *B. tillae* partially be involved in adhesion and serum resistance.

Our experimental data confirmed that *B. tillae* nicely survived in the presence of 50 % human serum. In agreement, no activated complement components could be detected on the spirochetal surface. Far Westernblot analyses identified distinct fibronectin-, plasminogen-, and factor H-binding proteins and further bioinformatics disclosed at least two genes encoding for host-interacting proteins in *B. tillae* on a megaplasmid. Of these, a potential fibronectin-binding protein, FbpC, sharing up to 26 % sequence identity to orthologous proteins of *B. duttonii* and *B. recurrentis* was recombinantly produced and then functionally characterized. Our data demonstrate binding of FbpC from *B. tillae* to fibronectin and plasminogen but not to complement C1r. These findings are in contrast to the binding properties of FbpC orthologs of *B. duttonii* and *B. recurrentis* known to strongly interact with C1r. Consequently, FbpC exhibits an attenuated inhibitory capacity on the classical pathway due to lack of C1r binding.

In conclusion, here we present first data on the capacity of *B. tillae* to evade innate immunity as an initial step to survive in the human host.

PS02.052

Application of Fourier-transform infrared spectroscopy to discrimination of _Escherichia_ coli O157:H7

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Background: Shiga-toxin-producing *Escherichia coli* (STEC) strains are zoonotic and waterborne pathogens, which represent a serious thread for public health. They can cause severe diseases like haemorrhagic colitis and haemolytic-uremic syndrome (HUS), especially in children and in elderly people. Worldwide, the most common STEC serotype is O157:H7, which may cause serious morbidity and large outbreaks worldwide, and has been linked to the consumption of contaminated plant foods (e.g., lettuce, spinach, tomato, and fresh fruits) and beef-based products. A prompt detection of O157:H7 serotypes of *E. coli* is of pivotal importance for food safety, as well as in clinical settings. In this study, we evaluated Fourier-Transform Infrared (FT-IR) spectroscopy to differentiate O157:H7 *E. coli* isolates from other serotypes.

Material/Methods: Overall, N=128 serologically or in-silico serotyped *E. coli* isolates were investigated (n=10 Stx-producing O157:H7 isolates and n=118 isolates belonging to 70 other different serotypes and pathovars). FT-IR analysis was performed applying the IR Biotyper® system (IRBT - Bruker Daltonics, Germany) following the manufacturer"s instructions. IR spectra were acquired from cultures grown on Columbia blood agar (Becton and Dickinson, UK) overnight at 37 ± 2 °C, in three independent biological replicates. Exploratory data analysis was performed by principal component analysis (PCA) and linear discriminant analysis (LDA). Predictive and machine learning models for the discrimination O157:H7 versus non-O157:H7 were designed, using the algorithms included in the IRBT software. The dataset was randomly split into two halves, comprising 64 isolates (n=5 O157:H7 and n=59 non-O157:H7) of each one. Two LDA models and two automated classifiers were set-up, using the two subsets alternatively as training set and as testing set.

Results: IRBT clustering showed that O157:H7 isolates are clearly separated from all the other assessed *E. coli* serotypes included in this study (Figure 1). The LDA predictive models showed a complete and clear separation of the two classes (Figure 2), and the automated classifiers provided 100% accuracy on the testing set.

Conclusion: IR biotyping showed the ability of discriminating O157:H7 isolates from a heterogeneous collection of non-O157:H7 *E. coli*, suggesting the suitability of this novel approach for infection control, public health interventions and epidemiological studies. Further investigations, including a higher number of well-characterized isolates and a large cohort of validation sets, are desirable to confirm and strengthen these promising preliminary results.

Fig. 1

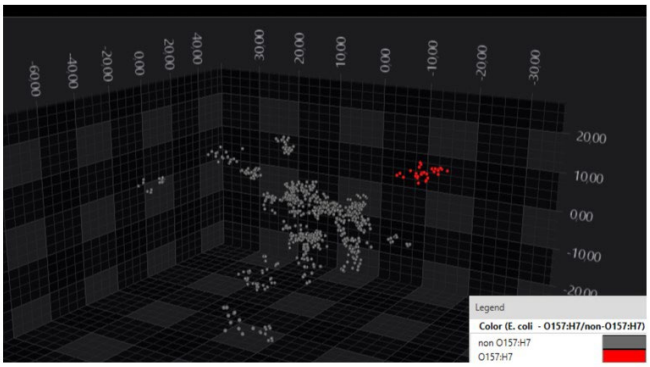

Figure 1. 3D LDA scatterplot showing the clustering of O157:H7 isolates (in red) and the other serotypes (in grey). Each single sphere represents a spectrum

Fig. 2

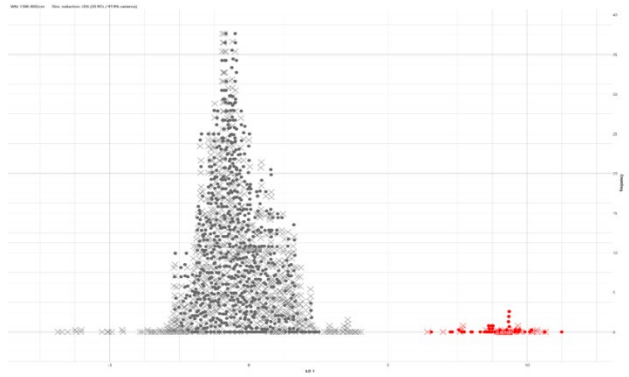

Figure 2. LDA predictive model with training spectra (indicated by spheres) and testing spectra (indicated by crosses). O157:H7 isolates are depicted in red, the other serotypes in grey.

PS02.054

Profiling of compound-induced cellular effects via high-throughput imaging and machine learning to gain early MoA insights

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The increased occurrence of resistant fungal pathogens and the lack of new antifungal agents reaching the market pose a significant threat to human health.

Basic phenotypic screens can rapidly identify promising compounds based on growth inhibition, but typically require extensive follow-up experiments to determine whether a compound acts through a novel mode of action (MoA). To address this limitation, we are establishing a high-throughput image-based profiling assay that can provide first insights into compound MoAs. This approach leverages our modularized robotics platform, JenXplor, enabling parallel testing of large compound libraries and prioritization of compounds with potentially new MoAs.

The assay utilizes fluorescent dyes to label key cellular substructures, enabling the observation of compound-induced morphological changes using high-throughput microscopy. These images are analyzed using a machine learning model extracting image features to cluster distinct phenotypes that can be attributed to specific MoAs, thus identifying compounds with potentially novel ones.

Historically, natural products have been a rich source of bioactive compounds in drug discovery, in part due to evolutionary selection for bioactivity, making them well-suited as test objects for our approach. We screened an in-house natural product library of over 9000 compounds against *Candida albicans,* yielding more than 70 potential hits. Their activities were determined via minimum inhibitory concentration assay, identifying several compounds with sub-micromolar activity. These promising compounds are now undergoing profiling to gain insights into their MoA.

This approach facilitates the rapid discovery and prioritization of antifungal compounds with potentially novel MoAs, accelerating their progression into the drug development pipeline.

PS02.056

Comparative analysis of efficiency and coverage of different selective agar media on the isolation of carbapenem-resistant *Enterobacterales* from wastewater

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Carbapenem-resistant *Enterobacterales* (CRE) in the environment pose serious public health challenges due to their resistance to a broad spectrum of antibiotic classes and inter-species transferability of carbapenem-resistance determinants. The effective detection and isolation of these organisms from environmental sources, such as wastewater, is vital for understanding their spread and implementation of control strategies. A variety of commercial selective agar media have been developed to facilitate the isolation of CRE, each differing in performance regarding bacterial species coverage and isolation efficiency.

This study aimed to compare the efficiency and selectivity of five commercially available chromogenic agar media in isolating CRE from wastewater samples (Table1; CM, CD, CB, CBSK and CBSO). Wastewater samples were collected at two time points from different locations in Leipzig, Germany, including three hospitals and the city's wastewater treatment system. Each sample was processed in duplicate by two independent analysts using standard cultivation methods. For each combination of sample and medium, 30 colored colonies were randomly selected by each analyst and identified using MALDI-TOF mass spectrometry. A subset of isolates underwent further characterization via PCR and whole-genome sequencing (WGS) to identify resistance genes and assess genetic relatedness.

A total of 1,949 isolates were identified, representing 732 species across 371 genera. The number of colonies analyzed (n=30 per plate) was generally sufficient to capture the species diversity present in most samples. However, CBSO medium consistently showed the lowest species richness. The six most prevalent genera among all isolates were *Aeromonas* (24.8%), *Pseudomonas* (17.2%), *Klebsiella* (14.7%), *Citrobacter* (11.7%), *Escherichia* (8.7%), and *Enterobacter* (7.5%). Recovery rates for these genera varied notably across the different media. For instance, *Aeromonas* was most frequently isolated on CM (32.8%), followed by CB (27.2%) and CD (22.4%). Notably, *Citrobacter*—a genus of particular public health concern—was significantly more prevalent on CM than on other media types.

The analysis also revealed significant discrepancies between the two analysts in terms of isolate composition. Analyst B identified a significantly higher proportion of *Aeromonas*, whereas Analyst A recovered more *Pseudomonas* isolates, highlighting the variability in colony selection as influencing factor for species coverage.

WGS of selected isolates revealed high strain diversity and the presence of various carbapenemase genes, including *bla*KPC-2, *bla*NDM-1, *bla*GES-5, *bla*OXA-48, and *bla*VIM-1. These findings underscore the critical role of selective media choice in the accurate detection of CRE from environmental samples. Moreover, the observed inter-analyst variability suggests that automated or standardized colony selection processes may enhance harmonization consistency in future surveillance efforts.

Fig. 1

Table 1. List of chromogenic agar media selective for CRE evaluated in this study.

Chromogenic agar	Abbreviation	Manufacturer
CHROMagar mSuperCARBA	CM	Mast Diagnostica GmbH, Germany
BD CHROMagar CPE	CD	Becton, Dickinson and Company, USA
CHROMID Carba	CB	bioMerieux Deutschland GmbH
CHROMID Carba SMART CARB	CBSK	bioMerieux Deutschland GmbH
CHROMID Carba SMART OXA	CBSO	bioMerieux Deutschland GmbH

PS02.058
Unexpected intracellular behavior of S. Paratyhi A
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Introduction: Typhoidal *Salmonella* serovars such as Typhi and Paratyphi A cause serious, chronic and systemic infections in humans. Typhoid toxin is an A_2B_5 toxin composed of PltA, PltB and CdtB, which plays a role as an important virulence factor of *S.* Typhi. Typhoid toxin is only expressed and secreted by intravacuolar bacteria, within the *Salmonella* Containing Vacuole (SCV)[1]. While it is known that translocation of the toxin across the peptidoglycan layer occurs due to the activity of TtsA at the bacterial poles, the mechanism and factors involved in the transport of the toxin across the outer membrane remain unknown. The mode of invasion by S. Paratyphi A has been suggested to influence the likelihood of vacuolar escape. In a study by Scharte and colleagues, it was found that invasion of S. Paratyphi A by a zipper-mediated mechanism facilitated less escape than the trigger mechanism[2]. A similar subpopulation of vacuolar escapers has previously been described in S. Typhimurium, undergoing hyper-replication once in the cytosol of epithelial cells[3].

Methods and Results: *S*. Paratyphi A was utilized as a model for typhoid toxin production in initial experiments due to its ease of use and genetic similarity to *S.* Typhi. To study toxin transport across the outer membrane, we generated tagged CdtB and TtsA subunits to facilitate the detection of typhoid toxin. Despite Western blots confirming the production of the tagged toxin when the bacteria were grown in TTIM medium, mimicking the intravacuolar environment, microscopy of intracellular bacteria showed a dispersed and unexpectedly faint signal corresponding to typhoid toxin. It was also noted that many of the infected cells appeared to have clustered bacteria which had potentially escaped the SCV. In contrast to intravacuolar Typhi, which shows strong, polar localisation of typhoid toxin and distinct LPS staining, these bacteria did not appear to produce toxin and had speckled LPS staining. Various MOIs, time points and strains were investigated to avoid this phenotype, however, it appeared that clustering occurred earlier than toxin expression was seen. Unlike the study by Scharte and colleagues using an *invA* mutant complemented with *Yersinia inv*, we used Acyl-homoserine lactones and TTIM medium to activate alternate invasion factors in the mutant, facilitating uptake by the zipper-mediated mechanism. This also resulted in a reduction of the clustering phenotype observed and in a distinct LPS staining.

Summary: *S.* Paratyphi A appears to either remain in or escape the SCV based on the mechanism by which it invades cells, further affecting the production of virulence factors such as the typhoid toxin. In the future, we hope to determine the location of these clustered bacteria using a specialised reporter system.

1. Fowler, C. C. *et al.* Emerging insights into the biology of typhoid toxin. *Current Opinion in Microbiology*(2017).
2. Scharte, F., Franzkoch, R. & Hensel, M. Flagella-mediated cytosolic motility of Salmonella enterica Paratyphi A aids in evasion of xenophagy but does not impact egress from host cells. *Molecular Microbiology*(2024).
3. Knodler, L. A. Salmonella enterica: living a double life in epithelial cells. *Current Opinion in Microbiology*(2015).

PS02.060
Evaluation of detection limits of different urinary antigen tests for *Legionella pneumophila* serogroups using standardized antigen concentrations
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Question: *Legionella* spp. is an intracellular pathogen, causing either a flu-like infection (also called Pontiac fever) or the more severe Legionnaires´ Disease (LD). In Germany, >2.000 cases where reported in 2024 (incidence increasing) with a case fatality rate of ~6 %.

Around 80 % of the LD diagnostic is conducted by urinary antigen tests (UAT). The broad majority of these tests detect lipopolysaccharide (LPS) of *Legionella pneumophila* serogroup (sg) 1. Currently, UATs are validated by spiking sterile urine with bacteria cells. However, clear limits of detection (LOD) of the antigen remain unclear.

A validation based on LPS could provide a standardized method to compare UATs. However, quantification of LPS remains challenging. As part of the LPS core region, 2-Keto-3-deoxyoctonate (KDO) is suitable for its indirect quantification. This can be done by MgCl2 precipitation of LPS followed by KDO extraction, offering a way to quantify LPS from gram-negative bacteria like *Legionella* spp. We evaluated in this small study i) whether the extraction and standardization of antigen helps to find clear LOD and ii) UAT might detect other serogroups of *L. pneumophila* besides sg 1.

Methods: Strains from *L. pneumophila* sg 1-15 (incl. all sg1 subgroups) were typed with the "Dresden-Panel". The strains were inoculated into 50 ml BYE liquid culture and harvested after 48 h incubation at 37 °C and 80 rpm. The LPS were precipitated using MgCl2, purified and dissolved in H2O. LPS quantification was performed indirectly by colorimetric KDO quantification.

Sterile urine was spiked with 0.1 ng/ml, 0.01 ng/ml and 0.001 ng/ml of LPS and tested by three different UATs (Liaison® Legionella AG from Diasorin, BinaxNOW™ from Abbott and RIDASCREEN® from R-Biopharm) according to manufacturer"s protocol. LPS isolated from *Escherichia coli* and *Pseudomonas aeruginosa* were used as negative controls. The tests were also checked for reactivity with other *Legionella spp.* (*e.g. Legionella longbeachae*).

Results: The RIDASCREEN® and Liaison® UAT were able to detect all subgroups of *L. pneumophila* sg 1 at a concentration of 0.1 ng/ml. Weak signals were observed with the BinaxNOW™ test for some subgroups at 0.1 ng/ml. *Legionella* spp. and serogroup 2-15 were only detectable with the Liaison® UAT.

Conclusion: A reliable protocol for LPS extraction and quantification for the standardize validation/evaluation of legionella UAT was established.

The Liaison® and Ridascreen® UATs have higher sensitivities compared to BinaxNOWTM. The Liaison® UAT was also able to detect *L. pneumophila* serogroups other that sg 1 and *Legionella* spp.

PS02.062
Cross-kingdom interaction between *Candida albicans* and *Akkermansia muciniphila*
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Overall well-being and health are strongly affected by the microbial composition of the gut, as shown in several studies. Various factors influence the gut colonization pattern, including the intake of antibiotics, which can lead to a shift towards dysbiosis. In this state of imbalance, the colonization with opportunistic pathogens such as the fungus *C. albicans* is no exception. Various cross-kingdom bacterial interactions with *C. albicans* have been studied. However, the interaction with the anaerobic bacterium *A. muciniphila*, which is considered a probiotic, remains understudied.

In this study we have investigated the effect of *A. muciniphila* on hyphae formation, the main virulence factor for *C. albicans*. Using *in vitro* intestinal cell model, viable and heat-inactivated *A. muciniphila* were cocultured with either *C. albicans* strain 101 or SC5314. Interestingly viable but not heat inactivated was *A. muciniphila* was able to impair hyphae formation and reduced host cell cytotoxicity. Such effect was independent of *C. albicans* strains. While prolonged inhibition of hyphae formation was observed in the presence of intestinal cell line, inhibition was observed in the absence of host cell lines. To determine the host cell response, IL-8 cytokine and antimicrobial peptide LL-37 were measured from cell culture supernatant. Interestingly there was a significant increase in LL-37 production after co-culture with viable but not heat inactivated bacteria. No significant difference was observed in terms of IL-8 production.

In conclusion, metabolically active *A. muciniphila* reduces *C. albicans* virulence by inhibiting hyphae formation via yet unknown mechanism. Further metabolomic and *in vivo* studies are planned to determine underlying mechanisms.

PS02.064
More than 15 years of the concept of complement inhibition in E. coli-associated haemolytic uraemic syndrome (eHUS)
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Following Otto Götze"s idea to detect C5, C6 and C7 on the surface of macrophages for biological functions, such as the "spreading" of these cells, Würzner and colleagues developed monoclonal antibodies directed against these complement proteins, employing the hybridoma technique which was established at the institute only months before, mainly by Horst Baumgarten und J. Hinrich Peters. Two anti-C5 (N19-8 and N20-9) and one anti-C6 (WU 6-4) were able to inhibit the terminal complement pathway. These studies of the years 1987 and 1988 were first made available to a restricted scientific community (applications for travel grants for the 7th International Immunology Congress in Berlin and the 13th International Complement Workshop in San Diego, in early 1989) and to the entire scientific community at both conferences in August and September 1989, respectively. These represent the first descriptions of inhibition of human complement C5 and C6 by monoclonal antibodies, more than 3½ decades ago.

Later, this anti-C5 was sold by Otto Götze, Dr. Würzner"s supervisor at that time, to Alexion. In the following months and years this antibody was a main focus of Alexion"s studies on the inhibition of complement lysis, until it was claimed that a superior monoclonal antibody, 5G1.1, was generated which then served as matrix for eculizumab, Alexion"s Soliris.

In Oxford and Cambridge Würzner continued his studies of complement inhibition by monoclonal antibodies and together with Dorothea Orth-Höller he discovered that Shiga toxin activates complement and binds complement factor H in Innsbruck more than 15 years ago. From this evidence for an active role of complement in E.coli associated hemolytic uremic syndrome (eHUS) they proposed that Eculizumab may be useful in severe forms of eHUS. In fact, this drug was then extensively used in the German 2011 EHEC outbreak. Today, use of Eculizumab in eHUS is still controversial, but favourable outcomes, especially in severe neurological involvement were reported. Recent meta-analyses and broad trials will be reviewed at the conference.

PS02.066
Exploring the persistence of respiratory virus-specific cellular responses across the 2024 summer season in Germany: a prospective study in healthcare workers
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Question: T cell-mediated immunity plays a critical role in the control and resolution of acute respiratory infections (ARI). Understanding the stability and seasonal waning of virus-specific cellular responses over time is essential for evaluating long-term immune protection, especially in populations with repeated exposure. This study aimed to assess the

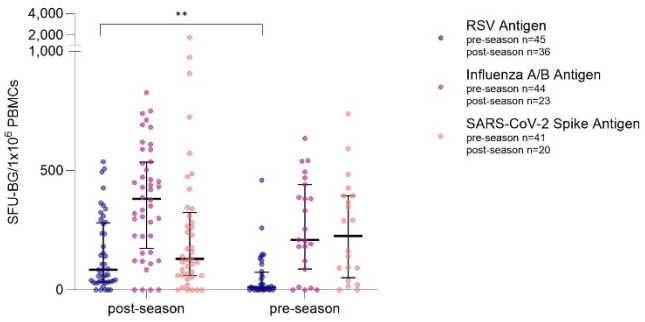
persistence of IFN-g-secreting cellular responses in healthcare workers (HCWs) between the end of the 2023/24 ARI season and the start of the 2024/25 season, focusing on RSV, Influenza A/B, and SARS-CoV-2.

Methods: As part of the prospective, longitudinal ARIPro cohort study investigating the incidence, risk factors, and preventability of ARI in HCWs, Cell Preparation Tubes (CPT) were randomly collected from participants within the cohort at two time points: After the 2023/24 ARI season (post-season: April 1–30, 2024, n = 45) and before the 2024/25 season (pre-season: October 1–31, 2024, n = 36). Peripheral blood mononuclear cells (PBMCs) were isolated from CPT samples and cellular reactivity was determined using a non-commercial IFN-g ELISpot assay following stimulation with RSV Antigen (Virion\Serion), a seasonal Influenza vaccine (Influvac Tetra 2024/25, Viatris) targeting Influenza A (H1N1) and B, and the SARS-CoV-2 Spike Ectodomain (S1-S2) Antigen (Virion\Serion). Spot-forming units (SFU) per 10^6 PBMCs were quantified after background subtraction (medium control). Non-parametric analyses were conducted using Mann-Whitney-U test. P-values were corrected according to Krüger and Yekutieli (α=0.05).

Results: Due to varying cell availability, the number of valid measurements differed between antigens (Figure 1). Cellular reactivity against RSV significantly declined from the post-season to the pre-season 2024 period (p = 0.001). A similar not significant trend (p = 0.24) was observed in response to Influenza Antigen. In contrast, cellular reactivity targeting the SARS-CoV-2 Spike Antigen remained stable across the same period (p = 0.98; Figure 1).

Conclusions: These findings indicate a marked seasonal decline in virus-specific cellular reactivity to RSV Antigen and to a lesser extent, Influenza A/B Antigens—from spring to autumn, whereas the spike-specific cellular reactivity was maintained. This may in part explain the seasonal risk of acquiring reinfection with RSV or Influenza, particularly as successful immune evasion mechanisms have been described for RSV in literature being successful to underwent cytotoxic T cell-mediated clearance.

Fig. 1



PS02.068

Case-based learning and artificial intelligence-based gamification to improve undergraduate students' motivation for One Health and climate change

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Background: The One Health (OH) approach, which acknowledges that human health cannot solely be looked at without also taking animal health and the health of ecosystems into account, is a critical concept for medical students and physicians. Yet, data on OH-specific teaching formats for medical undergraduates and their acceptance and effectiveness are scarce. We sought to investigate the feasibility of a new teaching format and its effects on student motivation.

Methods: We conceived a teaching format for the hygiene seminar using case-based learning (CBL), enriched with gamification elements partly based on artificial intelligence, for the topics One Health and climate change, for medical undergraduates at the University of Bonn, Germany. We measured intrinsic motivation of students using the intrinsic motivation inventory (IMI) by Deci and Ryan. We compared IMI subscale scores between students attending the CBL format to those attending non-CBL formats.

Results: During the summer term 2024, a total of 382 IMI forms were collected during 30 out of a possible of 42 seminar modules, of which 215 forms were from 14 CBL modules (mean 15.4 forms per module) and 167 forms from 16 non-CBL modules (mean 10.4 per module). We found higher scores for the CBL format across all IMI subscales, i.e., interest/enjoyment (Cohen's d between 0.27–0.49), perceived competence (Cohen's d 0-27–0.32), perceived choice (Cohen's d 0.43–0.55), but also for pressure/tension (Cohen's d 0.27–0.34). Ordinal logistic regression analysis yielded that having the CBL format was significantly associated with higher IMI scores in every item (odds ratios, OR, between 1.65 and 2.69).

Conclusions: We show the feasibility of a new, CBL-based format to teach medical undergraduates environmental medicine, including the One Health approach and climate change, and its effectiveness on students' motivation.

PS02.070

Treatment of newborns with probiotic *E. coli* strain Nissle 1917 (EcN) does not change the abundance of *pks+ E. coli* one year after last application

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Introduction: Facultative anaerobe microbes including *E. coli* play a crucial role as first colonizers of the human gut. Some of them and may persist in the host"s intestine for life. The species *E. coli* is highly heterogeneous, most members are beneficial commensals but some are highly pathogenic. Within the species *E. coli*, different phylogroups can be distinguished. Among these, members of group B2 as part of the intestinal microbiome have a rising incidence in high-income countries. 30 - 50 % of the B2 strains carry a 54-kb genomic island, the so-called *pks* island. It encodes the biosynthetic machinery for the production and transport of the hybrid non-ribosomal peptide-polyketide genotoxin colibactin. *pks+ E. coli* strains are present in 10 – 30 % of the

microbiomes of adult people in the Western world. Phylogroup B2 *E. coli* are particularly good at colonizing infants. The incidence of colonization of infants with *pks*+ strains reported in the literature varies between 15 % (France) and 18 % (Sweden) twenty years ago. E. *coli* strain Nissle 1917 (EcN) is a probiotic member of the B2 phylogroup. It is well-known for its anti-inflammatory, intestinal barrier stabilizing and antagonistic features.

Objectives: In order to investigate the prevalence for *pks* + *E. coli* strains and their persistence in infants in Germany and Poland, stool samples from participants in a randomized clinical trial, the so-called "RoNi" trial, were analysed 12 and 36 months after the last EcN administration at the end of the second year and fourth year of life, respectively.

Material and Methods: Newborns received EcN formulated as Mutaflor suspension or placebo for 14 or 10 days directly after birth, as well as after 6 and 12 months, respectively. One year after the last medication and a further time after 3 years, DNA was extracted from stool samples. The presence of beneficial microbes (*Akkermansia*, *Faecalibacterium* and EcN) and non-beneficial critical *E. coli* genes (*hlyA* and *pks*) was analysed by real time PCR.

Results: One year after the last administration of verum or placebo, the *pks* and *hlyA* genes were equally distributed in both groups (*pks* verum: 45%, placebo 40%; *hlyA* verum: 12%, placebo: 17%). The abundance for *pks* and *hlyA* genes decreased equally over time in both groups (*pks* verum: 26%, placebo: 29%; *hlyA* verum: 15%, placebo 17%). Although all children of the verum group had received EcN, this strain was detected in 19% of the stool samples collected one year after the last application only.

Summary: *E. coli* strains of phylogroup B2 and *pks*+ strains appear to be highly prevalent in infants in Germany and Poland. The frequency of *pks*+ strains or the occurrence of *hlyA*-positive strains was not affected by the use of the probiotic EcN strain. EcN is not a long-term colonizer in the infant gut. In children who had received EcN at the age of 1 year, it was no longer present in 81% of the recipients after 12 months.

PS02.074

First insights into the genetic landscape of carbapenem resistant *Pseudomonas aeruginosa* in tertiary hospital in the Ukraine

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Introduction: The spread of carbapenem-resistant organisms (CRO), such as carbapenem resistant *Pseudomonas aeruginosa* (*P. aeruginosa*), poses a significant threat to healthcare settings, especially in regions of conflict where infection prevention and control (IPC) strategies are difficult to implement. Here, transmission events with CRO among patients can further promote the increase of antimicrobial resistance (AMR) in hospitals and cause severe infections in vulnerable patient populations, such as burn victims.

Aims: Following an increase of carbapenem resistant *P. aeruginosa* isolates detected at a tertiary hospital in the Ukraine, isolates were characterized by long-read whole genome sequencing (lrWGS) to identify potential transmission events.

Methods: All samples were collected through routine clinical diagnostics and were identified as CRO based on phenotype. In total, seven carbapenem-resistant *P. aeruginosa* isolates were subjected to lrWGS using the PacBio® Sequel IIe system. lrWGS data analysis was performed utilizing Ridom SeqSphere⁺ and genotypic information e. g. multilocus sequence types (ST) and resistance genes was extracted. Genetic relatedness of isolates was assessed based on core genome multilocus sequence typing (cgMLST).

Results: Of the seven carbapenem-resistant *P. aeruginosa* isolates analysed, ST1047 (n=3), ST773 (n=3) and ST1071 (n=1) were detected. Isolates of ST1047 and of ST773 formed two distinct, genetically related cgMLST clusters. The ST1071 isolate was unrelated to all other isolates. Among the ST1047 isolates, the $bla_{\text{IMP-1}}$ gene was identified as the primary carbapenemase. Two ST773 isolates harbored the $bla_{\text{NDM-1}}$ gene. In addition, isolates in both clusters carried other resistance genes, including $bla_{\text{OXA-488}}$ and $bla_{\text{OXA-395}}$. All carbapenemases were encoded on the chromosome. All samples represent clinical isolates and all patients had to receive antibiotic treatment because of their *P. aeruginosa* infection. Evaluation of epidemiological data associated with patients showed that the majority of patient had contact to the intensive care unit (ICU) during their admission; two patients were treated for severe burns.

Conclusion: These results point towards two separate transmission events with carbapenem-resistant *P. aeruginosa* at a tertiary hospital in the Ukraine. This example illustrates the benefit of integrating sequencing technology in the surveillance of CRO to distinguish sporadic cases from genetically related isolates and detect ongoing hospital associated outbreaks. Establishing and supporting IPC strategies in regions of conflict will be essential to reduce the spread of AMR. This includes IPC measures in the ICU setting to protect critical ill patients from severe infection with CRO and improve patient outcome.

PS02.076

Glucose availability affects UPEC pathogenicity and infection dynamics

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Urinary tract infections (UTIs) are among the most common infections worldwide, most frequently caused by uropathogenic *E. coli* (UPEC). Around 25 % of all UTIs develop into recurrent infections. A key virulence strategy for this involves the formation of UPEC intracellular bacterial communities (IBCs) within bladder epithelial cells. This intracellular lifestyle enables the bacteria to evade immune responses and increases resistance to antibiotic treatment. The intracellular replication of UPEC is thought to place metabolic demands on host cells, particularly affecting central carbon metabolism and potentially increasing cellular glucose consumption. Thus, glucose availability could influence bacterial replication and egress.

We therefore investigated the effects of reduced glucose availability for the human bladder epithelial cell line RT-112 on different stages of the UPEC infection cycle—adhesion, invasion, intracellular survival and proliferation, and escape. At the same time, we tested the impact of different inhibitors of eukaryotic glucose import on UPEC growth and the

functionality of UPEC key virulence factors, including type I fimbriae and α-hemolysin.

Notably, we observed a strain-dependent influence of glucose availability to RT-112 cells on UPEC intracellular replication, and cellular egress. Our findings also demonstrate that some inhibitors of eukaryotic glucose transport can alter the functionality of UPEC virulence factors. This indicates side effects of eukaryotic glucose transport inhibitors, which affect the UPEC-host cell interaction independently of glucose availability.

Taken together, these results suggest that interference with host glucose transport pathways can impair UPEC virulence and alter the course of intracellular infection. These findings provide novel insights into the metabolic interplay between host and pathogen.

PS02.078
Analysis of *Bartonella bacilliformis* binding to erythrocytes via protein crosslinking and mass spectrometry
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Introduction: *Bartonella bacilliformis* is the etiological agent of Carrion"s disease, a vector-borne illness endemic to the Andean regions of South America, particularly Peru. During the acute phase of the disease, known as Oroya fever, *B. bacilliformis* infects erythrocytes, leading to severe hemolytic anemia with a mortality rate approaching 90%. Although the underlying molecular mechanisms remain poorly characterized, adhesion of the bacterium to erythrocytes is considered a critical step in pathogenesis. The erythrocytic binding partners of the trimeric autotransporter adhesins (TAAs) *B. bacilliformis* adhesin A and B (BbadA, BbadB) have yet to be identified.

Methods: Markerless deletions of the putative adhesins BbadA and BbadB were generated in *B. bacilliformis*. A novel protein crosslinking approach employing disuccinimidyl suberate (DSS) was utilized to identify erythrocyte binding partners of BbadA and BbadB by mass spectrometry. Furthermore, the contribution of BbadA and BbadB to bacterial virulence was assessed using the *Galleria mellonella* infection model.

Results: Preliminary mass spectrometry analyses revealed the presence of *B. bacilliformis* adhesins BbadA and BbadB, as well as several erythrocyte membrane proteins (e.g., glycophorin A) in the crosslinked samples. However, the high abundance of hemoglobin continues to impede comprehensive analysis. Infection assays revealed that *G. mellonella* were efficiently killed by *B. bacilliformis*, regardless of BbadA or BbadB expression.

Conclusion: These findings suggest that BbadA and BbadB are involved in the adhesion of *B. bacilliformis* to erythrocytes. Ongoing optimization of crosslinked protein purification aims to reduce hemoglobin contamination, thereby enabling more precise identification of host binding partners through mass spectrometry.

PS02.080
Resilience of chicken towards *Salmonella*: using surrogate infection models to define a protective microbiome (ChiSaRe)
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Salmonella enterica remains a major zoonotic threat in Europe, with over 60,000 human cases of salmonellosis reported in 2021. In addition to its public health impact, S. *enterica* poses a significant burden on poultry production. To mitigate these risks, a synthetic protective chicken microbiota is being developed to enhance host resistance and suppress pathogenic colonization. A defined oligo-chicken microbiota (OCM) was assembled based on an extensive literature review, selecting ten dominant, cultivable, and non-pathogenic bacterial species from the gastrointestinal tract of *Gallus domesticus*. These species represent six major bacterial orders, including Lactobacillales, Bacteroidales, and Bifidobacteriales. A commercially available complex microbiota product (Aviguard) serves as a positive control due to its known inhibitory effects on *S. enterica*. The protective capacity of the OCM is being assessed using two main experimental approaches. First, in vitro competitive growth assays (CGA) are conducted with *S.* Typhimurium ST4/74 expressing GFP. These assays are performed in microtiter plates under both aerobic and anaerobic conditions, with bacterial growth monitored via fluorescence plate reading and validated by colony-forming unit (CFU) enumeration. Preliminary results indicate that specific OCM strains can inhibit the growth of *S.* Typhimurium, with follow-up experiments underway to confirm these findings. Second, a *Galleria mellonella* infection model is used to evaluate the protective properties in-vivo. Larvae are orally inoculated with the OCM, followed by infection with *S.* Typhimurium or *E. coli*. Competitive colonization and survival assays are performed to determine the protective effects of the OCM. Furthermore, serovars of *S. enterica* are employed to identify potential fitness disadvantages in the presence of chicken commensal bacteria. To elucidate the molecular mechanisms underpinning these microbial interactions, multi-omics approaches -particularly transcriptomics- will be applied in future phases. This research aims to uncover key bacterial factors and host-microbe interactions that drive *Salmonella* suppression, supporting the development of microbiota-based interventions to reduce the burden of salmonellosis in poultry and along the food chain.

PS02.082
In the hot spot – transcriptomics and ecological interactions of *Aspergillus fumigatus* with the compost microbiota
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Introduction: Fungal infections pose a serious threat to human health with more than 3.5 million deaths each year worldwide. Among them, *Aspergillus fumigatus*, recently highlighted as a critical priority fungal pathogen by the WHO, causes a wide range of diseases, ranging from allergic reactions to lethal invasive infections, depending on the host's immune status. Despite the absence of selective pressure for fitness in humans, this fungal pathogen has developed virulence traits enabling human infection. *A. fumigatus* naturally thrives in soils and compost heaps, ecological hot spots in which it must compete with complex and diverse microbial communities composed of bacteria, fungi, and even amoebae predators. Emerging hypotheses suggest that such

environmental interactions may have shaped *A. fumigatus'* virulence to human and mammalian hosts.

Objectives: The aim of this study is to investigate the ecological interactions and transcriptomic response of *A. fumigatus* in its typical environmental niche, compost.

Methods & Results: First, co-occurence network analyses have been performed to identify bacterial and fungal taxa co-occuring with *A. fumigatus* in a wide compost and soil samples dataset. Preliminary results indicate that *A. fumigatus* co-occurs with over 200 and 140 different bacterial and fungal taxa, respectively. Then, based on the culturing, one compost sample containing *in-situ A. fumigatus* will be sequenced for bulk metatranscriptomics. Metagenomic analysis to characterize the community composition will be performed in parallel. Finally, the same selected compost sample will be autoclaved three times sequentially and spiked with a known concentration of *A. fumigatus* CEA10 conidia to have the transcriptome of *A. fumigatus* in a sterile compost as control.

Summary: This study will shed light on the gene expression of *A. fumigatus* in its native wild compost community, as well as the metatranscriptome of the whole community. This will help to identify key ecological functions that moonlight as virulence strategies during *A. fumigatus* infection in humans.

PS02.084

Exploring the spatial and temporal distribution of biofilm microbiomes on endotracheal tubes and their role in ventilator-associated pneumonia

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Ventilator-associated pneumonia (VAP) is a severe complication affecting patients under mechanical ventilation, often arising 48 hours or more after intubation. Biofilms forming on the surfaces of endotracheal tubes (ETTs) serve as persistent microbial reservoirs, shielding pathogens such as *Klebsiella pneumoniae* from immune clearance and antimicrobial treatment. Despite their clinical relevance, the temporal and spatial structure, composition, and treatment response of these microbial communities remain poorly understood.

Microbiome samples were collected from three defined regions of ETTs across a range of intubation times (24 to 600 hours). Extraction of DNA was conducted from each sample, followed by 16S rRNA gene sequencing using the Illumina iSeq 100 platform. The resulting data were analyzed to assess spatial and temporal changes in taxonomic composition.

Microbiome profiling based on 16S rRNA gene sequencing revealed distinct spatial and temporal patterns in both alpha and beta diversity along the endotracheal tubes (ETTs). Taxonomic classification provided insights into the relative abundance of microbial groups, while phylogenetic analyses further clarified evolutionary relationships and community structure. To contextualize these findings, we compared the ETT-associated microbiomes with reference datasets from healthy lung and gut microbiota, enabling a broader interpretation of microbial shifts under mechanical ventilation.

Since biofilms consist of a complex mixture of both live and dead microbial cells, and only viable cells contribute to infection risk, we focused our analysis on quantifying the living fraction. To assess viable microbial populations, we performed a serial dilution assay. The result suggests distinct spatial and temporal distribution of bacterial growth dynamics along the ETT.

To determine the source of the infection, we conducted a systematic outbreak analysis focused on the most common causative agent, *Klebsiella pneumoniae*. In parallel, *K. pneumoniae* strains were isolated from all three regions and subjected to whole-genome sequencing (WGS). Bioinformatic analysis included genome assembly and annotation, as well as the identification of resistance, mobile elements and virulence factors. Single-nucleotide polymorphism (SNP)-based phylogenetic analysis was performed to investigate clonal relationships among the isolates, revealing patterns suggestive of ecological and spatial segregation, likely influenced by the structural compartmentalization caused by the endotracheal tube cuff.

Together, these approaches provided detailed insight into the microbial ecology and structural dynamics of ETT-associated microbial communities. They highlight the dynamic and compartmentalized nature of biofilm-associated microbiomes on endotracheal tubes and underscore the importance of integrating phylogenetic and outbreak analyses to better understand infection risk and transmission dynamics under mechanical ventilation.

PS02.086

Antidepressants can induce antibiotic resistance and persistence in the gut microbiome of patients with major depression

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The rising global threat of antibiotic resistance is traditionally linked to the overuse of antibiotics. However, emerging evidence suggests that non-antibiotic pharmaceuticals, such as antidepressants, may also contribute to this phenomenon. This study investigates the potential of antidepressants to induce antibiotic resistance and persistence in the gut microbiome of patients with a major depressive disorder (MDD). The study was based on faecal samples of the ADAR study, which includes 53 patients treated with different kinds of antidepressants as well as untreated individuals. 16S rRNA gene sequencing was used for microbiome profiling complemented by selective culturing and antibiotic susceptibility assays on Enterobacteriaceae and Enterococcaceae. Preliminary findings indicate bupropion and sertraline treatment has been linked to the highest incidence of detected antibiotic resistance. Among the tested antibiotics, resistance was most frequently detected against ampicillin, ciprofloxacin and tigecycline. In this context, analyses are performed using a single bacterial isolate to assess the impact of these compounds on resistance dissemination. Moreover, it has been shown that bupropion and sertraline promote the spread of antibiotic resistance through horizontal conjugative

gene transfer. These findings suggest that antidepressant treatment may influence the distribution of antibiotic resistance in the gut microbiota, highlighting a need for further investigation.

PS02.088

Prophages in animal strains of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* is a common colonizer and pathogen in both, humans and animals. Many of its virulence-associated genes are localized on prophages and can be transmitted by phages.

This suggests that the host specifity of *S. aureus* strains and lineages might be predetermined by phages/prophages. Studying prophages in animal strains can, therefore, provide valuable insights into the evolution, host adaptation, and pathogenic potential of *S. aureus* across different host species.

Material and methods: We analysed *S. aureus* isolates obtained from a broad range of domestic, zoo, and wild animals, representing diverse ecological backgrounds. Two human isolates belonging to the "hedgehog-associated" lineage CC130 were also included for comparison. Isolates were characterized by microarray and nanopore sequencing. Genome assemblies were annotated and screened for prophage sequences. Comparative analyses were performed to assess the diversity and distribution of prophages and associated virulence factors.

Results: A total of 82 *S. aureus* genomes, representing 57 distinct clonal complexes and originating from 21 mammalian and three avian species, were analyzed. We identified 137 complete and 10 truncated/fragmented prophages. The complete prophages were classified into the genera *Biseptimavirus* (n=20), *Dubowvirus* (n=13), *Peeveelvirus* (n=16), *Phietavirus* (n=20), and *Triavirus* (n=42). In addition, five sequences were questionable Dubowviruses, seven could not be assigned to known genera, and 14 appeared to be chimeric. Notable phage-borne virulence factors included leukocidins (*lukF-PV83/lukM* in ruminant strains, *lukP/Q* in horse strains, *lukF/S-BV* in beaver strains), a chimeric leukocidin in a goat strain, enterotoxin E (*see*) in badger strains, enterotoxin *sea*-320E, staphylokinase, a horse-associated kinase gene, complement inhibitor (*scn*), equine *scn*, as well as an ornithine cyclodeaminase and a putative novel protease (D0K6J8+D0K6J9) in bird strains. A D0K6J8-like ornithine cyclodeaminase homologue was found in badger, lynx, and wild cat strains, and an enterotoxin-like gene in beaver strains. The distribution of these virulence factors was often host-specific, suggesting adaptation to particular animal hosts.

Discussion: Our findings reveal a remarkable diversity of prophages in animal-associated *S. aureus* strains, including several unclassified or chimeric elements, underscoring the dynamic nature of phage-mediated gene transfer. The host-associated distribution of key virulence factors, especially leukocidins, highlights the potential role of prophages in shaping host adaptation and pathogenicity. Ongoing studies include phage induction experiments followed by transmission electron microscopy. These results advance our understanding of the evolutionary mechanisms underlying *S. aureus* diversity and may inform strategies for monitoring zoonotic transmission and controlling the spread of virulence factors.

PS02.090

Detection of *Pseudomonas aeruginosa* from respiratory secretions of cystic fibrosis patients by qualitative Real-Time PCR

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Pseudomonas aeruginosa is one of the most important pathogens that can cause respiratory tract infections in people with cystic fibrosis (pwCF). Chronic infection can lead to a progressive deterioration in lung function, an increased frequency of hospitalizations and a reduced life expectancy. For this reason, routine microbiological monitoring is necessary in order to initiate timely an effective antimicrobial therapy. Culture-based methods are currently the goldstandard for microbiological examinations in CF. However, in the era of highly effective CFTR modulators, that improve CFTR function and lead to a reduced mucus secretion, the sensitivity of culture-based methods is under debate. Therefore, molecular-based diagnostics are increasingly recommended. However, validated *P. aeruginosa*-specific assays are hardly available in routine microbiological laboratories. Qualitative real-time PCR may be a promising approach to overcome this limitation. For this reason, we started to compare a published three-gene (*ecfX*/*gyrB*/*oprL*) qPCR assay (Boutin *et al.,* JCF, 2018) for the detection of *P. aeruginosa* from CF secretions with a four-gene based approach including *regA* gene. Initially, we collected sputum samples from pwCF treated at the university hospital Frankfurt (samples were processed according the recommended CF microbiology standard). We selected sputum samples of pwCF that were always negative, always positive or intermittently positive (<50% of the at least six samples positive collected over 12 months) for *P. aeruginosa* by microbiological culture. Then, the samples were investigated by qPCR targeting *oprL*, *ecfX*, *gyrB* and *regA* gene of *P. aeruginosa*. DNA from the reference strain *P. aeruginosa* PAO1 was used to create a calibration curve. To determine a cut-off value, clearly culture-positive samples

were compared with clearly culture-negative samples. All *P. aeruginosa* culture-positive samples could be confirmed at the molecular level. So far, 62 samples from intermittent patients were analyzed while 13 versus 26 samples were positive by culture or qPCR, respectively, which indicates a higher sensitivity of qPCR. Thus, qPCR may be complementary to standard culture especially in pwCF with less sputum production.

PS02.092

Type I interferons restrict *C. albicans* translocation through the intestinal epithelial barrier

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In healthy individuals, the microbiota and intestinal epithelial barrier supported by mucosal immune cells—acts as critical defences that prevent *Candida albicans* from causing invasive infections. However, when these defences are compromised, *C. albicans* can translocate from the gut into the bloodstream, leading to systemic candidiasis.

Type I interferons (IFN-I), a family of cytokines best known for their potent antiviral activity *via* IFNAR and the JAK-STAT1/2 pathway, are increasingly recognized for their role in antifungal immunity. Given the potential of type-I IFN responses to increase resistance of vaginal epithelial cells to *C. albicans* infection, we sought to explore whether the protective role of IFN-I, specifically IFN-β, extends to also the intestinal epithelial barrier.

Using an *in vitro* intestinal infection model, we found that IFN-β significantly reduces *C. albicans* translocation across the epithelial barrier. IFN-β treatment also triggered epithelial cytokine release, indicating an activation of epithelial immune defence. To understand how this antiviral response could translate to increased resistance to *C. albicans* infection we performed RNA sequencing on *C. albicans* infected intestinal epithelial cells in presence and absence of IFN-β treatment. Our analysis revealed over 200 differentially expressed genes, with a strong signature of antiviral response. Yet stratification to genes with roles beyond antiviral defence revealed mucins (*MUC13*, *MUC12A*, and *MUC3A*) and galectin-9 (*LGALS9*) to be significantly upregulated. These genes potentially play important roles in restricting *C. albicans* adhesion and invasion respectively.

Together, our findings reveal that IFN-β restricts *C. albicans* translocation through the intestinal barrier through inducing gene expression extending beyond antiviral defence. These genes may represent antifungal pathways to resist fungal invasion.

PS02.094

774 pathogen species across 4 domains: insights from over 10,000 clinical samples in over 5 years of routine metagenomics in Germany

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Clinical metagenomics emerges more and more from academic research to clinical practice. We"ve been spearheading this development in Germany evolving out of academia as a commercial metagenomic sequencing provider. We target the identification of pathogens from mainly blood but also other clinical specimens. Here we share data and insights from this >5 year journey.

Our DISQVER® test is a diagnostic algorithm based on next-generation sequencing of cell-free DNA for the detection of clinically relevant pathogens in blood and other specimens. Since starting our activities in Germany in 2017, with send-in service fully available since January 2020, we have processed >10,000 samples to date (May 2025) in routine service.

The test has been clinically validated for pathogen detection by more than 16 peer-reviewed clinical trials with approximately 3,400 patients across emergency/intensive care medicine, hematology or pediatrics. Additionally, several case reports or case series from diverse clinical backgrounds have highlighted its diagnostic use.

We transferred our wetlab processes to third-party laboratories to increase access, prove the robustness of the workflow and reduce shipping time. To date, our partners have processed over 2,000 samples. Across this consortium, including our own facility, >150 hospitals and health care institutions have been serviced.In these >5 years, we successfully demonstrated that our mNGS workflow enables a rapid turnaround time (median: 1 day) while detecting a very broad spectrum of pathogens with 774 unique species in total in this dataset.

Average positivity of blood samples was 48.0% and the most prevalent pathogens were bacteria (76.9%) followed by dsDNA viruses (13.0%), fungi (3%) and parasites (1.8%) based on species occurrence. Represented in the top 100 species from blood were 69 bacteria, 23 dsDNA viruses, 8 fungi and 0 parasites with the most frequently detected species (after Epstein-Barr virus with 1055 occurrences, common due to reactivation) being Enterococcus faecium (685) and Escherichia coli (543). Interestingly, besides the "expected species", a significant number of diagnostically challenging or rare pathogens were also detected such as e.g. Mucorales, Fusarium species, Enterocytozoon bieneusii or parasites such as Leishmania infantum/donovani, Schistosoma mansonii, Trichosporon asahii, Loa loa or Echinococcus multilocularis to name a few.

In conclusion, our long-term experience in Germany underlines that clinical metagenomics has emerged as a new tool within clinical microbiology and shows great potential to improve the diagnostic yield and consequently patient care substantially. Due to the novelty of the technology and lack of experience in selecting patients and interpreting results however, acceptance and clinical integration is gradual but quickly improving with user experience as clinical microbiologists begin integrating the method into their portfolio and guide its application.

PS02.096

The role of the pAA plasmid in mediating interaction and invasion of extraintestinal cell linages

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Introduction: Pathogenic *Escherichia coli* strains have traditionally been classified based on their ability to cause intestinal or extraintestinal infections, such as urinary tract infections or bloodstream infections. However, recent studies have revealed that some *E. coli* strains can cause diarrhea and extraintestinal infections within the same patient. One such strain belongs to the enteroaggregative *E. coli* (EAEC) pathotype, typically associated with diarrheal diseases, but has also been increasingly found in extraintestinal infection sites. This observation has raised important questions about whether EAEC-specific virulence factors might also contribute to extraintestinal pathogenicity.

EAEC pathogenicity is attributed to specific virulence factors encoded by the aggregative adherence plasmid (pAA), which plays a key role in the development of diarrheal disease. While these factors have a central role in defining the EAEC pathotype, the specific functions of most pAA-encoded genes and the genetic diversity among pAA plasmids remain poorly understood. Additionally, this topic has not been studied in the context of extraintestinal infections.

Goals: To investigate whether the presence of pAA plasmids could enhance extraintestinal virulence.

Materials and methods: The pAA plasmid from three EAEC strains, isolated from symptomatic urinary tract infections (HSP60, LSC52, and UPEC100), was transferred to non-pathogenic *E. coli* strain MG1655 through conjugation. The isogenic strains with and without pAA plasmids were quantitatively evaluated and compared for their capacity to interact with and invade eukaryotic cells from different sources. The tests were conducted on four extracellular-derived cell lineages: A549 (lung), T24 (urinary bladder), EA.hy926 (endothelium), and HK-2 (kidney), along with one intestinal epithelial cell line, Caco-2 cells.

Results: Our preliminary findings indicate that the acquisition of different pAA variants leads to distinct phenotypic outcomes. In some cases, the presence of specific pAA variants increases the capacity of *E. coli* MG1655 to adhere to and invade cells derived from extraintestinal sources but not from intestinal sources.

Conclusions: These results suggest that pAA plasmids may play a broader role in *E. coli* pathogenicity beyond their established roles in diarrheal disease. They could contribute to the emergence of hybrid strains capable of causing diverse infections.

Further characterization of the genetic content of the pAA plasmids will be critical to understanding their role in shaping the *E. coli* virulence. Additionally, it may provide new insights into the virulence and emergence of hybrid pathotypes that can potentially cause both intestinal and extraintestinal infections.

PS02.098

Infections with extensively antibiotic resistant *Shigella sonnei* and identification based on whole genome sequencing in Germany

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Introduction: Infections with *Shigella sonnei* can be fulminant and highly infectious, but most infections cause mild diarrhea. *S. sonnei* can be transmitted fecal-orally and sexually. Non-travel associated infections are mainly reported in men, with probable sexual transmission in men who have sex with men (MSM). Due to an increase in multi-drug-resistant (MDR) or extensively drug-resistant (XDR) infections, therapeutic options for more severe infections become limited.

After the United Kingdom (UK) reported on an unusual number of XDR- *S. sonnei* cases in February 2022, we analyzed isolates combined with notification data to determine a possible occurrence of such cases in Germany.

Methods: *S. sonnei* isolates sent to the National Reference Laboratory since 2021 were investigated using whole genome sequencing and an Enterobase *Escherichia/Shigella* cgMLST v1-scheme adapted to characterize *Shigella* strains.

Results: We analyzed 311 isolates (2021: 14; 2022: 45; 2023: 141 2024: 111). On basis of their cgMLST type and resistance associated gene profile we could assign 38/311 (2021: 4/14; 2022: 7/45; 2023: 13/141; 2024: 14/111) isolates to the same genetic cluster as the reference strain from the UK (internal classification SHO_2018_NGS_1). These strains were positive for CTX-M-27 plasmid. Since 2022 we observed a cluster of such XDR-Shigella isolates (internal classification SHO _2022_NGS _4). These 17 strains carried a CTX-M-15 plasmid (2022: 5; 2023: 12). A new clonal lineage with 83 isolates of XDR- resistant isolates (also characterized by a CTX-M-15 plasmid) was detected in 2023 in Germany and also identified in Netherlands and UK (internal classification SHO_2023_NGS_1). Most of these isolates were collected from men, data on sexual orientation of the patients was not available.

Conclusion: Cases of *S. sonnei* showing MDR-/XDR-resistance have been present for more than four years in Europe. The currently reported type of *S. sonnei* containing the plasmid CTX-M-27 and CTX-M-15 was identified in multinational outbreaks for the first time. Immunocompromised patients can experience a more severe clinical course of infection and outbreaks in MSM are sometimes prolonged. Therefore, physicians treating MSM presenting with gastrointestinal symptoms should be sensitized for *S. sonnei* and consider this pathogen as differential diagnosis. In case of such an infection appropriate prevention measures should be recommended to stop further transmission. MSM reporting sexual risk behavior should be sensitized with regard to a possible fecal-oral transmission of *Shigella* and encouraged to report a possible sexual transmission to their physicians in case of gastro-intestinal discomfort with sudden onset. Because resistance to antibiotics is becoming more important, antibiosis should be based on susceptibility testing.

PS02.100

A scheme to translate multilocus sequence typing sequence types derived from whole genome sequence data to PCR ribotypes in *Clostridioides difficile*

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Question: *Clostridioides difficile* is the most common bacterial pathogens isolated from nosocomial diarrhea. To study the epidemiology of *C. difficile*, PCR ribotyping (PCR RT) with differentiation into ribotypes (RT) is the current gold standard method for typing in Europe. With the increasing use of whole genome sequencing (WGS) in clinical diagnostic routine, the extraction of multilocus sequence types (MLST STs) gained importance as an alternative approach to group isolates and replaced in 2024 PCR RT as gold standard method for typing at the National Reference Centre (NRC) for *C. difficile*. The aim of this study was to directly assess STs and RTs forward and backward comparability.

Methods: Since 2017, the NRC for *C. difficile* maintains a database with WGS information about *C. difficile* isolates from clinical origin, prevalence screening and reference strains. From this database, we included WGS data of all isolates for which information on PCR RTs was available until 2024 and extracted the STs. Moreover, we included all available *C. difficile* WGS from the EnteroBase database (as per 23.10.2024) with information on RTs and added information from literature. All pairs of STs and associated RTs (ST/RT-pair) were summarized in a single dataset; only ST/RT- pairs with n≥ 3 were included. If one MLST ST was associated with more than one RT, we created "RT-groups" with all associated RTs if n≥ 3. Using 48 newly collected isolates, we validated the scheme by performing PCR ribotyping and WGS followed by MLST and determined the accuracy of the prediction of RT or RT-groups.

Results: In total, we included ST/RT-pairs of 5,293 *C. difficile* isolates (NRC-database: 1,234; EnteroBase-database: 3,575; literature 484) and consolidated them to 405 different ST/RT-pairs. Of these, 292 consisted of fewer than 3 isolates and were therefore excluded from the final scheme. The final scheme comprised 113 ST/RT-pairs (based on 4,943 isolates). Most frequent pairs were ST1/RT027 (n=943; 19%), ST11/RT078 (n=659; 13%), ST3/RT001 (n=339; 7%) and ST37/RT017 (n=300; 6%). Some associations, e. g. ST27/RT017, were even unambiguous. However, the majority of STs were paired with more than one RT, which led to RT-groups (e. g. ST1 and RT-group RT027/RT176/RT955). Vice versa, some RTs paired with more than one ST. The subsequent validation resulted in a correct RT/RT-group designation in 44 of 48 *C. difficile* isolates (accuracy: 92%). Of the remaining four isolates, for one (ST590/RT023) there was no RT/RT-group defined and in three isolates, PCR RT did not result in a known RT.

Conclusions: We present a translational scheme to derive RTs from STs based on WGS data for typing of *C. difficile* that is accurate for the most common STs. The availability of a translation tool between STs and RTs will facilitate the change from RT to ST nomenclature. To further reduce the gaps in our dataset due to new or rare STs and RTs, in the future more data have to be integrated.

PS02.102
The biological role of lytic polysaccharide monooxygenases in *Vibrio cholerae* pathogenicity
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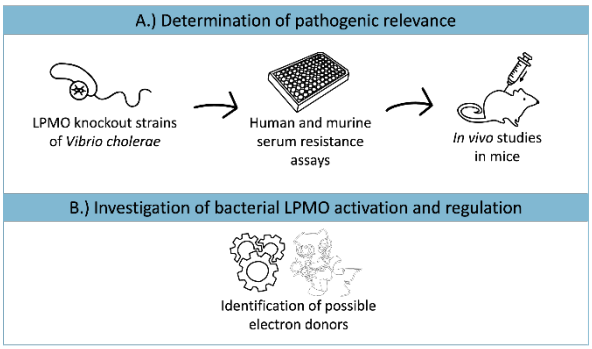
Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent redox enzymes primarily known for their role in the degradation of crystalline polysaccharides such as starch, chitin or cellulose [1]. In the past years, LPMOs have gained significant interest in biorefinery research and have become essential components of commercial enzyme mixtures employed for industrial lignocellulose processing [2]. Furthermore, LPMOs found in bacteria, fungi, insects and ferns have been shown to fulfil a variety of biological functions that go well beyond biomass degradation [3]. Notably, recent research has linked LPMO activity to bacterial pathogenicity. For example, in *Pseudomonas aeruginosa,* the LPMO CbpD has been shown to play a crucial role in the bacterial survival in blood during the infection [4]. Likewise, LPMOs from *Aliivibrio salmonicida* were shown to be relevant for the bacterium"s pathogenicity to Atlantic salmon [5]. These and similar findings raise the question whether in other bacteria, such as *Vibrio cholerae (V. cholerae),* LPMOs might also be involved in pathogenic processes.

Within this ongoing project, *V. cholerae* LPMO knockout strains are generated for subsequent testing in serum resistance assays and *in vivo* assays to determine the pathogenic relevance of the 2 *lpmo* genes found in *V. cholerae* (Figure 1A). Eventually, this project aims to clarify whether LPMOs in pathogenic bacteria facilitate infectious processes or if their primary role is to support survival outside the host.

In addition to that, the project aims to investigate how LPMO activation is enabled in bacteria. Since LPMO activity strictly relies on the supply of external reductants and H2O2, a variety of proteinogenic and other redox-active compounds are being investigated as possible sources of electrons (Figure 1B). For that purpose, a panel of predicted heme c binding proteins of *V. cholerae* was selected from literature for preliminary *in silico* analysis, yielding promising results for several candidates. Further investigations employing these candidates will be conducted to shed light on how bacteria facilitate activation of LPMOs.

[1] Y. Gaber et al., *Biotechnol. Adv,* **2020**, *43*, 107583. **[2]** P. Chylenski et al., *ACS Catal,* **2019**, *9*, 4970-4991. **[3]** T.M. Vandhana et al., *New Phytol,* **2022**, *233*, 2380-2396. **[4]** F. Askarian et al., *Nat commun,* **2021**, *12*, 1230. **[5]** A. Skåne et al., *BMC Microbiol,* **2022**, *22*, 194.

Fig. 1



PS02.106
Porin-A and α/β-hydrolase are necessary and sufficient for hemolysis induced by *Bartonella bacilliformis*
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Introduction: *Bartonella bacilliformis* is the etiological agent of Carrion"s disease, a vector-borne illness endemic to the South American Andes. In the acute phase, the bacteria infect erythrocytes, causing severe hemolytic anemia ("Oroya fever") with a case-fatality rate of up to 88%.

Objectives: This study aims to provide a detailed understanding of the hemolysis process, which is essential for the development of targeted anti-virulence therapies.

Materials & Methods: To identify genetic determinants of hemolysis, a transposon mutant library was screened for non-hemolytic mutants, revealing two key virulence factors: a porin (porin-A) and an α/β-fold hydrolase. *In silico* analyses were conducted to predict conserved functions and three-dimensional structures. Markerless deletion and complementation mutants were tested *in vitro* using a human erythrocyte hemolysis assay. To evaluate the α/β-hydrolase as a therapeutic target, site-directed mutagenesis of its catalytic triad was performed, and a phospholipase inhibitor library was screened for hemolysis inhibition.

Results: A genetic screen identified porin-A and an α/β-fold hydrolase as essential factors for *B. bacilliformis* hemolysis. *In silico* modeling predicted conserved functions, and functional assays with deletion and complementation mutants confirmed their role in erythrocyte lysis. Targeted mutagenesis of the hydrolase catalytic triad (Ser-His-Asp) highlighted its functional importance for hemolysis. Compound screening identified compound 48/80 as an inhibitor of hemolytic activity at micromolar concentrations.

Conclusion: Porin-A and the α/β-fold hydrolase are both necessary and sufficient for the hemolytic activity of *B. bacilliformis*. The identification of a small-molecule inhibitor highlights the feasibility of an anti-virulence approach targeting hemolysis as a central pathogenic mechanism in Carrion"s disease.

PS02.108
Carbapenem-resistant *Salmonella* Typhi infection in a traveler returning from India, Germany 2024

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Introduction: Expanding antibiotic resistance in *Salmonella* (*S.*) Typhi, in particular extensively drug-resistant (XDR) strains, pose a significant challenge to typhoid fever treatment, limiting therapeutic options mainly to macrolides and carbapenems. The XDR *S.* Typhi lineage first described in Pakistan is meanwhile reported in many countries. Furthermore, IncX3 plasmids encoding *blaNDM-5* carbapenemase have been recently detected in endemic regions in XDR *S.* Typhi from Pakistan and in a non-XDR strain from India. *blaNDM-5* positive strains were also described in non-typhoidal *Salmonella* and other enterobacterial genera in nosocomial settings, food, and animals, thus emphasizing the potential for interspecies and intersectoral transmission.

Objectives: We analyzed a carbapenemase producing *Salmonella* Typhi strain of a patient returning from India. To place the strain characteristics in a broader context, we explored its phylogenetic environment and compared its resistance determinants with published *S.* Typhi strains from Pakistan and India.

Materials & Methods: Serotype *S.* Typhi was confirmed by slide agglutination according to the White-Kauffmann-LeMinor scheme. Antimicrobial susceptibility testing was performed using broth microdilution in accordance with EUCAST guidelines. Core genome MLST, ResFinder and SNP analysis were conducted from whole genome data in comparison to other *S.* Typhi strains from Pakistan and India.

Results: A *S.* Typhi strain isolated from a patient in Germany with travel history to India by means of phenotypic antimicrobial susceptibility testing revealed resistance to fluoroquinolones, tetracyclines, beta-lactam antibiotics including penicillins, third generation cephalosporins, and carbapenems. The strain was susceptible to chloramphenicol, co-trimoxazole, and azithromycin, therefore the strain does not belong to the *S.* Typhi MDR or XDR class. Resistance determinants *blaNDM-5*, *blaCTX-M-15*, and *qnrS1* are localized on two plasmids of IncFIB and IncX3 replicon types, respectively. Phylogenetic analyses placed the strain in close vicinity to a recently published NDM-5 positive *S.* Typhi strain from India and clearly distinguishes it from XDR strains of the Pakistan XDR lineage.

Summary: Emergence and spread of XDR, ESBL and carbapenemase producing *S.* Typhi strains due to plasmid-mediated transfer of resistance determinants across bacterial species underlines the need for antimicrobial susceptibility testing of *S.* Typhi isolates. This enables appropriate patient treatment, while avoiding last line antibiotics where they are not needed. It also highlights the importance of prevention measures, including typhoid (re)vaccination for travelers and individuals visiting endemic regions. Our study further shows that *blaNDM-5* positive *S.* Typhi strains can be transmitted globally.

PS02.110
Agama lizard feces – a source of antibiotic-resistant ESKAPE pathogens?

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Introduction: Animals are an integral part of the one health continuum. If they carry antibiotic-resistant pathogenic bacteria in their intestinal system, these bacteria might be released into the environment via animal fecal matter. Consequently, these animal droppings might be a source of such antibiotic-resistant bacteria and the corresponding resistance genes. As data on the role of lizard feces as a source for antibiotic-resistant bacteria is limited for South Africa, we analyzed agama lizard droppings collected in Pietermaritzburg (KwaZulu-Natal, South Africa) for the presence of antibiotic-resistant *Pseudomonadota*.

Methods: Agama lizard droppings were collected and decimally diluted in sterile saline, and samples were spread-plated directly onto solid media containing carbenicillin or, after pre-enrichment, onto solid media containing cefalexin to obtain candidate colonies. Randomly selected candidate colonies were purified and characterized phenotypically (microscopy, biochemical testing) and by whole genome sequence analysis. Reads generated by Illumina sequencing were processed, trimmed, and *de novo* assembled using the BV-BRC genome assembly pipeline. The established draft genomes were further analyzed using available bioinformatics software tools (e.g., BUSCO, MLST, Resfinder). In addition, the antibiotic resistance profiles of the selected isolates were established by employing the EUCAST disk diffusion procedure.

Results: Three selected antibiotic-resistant isolates representing the genera *Pseudomonas* (one isolate) and *Enterobacter* (two isolates) were identified based on phylogenomic analyses as *P. aeruginosa* A191, *E. hormaechei* subsp. *steigerwaltii* AKK1, and *E. hormaechei* subsp. *steigerwaltii* AKK2, confirming that the agama lizard feces contained members of the so-called ESKAPE pathogens. Strain A191 carried antibiotic resistance genes representing class C and class D β-lactamases, and growth was not inhibited by antibiotics such as amoxicillin-clavulanic acid and cefotaxime. The two *Enterobacter* strains, AKK1 and AKK2, exhibited resistance against various β-lactam antibiotics (e.g., ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime) and showed AmpC-type β-lactamase activity, with a matching resistance gene (*bla*ACT15) detected.

Conclusion: Our results demonstrate that agama lizard feces can contain antibiotic-resistant pathogenic bacteria representing the so-called ESKAPE group. Therefore, defecation by lizards can contribute to the presence of such antibiotic-resistant bacteria and matching resistance genes in the environment.

PS02.112
Development of monoclonal antibodies targeting pneumococcal surface proteins for functional applications

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Background: *Streptococcus pneumoniae* is a major human pathogen responsible for diseases such as pneumonia, meningitis, and sepsis. Pneumococci express a broad array of surface-associated proteins involved in colonization, immune evasion, and pathogenesis. These proteins represent attractive targets for analytical, diagnostic, and potentially therapeutic applications. To enable targeted detection and characterization of these factors, we aimed to generate a comprehensive panel of monoclonal antibodies (mAbs) against selected pneumococcal surface proteins.

Objectives: The objective of this study was to generate and validate antigen-specific monoclonal antibodies targeting a diverse set of surface-associated pneumococcal proteins for use in downstream analytical and functional studies.

Methods: A comprehensive panel of 73 recombinant pneumococcal proteins – predominantly surface-localized – was selected for mouse immunization. Hybridomas were generated via classical hybridoma technology. A tiered screening approach based on indirect ELISA was applied to select hybridoma clones according to antigen specificity and likely epitope accessibility: (I) Initial screening on intact *S. pneumoniae* cells to enrich for surface-specific binders, (II) Screening with pneumococcal lysates to capture additional antigens, including cytoplasmic and partially exposed proteins, (III) Validation with purified recombinant proteins to assess specificity and cross-reactivity. Hybridoma clones of interest were subcloned, and monoclonal antibody production was established. Initial validation included ELISA, flow cytometry, and fluorescence microscopy to assess specificity and target binding.

Results: To date, 38 monoclonal hybridoma lines producing antibodies against 17 pneumococcal proteins have been established. These include six lipoproteins, two choline-binding proteins, three sortase-anchored proteins, two cytoplasmic proteins, one non-classical surface protein, and three proteins with alternative surface association mechanisms. Monoclonal antibodies for four targets could be produced at preparative scale and validated in multiple detection formats. Binding profiles confirmed antigen specificity and indicate suitability for various applications in pneumococcal research, including localization, detection, and protein-interaction studies. Cross-reactivity with unrelated antigens was not observed in the initial screening assays, though further validation is ongoing.

Conclusions: The generated monoclonal antibodies expand our toolkit for pneumococcal research and offer new opportunities for studying the role of individual surface proteins in bacterial biology and host interaction. Ongoing efforts aim to broaden and further functionally characterize the existing panel of monoclonal antibodies. These antibodies may also support the development of targeted detection methods and provide a basis for exploring therapeutic strategies.

PS02.114
TLR7 senses microbial viability of *Orientia tsutsugamushi* in plasmacytoid and myeloid dendritic cells

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The intracellular bacterium *Orientia tsutsugamushi* (OT) causes scrub typhus, a potentially life-threatening zoonosis with growing global relevance. Severe scrub typhus is characterized by elevated cytokines and activation of dendritic cell subsets, yet the innate immune sensors responsible for initiating these responses remain unknown. We investigated how murine myeloid and plasmacytoid dendritic cells detect OT and discriminate between viable and non-viable bacteria.

Primary BMDCs and pDCs were differentiated from murine bone marrow using GM-CSF and Flt3-ligand, respectively. Cells were infected with live OT, stimulated with 70°C heat-inactivated OT (hkOT), or transfected with RNA from live OT (OT-RNA). After 24 hours, TNF, IL-12p40, and IFN-α were measured in supernatants via ELISA.

BMDCs secreted TNF and IL-12p40 in response to OT, while pDCs mounted a massive IFN-α response. Titration with varying MOIs (40–0.04) showed that pDCs maintained

maximal IFN-α production down to MOI 1, highlighting their high sensitivity to OT.

To assess whether immune recognition depends on bacterial viability, we compared responses to live versus hk OT. BMDCs showed comparable TNF secretion in response to both forms. Contrarily, pDCs showed a ~10-fold reduction in IFN-α secretion in response to hkOT compared to live OT, suggesting that pDCs function as sensors of microbial viability.

As OT lacks classical pathogen-associated molecular patterns (e.g. LPS, flagellin, and conventional peptidoglycan), we hypothesized that immune recognition could rely on detection of nucleic acids, particularly of RNA. Given the robust type I interferon response in pDCs and its sensitivity to bacterial heat inactivation, we investigated the role of the endosomal RNA-sensing receptor TLR7. pDCs were differentiated from wild-type (WT) and Tlr7−/− mice and stimulated with live OT and hkOT. The IFN-α response to live OT but not to hkOT was significantly reduced in Tlr7−/− pDCs, confirming that TLR7 plays a critical role in recognition of viable OT. Additionally, transfection of pDC with OT-RNA extracted from viable bacteria triggered a TLR7-dependent IFN-α induction and confirmed bacterial RNA as the TLR7 ligand.

To assess TLR7's role in BMDCs, we performed experiments in WT and Tlr7−/− BMDCs. While TNF and IL-12p40 responses to live OT were reduced in Tlr7−/− BMDCs, responses to hkOT and OT-RNA were unaffected, indicating TLR7-independent recognition to non-viable bacteria.

Our findings uncover TLR7 as the primary sensor enabling pDCs to detect viable OT, highlighting a cell type–specific strategy for microbial viability sensing. This reveals a novel innate recognition mechanism for an obligate intracellular bacterium devoid of classical PAMPs.

PS02.116

Jep, a novel serine protease common to murine *Staphylococcus aureus* isolates, modulates *S. aureus* virulence by cleaving staphylococcal virulence factors

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Introduction: *Staphylococcus aureus* can colonize an array of different hosts, including humans and livestock. Adaptation to these different hosts is mediated, for instance, by host-specific virulence factors such as toxins, and extracellular enzymes, including proteases. Both the inactivation of existing proteases as well as the acquisition of novel proteases have been described as host adaptation mechanisms. In principle, extracellular proteases could contribute to *S. aureus* host adaptation by inactivating *S. aureus* virulence factors or host-derived proteins.

Our group has reported, that *S. aureus* is a frequent colonizer of wild and laboratory mice, with the CC88 and CC49 lineages being the most common in laboratory mice and wild mice, respectively. The CC88 prototype strain JSNZ is now commonly used in murine persistent colonization models. Interestingly, isolates from both lineages encode a novel phage-encoded secreted serine protease, named Jep (JSNZ extracellular protease). This protease is common in murine *S. aureus* isolates, but rarely found in human isolates. It is closely related to the *S. aureus* Spl proteases (45-48 % sequence identity) and is produced at very high levels *in vitro*.

Aim: We aimed to investigate the role of the serine protease Jep in murine *S. aureus* infection and in particular its function as a possible *S. aureus* virulence regulator.

Methods: To investigate the role of Jep in *S. aureus* infection, the virulence of the JSNZ wild type strain, a JSNZ∆*jep* deletion strain and a JSNZ ∆*jep:jep* complementation strain were compared in a murine bacteraemia model. N-terminomics analysis was performed for substrate identification and cleavage motif discovery. Substrate cleavage was additionally analysed by SDS-PAGE followed by silver staining. To investigate the activity of the Jep-processed coagulases, a coagulation assay with murine plasma was performed.

Results: The JSNZ∆*jep* deletion strain showed enhanced virulence in the murine bacteraemia model compared to the wild-type strain, reflected in an increased lethality and higher bacterial loads in spleen, lung and blood. N-terminomics showed several *S. aureus* virulence factors (e.g. lipases, coagulases, IsdB) as substrate candidates of the Jep protease. Indeed, silver staining showed that two lipases (Lip, Geh), the pro-coagulatory factor Von-Willebrand-factor binding protein (vWbp) and the iron acquisition protein IsdB were directly cleaved by the Jep protease. Further analysis identified several cleavage sites within vWbp and Lip, which are mostly related to arginine or lysine. The coagulation assay with murine plasma showed reduced coagulation after incubation of the vWbp with the Jep protease, but no effect when incubated with an enzymatically inactive Jep mutant.

Summary: The secreted serine protease Jep acts as a virulence regulator by cleaving several secreted *S. aureus* virulence factors and thereby balancing the virulence of the mouse-adapted *S. aureus* strain JSNZ.

PS02.118

Improved molecular surveillance and assessment of host adaptation and virulence of *Coxiella burnetii* in Europe

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Coxiella burnetii, a small gram negative and obligate intracellular bacterium, is the causative agent of the zoonotic disease Q fever. The bacteria display a broad host range and clinical manifestations of Q fever in ruminants are diverse and mainly associated with infertility in cattle, abortion in goats and asymptomatic infections in sheep. Despite these differences, human Q fever outbreaks mostly originate from *C. burnetii* shedding by sheep and goats. The extent to which virulence varies between isolates and if it is the same across host species remains to be clarified. We hypothesize that the zoonotic potential and clinical relevance of *C. burnetii* isolates can be deduced from yet undisclosed differences in host-specificity and genomic traits. First, we aimed at increasing the availability of isolates currently circulating in Europe. To

this end, an improved isolation protocol using enzymatic digestion, homogenization and sequential filtration was established. Treatment with ampicillin, vancomycin, nystatin and amphotericin B as post isolation option was found to be applicable to limit contaminations. For phenotypic characterization, an *ex vivo* whole blood model was established. Whole blood from cattle, sheep, goats and humans was inoculated with *C. burnetii* isolates with a multiplicity of infection (MOI) 100 from different hosts and disease manifestations. Lipopolysaccharide (LPS, 5 µg/ml) from *Escherichia coli* (*E. coli*) O111:B4 and Concanavalin A (ConA, 100 µg/ml) from *Canavalia ensiformis* were used as stimulation controls. Transcription of marker genes was analyzed by qPCR ($\Delta\Delta$Cq). Interleukin (IL) 6 and IL-10, inducible nitric oxide synthase 2 (iNOS2), tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) served as initial markers to assess the immune response. Relative gene expression was calculated by comparing mock inoculated blood with stimulated or bacterial inoculated blood. Preliminary data suggest that the whole blood model provides reliable results and is suitable for virulence testing of isolates. Testing with field isolates is ongoing.

PS02.120

Targeting bacterial adhesion with Sybodies: insights from a *Bartonella* adhesin A domain

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Introduction: Bacterial infections pose a significant threat to global health. A critical initial step in the pathogenesis of these infections involves bacterial adhesion to host cells. Trimeric autotransporter adhesins (TAAs) are key virulence factors in many Gram-negative bacteria. Among these, *Bartonella* adhesin A (BadA), a prototypic TAA, mediates adhesion of *Bartonella henselae* to endothelial cells (ECs) by interacting with extracellular matrix (ECM) components, particularly fibronectin (Fn). This study aimed to develop novel anti-adhesion agents to inhibit TAA interactions with Fn and ECs.

Material and methods: A key Fn-binding domain of BadA, designated D27, was expressed and purified. Single-domain antibodies (sybodies) targeting D27 were selected using a combined ribosome and phage display platform. The affinity and specificity of the selected sybodies to D27 were assessed via ELISA assays. Sybodies were further evaluated for their ability to inhibit bacterial adhesion in assays using whole-cell bacteria. As a proof of concept, D27-coated magnetic beads were employed to simulate bacterial binding in inhibition assays with ECs.

Results: From a library of $4x10^{12}$ candidate sybodies, eight were shown to interact with D27. Functional assays confirmed the binding capability of these sybodies to the BadA domain. Four sybodies demonstrated interaction with BadA in whole-cell bacterial assays, and two showed a slight reduction in binding between whole-cell bacteria and Fn. Inhibition assays using D27-functionalised beads provided preliminary data about the use of sybodies to impede bacterial adherence to ECs.

Conclusion: This study demonstrated that two sybodies targeting a specific domain of BadA reduce TAA-mediated binding to Fn and ECs. These findings highlight the potential of sybodies as anti-adhesion agents, supporting their future development as therapeutic candidates against TAA-expressing pathogens.

PS02.122

Establishment of a blood-brain barrier infection model for Lyme neuroborreliosis

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Introduction: The blood–brain barrier (BBB) is a selective and semipermeable interface that separates the central nervous system (CNS) from intruding pathogens. Lyme neuroborreliosis is caused by neuroinvasive spirochetes of the *Borrelia burgdorferi* sensu lato complex. While some *Borrelia* species exhibit pronounced neurotropism, the specific factors that determine neuroinvasive capacity remain poorly understood. This study aimed to establish an *in vitro* BBB model to elucidate the pathomechanisms by which *Borrelia* spp. interact with and cross the BBB.

Material and methods: An *in vitro* BBB model was established using human brain microvascular endothelial cells (HBMECs) cultured in a transwell system. A collection of *Borrelia* isolates from both humans and ticks was used to assess bacterial adhesion and endothelial translocation. Adhesion to HBMECs was quantified by immunofluorescence microscopy and quantitative PCR (qPCR). Spirochetal transmigration across the BBB model was quantified via qPCR, while barrier integrity post-infection was assessed by measuring paracellular permeability using fluorescent dextrans.

Results: The integrity of the BBB model was confirmed by tight junction localisation of ZO-1 protein and by paracellular permeability assays using dextran tracers. Infection assays revealed strain-specific differences in adhesion to HBMECs, with clinical isolates displaying significantly higher adhesion than tick-derived strains. Preliminary data suggest differential capacities among *Borrelia* strains to cross the BBB.

Conclusion: Our findings demonstrate that *Borrelia* adhesion to brain endothelium is strain-specific and may contribute to neuroinvasive potential. The established BBB model provides a reliable platform to investigate the molecular interactions between neuroinvasive *Borrelia* spp. and the CNS barrier.

PS02.124

Extracellular vesicles of immune cells affect the human pathogenic fungus *Aspergillus fumigatus*

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Introduction: The ubiquitously distributed saprophytic and opportunistically pathogenic fungus *Aspergillus fumigatus* can cause a variety of diseases in immunosuppressed patients, including life-threatening invasive aspergillosis. Asexual, airborne spores called conidia are inhaled daily by humans upon which they can reach the alveoli of the lungs due to their small size. In the lungs they come in contact with lung epithelial and immune cells. All of these cells can produce

extracellular vesicles (EVs), membrane-surrounded nanoparticles which contain proteins, lipids and nucleic acids. EVs have been shown to play a role in different processes such as cell-cell communication, immune response, cellular homeostasis and interspecies communication. It was previously shown that polymorphonuclear leucocytes-derived EVs have an antifungal activity against *A. fumigatus*.

Objective: Our aim is to characterise immune cell-derived EVs and investigate their interaction with *A. fumigatus* as well as their potential antifungal capacity.

Material/Methods: To analyse the potential antifungal capacity of immune cell-derived EVs, we isolated EVs of macrophages and neutrophils and treated *A. fumigatus* conidia with different concentrations of EVs. For visualisation by confocal laser scanning microscopy, EVs were stained with different lipophilic dyes and as their target a fluorescent *A. fumigatus* strain was used. For characterisation of the EVs nano particle tracking analysis (NTA) and western blot analysis of specific EV marker were carried out.

Results: Different extracellular vesicle markers like CD63 and annexin A1 were detected via western blot. Whereas as negative control lamin A and calnexin were not detectable in the EVs isolated from immune cells. NTA measurements showed that the size of the immune cell- derived EVs was depending on the parental cells as well as the isolation method. The size thus varied between 80 nm and 500 nm. When *A. fumigatus* was treated with immune cell-derived EVs hyphal growth was inhibited in a concentration dependent manner.

Conclusion: We characterized the EVs of different immune cells and could show their characteristic markers and size. We showed that immune cell derived EVs interact with *A. fumigatus* and decreased the growth of the fungal hyphae in a concentration dependent manner.

PS02.126

Food borne-outbreak analysis – direct identification of cluster strains in complex food matrices

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Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne-pathogens which cause mild to severe diarrheagenic infections in humans including the haemorrhagic uremic syndrome (HUS). Isolation of STEC from contaminated food matrices require extensive and time-consuming lab methods including several enrichment steps and PCR analyses of mixed cultures and pools of colonies. In case of outbreak situations, it is necessary to identify a contamination source or a vehicle for infections as fast as possible to prevent spread of those strains. Therefore, we investigated the application of outbreak cluster-specific PCR methods for complex food matrices to shorten lab hands on time and to identify the respective contamination source as fast as possible.

In our study, we contaminated mozzarella, raw milk cheese and mixed minced meat with two *E. coli* strains of which one strain was assigned as a human outbreak strain (OS) and the other as close related non-outbreak strain (NOS). We used two different spiking concentrations (104 and 10^2 cfu per 25g sample) of the OS, the NOS as well as a combination of both strains and a negative sample. Samples were taken for investigation of outbreak-cluster specific PCR methods at three different time points during microbial enrichments (t0, t24, t48). Outbreak-cluster specific primer and probes were designed based on whole genome sequences of the strains to detect single nucleotide polymorphisms or the identification of unique core sequences of the outbreak-cluster and were applied in conventional PCR, Taq-Man real-time PCR and Melt analysis of mismatch amplification mutation assays (Melt-MAMA).

In mozzarella cheese samples the applied PCR methods revealed a distinct differentiation of OS and NOS strains also in the mixed samples after two enrichment steps (t48). There was no cross-reaction observed with possible microbial background of the mozzarella cheese. For raw milk cheese a distinct identification of the OS and NOS strains was possible after two enrichment steps (t48) and the applied methods also identified other *E. coli* strains present in the raw milk cheese. In mixed minced meat the OS strain was already identified after the first enrichment step (t24). Besides detection of the NOS and other background *E. coli* we also observed some unspecific binding in the conventional PCR. Furthermore, the evaluation of the melt-MAMA assays using SYBR-green melting curves was mostly inconclusive in case of mixed contaminations for raw milk cheese and mixed minced meat samples.

In summary, we successfully applied outbreak cluster-specific PCR methods on complex food matrices. This is a promising tool which needs further evaluation as the results seems to be food matrix depended.

PS02.128

Immune response of the Monocytic Cell Line THP-1 against wild-type *Staphylococcus aureus* and small colony variants to monitor host-pathogen interaction

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Small colony variants (SCVs) of *Staphylococcus aureus* represent a distinct subpopulation of bacteria characterized by their slow growth and auxotrophic nature, typically reaching only 10% of the colony size compared to wildtype isolates. These variants arise from genetic mutations that affect essential nutrient biosynthesis pathways, particularly those involving thiamine, menadione, hemin, or thymidine. Due to these metabolic adaptations, SCVs exhibit altered interactions with innate immune cells, which are modelled using the THP-1 cell line.

In this study, clinical wild-type (WT) and SCV strains underwent whole-genome sequencing and bioinformatics analyses, revealing mutations in the menaquinone biosynthesis pathway (*menB*), shikimate pathway (*aroA*), and the TCA cycle (*citZ*). These genetic changes impact SCV growth kinetics, with some exhibiting prolonged lag phases or biphasic growth in Mueller Hinton Broth 2 (MH2) media. SCVs demonstrated an enhanced ability to form aggregates as well as biofilms, assessed via crystal violet staining, potentially contributing to their persistence and resistance to host defences. The immune response of THP-1 cells to WT and SCV strains was examined using an in vitro infection model, and RT-qPCR quantification of apoptosis- and pyroptosis-

related genes (e.g., *TLR4, TNF-α, CCL2, CCL20, JUN, RELA, BAX, TP53, CASP3, NLRP3,* and *IL-1β*) indicated differential immune activation between strain types. This study integrates phenotypic, genetic, and functional analyses to elucidate the unique immunological interactions of SCVs, providing valuable insights into their immune evasion strategies and persistence in host environments.

PS02.130
Comparison of the interaction of *S. schweitzeri* and *S. aureus* with human neutrophils
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The One Health approach emphasizes the interconnectedness of human, animal, and environmental health, highlighting the need to monitor zoonotic risks. *Staphylococcus schweitzeri* is mainly found in bats and non-human primates in Africa. However, only three cases of colonization in humans have been reported, and no human infections have been detected. *S. schweitzeri* possesses virulence factors similar to those of *Staphylococcus aureus*, including enterotoxins and haemolysins. We demonstrated that *S. schweitzeri* interacts with epithelial cells from monkeys and humans similarly to *S. aureus*.[1] However, whole genome sequencing shows that *S. schweitzeri* lacks immune evasion factors such as staphylococcal chemotaxis inhibitory protein and complement inhibitor, which protect *S. aureus* from the human immune system.[1] It is conceivable that the absence of these factors prevents *S. schweitzeri* from evading neutrophil killing and overcoming human immunity. Therefore, in this preliminary study, we investigated differences in the interaction of *S. schweitzeri* and African *S. aureus* strains with human neutrophils.

We studied the interaction of seven *S. schweitzeri* strains and four African *S. aureus* isolates with the human neutrophil cell line HL-60. Before infection, bacteria were opsonised with 10% human serum. After 30 and 60 minutes of incubation, extracellular bacteria were removed with lysostaphin, and neutrophils lysed. Intracellular bacteria were quantified after serial plating. Neutrophil number and cell death (uptake of propidium iodide (PI)) were assessed by flow cytometry. Bacterial survival in the presence of HL-60 cells was determined two hours post infection after lysing the neutrophils.

No difference in phagocytosis between *S. schweitzeri* and *S. aureus* was observed at 30 minutes. However, at 60 minutes, significantly more *S. aureus* per cell were detected. Cell death of HL-60 cells was induced by bacterial uptake. At both time points, *S. schweitzeri* caused significantly higher HL-60 death than *S. aureus*, but overall cell death remained low, with a maximum mean of 17% PI-positive cells (60 minutes, *S. schweitzeri*). After two hours of neutrophil infection with the bacteria, fewer *S. schweitzeri* than *S. aureus* were recovered, though the difference was not significant. For both species, the number of colony forming units increased compared to baseline across most isolates.

The neutrophil response was minimal in some experiments, possibly due to the cell line used. However, in this preliminary study, we provide the first evidence of significant differences in *S. schweitzeri* interaction compared to *S. aureus* with human cells. Further work will use primary neutrophils and more isolates. Understanding *S. schweitzeri*"s pathogenic potential is crucial to assess whether it could one day—by e.g. acquiring immune evasion factors via phages—emerge as a new zoonotic pathogen.

1 Grossmann et al, 2021, Sci Rep, 11(1):1157.

PS02.132
Function of the serum opacity factor of *Streptococcus canis* and of the pneumococcal pneumolysin in cell culture infection analyses under defined microfluidic
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Introduction: The zoonotic pathogen *Streptococcus canis* is known to mediate local and systemic infections in dogs, cats, and also in humans. Similar to *Streptococcus pneumoniae* (the pneumococcus), *S. canis* causes severe septicemia, which leads to multifocal vascular injury and is also able to colonize pre-damaged heart valves thereby inducing infective endocardites. Since systemic blood stream infections are affected by flow parameters of the blood flow, cell culture infection analyses under defined microfluidic is perfectly suited to assess the function of pneumococcal and streptococcal attachment to endothelial cells. In this respect, the impact of the pneumococcal cytotoxin pneumolysin (Ply) and of the streptococcal serum opacity factor (SOF) of *S. canis* in bacterial attachment to primary human endothelial cells was functionally analyses using different cell culture infection models and a human *ex vivo* heart valve explant model.

Methods: A transposon-based genome library of a clinical *S. canis* isolate was applied in cell attachment analyses under defined laminar flow of 10 dyn/cm2 to identify novel *S. canis* adhesins. After cultivation and infection of primary human endothelial cells (HUVEC), bacterial cell attachment was quantified by differential immuno-fluorescence microscopy. The function of SOF as adhesin was confirmed using a *sof*-deleted isogenic mutant (Sc∆*sof*), which was employed in a chamber separation cell migration assay (CSMA). This assay was also applied to analyse the impact of pneumococcal pneumolysin on endothelial wound healing processes simulating blood flow conditions. For visualization and quantification, differential immunofluorescence staining followed by confocal laser scanning microscopy was performed in addition to electron microscopic approaches.

Results and Conclusion: After transposon-directed insertion site sequencing (TraDIS), bioinformatical analyses identified the SOF of *S. canis* as novel bacterial adhesin. Cell culture attachment analyses confirmed that the SOF protein mediates bacterial adherence to HUVEC as well as to primary small muscle cells under flow of 10 dyn/cm2, which mimics the average shear stress present in the vascular system. This findings were further confirmed by electron microscopic imaging. Moreover, cell regeneration capacity of HUVEC is significantly increased in CSMA infected with *sof*-gene-deleted *S. canis* compared to infections with SOF-expressing

bacteria. Interestingly, incubation of HUVEC with pneumococci resulted in significantly reduced endothelial cell regeneration and similar results were determined by employing purified pneumolysin protein, which identifies this cytotoxin as a potent inhibitor of endothelial cell migration. The establishment of a human *ex vivo*-heart valve explant model for streptococcal infection analyses under physiological blood flow conditions enabled for the first time microscopic visualization of bacterial heart valve colonization.

PS02.134

Impact of different flow profiles and flow velocities on adherence of *Streptococcus canis* to human vasculature using microfluidic cell culture infection

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Introduction: *Streptococcus canis* is a zoonotic pathogen transmitted via companion animals to the human host thereby causing local and systemic infections such as septicemia. Depending on the vessel diameter, the human vascular system is characterized by shear forces ranging from 0.1 dyn/cm2 in smallest capillaries to 70 dyn/cm2 in the aortic vessel. In addition to different flow velocities, vascular branches and functionally compromised heart valves induce a turbulent flow pattern which might affect bacterial attachment to the vasculature and to the endocardial tissue.

Material and Methods: The microfluidic pump system of ibidi was employed for simulation of the different physiological blood flow parameters. Primary human endothelial cells were cultivated in microslides composed of a y-shaped channel with two different branching angels of the flow channels. The specific channel architecture of the slide creates defined flow areas with at least four different flow properties in which the flow is either laminar or non-laminar and the flow velocity reaches 100% or only 50% of the programmed flow. Microscopic analyses of bacterial attachment to HUVEC seeded within these y-shaped microslides was performed after nucleic acid staining using DAPI. In additional approaches, a serum opacity-factor-negative *S. canis* deletion mutant (*Sc*Δ*sof*) was employed in this analyses with the aim to decipher the function of SOF as bacterial adhesion in conditions simulating different physiological blood flow conditions.

Results & Conclusion: Infection of endothelial cells in y-shaped slides revealed significantly different attachment capacity of the bacteria. Cellular attachment of *S. canis* was highest in the area of non-laminar flow and 100% velocity, whereas it was lowest at the same speed but at a laminar flow. Moreover, reduction of flow speed to 50% did not resulted in significantly altered bacterial attachment. These results indicate that a non-laminar flow profile resembling blood flow turbulences at a heart valve has major impact in supporting bacterial attachment to endothelial vasculature even at high flow rates. Furthermore, this experimental cell culture infection setup also confirmed the function of surface-expressed SOF of *S. canis* as adherence factor mediating attachment to endothelial cells even under high shear forces.

PS02.136

Ciprofloxacin susceptibility in *E. coli* from infectious diseases in animals collected within the GE*RM*-Vet resistance monitoring

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Introduction: The occurrence of bacterial infectious diseases might be a problem in the human and also in the veterinary health sector and has to be considered within the "One Health" context. Especially resistances in disease causing bacteria towards antibiotics that are critically important for human medicine, such as ciprofloxacin, has to be monitored. These analyzes aimed to gain knowledge on the antimicrobial resistances in *E. coli* as veterinary pathogen.

Objectives: Susceptibility towards ciprofloxacin in *E. coli* causing infections in pigs, cattle, poultry and companion animals was investigated within the German antibiotic resistance monitoring of veterinary pathogenic bacteria. The occurrence of further resistance and ESBL phenotypes in *E. coli* with increased MIC values against ciprofloxacin was evaluated.

Materials & Methods: *E. coli* isolates from pigs (n=1407), cattle (n=1578), poultry (n=1830) and companion animals (n=1058) were collected between 2019 and 2023 within the GE*RM*-Vet national monitoring. Antimicrobial susceptibility testing was performed via broth microdilution and evaluated according to CLSI VET01S 7th ed., which provides human clinical breakpoints for 6 antibiotic substances. An MIC of >1µg/ml against cefotaxime (which is not a clinical breakpoint) indicated an ESBL phenotype. A veterinary clinical breakpoint for ciprofloxacin was not available. Isolates with MICs of >0,5µg/ml were categorized as non-susceptible to ciprofloxacin.

Results: Decreased susceptibility towards ciprofloxacin (MIC >0,5µg/ml) was observed in 7% of pig isolates, 6% of poultry isolates and in 10% of *E. coli* from cattle and companion animals, respectively. Isolates with increased MIC values against ciprofloxacin were found to also exhibit an ESBL phenotype (cefotaxim MIC >1µg/ml). This was detected in 6% of poultry, in 18% of porcine, in 34% of cattle and in 38% of companion animal *E. coli* with ciprofloxacin non-susceptibility. The resistance pattern assessed for amoxicillin, amoxicillin-clavulanic acid, gentamicin, trimethoprim-sulfamethoxazole, tetracycline and doxycycline resistances often revealed multiple resistances (resistances towards ≥3 antimicrobial classes) among the veterinary *E. coli* next to decreased ciprofloxacin susceptibility. Above 70% of *E. coli* from companion animals being non-susceptible to ciprofloxacin harbored a multiple resistance pattern. Originating from infected cattle, 81% of those isolates were multi-resistant.

Summary: Ciprofloxacin is not intended for veterinary use (whereas enrofloxacin is). *E. coli* with decreased susceptibility towards ciprofloxacin were isolated rarely from veterinary infections. However, when these isolates occurred, most of them were detected to have multiple resistances from the veterinary perspective. Since ciprofloxacin plays an important role in infectious disease treatment in human medicine, veterinary surveillance provides an important contribution to detect resistances.

PS02.138

Cobalt ion-induced adaptation and antibiotic resistance in PJI-associated pathogens

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Background: Metallic biomaterials such as cobalt-chromium alloys are widely used in orthopedic implants [1]. The corrosion-related release of cobalt ions (Co^{2+}) has been linked to altered host immune responses [2]. However, the influence of these ions on implant-associated pathogens, such as *Staphylococcus S. aureus*, *S. epidermidis* and *E. coli*, is not well understood. As these pathogens are a key contributors to prosthetic joint infections (PJIs) [3], it is important to understand the microbial response to metal ions. This study is part of the DFG Research Training Group 2901 SYLOBIO.

Objective: This study aimed to investigate the effect of cobalt ions on the growth, viability, biofilm formation and antibiotic susceptibility of *S. aureus*, *S. epidermidis* and *E. coli in vitro*.

Materials & Methods: The aforementioned bacteria were exposed to $CoCl_2$ concentrations of up to 3000 µM. Growth was monitored via OD_{600} and CFU/mL. Adaptation was assessed through serial passaging in media containing Co^{2+}. The impact of cobalt on antibiotic susceptibility was determined using minimum inhibitory concentration (MIC) and checkerboard assays. Biofilm formation was evaluated using crystal violet staining, raster electron microscopy and determination of viable counts.

Results: Exposure to at least 1000 µM Co^{2+} significantly impaired bacterial growth, but serial passaging in media containing Co^{2+} lead to strain-specific adaptation and recovery of the growth. *S. epidermidis* showed biofilm-like aggregation under cobalt stress. All strains had increased MICs for gentamicin and/or levofloxacin following cobalt exposure or pre-adaptation. While the biofilm formation of *S. aureus* was only slightly impaired, *E. coli* and *S. epidermidis* initially showed reduced biofilm formation, which recovered within 72 h.

Conclusion: PJI related bacteria seem to overcome adverse effects of elevated Co^{2+} concentrations by adaptation processes that render them less susceptible to certain antibiotics. This may complicate the treatment of PJIs. Further studies are needed to elucidate the underlying mechanisms, including efflux pump activation and stress-induced biofilm responses.

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PS02.140

Comparative analysis of genomes and pathogenic potential of *Chlamydia avium* – a new player in avian chlamydiosis

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Introduction: *Chlamydia (C.) avium* is a recently described novel species of *Chlamydia* found in pigeons and psittacines and potentially zoonotic. While infections in pigeons remain mostly asymptomatic, psittacines often present with severe symptoms, even leading to death. The aim of this study was to characterize *C. avium* as a new agent of avian chlamydiosis by performing comparative genomics and investigating its pathogenicity in different infection models.

Materials & Methods: Whole genome sequences served as the basis for the comparison of 13 *C. avium* strains. Comparative genomics included classical MLST-typing, identification and evaluation of SNPs with subsequent phylogenetic tree construction, and pangenome analysis. Survival analysis was performed in the embryonated chicken egg model to assess the virulence of twelve *C. avium* strains *in vivo*. In addition, the growth and proliferation of four *C. avium* isolates were studied *in vitro* using BGM cell culture. Parameters observed were proliferation, number and size of inclusions, and formation of infectious progeny.

Results & Discussion: Compared to the well-known *C. psittaci*, *C. avium* lacks certain virulence-associated genes, such as several genes of the plasticity zone or some genes encoding polymorphic membrane proteins (Pmps), which may reflect a high degree of host adaptation. A phylogenetic tree based on a core-SNP analysis of *C. avium* strains shows two distinct clades corresponding, with two exceptions, to host species and disease manifestation. Variant scoring revealed 1238 non-synonymous variants coinciding with the two phylogenetic clades. Most variants affect genes involved in energy metabolism or encoding Pmps, the latter potentially conferring host specificity at the subtype level. Despite genetic differences in genes involved in energy metabolism, no differences in *in vitro* growth in a heterologous cell culture model were observed between *C. avium* clades. However, compared to *C. psittaci,* growth characteristics differed significantly, both *in vitro* and *in vivo*. *C. psittaci,* which is the classical causative agent of avian chlamydiosis and human psittacosis, showed faster proliferation and earlier formation of infectious particles *in vitro* and was more virulent in the embryonated chicken egg model. While *C. psittaci* caused 100 % mortality after only five days, *C. avium* isolates even at a very high infection dose of 1*10^7 IFU per egg, caused only low mortality of 10-40 % or no mortality at all over an observation period of eight days. The virulence of the strains varied in this model, regardless of whether the strains were obtained from sick parrots or healthy pigeons. Chickens are not natural hosts for *C. avium* which may prevent efficient initialization of infection, and certain effects of differing genetic traits may only be seen in the natural host system.

PS02.142

Non-carbapenemase mediated carbapenem resistance in *Acinetobacter baumannii*

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Objectives: *Acinetobacter baumannii* is a Gram-negative nosocomial pathogen that plays a major role in the context of bacterial multidrug resistance. The increasing resistance to carbapenems is of particular therapeutic concern, as only a limited number of treatment options remain in such cases. While carbapenem resistance in *A. baumannii* is primarily mediated by carbapenemases, alternative mechanisms such as porin loss, efflux pump activity, and alterations in penicillin-binding proteins (PBPs) have so far been insufficiently studied.

Methods: The *A. baumannii* reference strain ATCC 17978 was subjected to stepwise selection pressure using increasing concentrations of meropenem until stable carbapenem resistance was achieved. Growth analyses were performed on the resulting mutants, and minimum inhibitory concentrations (MICs) were determined for relevant antibiotics. Subsequently, whole genome sequencing (WGS) of the mutants was performed and compared to the wild-type strain.

As multiple targeted mutagenesis attempts to reconstruct the observed resistance phenotype were unsuccessful, a strain collection provided by the NRC was screened for clinical *A. baumannii* isolates that exhibited carbapenem resistance in the absence of detectable carbapenemase genes. These isolates were also subjected to WGS and analysed for mutations in PBP and porin genes in comparison to ATCC 17978 and to carbapenemase-positive isolates.

Results: In three of the ATCC 17978-derived mutants, a point mutation in the *mrd*A gene was identified, resulting in a W366L amino acid substitution in PBP2. These mutants demonstrated resistance to carbapenems and were unaffected by the β-lactamase inhibitor avibactam, in contrast to the wild-type strain. Growth curve analyses revealed a measurable fitness cost associated with the mutation.

Although the W366L mutation was not observed among the clinical isolates, other genetic alterations were identified, particularly in genes associated with carbapenem resistance. Mutations were predominantly found in PBP genes, with a substitution in PBP3 (A515V) occurring at notably high frequency.

Conclusion: The findings of this study support the emerging hypothesis that mutations in PBPs can contribute to carbapenem resistance in *A. baumannii*. Given that this resistance mechanism has been poorly characterized to date and is often based on dated methodologies, our results provide important new insights. To further elucidate the role and functional consequences of these PBP mutations, additional targeted mutagenesis experiments are necessary. Initial attempts to perform such experiments have so far been unsuccessful.

PS02.144

Subclinical mastitis in dairy cows in the Upper Cheliff Region, northern Algeria: prevalence, associated risk factors and antimicrobial resistance of causative agents

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Introduction: Subclinical mastitis (SCM) is a common and economically significant infection in dairy cows worldwide. The antibiotic resistance of the main causative pathogens poses a serious threat to both animal and human health. Addressing SCM and antimicrobial resistance within the One Health framework is essential to safeguard public health, ensure food safety, and promote environmental sustainability.

Aims: This study aimed to assess the prevalence of SCM in dairy cows in the Upper Cheliff region, northern Algeria, identify the causative agents, and evaluate their antimicrobial susceptibility.

Material & Methods: A total of 263 cows from 23 farms were screened for SCM using the California Mastitis Test (CMT). CMT-positive milk samples were cultured for bacterial isolation. Microbial identification was done by MALDI-TOF mass spectrometry and antimicrobial susceptibility testing (AST) of isolates was performed by the VITEK 2 system. The AST results were interpreted according to the breakpoint criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), version 15.0, 2025.

Results: The results revealed a 58.9% prevalence of SCM at the cow level (155/263) and 31.1% (317/1020) at udder quarter-level. The most prevalent microorganisms identified were *Enterococcus faecium* (24.4%) and *Enterococcus faecalis* (20.5%), followed by non-*aureus* staphylococci (16.9%) and *Staphylococcus aureus* (7.5%). All enterococcal isolates were susceptible to vancomycin. All isolates of *S. aureus* were methicillin-susceptible (MSSA), however 18.6% (8/43) coagulase-negative staphylococcal were methicillin-resistant. Risk factors significantly associated with the prevalence of SCM included parity, lower milk production (vs. 102.7 days; $P < 0.0001$), more services per conception (2.5 *vs*. 1.9; $P < 0.0001$), and a lower pregnancy rate at first service (24.5% *vs*. 48.1%; $P < 0.0001$).

Summary: SCM is prevalent in the area of study with significant negative impacts on milk production and reproductive performance. Effective management strategies are essential to control SCM and improve dairy farm productivity. Further work is recommended to investigate the genetic mechanisms underlying antimicrobial resistance. Integrating the One Health concept into surveillance and intervention strategies could help mitigate the broader risks associated with mastitis and emergence of antimicrobial resistance.

PS02.146

Targeting host cell factors to combat UPEC infection: the role of S100A10 in urinary tract infections

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Urinary tract infections (UTIs), primarily caused by uropathogenic Escherichia coli (UPEC), represent one of the most prevalent bacterial infections worldwide and disproportionately affect women. Rising antibiotic resistance among UPEC strains is diminishing the efficacy of conventional treatments, highlighting the urgent need for alternative therapeutic strategies. Targeting host cell factors essential for bacterial invasion and replication offers a promising approach that bypasses direct selective pressure

on the pathogen.

In this study, we identified the host protein S100A10 (p11), a member of the S100 family of Ca^{2+}-binding proteins, as a key factor in UPEC pathogenesis. Using a CRISPR/Cas9-engineered human epithelial model cell line lacking S100A10 expression, we observed a significant reduction in UPEC invasion and intracellular replication. These findings position S100A10 as a novel and druggable host target for anti-UPEC strategies.

Our work contributes to the development of host-directed therapies aimed at mitigating antibiotic resistance and improving clinical outcomes in recurrent or complicated UTIs, especially in female patients.

PS02.148

Fournierella genus and lipid pathways of the gut microbiome are associated with the extent of improvement of treatment expectations by pill intake

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Introduction: Clinical and experimental studies indicate that the gut microbiome influences brain function, including affective behavior, social behavior, eating behavior, and stress response. The production of metabolites or neurotransmitters by the microbiota, vagal nerve signaling, and immune modulation by influencing immune cell maturation have been proposed as underlying mechanisms. However, data are lacking investigating possible links between the gut microbiome and treatment expectations.

Methods: In a cohort of 108 participants, we analysed the gut microbiome associated with the extent of expected discomfort following an *E. coli* lipopolysaccharide (LPS) injection and the expected benefit of a pill taken to reduce symptoms. We analysed the gut microbiome using *16S rRNA* sequencing and used the QIIME2 pipeline for data analysis, MaAsLin3 for differential abundance analysis and PICRUSt2/MetaCyc database for predicting metabolic pathways of the gut microbiome.

Results: Alpha diversity of the gut microbiome was higher in participants who had lower expectations of improvement in pill intake (p=0.049) and PCoA of Jaccard distance matrix was significantly different between both groups (p=0.028). Fournierella, a SCFA producer that synthesizes propionate, was the only genus that differed significantly between individuals with higher compared to those with lower expectations for improvement of pill intake following LPS treatment. PICURSt2 identified six pathways that differed between the two groups, including the Acetyl-CoA fermentation to butanoate and three belonging to the class of fatty acid and lipid biosynthesis.

The differences in the gut microbiome composition associated with expected discomfort after LPS treatment were insignificant for alpha and beta diversity, for relative abundance of individual taxa using MaAsLin3 and for metabolic pathways analysed by PICRUSt2.

Conclusions: The taxonomic and functional differences of the gut microbiome associated with the extent of improvement of pill intake were associated with the production of SCFA; metabolites that cross the blood brain barrier and play a role in neuronal communication and metabolic processes in the brain.

PS02.150

Agar matters – enhancing *Bordetella* spp. isolation from PCR-positive samples

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Objectives: PCR has become the primary method for diagnosing pertussis due to its higher sensitivity and faster turnaround time compared to culture. Therefore there is a growing shortage of clinical *Bordetella* isolates, which are essential for resistance testing and genomic surveillance. Currently, limited data are available on factors influencing the successful recovery of bacterial strains from PCR-positive samples, as well as on possible improvements to culture media. The primary aim of this study was to evaluate whether a modified Regan-Lowe agar improves the recovery of *Bordetella* spp. A secondary aim was to assess the effects of transport medium, transport time, and Ct values on culture positivity.

Methods: A total of 500 PCR-positive respiratory samples from Austria and Germany were prospectively cultured using both standard Regan-Lowe agar and an in-house modified version containing vancomycin in addition to cephalexin. Plates were examined for *Bordetella* spp.-suspicious colonies, which were confirmed via MALDI-TOF mass spectrometry. Differences in Ct values, transport time, and transport media were analyzed between culture-positive and culture-negative samples.

Results: *Bordetella* spp. were recovered from 167 (33%) of the 500 PCR-positive samples. The standard agar yielded 82 isolates (16%), while 160 isolates (32%) were obtained from the modified agar. Culture-positive samples had lower average Ct values (23.1 vs. 29.2) and shorter transport times (3.9 vs. 7.9 days) than culture-negative samples. The highest culture recovery was observed with flocked swabs in Amies transport medium (eSwab®), while Universal Transport Medium (UTM®) performed comparably well for samples with shorter transport times. Multivariable analysis showed that Ct value, transport time, and transport medium were independently associated with culture positivity.

Conclusion: The addition of vancomycin to Regan-Lowe agar doubled the probability of isolation of *Bordetella* spp. from PCR-positive respiratory samples. Furthermore, this study evaluates Ct value, transport time and medium as key factors that independently influence culture positivity rates. The improved strain recovery is a critical prerequisite for molecular epidemiology and surveillance.

PS02.154

Impact of pneumolysin, hydrogen peroxide, and *Streptococcus pneumoniae* strains on blood–CSF barrier integrity in a human choroid plexus co-culture model

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Introduction: *Streptococcus pneumoniae* (pneumococcus) is a Gram-positive human pathogen crossing the blood-brain barrier and causing pneumococcal meningitis (PM). Key factors are the capsular polysaccharide (CPS), hydrogen peroxide (H_2O_2), and the pore-forming toxin pneumolysin (Ply). In this study, we utilized a physiologically relevant transwell co-culture model comprising human choroid plexus endothelial (iHCPEnC) and epithelial (HIBCPP) cells to investigate the impact of pneumococcal virulence factors on barrier integrity. This model enables the study of cellular interactions at the blood–brain interface.

Objectives: We aimed to assess the effects of pneumococcal virulence factors Ply and H_2O_2 on barrier function and compared therefore the barrier-disruptive potential of non-invasive (19F), invasive wild-type (D39), and mutant strains (Δ*cps*, Δ*ply*, Δ*spxB*) of *S. pneumoniae*. Barrier integrity was evaluated using transepithelial electrical resistance (TEER) measurements and immunofluorescence-based analysis of tight junctions.

Results: Viability assays demonstrated that relatively high concentrations of both Ply or H_2O_2 were required to induce cytotoxic effects in HIBCPP and IHCPEnC cell lines. Specifically, cell lysis was observed at 25 µg/mL Ply after 10 minutes of incubation, and at 5 mM H_2O_2 after 15 minutes. These findings suggest that both cell lines exhibit a notable resistance to cytotoxicity. TEER measurements demonstrated that both Ply and H_2O_2 individually compromised barrier integrity over time, as indicated by a progressive decrease in resistance. Notably, the combination of Ply and H_2O_2 led to a more pronounced and sustained reduction in TEER values, suggesting an additive or synergistic effect on barrier disruption. Consistent with these findings, FITC-Dextran flux assays revealed significantly increased permeability after 6 hours in the combined treatment group compared to either agent alone, further supporting the enhanced disruptive effect on the epithelial barrier when both stimuli were present. Building upon these findings, we intend to systematically evaluate the barrier-disruptive capacities of non-invasive (19F), invasive wild-type (D39), and mutant (Δ*cps*, Δ*ply*, Δ*spxB*) strains of *S. pneumoniae* employing the same methods. This comparative analysis is currently in progress as part of our experimental framework.

Conclusions: Both Ply and H_2O_2 required relatively high doses to affect cell viability but were capable of progressively weakening barrier function when applied separately. Their combined application resulted in a significantly greater and sustained disruption of barrier integrity, as evidenced by TEER measurements and permeability assays. Following these observations, ongoing work aims to compare the effects of various pneumococcal strains, including invasive and mutant types, on barrier integrity using the established co-culture model.

PS02.156

Arginase 1 promotes colitis due to L-arginine depletion and intestinal dysbiosis

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Introduction: The metabolism of the semi-essential amino acid L-arginine in the alimentary tract is altered in animal models of colitis and in patients with inflammatory bowel disease (IBD). Furthermore, the expression and activity of arginase 1 (Arg1) which utilizes L-arginine as substrate are enhanced in mucosal tissues of IBD patients.

Aims: As dysbiosis is a signature of both, IBD patients and colitis models, we characterized the impact of L-arginine and Arg1 on intestinal microbiota and microevironmental parameters, which mediate the induction, perpetuation and resolution of colitis.

Material and Methods: We fed L-arginine-deprived and -supplemented chow to DSS- or oxazolone-treated Tie2-Cre+/-Arg1fl/fl mice lacking Arg1 in hematopoietic and endothelial cells and respective Arg1-expressing littermate controls. Whole-genome transcriptomic patterns, the intestinal metabolome and microbiome as well as the extent of intestinal inflammation were assessed using immunohistochemistry, high resolution endoscopy, qPCR, RNA sequence analyses, 16S rRNA sequencing, confocal laser scanning microscopy and HPLC.

Results: In contrast to the widely accepted immunosuppressive function of ARG1, we have made the unexpected observation that ARG1 acts as anti-resolvin in mouse models of DSS-induced or oxazolone-driven colitis. In both models, Tie2-Cre+/-Arg1fl/fl mice lacking Arg1 in endothelial and hematopoietic cells recovered faster from experimental colitis than Arg1-expressing littermates. The pro-resolving effect of ARG1-deletion was reduced by an L-arginine-free diet, but rescued by simultaneous deletion of other L-arginine-metabolizing enzymes such as NOS2, demonstrating that protection from colitis requires L-arginine. Accordingly, dietary supplementation of wildtype mice with L-arginine promoted the resolution of intestinal inflammation. Protection from disease was associated with an accumulation of intraluminal polyamines and compositional changes in the intestinal microbiota in L-arginine-supplemented wild-type litters, similar as observed in control chow fed Tie2-Cre+/-Arg1fl/fl mice. 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 Tie2-Cre+/-Arg1fl/fl donors, suggesting the microbiota as source for protective polyamine production.

Summary: Due to its high expression and acitivity in colitic tissues Arg1 might thus, serve as novel target for clinical intervention in patients suffering from inflammatory bowel disease. Dietary L-arginine restriction abolished the protective effect of Arg1-deletion, suggesting that protection is related to an increased availability of L-arginine and the expansion of an anti-inflammatory microbiota.

PS02.158

Genomic insights into plasmid mediated NDM-5-producing gram-negative pathogens in a patient with nosocomial sepsis: a case report

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Introduction: Antimicrobial resistance (AMR) poses a threat to healthcare settings worldwide, with treatment failure due to resistant pathogens affecting patient outcomes. Therefore, rapid identification of AMR bacteria is essential for effective therapy.

Case Description: A 75-year-old patient underwent revision surgery to replace spinal implants due to mechanical failure and pain six months after placement, caused by a spinal infection. No signs of recurrent infection were detected during surgery. The postoperative course was complicated by a deep surgical site infection caused by *Enterococcus faecium*. Afterwards the patient developed sepsis during treatment with linezolid, most likely originating from a pulmonary focus. Two carbapenem-resistant Enterobacterales were identified in blood cultures: *Klebsiella aerogenes* and *Enterobacter cloacae* complex.

Methods: Microbiological diagnostics were performed using standard culture techniques and EUCAST guidelines for antimicrobial susceptibility testing. Isolates underwent long-read whole-genome sequencing (lrWGS) on the PacBio® Sequel IIe platform. Bioinformatic analyses were conducted using Ridom SeqSphere⁺ software. Core genome multilocus sequence typing (cgMLST) and resistome profiling were used to assess genetic relatedness and resistance determinants. Plasmids were compared based on Mash distance.

Results and Discussion: Genomic analysis revealed the presence of an IncC plasmid harboring a *bla*$_{NDM-5}$ positive AMR cassette in both isolates with high similarity (adj. mash <0.0001). Notably, an increase in the AMR cassette copy number containing *bla*$_{NDM-5}$ — from one to five — was associated with elevated minimum inhibitory concentrations (MIC Meropenem: 16 mg/L vs >32 mg/L). No further carbapenemase genes were found. Targeted antibiotic therapy with aztreonam/avibactam was initiated after phenotypic proof of susceptibility. An indwelling port was explanted, to eliminate a potential focus. Transoesophageal echocardiography (TEE) revealed a mobile vegetation on the native mitral valve, raising suspicion of infective endocarditis (IE). Repeat blood cultures were negative and control TEE revealed no change in the morphology of the putative vegetation. Following interdisciplinary consultations, the diagnosis of IE was rejected. The gram-negative therapy was stopped after 14 days, with nonrecurrence of infection within six weeks.

Conclusion: This case illustrates the challenges of AMR infections. Distinguishing IE from bloodstream infections requires an interdisciplinary team, as well as careful consideration of the clinical response to anti-infective therapies. Clinical decision-making in AMR infections is based on phenotypic testing. Whole genome sequencing adds the advantage of precise resistance profiling, such as AMR gene-copy number analysis that explain resistance phenotypes.

PS02.160

Characterization of *Candida albicans* sucrose utilization mutants

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An unbalanced diet rich in carbohydrates is associated with diseases such as cardiovascular disease and type 2 diabetes [Temple *et al.* 2018]. It can alter the gut microbiota and promote the growth of facultative pathogenic microorganisms, including the yeast *Candida albicans*. We have observed an increase in *C. albicans* under high sucrose conditions in mice, while the mechanism behind this phenomenon remains unclear.

This project investigated the role of sucrose metabolism for *C. albicans colonization capacity* with competition experiments using mutants deficient in sucrose utilization. Both SPF and germ-free mice were fed a fiber-rich grain-based diet or a fiber-poor purified diet and colonized with a wild-type (SC5314) and a sucrose utilization mutant. By analyzing the fungal burden in the feces, we identified fitness disadvantages of the mutant compared to the wild-type. Additionally, the growth of the mutant strains was systematically tested *in vitro* on a range of carbon sources, including different mouse diets.

The competition experiments revealed that a *C. albicans* mutant unable to hydrolyze sucrose displayed a significant fitness disadvantage in competition with the wild type in mice fed a grain-based diet. This finding highlights the ability of *C. albicans* to effectively exploit disaccharides derived from grains in the murine gut. In contrast, a mutant deficient in sucrose transport, only exhibited fitness defects under high-sucrose conditions during competition with bacteria, indicating that rapid sucrose uptake provides *C. albicans* with a competitive advantage in such environments.

The *in vitro* testing confirmed that mutants unable to metabolize sucrose exhibited a pronounced growth defect when sucrose was the sole carbon source, while their growth on other carbon sources was largely unaffected. In mouse diet experiments, these mutants were able to grow without any noticeable disadvantage when fed standard mouse chow. However, under sugar-rich dietary conditions, the ability to utilize sucrose became important for optimal fungal growth.

In addition, future studies will compare the oral isolate *Candida albicans* 101 with SC5314 to further elucidate strain-specific differences in sugar utilization. This comparison may provide valuable insights into the metabolic diversity and adaptation of *C. albicans* in different host environments.

In summary, this project provides new insights into the role of sucrose utilization in *C. albicans*. The findings demonstrate that the fungus needs to effectively exploit dietary sucrose for colonization success, in sugar-rich as well as grain-based environments. These results contribute to a better understanding of the interplay between diet, microbiota, and fungal pathogenicity, and may inform future strategies for the prevention or treatment of fungal infections related to dietary habits.

Temple, N. J. (2018). Fat, Sugar, Whole Grains and Heart Disease: 50 Years of Confusion. *Nutrients*, *10*(1), Article 1. https://doi.org/10.3390/nu10010039

PS02.162

Analysis of the role of *Yersinia enterocolitica* YopP phosphorylation

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Introduction: Pathogenic *Yersinia spp*. rely on a major virulence system, a type III secretion system, to translocate effector proteins (Yersinia outer proteins, "Yops") into infected cells. These Yops interact with host proteins and modulate host cell immune responses. YopP possesses an acetyltransferase activity, which disrupts central innate immunity pathways within the host cell by acetylating NF-kB and MAPK pathway-related kinases. Phosphorylation is a common post-translational modification, which can alter the function or activity of a protein. Our results suggest that *Y. enterocolitica* YopP itself is subjected to phosphorylation by host cell kinases after injection into the host cell via type III secretion and its function might therefore be altered.

Materials & Methods: A fusion construct was created by combining a newly developed helical peptide tag (ALFA-Tag) with YopP. This construct was then introduced into a corresponding knockout Yersinia lineage. J774 macrophages were infected with this strain, and cell lysates were analysed by immunoblotting with an anti-ALFA-tag antibody. Subsequently, ALFA-tagged YopP was immunoprecipitated and analysed via Mass spectrometry (MS) to identify potential phosphorylation sites. Point mutations of these identified sites were subsequently inserted into YopP to study the relevance of the phosphorylation of YopP during host cell infection.

Results: Immunoblotting of J774 macrophage cell extracts following Yersinia infection revealed the appearance of additional YopP bands with slower electrophoretic mobility. These additional YopP bands disappeared upon phosphatase treatment, but were stabilised by the phosphatase inhibitor Calyculin A. This suggested phosphorylation as the cause of these modifications. Subsequently, a protein band corresponding to phosphorylated YopP was purified by SDS-PAGE separation and analysed via MS. This approach identified Serine-6 and Serine-10 of YopP as being phosphorylated by the host cell, starting as early as 15 minutes post infection. Phospho-mimicking (S6/10D) and phospho-nil (S6/10A) YopP mutants were then developed. These mutants exhibited the expected altered electrophoretic mobility pattern in western blot experiments. The effect of phosphorylation on YopP activity is further being investigated.

Summary: Our results show that *Y. enterocolitica* YopP is phosphorylated at S6 and S10 by yet unknown host cell kinases very early post infection. Studies are ongoing in order to assess the physiological relevance of these phosphorylation events on YopP functions and their consequences for the host cell immune response.

PS02.164

Free ISG15 dampens neutrophil hyperactivation by *C. albicans*

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Vulvovaginal candidiasis (VVC) is one of the most common fungal infections affecting women in their reproductive years. The interplay between the immune system and the virulence mechanisms of *C. albicans* drives the pathogenesis of VVC, leading to a hyperinflammatory response, neutrophil recruitment and activation as hallmarks of the infection. Type I interferon signalling and stimulation of interferon-stimulated genes (ISGs) has been identified as a common signature of early vaginal epithelial cell responses to infection with *Candida* species. This response improves epithelial resistance to *Candida* infections. While a myriad of ISGs are regulated by interferon signalling, *ISG15* warrants further exploration given its role as both an intracellular and extracellular mediator controlling certain viral and bacterial infections.

We investigated the localisation of ISG15 during *C. albicans* infection of vaginal epithelial cells (VECs). Upon infection, we observed an intracellular accumulation of ISG15 using fluorescence microscopy. Our measurements of intracellular freeISG15 showed a decrease at 3 h post-infection, without an increase in release, which suggests conjugation to other proteins. Silencing of *ISG15 mRNA* expression was associated only with slightly decreased epithelial damage, compared to infection with non-targeting siRNA. This could imply a subtle role for ISG15 in epithelial resistance to infection. Knowing the localisation of ISG15 determines its function, we used recombinant ISG15 to study its effects on neutrophil function. We observed that ISG15 modulates neutrophil function by dampening IL-8 release, ROS production while increasing NETosis and improving the neutrophil lifespan. However, *C. albicans* clearance was not negatively impacted.

Collectively, our data suggest that while ISG15 is expressed within vaginal epithelial cells upon fungal infection, its secretion may be tightly regulated or dependent on additional factors. Extracellular ISG15 in the context of neutrophil effector mechanisms could act in an effector-function dependent manner, either activating or dampening the anti-fungal response. Thus, ISG15 may play a role in dampening the inflammatory responses driving immunopathology in VVC, which warrants its investigation in patient cohorts.

PS02.166

Bridging the gap: addressing the demand for tropical diseases education in medical school curricula of microbiology and hygiene in Germany

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Introduction: Globalization, climate change and increasing migration have made tropical diseases relevant on a global scale. This shift demands updates to traditional microbiology and hygiene curricula in German medical schools to adequately prepare future doctors.

Aim: This study aims to evaluate the interest of the medical university community in tropical microbiological topics and to explore the need for their integration into medical school curricula. Furthermore, to prove the medical community"s incentive to gain knowledge about these topics outside of regular curricula.

Methods: To explore the need for further coverage of tropical diseases in microbiology and hygiene education, the participants of some nationwide, online extracurricular lecture series on tropical medicine and global health in Germany were asked to rate the usefulness of each lecture topic for their own studies or clinical work. The lecture series was held over two consecutive study terms between 04/2023 and 02/2024. Data for engagement in the topics was acquired via Microsoft Forms and respondents were recruited by the distribution of a QR-code after each lecture to tell us if they were present. At the end of the series a QR-code to a questionnaire was distributed to evaluate the usefulness. Descriptive analyses were performed separately for medical students and professionals to account for different levels of experience and depth of knowledge that might influence their decision on usefulness or to attend a specific lecture. Both study terms were evaluated in parallel to account for unbalanced participation in total and between the specific subgroups. Data was visualized in a graph of usefulness versus attendance and normalized to the highest value within subgroup and semester to show simultaneously.

Results: The maximum number of participants that reported their presence via the QR-code feedback system in the summer term were 152 students and 63 academics, and 103 and 34 in winter. Both groups showed a high engagement in lectures focusing on infectious diseases of rising concern namely chagas, malaria, tuberculosis, and schistosomiasis.

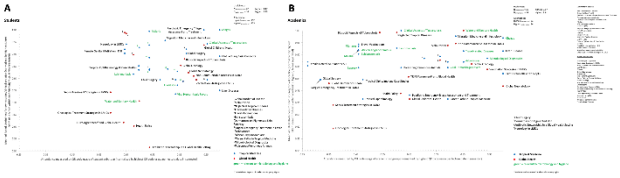
Compared to other subjects within the tropical medicine or global health domain, microbiology focused lectures were among the highest rated for clinical usefulness.

Professionals rated microbiological topics uniformly higher than students and notably also the hygiene-focused topic of water and sanitary health.

Conclusion: This study demonstrates a strong interest in basic education on the microbiology of tropical infectious diseases confirmed by a high engagement with these topics in the lecture series by both subgroups that seek knowledge through extracurricular learning platforms.

Professionals rated microbiological topics and a hygiene-focused topic uniformly higher than students confirming an emerging clinical relevance which highlights the need for more integration of such topics in existing medical school curricula.

Fig. 1



PS02.170

Differentiation of *Cronobacter sakazakii* from other Cronobacter species via lipid profiling using MALDI-ToF MS in negative ion mode

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Background: *Cronobacter sakazakii* (CS) is an opportunistic foodborne pathogen frequently associated with post-processing contamination in dairy-based products. It is implicated in severe neonatal infections, including necrotizing enterocolitis, septicemia, and meningitis. This can be fatal for neonates and immunocompromised individuals [1]. Consequently, regulatory frameworks within the European Union mandate routine testing for CS in infant formula and related products [2]. Current identification protocols rely on selective culturing followed by PCR-based confirmation, which are labour-intensive and time-consuming. MALDI-ToF MS offers a rapid and cost-effective alternative for colony screening and species-level discrimination. While a characteristic secondary Lipid A peak has been reported for CS [3], comprehensive lipidomic profiling of the remaining six Cronobacter species is lacking. Moreover, comparative analyses of lipid spectra between CS and other Cronobacter spp. have not been systematically performed.

Methods: A total of 807 lipid were analysed originating of the seven *Cronobacter* species cultured on three different solid media at two different temperatures (35°C and 44°C) to assess variability in lipid expression. Membrane lipids were extracted using the MBT Lipid Xtract™ Kit (Bruker, Germany), and spectra were acquired in negative ion mode using the MALDI Biotyper sirius™ system (Bruker). Both the extraction kit and software employed are intended for research use only and are not approved for diagnostic applications.

Results: The second Lipid-A (1824 m/z) peak of CS is in greater than the primary peak of Lipid-A (1796 m/z), independent of growth medium or incubation temperature, whereby the second Lipid-A-Peak has lower intensity within the other six species. Both visual and computational analysis of the spectra lead to a reliable discrimination in 100% of the tested *Cronobacter* species.

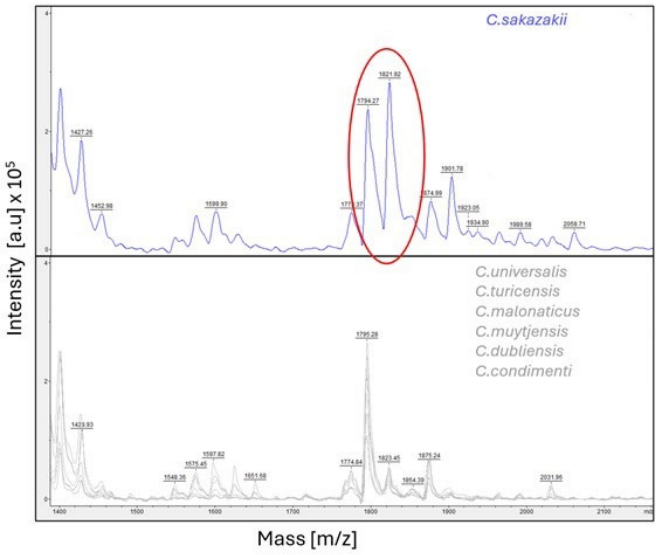
Conclusion: The here presented subtyping would allow a quick discrimination and identification of the pathogen. MALDI-ToF MS offers a significant advantage by providing faster and more resource-efficient identification. This method allows for the simultaneous identification and subtyping of *Cronobacter spp.* directly from colonies, streamlining the process and reducing the need for multiple testing steps.

[1] 10.3168/jds.2017-12969

[2] DIN EN ISO 22964:2017-08

[3] 10.1255/ejms.1074

Fig. 1



PS02.172
The interaction of two micro worlds: the hidden role of microbiome in Microglia biology during stroke
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Stroke is a leading cause of mortality globally, accounting for approximately 8% of all deaths annually and ranking as the second most frequent cause of death worldwide. Stroke prevention is complicated due to the multifaceted nature of stroke pathogenesis, involving complex interactions between vascular, inflammatory and neuronal mechanisms. Treatment options are limited because of a narrow time-window when the therapy can be applied and strict inclusion criteria. Currently, researchers try to understand detailed processes involved in stroke pathophysiology. Microglia - resident brain macrophages, which control local inflammation, clearance of dead cells and excitotoxicity play an important role in the course of stroke. Interestingly, it is already known that there are multiple microbial metabolites, which can affect microglia, like trimethylamine N-oxide, for example, leading to glial activation; or bile acids, which have neuroprotective effects and reduce production of NO by microglia. Generally, however, the topic of the effects of microbial signals on microglia remains poorly explored. Recently, our group has identified time-dependent fluctuations in a microbiota member - *Faecalibalulum rodentium* in an experimental mouse stroke model. We further investigate the effect of *F. rodentium* metabolites on EOC 20 microglial cells, using live-cell imaging to asset changes in their phagocytic activity, proteomics to characterize microglial responses and RT-qPCR to profile changes of markers of interest, such as neurotrophic factors (BDNF), cytokines (IL-10, IL-6) and membrane receptors (TLR4, TREM2). Investigating microglia-microbiota interplay may allow us to identify novel therapeutic targets for stroke prevention and treatment.

PS02.174
Surveillance of invasive meningococcal disease in Germany 2023/2024
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Introduction: In Germany, invasive meningococcal disease (IMD) must be notified to the Robert Koch-Institute, whereas submission of meningococcal isolates and clinical samples to the German National Reference Laboratory for Meningococci and Haemophilus influenzae (NRZMHi) is voluntary.

After the COVID-19 pandemic, the number of IMD cases started to increase at the end of 2022.

Goal: To present laboratory surveillance data of IMD in Germany 2023/2024

Materials and Methods: The data of the NRZMHi were analysed according to serogroup, genome sequencing, patients" age and antimicrobial susceptibility.

Results: IMD cases in Germany reached the pre-pandemic level in 2023 and further increased in 2024. MenB predominated in both years with comparable proportions of MenY whereas MenC and MenW were at very low levels.

Infants, adolescents, young adults and the elderly aged 60 years and older were most affected by IMD. MenY was less prevalent than MenB among infants and toddlers but most prevalent among the elderly.

Almost all MenY isolates belonged to cc23 whereas MenB isolates were assigned to ccs 213, 269, 32, 41/44 and 60.

Most meningococcal isolates were susceptible to cefotaxime, ciprofloxacin and rifampicin, but the proportion of penicillin-resistant isolates increased. Few isolates harboured a beta-lactamase or were resistant to multiple antibiotics.

Summary: After the COVID-19 pandemic, the IMD epidemiology in Germany changed significantly with an increase of MenY. Thus, the question arises whether the German immunization programme needs to be adapted.

Due to an increase of penicillin resistant meningococcal isolates ongoing surveillance is important.

PS02.176
E3 ubiquitin ligase Skp2 limits autophagy during *Staphylococcus aureus* infection
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Introduction: Ubiquitination is a post-translational modification that regulates protein function, localization, and turnover, playing a central role in maintaining cellular homeostasis. During microbial infection, the host ubiquitin system becomes a regulator of intracellular signalling pathways, coordinating pathogen recognition, immune activation, and clearance mechanisms. *Staphylococcus aureus* is a versatile opportunist capable of invading and persisting within both professional vs. non-professional phagocytes, including epithelial and haematopoietic cell types. The host counters such intracellular threats through selective autophagy, a defence strategy that tags and degrades pathogens via ubiquitin-dependent pathways. In this study, we investigated the role of the E3 ubiquitin ligase

component Skp2, as part of the Skp1-Cullin1-F-box complex, in regulating host responses to *S. aureus* infection. We investigated the role of Skp2 in regulating selective autophagy and its impact on intracellular bacterial survival in human alveolar epithelial cells and macrophage-like cells.

Methods: We employed *in vitro* infection models using A549 epithelial cells and differentiated THP-1 macrophage-like cells infected with the *S. aureus* strains HG001 and USA300. Total Skp2 expression and posttranslational modifications were analysed on protein level via immunoblotting during infection. Further changes in intracellular Skp2 localization were monitored by immunofluorescence and subcellular fractionation. In functional assays, we monitored intracellular bacterial survival upon Skp2 knockdown, along with the induction of autophagy markers, which were evaluated through analysis of the expression levels of LC3-II and p62/SQSTM1.

Results: We observed a marked increase in Skp2 protein levels following *S. aureus* infection attributed to acetylation-mediated stabilization. Notably, Skp2 was translocated from the nucleus to the cytoplasm upon infection. Cytoplasmic Skp2 was found to limit autophagy induction. Skp2 supported the restriction of *S. aureus* replication within host cells. The loss of Skp2 removed inhibitory regulation on autophagy and impaired effective targeting of the pathogen, leading to increased bacterial persistence.

Conclusion: Our study identifies Skp2 as a critical modulator of the host autophagic response during *S. aureus* infection. Infection-induced acetylation enhances Skp2 stability and cytoplasmic localization, where it promotes autophagy-mediated restriction of intracellular bacteria. These insights underscore a role for Skp2 in the ubiquitin-autophagy axis and emphasize the importance of deepening the understanding of autophagy in host-pathogen interactions, particularly against pathogens like *S. aureus* that can exploit this host defence mechanism to its advantage.

PS02.178

Genomic surveillance of MRSA and MRSE from blood cultures – a key to outbreak detection?

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Background: In recent years, Germany and parts of Europe have seen a steady decline in MRSA (methicillin-resistant *Staphylococcus aureus*) rates in both hospitals and outpatient settings. This trend is also reflected in the number of MRSA bloodstream infections (BSIs) reported to the Robert Koch Institute (RKI), which has remained at around 1,100 cases per year since 2020. The NRC received approximately 100 to 250 MRSA blood culture isolates annually, along with a steadily increasing number of MSSA and multidrug-resistant *S. epidermidis* isolates from BSIs.

Materials and Methods: The NRC for staphylococci and enterococci analyzes 2,500 to 4,000 staphylococcal isolates annually. All isolates undergo antimicrobial susceptibility testing using broth microdilution according to EUCAST guidelines. *S. aureus* isolates are additionally subjected to *spa* typing. Since 2020/2021, all staphylococcal blood culture isolates have been analyzed via whole-genome sequencing (WGS) and examined for genetic relatedness using core genome multilocus sequence typing (cgMLST), based on both published and inhouse ad hoc schemes. This report includes data on MRSA (n = 675), MSSA (n = 186), and MRSE (n = 380) blood culture isolates submitted to the NRC between 2020 and 2024.

Results: MRSA and MSSA BSI isolates belong to a wide range of distinct clonal lineages while multidrug-resistant MRSE isolates are predominantly assigned to a single *S. epidermidis* lineage (ST2). cgMLST analysis **s**howed that 74 MRSA isolates formed 32 clusters, mostly consisting of 2–3 isolates from different patients; only two clusters contained four and six isolates, respectively. Clusters mainly comprise common hospital-associated MRSA lineages, with isolates typically originating from the same hospital or region. Some MRSA BSI isolates were part of larger submissions related to outbreak investigations, suggesting that some of the clusters identified here may indicate previously undetected outbreaks. In contrast, the cgMLST analysis of MRSE isolates revealed significantly larger clusters including isolates from different facilities and regions, supporting the hypothesis of widespread dissemination of hospital-adapted *S. epidermidis* lineages throughout Germany.

Conclusion: The findings highlight that MRSA blood culture isolates represent only the "tip of the iceberg" of nosocomial MRSA infections and potential outbreaks. To investigate further, we are retrospectively sequencing all MRSA BSI isolates dating back to 2000, aiming to identify clusters spanning longer timeframes that indicate persistent transmission within specific facilities. Such insights could help refine strategies to combat MRSA infections and further strengthen the success achieved in controlling nosocomial MRSA. The widespread spread of multidrug-resistant, hospital-adapted *S. epidermidis* strains is concerning, as it hampers outbreak detection and may hinder infection control efforts.

PS02.180

Elimination of SARS-CoV-2 by high affinity virus binding conjugates

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In 2019 the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is the causing agent of the disease COVID-19, triggered a pandemic. Compared to the known SARS-CoV virus, the virus was characterized by an easy and fast transmission, an increased virulence and in case of COVID-19 severe to life-threatening outcomes, in part due to limited therapeutic options. In addition to life-threatening COVID-19 infections, a number of patients with mild disease symptoms developed the Long- or Post-COVID-syndrome. Our work focuses on the development of an innovative therapy concept, which uses the high affinity of SARS-CoV-2 to the human ACE-2 receptor in combination with nanoparticle technology to reduce disease symptoms as well as the duration of illness.

Due to their small size the uptake of virions by immune cells is driven by pinocytosis or endocytosis but not by the degradative phagocytosis pathway. Functionalization of biodegradable nanoparticles with a high affinity ACE-2 homolog, called LCB1-minibinder, leads to complexation of viruses and in consequence to a significant increase of their size. The treatment of infected host cells with these nanoparticles then resulted in a significant decrease of the virus titer most likely due to the phagocytosis of nanoparticle-virus-complexes by macrophages. By the development of a software-based evaluation algorithm we were able to follow and quantify the uptake and the acidification of particle-containing phagosomes proving their direction to degradative phagosomes. This was supported by the detection of marker proteins specific for maturing phagosomes like Lamp-1.

The established concept can be adopted to other respiratory viruses like influenza or RSV. Furthermore, the modular synthesis principle allows the fast adaptation of the particles for other viruses or mutant viruses. Our concept of directing virus-binding conjugates to the degradative phagocytosis could form the basis of innovative therapies against virus epidemics or pandemics.

PS02.182

Wastewater-based pathogen surveillance is also an important capability in a military environment

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Although testing wastewater for infectious agents is not a new concept, its use increased dramatically during the global COVID-19 pandemic, providing valuable insights into the dynamic SARS-CoV-2 infection rate and enabling the adaptation of prevention and control measures. The German Armed Forces established and used this innovative method to support operational readiness during the pandemic. From 2021 until the end of the mission in 2023, daily SARS-CoV-2 wastewater monitoring was carried out at the Camp Castor field camp in Mali, Africa, as part of an EU support mission. The samples were processed on site in a specially developed laboratory container and analysed using digital PCR. This enabled infection clusters to be recognised and localised several days before the onset of symptoms. The preventive medical measures introduced as a result were able to prevent or minimise major infection incidents in good time. Despite the cramped working and living conditions in a military field camp, the mission was fulfilled throughout, even under the special circumstances of a pandemic. Operations and workability never came to a standstill.

The experience gained from the successful implementation of pathogen surveillance using wastewater was subsequently incorporated into and developed further in a pilot project in Germany. For 1.5 years, wastewater has been analysed twice a week for SARS-CoV-2, influenza and RS viruses at five locations, and the results have been compared with the soldiers' infection and health data in the barracks. This data is regularly used to inform outpatient medical care, enabling targeted detection of infections and minimising the impact on teaching and training operations. This important new method of preventive medicine is now an integral part of Force Health Protection and will be considered in future missions.

PS02.184

Label-free spectroscopic characterization of macrophages: from defined *in vitro* experiments to an *ex vivo* lung infection model

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Introduction: Macrophages are essential immune cells that play central roles in maintaining immune homeostasis and orchestrating responses to infection, injury, and disease. They can adopt distinct functional states — pro- or anti-inflammatory phenotypes. Label-free methods that can visualize this so-called macrophage polarization also in complex tissue environment in a label-free manner are of utmost importance.

Objectives: We aim to apply Raman spectroscopy as a powerful, non-destructive analytical technique to characterize macrophage function (e.g. phagocytosis) as well as to reveal the macrophage polarization state, both in isolated cell populations as well as within complex tissue environment, in particular within an ex vivo precision-cut lung slice infection model.

Materials & Methods: THP-1 macrophages were challenged with polystyrene beads. [1] Defined macrophage phenotypes (M0, M1 and M2) were generated from monocytes from peripheral blood of healthy donors. [2] Human ex vivo precision-cut lung slices were infected with SARS-CoV-2. [3]

Raman spectroscopic imaging was used for characterization. Data were analysed using in-house written scripts. Fluorescence-based methods were used to support and validate Raman spectroscopic findings.

Results: In a label-free and non-invasive manner specific interaction of macrophages with foreign material, such as microplastics, was visualized in false colour Raman images, proving the cellular uptake. [1] Furthermore, single cell Raman spectroscopic imaging was used to differentiate between pro- and anti-inflammatory macrophage phenotypes with high accuracy. Spectral features responsible for the differentiation pointed to characteristic chemical differences. [2] The translational potential of the method was proven with the characterization of alveolar macrophages directly within intact human ex vivo precision cut lung slices. Molecular changes upon interactions with vial pathogens, in particular SARS-CoV-2, revealed by Raman spectroscopy, point to variations in intracellular RNA, carotenoid, triacyl glyceride, and glucose levels. [3] Those spectral differences are similar to the ones observed between pro- and anti-inflammatory macrophages in the cellular in vitro experiments.

Summary: Raman spectroscopy is a powerful tool for characterizing macrophage immune responses and phenotypic plasticity. It applies to both single cells and complex tissues, revealing biochemical changes from pathogen interactions in a label-free, non-invasive way and enabling automated cell differentiation through statistical analysis.

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PS02.186
Models of blood microbiota proliferation
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Introduction: The blood microbiome is still an enigma. The existence of blood microbiota in clinically healthy individuals was proven during the last 50 years. Indirect evidence from radiometric and sequencing analysis suggested the existence of living microbial forms in blood cells. The morphology and reproduction cycle of blood microbiota in healthy individuals are not sufficiently well studied. Aim of this study was to investigate the morphology and life cycle of blood microbiota in healthy individuals.

Methods: For the purpose of the study, we used freshly collected peripheral blood from healthy volunteers. Part of the samples were cultivated under stressful conditions. Blood samples were cultured at 43°C in the presence of vitamin K (1 mg/ml). Native and cultured blood preparations were examined by light and electron microscopy.

Results: In native blood, by light and electron microscopy methods, we have observed free-circulating microbiota that possess a well-defined cell wall and divide by budding or extrusion of progeny cells. Microbiota in stressed blood samples proliferate as electron-dense and electron-transparent bodies. Electron-dense bodies divide by forming simple fission or by forming chains of Gram-negative daughter cells or grow and then burst and release progeny cells 180 – 200 nm in size. A novel proliferation mechanism of the blood microbiota has been observed, which we call "a cell within a cell" or the "Matryoshka" model.

Conclusions: The demonstrated rich diversity of eukaryotic and prokaryotic microbiota in blood by the next-generation sequencing technique and our microscopic results suggest different mechanisms of blood microbiota division in native and cultured blood. Our results significantly enrich the current understanding of the presence and proliferation mechanisms of blood microbiota in healthy individuals.

Key words: blood microbiota, light microscopy, electron microscopy

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PS02.188
A primary human bronchus-on-chip model for studying Aspergillus fumigatus airway infection
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Introduction: Invasive pulmonary aspergillosis (IPA), primarily caused by *Aspergillus fumigatus*, poses a serious threat to immunocompromised individuals. Patients with underlying respiratory diseases such as chronic obstructive pulmonary disease (COPD), cystic fibrosis, and severe asthma are especially susceptible due to impaired mucus clearance mechanisms. However, current experimental models inadequately replicate human-specific lung responses, limiting insights into IPA pathogenesis. Organ-on-chip (OOC) technology has emerged as a promising approach to overcome these limitations.

Objectives: The objective of this study was to develop a physiologically relevant human bronchus-on-chip model that mimics the architecture and function of human bronchial tissue under air-liquid interface (ALI) conditions. This model aims to provide a robust platform for studying the transport dynamics of *A. fumigatus* conidia in healthy and compromised airways, and to support antifungal drug testing and personalized medicine applications.

Materials & Methods: Building upon a previously established invasive aspergillosis chip (IAC) model [1], a dual-compartment bronchus-on-chip system was engineered. It features a differentiated bronchial epithelial layer and a perfused vascular compartment separated by a porous membrane. The bronchial epithelium was cultured at ALI and

assessed for key cell types using cell-specific markers (figure 1): MUC5AC for goblet cells, acetylated-α tubulin for ciliated cells, and CC10 for secretory club cells. Ciliary coverage was quantified through image analysis. To study conidial transport, *A. fumigatus* conidia were introduced onto the epithelial surface, and their movement was tracked over time.

Results: The bronchial epithelium developed in the chip model included major cell types found in vivo and achieved ciliary coverage of 60–70%, closely resembling native bronchial tissue. Preliminary tracking of *A. fumigatus* conidia demonstrated directional transportation of conidia in the healthy airway model, suggesting active mucociliary clearance.

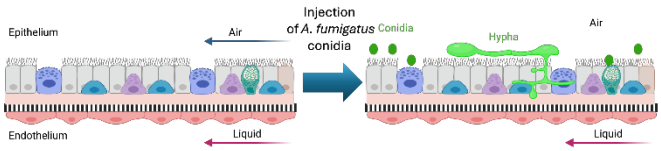
Summary: This human bronchus-on-chip model successfully replicates the structural and functional features of the bronchial airway and supports real-time analysis of *A. fumigatus* conidia transport. It presents a valuable platform for investigating IPA pathogenesis in obstructed airways and offers potential for therapeutic screening and personalized treatment strategies.

Figure legend: After a four-week differentiation period, a highly differentiated bronchial epithelium was established in the upper channel of the chip, along with a bottom (vascular) channel containing pulmonary endothelial cells. The chip was subsequently utilised for an *A. fumigatus* infection study.

Reference

[1] T.N.M. Hoang, Z. Cseresnyés, S. Hartung, *Biomaterials* 283: 121420 (2022)

Fig. 1



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