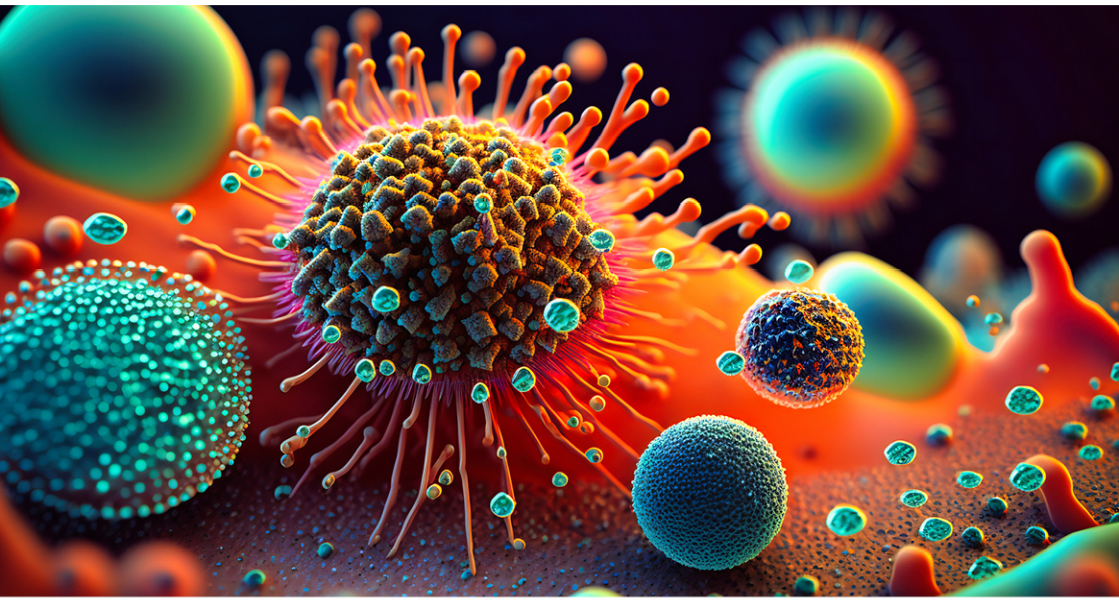

Abstractbook

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O001

Detection of Fluconazole Resistance in Candida albicans Using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) Based on Lipid Profile

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The emergence of fungal infections caused by Candida albicans in immunocompromised people represents a major challenge for healthcare systems. This phenomenon is amplified by increasing resistance to antifungal treatments, particularly fluconazole, the first-line antifungal drug. These infections lead to systemic complications, with a significant increase in mortality rates up to 40% in the case of systemic infection. In addition, current methods of resistance detection in clinical microbiology laboratories are costly, time-consuming and require expertise. The aim of our study is to present a novel approach using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry in positive ion mode. This method could enable rapid and cost-effective detection of fluconazole resistance by analyzing lipid variations, focusing in particular on ergosterol, a biomarker exclusive to fungi and the biosynthesis of which is targeted by fluconazole.

The fluconazole treatment protocol was optimized to assess its impact on ergosterol synthesis, followed by a lipid extraction protocol to recover lipids from the samples. The lipid profiles were then analyzed by MALDI-TOF MS, paying particular attention to variations between fluconazole-sensitive and fluconazole-resistant strains. Finally, the mass spectrometry data was subjected to various quality controls before being statistically analyzed to interpret the nuances observed in the lipid profiles.

The first results show that the use of MALDI-TOF in positive ion mode with a fluconazole concentration of 4 µg/mL and a 2-hour incubation on sensitive and resistant clinical strains (DSY294 and DSY296 respectively) reveals significant differences in peak masses between fluconazole-treated and untreated strains. These results correlate with the fluconazole sensitivity and resistance of strain DSY294 and DSY296 respectively.

Preliminary research into the application of MALDI-TOF in positive ion mode shows promising potential for rapid and cost-effective detection of fluconazole resistance in Candida albicans, a crucial element in patient management.

O002

Time-calibrated WGS-based phylogeny and mobile genetic element analyses of international Staphylococcus aureus CC398 strains revealed geographical and host-related evolution.

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Aims. To determine the geographical and evolutionary phylogeny of Staphylococcus aureus strains of clonal complex (CC) 398 as well as to identify mobile genetic elements (MGEs) carrying antimicrobial resistance and virulence genes.
Methods. The genomic data of an international collection of S. aureus CC 398 from diverse host origins spanning all continents and a period of 30 years were included. A time-calibrated phylogeny was reconstructed using BEAST2 based on a recombination-free core genome single nucleotide polymorphisms (cgSNPs) called using Snippy and Gubbins. Acquired mobile genetic elements were identified using a novel manual approach that we developed and termed “nested screening”.
Results. The time-calibrated phylogeny revealed the presence of distinct phylogroups present in Asia, North and South America and Europe. European methicillin-resistant S. aureus (MRSA) diverged from methicillin-susceptible S. aureus (MSSA) at the beginning of the 1950s. Two major European phylogroups (EP4 and EP5), which diverged approximately 1974, were identified as the main drivers of MRSA CC398 spread in Europe; specifically, an emergent lineage spreading among the European horse population (EP5-Leq), which also contains human-related strains. The major MRSA lineages EP5-Leq, characterized by staphylococcal cassette chromosome mec (SCCmec) IVa and spa type t011 (CC398-IVa-t011), and EP5-Lpg (prevalent among pigs) by CC398-SCCmecVc-t011 diverged approximately in 1996. The lineage-specific antibiotic resistance and virulence gene patterns were mostly mediated by the acquisition of MGEs such as the SCCmec, S. aureus Genomic Islands (SaGIs), prophages and transposons. Different combinations of virulence factors were identified on S. aureus pathogenicity islands (SaPIs), and novel antimicrobial resistance gene (ARG)-containing elements were detected in certain lineages expanding in Europe.
Conclusion. This WGS-based analysis revealed the actual evolutionary trajectory and epidemiological trend of the international MRSA CC398 population considering temporal, geographical and molecular factors and provided a baseline for future global WGS-based One-Health monitoring studies of the adaptive evolution of MRSA CC398 as well as for local outbreak investigations.

O003

Plasmid-driven epidemiology: unravelling carbapenem resistance transmission in hospital setting

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Aim
The dissemination of antimicrobial resistance, particularly carbapenem resistance, is predominantly attributed to plasmid transmission among bacterial cells, within the same or different species. In hospital settings, this phenomenon may contribute to outbreaks of multidrug-resistant pathogens. Unfortunately, epidemiological surveillance often focuses solely on bacteria and neglects plasmids. Long-read sequencing has made plasmid sequencing cheap, feasible, and reliable. However, the absence of a standardized plasmid typing methodology and varying accuracy levels based on study objectives remain challenges. This study aims to establish a methodology for delineating epidemiological links and plasmid transmission between strains, demonstrated through two carbapenem-resistant strain outbreaks in our hospital.

Method
The first dataset comprised 7 blaNDM-1 Klebsiella pneumoniae isolates from a single transmission cluster, while the second dataset involved 8 blaKPC-2 Serratia marcescens isolates from another transmission cluster. For both datasets, isolates originated from patients and from the environment (sinks traps). Additionally, 4 blaNDM-1 and 8 blaKPC-2 isolates (K. pneumoniae, S. marcescens and others) unrelated to these clusters and recovered during the same period were sequenced. All strains underwent Nanopore MinION sequencing with R10.4.1 chemistry, followed by assembly using Flye and resistance gene detection using ResFinder. Plasmid sequences were initially analyzed using mob-suite, and plasmid clusters were defined using MGE-cluster. These plasmids were further characterized using core SNPs analysis and accessory genome genes distance.

Results
Two plasmids were found circulating among blaNDM-1 K. pneumoniae strains and two plasmids harboring blaKPC-2 were also found among the S. marcescens outbreak isolates. At the plasmid level, both outbreaks could be extended, as corresponding plasmids were identified in isolates beyond the outbreak in genetically distant strains or in different species. Sink traps not only acted as reservoirs for pathogenic species but also housed a pool of different plasmids carrying the same antimicrobial resistances.

Conclusion
This study revealed the involvement of numerous plasmids associated with transmission clusters. This study underscores the applicability of this methodology in an epidemiological context, allowing the extension of transmission cluster definition to the plasmidic level.

O004
Eukaryote-like tubulins in a Verrucomicrobiota epibiont of ciliates

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Microtubules are composed of α and β tubulin heterodimers and are only found in eukaryotes. However, bacterial tubulins have been reported in the Prosthecobacter species (1,2) and odin tubulin in Odinarchaeota (3). Uniquely, transmission electron microscopy images have revealed bundles of eukaryote-like microtubule structures in epixenosomes, members of Verrucomicrobiota (4). Yet, it remains unknown whether epixenosomes encode tubulins that form these microtubule-like structures.
Here we report the presence of two tubulin-like genes (etubA and etubB) in the epixenosome genome which cluster with eukaryotic tubulins during phylogenetic analysis. Preliminary genome analysis suggests the absence of known tubulin chaperones necessary for the folding of eukaryotic tubulins. Nevertheless, attempts to heterologously express epixenosome tubulins (Etubs) in a bacterial system were unsuccessful. However, using a eukaryotic system, we have successfully expressed, purified and polymerised the Etubs into short curved protofilaments. Despite a wide screen of polymerisation conditions, we have not been able to reconstitute eukaryote-like microtubule structures in vitro but the Etub polymers do respond to taxol, a microtubule binding drug.
Currently, using single particle cryo electron microscopy, we are resolving a high-resolution structure of the Etub heterodimer to analyse its structure at molecular detail. Additionally, by combining cryo-focused ion beam milling and cryo electron tomography, we are developing a workflow to visualise the in situ architecture of Etubs in a near-native state.
Overall, our study shows the presence of the only reported eukaryote-like tubulins within bacteria and then aims to characterise them and any higher order structures they form both in vitro and in situ. Their level of similarity to eukaryotic tubulins suggests they are likely products of horizontal gene transfer but due to their eukaryote-like nature, it is puzzling how Etubs can be natively produced in bacteria.

O005

Enhancing Methanogenesis Performance in the PtM process: Addressing H_2 and CO_2 Intermittency

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EPFL

Aims
Power-to-Methane (PtM) technology stands at the forefront as a promising solution for the storage of surplus renewable energy aligned with CO2 reduction. In the biological PtM process, methanogens utilize H2 generated from water electrolysis powered by renewable energy and CO2 from industrial waste gas to produce CH4, namely methanogenesis. Our work aims to enhance the robustness of PtM processes by optimizing this methanogenesis step, addressing challenges related to intermittent gas feeding.
Methods
The model strain Methanococcus maripaludis MM901 was cultured in batch cultures to study its starvation-revival dynamics under H2- vs. CO2- starvation by monitoring OD600nm and specific CH4 production rate. To obtain mechanistic understanding of the observed difference, we also (1) measured NAD+ and NADH in the cells during starvation using bioluminescent assays, as well as (2) performed oxygen exposure experiments, where cultures were exposed to air for 1h during starvation followed by monitoring their revival dynamics after O2 removal.
Results
M. maripaludis MM901 exhibited a shorter lag time and higher metabolic activity after H2 starvation compared to CO2 starvation, and an increase in starvation time amplified the difference. We hypothesize that cells experience a reductive stress under CO2 starvation. We measured NAD+ and NADH in cells during starvation and observed significantly higher NADH/NAD+ ratio in CO2 starved cells indicating accumulation of reducing equivalence. O2 exposure also exerts greater inhibition on the revival of CO2-starved cultures over H2-starved cultures shown by a significantly longer lag phase.
Conclusions
In CO2-starved cells where there is H2 leftover, it is possible that O2 reacts with accumulated reducing equivalence, resulting in the production of highly oxidative reactive oxygen species, and subsequently damaged enzymes and DNA in cells. To ensure the process stability and gas product quality, where H2 leftover is preferred over CO2 leftover, further efforts are needed to adapt methanogens to starvation under CO2 limitation.

O006

Unveiling the metabolic profile of Bdellovibrio bacteriovorus during its predation cycle in Escherichia coli

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Aim: B. bacteriovorus is a predatory bacterium capable of attacking and lysing E. coli regardless of its antibiotic resistance (AMR) profile. This predator is particularly interesting for its natural ability to detect and eliminate other Gram-negative bacteria. The aim of this study is to get a holistic overview of the metabolites present and released during the predatory life cycle of B. bacteriovorus on E. coli.

Methods: B. bacteriovorus and E. coli were cultured independently, at 29°C and 37°C with continuous shaking. Subsequently a predation experiment to follow the whole predatory life cycle was initiated. The samples were taken from B. bacteriovorus or E. coli separately, before mixing, and from the interaction of the two (lysis) at regular intervals throughout a six hours timeframe. Each collected sample (pellet or supernatant) was snap-frozen in liquid nitrogen and lyophilized, pellets were additionally resuspended in 80% methanol and dried down. Finally all samples were suspended in acetonitrile for analysis by liquid chromatography–mass spectrometry (LC/MS) to identify the metabolites. An untargeted metabolomics approach was chosen to identify the broadest range of compounds.

Results: A complete overview of the metabolites in the different stages of the predation process was obtained, which allowed to reveal the major metabolic pathways involved in the predation process. A total of 953 compounds were detectable from pellet samples by LC/MS analysis, 460 of which were identifiable based on the reference databases mzVault, myCloud and ChemSpider. The results from the predation experiments showed as most abundant categories, compounds belonging to: central metabolic pathways, secondary metabolites biosynthesis, cofactors, ABC transporters, nucleotide degradation, purine and pyrimidines metabolism.

Conclusion: This study is the first investigation on the overall metabolites present during the predation cycle of B. bacteriovorus. Our investigation elucidates the landscape of essential metabolic pathways that play a crucial role in the destruction of the prey and leading to the production of novel predatory cells. Further, it is paving the way to a deeper understanding of how these specific metabolites could aid in the fight against AMR bacteria.

O007

Phage predation accelerates the spread of plasmid-encoded antibiotic resistance

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Phage play a central role in influencing the structure, functional dynamics and evolutionary trajectory of ecosystems, with significant implications in diverse areas including human health, biotechnological applications, biogeochemical cycles and environmental remediation efforts. In the field of phage therapy, phage are typically used as biological agents to target and mitigate bacterial populations that have developed resistance to conventional antibiotics. It is widely believed that predatory phage to control microbial proliferation is generally assumed to not contribute to the spread of antibiotic resistance.
However, our research challenges this assumption by providing evidence that phage predation can create conditions conducive to the spread of antibiotic resistance, particularly in scenarios involving surface-associated microbial growth. We conducted experiments utilizing two strains of Escherichia coli– one serving as a donor of plasmid-encoded antibiotic resistance genes, and the other as a potential recipient. Our findings reveal that the presence of phage predation impedes the spatial segregation of these bacterial strains. This reduced segregation effectively increases the frequency of direct cell-to-cell interactions, thereby amplifying the rate and extent of plasmid-mediated transfer of antibiotic resistance. The primary mechanism driving this phenomenon is a shift induced by phage predation in the locus of maximum bacterial growth. Under predatory pressure, the peak growth area moves from the outer edges of the bacterial biomass towards its interior. This relocation to a more confined space restricts bacterial movement, resulting in the formation of straighter interfaces between the distinct bacterial strains. These straighter interfaces are less prone to merging with adjacent interfaces, which in turn slows down the process of spatial segregation and augments the likelihood of plasmid transfer.
In conclusion, our study reveals an important consequence of phage predation in the microbial environment: it can promote the spread of antibiotic resistance. This finding is particularly important in the context of phage therapy, suggesting a potential unintended consequence that must be carefully considered in its application.

O008
Nutrient acquisition through contact-dependent antagonism between bacteria

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[1] ETH Zurich; [2] University of Rennes; [3] University of Southern California; [4] University of Basel; [5] Eawag: Swiss Federal Institute of Aquatic Science and Technology; [6] Eawag, Swiss Federal Institute of Aquatic Science and Technology and ETH Zurich; [7] ETH Zürich

In natural habitats, nutrient availability limits bacterial growth. We discovered that bacteria can acquire nutrients by lysing neighboring cells through contact-dependent antagonism. Specifically, the type VI secretion system increases the growth of antagonistic bacteria during starvation through the uptake of nutrients from lysed cells. Slow lysis, where cells leak nutrients over time before bursting, enhances the nutrient uptake by surrounding cells. The genomic adaptations in antagonists, which show a reduced metabolic gene repertoire, and the prevalence of the type VI secretion system in environmental bacteria from nutrient-limited environments like the global oceans, suggest that bacterial antagonism plays a significant role in nutrient transfer within microbial communities across various ecosystems.

O009

Historical contingencies and phage induction diversify bacterioplankton communities at the microscale

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1 Massachusetts Institute of Technology; 2 ICPT

In many natural environments, microorganisms decompose microscale resource patches made of complex organic matter. The growth and collapse of populations on these resource patches unfold within spatial ranges of a few hundred micrometers or less, making such microscale ecosystems hotspots of heterotrophic metabolism. Despite the potential importance of patch-level dynamics for the large-scale functioning of heterotrophic microbial communities, we have not yet been able to delineate the ecological processes that control natural populations at the microscale. Here, we address this challenge by characterizing the natural marine communities that assembled on over 1,000 individual microscale particles of chitin, the most abundant marine polysaccharide. Using low-template shotgun metagenomics and imaging, we find significant variation in microscale community composition despite the similarity in initial species pools across replicates. Chitin-degrading taxa that were rare in seawater established large populations on a subset of particles, resulting in a wide range of predicted chitinolytic abilities and biomass at the level of individual particles. We show, through a mathematical model, that this variability can be attributed to stochastic colonization and historical contingencies affecting the tempo of growth on particles. We find evidence that one biological process leading to such noisy growth across particles is differential predation by temperate bacteriophages of chitin-degrading strains, the keystone members of the community. Thus, initial stochasticity in assembly states on individual particles, amplified through ecological interactions, may have significant consequences for the diversity and functionality of systems of microscale patches.

O010

Toward higher-efficiency metabolic exchanges: mechanisms of maintaining spatial proximity between interacting cells in microbial communities

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The ability of bacteria to interact and exchange metabolites with one another is indispensable for their survival in changing environments. Previous studies suggest that such metabolic interactions only occur among cells within a very limited spatial range. These observations motivated us to explore mechanisms that can increase the spatial proximity of interacting cells, and, in this presentation, we will highlight two such critical mechanisms. First, spatial proximity can be maintained by direct cell-cell connection. We found that cells of dimorphic prosthecate bacteria (DPBs) use their stalks to establish direct connections to the cells of diverse 'host' organisms, including bacteria, archaea, eukaryotic algae, and fungi. Quantitative analyses through microfluidic experiments and nanoscale secondary ion mass spectrometry (nanoSIMS) indicated that direct cell-cell connections between DPB and 'host' cells facilitate metabolic exchanges, consequently improving the growth of the DPB cells. Second, cell motility could improve the spatial proximity of different bacterial populations. To test this hypothesis, we combined individual-based modeling and microfluidic experiments using synthetic consortia composed of motile or non-motile Pseudomonas aeruginosa strains that exchange metabolites for growth. We found that cell motility reduced the clustering of cells from the same lineage, fostering improved spatial proximity between interacting strains and thus facilitating the growth of the consortium. In summary, our findings shed light on the crucial roles of direct cell-cell connection and active cell motility in improving spatial proximity and therefore the metabolic exchange of interacting cells. These mechanisms offer novel perspectives into the survival strategies of microorganisms in ever-changing environments.

O011

Do metabolic interactions explain spatial patterns of dual-species microbial communities?

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Microbes often live in surface-attached formations known as biofilms, where they form complex spatial patterns. Once established, these patterns entirely govern a cell's local ecology, impacting its growth and evolution. A large body of literature addresses how metabolic interactions affect spatial patterns in microbial biofilms. However, most of these studies used strains of the same species that are identical in every aspect except for single mutations in a metabolic pathway. In natural, multispecies communities, patterns could also be affected by non-metabolic properties of species, such as their shape, friction with the surface, or motility, that could play a relevant role in the pattern formation process. Our aim is to understand to what extent we can understand spatial patterns in dual species biofilms from the knowledge of their metabolic interactions. We grew two-species colonies of Ochrobactrum anthropi (Oa) together with one of a collection of 28 bacterial interaction partners. Oa is auxotrophic for an intermediate of the Thiamine synthesis pathway. Therefore, if Thiamine is absent from the media, Oa can grow only if it is fed Thiamine (or a precursor) by the partner species. We typically observed that the partner species facilitates Oa if Thiamine is absent from the media and competes with it when Thiamine is added. We also observed that Thiamine cross feeding generally increases mixing of the two species, however, the absolute degree of mixing strongly differs between species pairs. We are currently investigating which features of the patterns can be predicted by knowing the metabolic interactions (cross-feeding or competition) as well as which other effects are important to explain them. We hope that our results will contribute to finding general principles for the process of spatial pattern formation.

O012

A Paramyxovirus V Protein Enigma: Suppression of a Host Transposable Element-mediated Antiviral Response?

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Tripartite Motif Protein 28 (TRIM28) is a key host transcriptional regulatory protein and repressor of transposable elements (TEs). Previous research implicated influenza A virus (IAV) infection-induced loss of SUMO-modified TRIM28 in de-repression of immunostimulatory TEs and the subsequent augmentation of host antiviral immunity. We therefore hypothesised that other viruses may have evolved antagonistic mechanisms to control and counteract the TE-derived Antiviral Response (TEAR) if it is indeed a broadly important component of host defences. In this regard, a high-throughput proteomics screen suggested that the Measles virus innate immune antagonist protein, V, interacts with TRIM28, but the significance of this interaction and its function have yet to be explored.

Here, we show that the V proteins from 11 different clinically important paramyxoviruses, including Parainfluenza, Measles, and Nipah viruses, all interact with human TRIM28 in co-immunoprecipitation assays. Remarkably, TRIM28 co-precipitation efficiency with V differs between viruses, and using a chimeric protein approach we could map this property to the unique C-terminal domain of V, and ultimately to a single residue. At a functional level, using Parainfluenza viruses 2 and 5 (PIV2, PIV5) as model systems, we could also show that paramyxovirus infection induces the stress-response activated phosphorylation of TRIM28, similar to IAV infection. However, subsequent phospho-regulated loss of SUMO-modified TRIM28 only occurs with IAV infection, while SUMO-modified TRIM28 is maintained during paramyxovirus infection. The precise mechanism is unknown, but we could show that V proteins do not block deSUMOylation of TRIM28 caused by host SUMO-proteases (SENPs) or by XAF1, a factor recently linked to TRIM28-associated immune responses. Nevertheless, while IAV infection induces TE expression during infection, TE induction during paramyxovirus infection is minimal.

Given that viral co-infections can lead to more severe disease, we are currently assessing the interplay between parainfluenza viruses and IAVs with respect to the TEAR response and viral replication. The aim is to uncover whether potential suppression of TE-induction by co-infecting parainfluenza viruses might modulate the TEAR response to IAV and thereby impact pathogenicity.

Taken together, our results substantiate the emerging concept of the TEAR pathway, and highlight the first description of a potential viral antagonistic strategy.

O013
Investigating the role of (stress) granules during virus infections

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Stress granules (SGs) are membraneless accumulations of ribonucleoproteins which form in the cytosol of eukaryotic cells upon exposure to a variety of stress stimuli. These granules are proposed to store untranslated mRNA during stress and support the dynamic reprogramming of translation towards stress resolving pathways. Growing evidence of many viruses specifically interfering with SG formation, and the identification of antiviral sensor and effector proteins in SGs suggests an involvement of these condensates in the antiviral host response. However, the characteristics of virus-induced SGs are poorly understood.
Our lab previously showed that SGs induced by the model coronavirus mouse hepatitis virus (MHV) exhibit a changed composition compared to canonical SG. To assess whether these differences are common for virus-induced SGs or rather virus-specific, we extended the characterization of virus-induced SGs to the Semliki Forest virus (SFV), serving as a reference. Microscopy-based investigations, including live-cell imaging, indirect immunofluorescence, and single molecule RNA fluorescence in-situ hybridization, revealed differences in SG dynamics during infection and the abundance of specific SG components between MHV and SFV. To comprehensively compare the SG protein composition, we further applied proximity labelling and quantitative proteomics at different time points of infection. These techniques provided insight into the SG proteome and the temporally resolved microenvironment of our SG bait protein revealing profound differences in the SG protein composition between MHV and SFV. Our results showed a reduced connection of MHV-induced SGs to canonical SG themes when compared to SFV-induced ones. As this observation is similar to our previous comparison between MHV-induced and canonical stress granules, the question arises if the firmer are SGs at all. Taken together, our findings revealed different aspects of these virus-induced granules to be strongly virus-specific, which might reflect different roles of the condensates during infection.
Our SG proteome screen provides a base for further investigations into the role of SGs during virus infection and the interplay between these condensates and different viruses.

O014

Population genetics and molecular epidemiology of wheat powdery mildew in Europe

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University of Zurich

To control pathogens, modern agriculture relies on pesticide treatments and breeding of resistant crop varieties. However, pesticides can be harmful to the environment and to human health, and pathogen populations can quickly develop resistance to pesticides and gain virulence on previously resistant varieties. Large scale real-time population genomics and molecular epidemiology studies can improve our understanding of pathogens epidemics and contribute to the development of novel control strategies. While these approaches are commonly used for research, diagnostic and monitoring of human infectious diseases, they are rarely and only partially applied to agricultural pathogens.

In this project we sampled the wheat pathogen Blumeria graminis f.sp. tritici for two consecutive years in Europe and in the Mediterranean region. We used whole genome sequencing to study its population genetics and molecular epidemiology. Overall we assembled a data-set of 416 samples collected from 20 different countries. We found that wheat powdery mildew does not constitute a single panmictic population over the studied region, with the major differences between Northern Europe, Southern Europe and the Middle East. The population in Northern Europe stood out because of it homogeneity over a lager geographic region, which was caused by higher rates of gene flow. We found that both geographic distance and climate shaped the genetic diversity of the pathogen and that the adaptation to tetraploid or hexaploid wheat might also play a role in the differentiation of different populations. In addition, genome scans for selection revealed that different fungicide targets and avirulence loci have been selected in different regions, reflecting heterogeneous agricultural practices and breeding programs. Finally, we investigated the spatio-temporal dynamics of the epidemic, including the direction and magnitude of dispersal, and the evolution and spread of mutations conferring resistance to fungicides or virulence onto wheat varieties containing resistance genes.

O015
The use of biological soil monitoring data to track fungal plant pathogenic taxa

KE Sullam; F Gschwend; C Panzetti; J Enkerli; F Widmer

Agroscope

Aims
High throughput sequencing (HTS) has revolutionized biodiversity monitoring, enabling extensive data collection on various fungal genera without targeted sampling. Our aim was to test how monitoring data can be used to track soil-borne plant pathogenic taxa, gain information about their spatial and temporal trends, and identify the abiotic and biotic factors that may influence their distributions.

Methods
We compiled data on eight fungal genera (i.e., Armillaria, Fusarium, Gaeumannomyces, Heterbasidion, Paraphoma, Rhizoctonia, Sclerotinia, and Verticillium) that were selected because they include soil-borne plant pathogenic fungi with broad host ranges found in Switzerland. We investigated their occurrence at 30 sites included in the Swiss Soil Monitoring Network using amplicon sequence variants (ASVs) obtained from ITS-based metabarcoding. Samples were collected yearly (2012 to 2016) from ten sites of the land use types (LUTs) forest, permanent grassland, and arable land. The ASVs were analyzed in relation to their geographic location and LUT-preference. Category discrimination analyses were used to explore the associations among the presence of these taxa and the abiotic and biotic factors at each site.

Results
The eight selected pathogenic taxa included 2 to 110 ASVs, of which 44% displayed a widespread distribution and appeared in more than one LUT. Most of the ASVs shared among LUTs (65%) were found in both arable and grassland sites. Fusarium, Rhizoctonia, and Paraphoma were relatively more abundant at arable and grassland sites, and Verticillium was only found at arable and grassland sites. These genera showed negative associations with soil organic carbon, total carbon, and C:N ratio, but a positive association with bulk density. Armillaria was found exclusively in forest sites and showed the opposite trends in its associations with soil nutrients and density.

Conclusion
These analyses highlight how biodiversity monitoring can contribute information on plant pathogen ecology, including the habitat breadth and specificity of the selected genera. The spatial and temporal patterns of potentially pathogenic taxa derived from HTS analyses could aid in more targeted studies, and ultimately, contribute to the development and implementation of sustainable control strategies.

O016
Volatile-mediated interaction between plant-associated beneficial microorganisms and phytopathogenic fungi

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UNI FRIBOURG

Many beneficial microorganisms contribute to plant resistance towards biotic and abiotic stresses. Recently, there has been growing evidence that plants are protected from diseases by their microbiome and the volatile organic compounds (VOCs) they emit could have a major role in this process.
Emission of VOCs is an important means of communication among microorganisms. These volatiles have various effects, they contribute to the stabilization of microbial communities, they can attract or repel different species, promote growth or display antimicrobial properties. Recently, several pieces of evidence have shown that the volatilome emitted by a microorganism depends on the volatiles of its surrounding. Thus, the volatilome of two different microbes grown together is different from the sum of the two individuals grown separately. These differences may include inhibition and promotion of various compounds, and the production of new compounds. However, the set up used so far to highlight these kinds of interactions has several drawbacks, including artificial volatile overaccumulation, potential oxygen limitation and the impossibility to assign a producer to the compounds newly emitted during the interaction.
To solve this problem, we have developed a solution enabling us to trap the whole volatilome of an organism and to use it to expose another one unilaterally. By combining this method with comparative Gas Chromatographic–Mass Spectrometry (GC-MS), it is thus possible to study the volatile-mediated interactions more precisely by identifying more easily the compounds responsible for the changes in the volatilome and the emitter of any newly produced compound.
We have used this new procedure to study volatile-mediated interactions between the biocontrol fungus Trichoderma simmonsii and the two plant pathogens Botrytis cinerea and Fusarium oxysporum. Our results show that the perception of each pathogen's volatilome triggered a specific response, resulting in significant changes in the VOCs emitted by Trichoderma. Trichoderma's volatilome modulation was overall stronger when exposed to the VOCs from Fusarium than to the VOCs from Botrytis, which correlated with increased siderophore production when co-incubated with this fungus. Our newly developed method will not only help to better understand volatile-mediated interactions in microbes but also to identify new molecules of interest that are induced by VOCs, as well as the putative inducing signals themselves.

O017
Emergence of OXA-23-producing Proteus mirabilis in Switzerland

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Aims
Proteus mirabilis are Gram-negative bacteria that are found in the environment and also forms part of the commensal flora in the gastrointestinal tract of both humans and animals. This species does not harbour any intrinsic β-lactamase genes, explaining the natural full susceptibility to β-lactams. OXA-23 enzymes are carbapenem-hydrolyzing class D β-lactamases originating from Acinetobacter radioresistens and are the main source of acquired resistance to carbapenems in Acinetobacter baumannii but are rarely reported in Enterobacterales. Here, we report the emergence of OXA-23-producing P. mirabilis clinical isolates in Switzerland.
Methods
Between 2018 and 2023, nine non-duplicate P. mirabilis isolates were submitted to the Swiss National Reference Center for Emerging Antibiotic Resistance for the investigation of antimicrobial resistance. Susceptibility testing was performed by disk testing and PCR analyses were used to detect the OXA-23 allele. Whole genome sequencing (WGS) was performed using the Illumina platform.
Results
Isolates were submitted from 6 laboratories, across six Swiss cantons, and all were obtained from urines. All isolates exhibited a similar phenotype with resistance to amoxicillin, amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanic acid, and exhibited intermediate susceptibility to temocillin. WGS analyses showed that the isolates could be divided into two groups; group 1 (n=7) had snp differences ranging from 2-41 snps, and the isolates in group 2 (n=2) differed by just a single snp. Unsurprisingly, 3/4 isolates submitted from a single hospital differed by ≤6 snps, suggesting a dominant clone in this hospital environment. The blaOXA-23 gene was found to be located within a 41.2 kb genomic island, flanked by the IS15DII IS6-family transposon. Similarly that observed isolates from France, the blaOXA-23 gene was located within a transposon, Tn6704, which also contained the ISAba-type insertion sequences, ISAba1, ISAba125 and ISAba14.
Conclusions
This study identified the emergence of two closely related clones in Switzerland which were closely related to that previously identified in France, suggesting cross-border dissemination of OXA-23-producing P. mirabilis. The increasing identification of OXA-23-positive P. mirabilis in Switzerland is concerning since these strains may serve as a reservoir to aid the silent dissemination of the blaOXA-23 gene in other bacterial species.

O018

Rapid detection of expanded-spectrum β-lactamase and carbapenemase producers in Enterobacterales : The Rapid Combo ESBL/Carba NP test

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Aims. The increase of multidrug resistance in Enterobacterales constitutes a major global health threat. Acquired resistance to broad spectrum □-lactams is dominated by the emergence of broad-spectrum ß-lactamases (ESBLs) and carbapenemases. The rapid detection of these major antibiotic resistance mechanisms is critical to ensure a successful clinical outcome and to prevent their dissemination. Here, we developed a rapid biochemical test for a concomittant identification of ESBLs and carbapenemases in Enterobacterales.

Method. The rapid Combo ESBL/Carba NP test is a colorimetric test based on citrate-capped gold nanoparticles (AuNPs) aggregation in the presence of acid products resulting from cefotaxime or imipenem hydrolysis. The ESBL and the carbapenemase activity were evidenced by a color change of the AuNPs solution from red to purple, blue, or green in the presence of cefotaxime (reversed by tazobactam) and imipenem, respectively. A total of 76 characterized clinical enterobacterial isolates of worldwide origin were used to evaluate the test performance, among which 22 carbapenemase producers, 7 carbapenemase + ESBL, 29 ESBL, 3 ESBL + cephalosporinase, 8 cephalosporinase, 4 penicillinase and 3 wild type isolates.

Results. The sensitivity and specificity were found to be respectively 98.4% (95% CI 91.4% - 99.7%) and 92.9% (95% CI 68.5% - 98.7%). Of note, none of the cephalosporinase producers was detected as ESBL producers. All results were obtained within 60 minutes, with all ESBL detection within 30 minutes.

Conclusion. The rapid Combo ESBL/Carba NP test is rapid, highly sensitive, specific, easily interpretable, and easy to implement in routine. This is the first rapid phenotypic test providing a combined detection of all types of ESBL and carbapenemases, regardless of the gene types.

O019
Induction, characterization, and resuscitation of viable but non-culturable Legionella pneumophila

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Numerous bacteria, including the opportunistic human pathogen Legionella pneumophila, respond to various stresses by entering a dormant state known as "viable but non-culturable" (VBNC). In this state, the cells display viability markers but cannot be grown on standard laboratory media, rendering them undetectable by conventional diagnostic tools reliant on cultivation.

L. pneumophila is a widespread bacterium found in fresh water sources, both natural and artificial, where it can infect and replicate inside amoeba and other protozoa. Utilizing mechanisms similar to those used in amoeba, L. pneumophila can infect and destroy human macrophages. Upon inhalation of pathogen-laden aerosols, L. pneumophila replicates within alveolar macrophages, leading to a severe pneumonia known as Legionnaires' disease.

This project investigates the induction of VBNC L. pneumophila through heat stress and their “resuscitation” upon encountering different amoeba species. Incubation of L. pneumophila at 60°C, 55°C, or 50°C for 30 min, 5 h, or 30 h, respectively, caused a complete loss of culturability. We found that this treatment rapidly inactivated the Icm/Dot type 4 secretion system (T4SS), and the inactivation prevented the resuscitation of heat-induced VBNC L. pneumophila by amoeba. Neither the formation of a Legionella-containing vacuole (LCV) nor intracellular replication was detected in A. castellanii, A. polyphaga or D. discoideum amoeba upon infection with VBNC L. pneumophila. Intriguingly, already the uptake rate of VBNC L. pneumophila was severely diminished in amoeba. Current research aims at characterizing bacterial and host factors involved in the VBNC state, with an emphasis on the pivotal role of ribosomes during dormancy/hibernation.

O020
Biochemical and structural characterization of Mycobacterium tuberculosis Rv2783c (GpsI) as novel drug target

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Aim: Whole-cell screening of a comprehensive chemical library containing 400’000 compounds for anti-mycobacterial activity revealed 9 promising drug lead candidates (1). The compound, abbreviated here as X1, piqued our interest, due to its effectiveness in the low µM range against internalized M. tuberculosis (MTB), without exhibiting any host cell toxicity. WGS analysis of spontaneous compound X1 resistant Mtb showed three independent SNP in gpsI a gene involved in mRNA metabolism. We aim to confirm gpsI as target of X1 and investigate its mode of action and mode of resistance.
Methods: We used biochemical methods to investigate the target interaction of X1. We cloned wild type gpsI and mutated gpsI in expression vectors and purified it. Poly(A) degradation and synthesis in presence and absence of compound was monitored in real time using Thioflavin T as RNA probe. Reaction parameters were assessed by Michaelis-Menten kinetic and bactericidal properties of X1 were determined by dose and time-dependent kill curves. Further, drug target interaction was investigated by Cryo-EM.
Results: Recombinant Mtb GpsI showed PNPase activity (RNA degradation and Synthesis). In presence of 4 µM compound X1, enzymatic activity was completely abolished. Titration of X1 showed dose dependant inhibition, half maximal inhibition was reached at 2 µM. Michaelis Menten Kinetic revealed that Vmax but not Km value was reduced, pointing towards a non-competitive inhibitor. Mutant enzymes showed complete and partial X1 resistance profiles. Bactericidal activity of X1 against MTB was investigated, revealing that X1 kills 99% of MTB bacilli within 48 hours at concentration of 4.4 mg/L. The homotrimeric core structure of GpsI-X1 complex was resolved by Cryo-EM at resolution of 2A. X1 binds to the phosphate binding site, forming two H-bonds and several weak interactions with GpsI.
Conclusion: GpsI is a functional PNPase. Compound X1 targets and blocks GpsI activity in a dose dependant matter. Mutant enzymes show similar in vitro activity as wt GpsI and exhibit different resilience profile (half to full tolerant) to inhibitor X1. X1 possesses potent bactericidal properties, killing 99% of bacteria within 3 days. X1 binds to the phosphate binding site, stabilizes RNA and thereby blocks GpsI activity.

O021

Enhancing therapy of Pseudomonas aeruginosa in urinary tract infections with combinatorial anti-biofilm approaches in human bladder microtissues

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Urinary tract infections (UTI) are one of the most common human acquired and recurring infections. With a recurrence rate of 30% within the first 6 months, UTIs lead to high antibiotic prescription, which in turn exacerbates the current antimicrobial resistant crisis.
Existing research models investigating UTI are murine in vivo models or in vitro models based on cancer cell lines. These do not accurately reflect many of the human bladder's features including physiology, immunity and the tissue's structure, making it difficult to effectively develop new adequate therapies and study the conditions that uropathogens face.
Here, we employ the newly developed 3D-UHU microtissue model (PMID:37939183), recapitulating key human bladder features, including full stratification into 7 cell layers, differentiation into three urothelial cell subtypes and urine tolerance. This model allowed us to test the bladder infection strategies employed by uropathogenic Pseudomonas aeruginosa (PA). We showed that PA preferentially forms biofilms in this human-like microenvironment. Aiming at classifying the heterogeneity underlying PA biofilms, we monitored their ability to form biofilms, while infecting our bladder model with 8 strains isolated from the University Hospital Basel. Additionally, we are investigating the effectiveness of two novel anti-biofilm molecules in combination with standard-of-care antibiotics to clear PA infection. Preliminary tests at high concentrations of the anti-biofilm agents (100µM) have proven a strong effect in biofilm disruption.
Overall, this combinatorial approach will pave the way for a better understanding of P. aeruginosa pathophysiology in UTIs, as well as for the development of a more effective course of treatment.

O022

Uncovering Cyanobacterial Growth Drivers through State-Dependent Dynamics Reconstruction using High-Frequency observational data

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Understanding complex systems dynamics entails the discerning of the underlying mechanisms governing interactions between species and with their environment. Here, we aim to infer the mechanisms that drive community dynamics using high-frequency time series data and an established equation-free modelling framework that addresses the complexity of high-dimensional interconnected systems. To reach this goal we are using a natural planktonic community as our study system and focus on understanding how changing interactions are linked to the emergence of cyanobacterial blooms. We conduct in situ investigations of cyanobacteria and their growth rate within the plankton community of primary producers and consumers, utilizing daily observational data spanning 5 years. Our focus lies on identifying the key drivers governing cyanobacteria growth rates, comparing responses across four different blooming taxa and study how interactions affect bloom initiation. To accomplish that we analyze daily cyanobacterial abundance data from Lake Greifensee, a eutrophic Swiss lake, calculate growth rates (cell divisions minus losses), and reconstruct the attractor manifolds of the system to estimate the magnitude and sign of interactions between species and biotic / abiotic factors in state space using locally weighted multivariate linear regressions. These factors include nutrient supply, physical parameters of the water column, meteorological conditions, grazer abundance, and competing phytoplankton densities, all recorded in situ. Our analysis reveals the complex interactions of biotic and abiotic environmental factors that facilitate cyanobacterial blooming. This study highlights a knowledge gap: the importance of biotic interactions (e.g. grazing, competition, facilitation) in cyanobacterial bloom initiation.

O023

Dispersal shapes compositional and functional diversity in aquatic microbial communities

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Dispersal processes shape aquatic microbial communities, but their respective role is difficult to disentangle in field studies. While dispersal limitation enhances colonization by random taxa, the dissimilarity among newly formed communities is reduced by high dispersal rates (mass effect). By contrast, the subsequent development of such communities will mainly be determined by biotic interactions. To disentangle the importance of these factors, we studied compositional and functional changes of freshwater bacteria in 20 parallel experimental microcosm communities during contrasting dispersal regimes. The Elo-rating, an index used in competitive chess was applied to assess the success of bacterial taxa during the incubations. Dispersal limitation generated high beta diversity and the emergence of replicate community ‘types’ dominated by Acidovorax, Pseudomonas, or Aeromonas genera. Compositional stability and evenness of these types varied over subsequent growth cycles, reflecting differences in functional properties. Overall, carbon use efficiency increased during cultivation, with some communities of unique composition outperforming the dominant ‘types’. Homogenizing dispersal led to high compositional similarity and reduced gamma diversity. While neutral and competitive models (Elo-rating) together largely explained community assembly, Pseudomonas outperformed predictions, possibly due to exclusive genomic traits. Thus, high beta-diversity caused by initial dispersal limitation can subsequently be ‘purified’ into community types by deterministic processes. However, dispersal limitation may represent an insurance for highly productive microbial taxa that are competitively excluded during community coalescence.

O024

Microbes 4 future: how can we use algae-bacteria co-metabolism to help feed and fuel the world?

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Farming of phytoplankton (both eukaryotic microalgae and cyanobacteria) has the potential to revolutionize agriculture. Algae fix carbon into proteins, lipids, and other biopolymers at faster rates than any other autotrophs on the planet. The ancient association between algae and heterotrophic bacteria is strong yet nuanced, and its implications are only beginning to be understood. Despite the ubiquity of algae-bacteria interactions, commercial scale cultivation of algae and bacteria has tended to focus on each group in isolation. My work at Eawag makes links between algal taxonomy, algal exometabolites, and bacterial communities, with the goal of making fundamental insights that can inform industrial cultivation of microbes for sustainable bioproducts. I will outline ongoing work to characterize algal exometabolomes using a broad high-throughput screening, as well as our exploratory work to characterize the microbiomes of these 84 algae strains under investigation and a targeted survey for genes related to bioplastic production by microbes. A more holistic overview of how mineralized carbon flows into algal and bacterial biomass will help us more effectively and economically grow such microbes at the massive scales required to address pressing issues at the climate-energy-water nexus.

O025

The Drivers of High Arctic Tundra Soil Microbial Communities: A Biogeography Study in Light of Arctic Greening

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The Arctic has been warming at an unprecedented rate. In response, Arctic plants are experiencing prolonged growth seasons and higher productivity. On the global scale, the plant response to these changes is commonly referred to as “Arctic Greening” but above ground changes are not homogeneous. While soil microorganisms are known to play an important role in many ecosystems, their connections to the above ground vegetation in the greening Arctic is largely unknown. To evaluate the role that microorganisms play in Arctic Greening, we sampled and analysed different sites on Svalbard. These sample sites consist of (i) native tundra soils that are characterized by low nutrient input, (ii) soils around bird cliffs that are amended with nutrients such as nitrogen by bird droppings, and (iii) soils with influence and disturbance from humans and agriculture. 31 topsoil samples from the three site types were sequenced for 16S rRNA and a quantitative PCR of 16S rRNA and ITS was performed. This study shows that these three high Arctic tundra ecosystems have specific impacts on the soil microbial communities. Anthropogenic disturbance leads to a microbial community with more cells, that is lower in diversity and that has a very steep distance decay of community similarity. This is due to a few dominant groups such as Clostridia and Bacteroidia and the phylum of Desulfobacterota profiting from high nutrient input and outcompeting other community members. Soils surrounding bird cliffs have a higher abundance of fungi over procaryotes, a significantly more diverse soil community and very slow distance decay. This is likely due to less selective pressures and more dispersing effects caused by the spread bird droppings, the large water flow in the beginning of the growing season and potentially by fungi. Important biological above ground associations with community composition are the graminoids that are strong drivers of greening observed in sites (ii) and (iii) and the dwarf shrubs that were dominant in (i). Below ground parameters affected by climate change such as the soil moisture and deep soil temperature also associated with the communities, while signs of exogenous input such as total nitrogen, total organic carbon and manganese correlated as well. A more comprehensive understanding of the biogeographical processes that influence these microbes is crucial for clarifying their role in Arctic greening and predicting how this will evolve with climate change.

O026
Investigating coronavirus recombination and the role of the replicase-transcriptase complex

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1. Introduction
Coronaviruses are a family of enveloped spherical viruses, with a monopartite linear ssRNA+ genome. They have been described to cause from mild to lethal infections in mammals and birds, and more recently have been put at the forefront of global attention after the COVID-19 pandemic. Mouse Hepatitis Virus (MHV), similar to SARS-CoV and MERS-CoV, is classified within the Betacoronavirus genus, thus it provides a model system to study coronavirus tropism, pathogenicity and lifecycle.

2. Aims
This project is focused on studying coronavirus recombination, a process thought to be a driver of coronaviruses spillover, emergence and evolution. Despite its important role, it is poorly understood. Additionally, we want to investigate the Replicase-Transcriptase Complex (RTC) organization and environment.

3. Materials & methods
We are studying coronavirus recombination via two different strategies. First, we produced fluorescent-labelled viruses by TAR cloning technology to visualize their localization within the infected cells. We plan to combine this method with High Resolution Microscopy to enhance the visualization.
And second, by Cryo Electron Microscopy (CryoEM) we plan to investigate what happens in the cells upon coinfection with two different viruses.

4. Results
Preliminary data has shown overlap of fluorescence signal from two differently labelled viruses in the same areas of the cell, thus suggesting viral replication of two different viruses in close proximity. Currently, we are at the stage of optimizing the methods to combine these results with High Resolution Microscopy. Additionally, to improve our resolution, with CryoEM we are working on obtaining tomograms of the Double Membrane Vesicles (DMVs) in single infection and coinfection settings during a MHV infection.

5. Conclusion
The results of this project will shed light on coronavirus co-infection and recombination. Understanding this complex mechanism is crucial for deepening our knowledge and understanding on virus infections, as well as developing diagnostic methods and therapeutics for both humans and animals for future pandemics prevention.

O027

Investigating the role of lake microbial enzymes on virus inactivation using an open culture system

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Enteroviruses are a group of single-stranded RNA virus that are associated with multiple diseases in humans and animals. Their transmission relies on their ability to persist in the environment. In aquatic environments, multiple abiotic and biotic factors contribute to viral inactivation. While abiotic factors such as temperature, pH and UV-light exposure have been studied, biotic factors remain uncharacterized. Our lab has previously shown that lake water microbial communities reduce the infectivity of Echovirus-11 (E11) and Coxsackievirus-A9 (CVA9), and that this reduction is associated with the production of bacterial proteases. Here, we aim to further characterize these bacterial communities and their role in viral inactivation. We have developed a pipeline to isolate and grow bacterial communities from Lake Geneva using chemostats. Chemostats are open culture systems with a continuous inflow of nutrients and removal of microbial metabolic waste. This culture system allows bacterial cultures to be maintained in exponential growth phase for an extended period of time. Once the bacterial communities are established, we will characterize their viral inactivation capacity, and their community structure and metabolism. To characterize bacterial capacity to inactivate enteroviruses, E11 and CVA9 will be inoculated into the chemostat communities and viral infectivity will be monitored over time. In addition, to characterize our lake communities and their metabolism, a combination of “-omics” and biochemical approaches will be used. Metagenomics will be used to infer the taxonomic profiles of the communities, metatranscriptomics their gene expression profiles, and metabolomics their metabolite production. Data analysis will be focused on identifying bacterial taxa and metabolic processes responsible for viral inactivation in the chemostat communities. All in all, our research provides a foundation to better understand the fate of enteroviruses in the environment and their interaction with environmental microbial communities.

O028
Lignocellulosic wastes degradation for biopolymer biosynthesis

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HES-SO Valais-Wallis

Aims: Lignocellulosic biomass and its associated wastes are available sources of carbon and energy for microorganisms. These materials are however complex to be degraded into useful molecules, requiring physical or chemical pre-treatments before an enzymatic treatment. After a final detoxification step, the generated building blocks (glucose, xylose, etc.) may become suitable substrates for microorganisms to produce added-value products. Here a microorganism, Trichoderma reesei was coupled with at least another microorganism strain to produce an intracellular biopolymer called poly(3-hydroxyalkanoate) (PHA) using solely cardboard as substrate.

Methods: A minimal medium was designed for supporting the growth of T. reesei and the following strains: Pseudomonas putida, Wickeramomyces anomalus, and an isolate from a composting unit. Cardboard was used as only carbon and energy source. Shake flasks tests were conducted for each microorganism alone and for combinations of microorganisms (synthetic consortia). A benchtop bioreactor culture was conducted with T. reesei and P. putida for evaluating the feasibility of such a process at the larger scale.

Results: Shake flask tests showed growth for all cultures in presence of T. reesei and no growth in its absence, showing that its presence was essential for usage of carbon from the cardboard. P. putida accumulated low amounts of PHA (4.18 wt% of the total dry cell weight). Enzymatic tests evidenced the presence of several enzymes, endo- and exocellulases, beta-glucosidase, and endoxylanase, in the medium. In the bioreactor, T. reesei grew well ($\mu = 0.1$ h-1), but P. putida was not able to grow or to significantly accumulate PHA, probably due to an unsuited pH. The yield Y(X/C) for T. reesei was determined to be 0.33 g/g.

Conclusion: A synthetic consortium based on two microorganisms, a medium, and growth conditions were designed and evaluated for use of lignocellulosic materials such as cardboard to produce PHA. The product was indeed obtained in a reproducible manner, indicating the feasibility of the proposed concept. Based on these preliminary results, further efforts will be conducted on the optimization of the PHA productivity. This involves a careful balance bewteen the growth of T. reesei and the PHA production.

O029

Genetic structure and long-term genetic diversity assessment of Burgundy truffle populations in central Europe

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WSL Birmensdorf

The Burgundy truffle is an abundant ectomycorrhizal symbiont that occurs in a wide range of temperate climates. Despite its prized underground ascocarps, its complex lifecycle and possible response to anthropogenic climate change are largely known.We ran a citizen science monitoring of Burgundy truffle ascocarp production with a 3-week resolution in 23 natural populations at the center of its European distribution over up to 11 years. We genotyped more than 3000 truffles using microsatellite markers to assess the genetic structure and diversity of these populations in space and time. Preliminary results support well-differentiated genetic groups on a small spatial scale and throughout the study area, with often low levels of individual admixture. Within populations, genotypes from different genetic groups co-occurred, while only a few perennial genotypes dominated, producing most of the ascocarps. Further analysis indicate that genetic diversity was generally low in most populations and fluctuated over time with unpredictable trend. Our data suggest that among-population gene flow in Burgundy truffles is limited, even if they grow only a few kilometers apart. This restricted dispersal ability could prevent the northward migration of advantageous alleles from more heat- and drought-tolerant southern populations.
Conclusion – Given that ascocarp production in truffle populations is highly sensitive to dry conditions, our data call for genomic studies on local adaptation of this valuable ectomycorrhizal fungus, which could guide the use of well-adapted provenances for truffle cultivation and restoration.

O030

Importance of drought conditions in structuring root fungal communities of healthy and decaying juvenile beech trees

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Aims
Soil fungal communiUes associated with trees play an essenUal role in forest ecosystems by
providing water, nutrients, and protecUon against bioUc and abioUc stress. However, it
remains unclear how these communiUes respond to drought stress when associated with their
host forest tree species.

Methods
At six sites across Switzerland severely affected by drought condiUons, following a water stress
gradient, and well characterized environmentally over the past 10 years, we tracked the fungal
communiUes associated with healthy and decaying beech root systems using DNA metabarcoding.

Results
Fungal richness and diversity correlated posiUvely with an increasing water stress and slightlybetween beech health status. Fungal communiUes were dominated by ectomycorrhizal (EcM)and saprotrophic fungi, and their relaUve abundance was altered by beech health status butnot by drought. The EcM fungi Lactarius was significantly associated with drier sites and healthy beech root systems, while saprotrophic fungi were mainly associated with decaying beech. Fungal community assemblies were more determinisUc in decaying beech than in healthy beech root systems. Specifically, the stochasUcity of fungal communiUes' assembly
increased with a rising water stress only for healthy beech, and was mainly driven by saprotrophs.

Conclusions
Drought-related condiUons rather than other environmental parameters and health status of the beech trees were the major drivers of fungal communiUes, even though their assembly processes differed between fungal funcUonal traits. This study highlights the importance of drought condiUons for the assembly of fungal communiUes. It also points to an interplay between fungal funcUonal traits and host health status when facing environmental constraints.

Key words
Water stress, fungal community, stochasUcity, EcM, Lactarius

O031

First complete genome sequence of an Enterococcus devriesei isolated from Zophobas morio larvae

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Aims

Only five genomes of E. devriesei strains from different human, animal, and environmental sources are available at the contig or scaffold levels on the NCBI database. Here, we describe the first complete genome of an E. devriesei from larvae.

Methods

E. devriesei strain Ed-CK-24 was isolated from homogenized tissues of Z. morio larvae, which we have been using to establish a new in vivo model of gut colonization (https://data.snf.ch/grants/grant/206400, https://doi.org/10.3389/fmicb.2024.1381051). Species identification (ID) was achieved using the MALDI-TOF MS. The strain was sequenced and assembled using a combined short-read (Illumina) and long-read (Nanopore) sequencing approach to obtain the complete de novo genome. Species ID was confirmed using JSpeciesWS and OrthoANIu software. Antimicrobial susceptibility tests (ASTs) were performed using a microdilution MIC system and antimicrobial resistance genes (ARGs) were identified with AMRFinder and CARD-RGI. Phylogenetic analyses based on the 16S rRNA gene sequence and a core-genome alignment were also performed.

Results

The genome of Ed-CK-24 consisted of one circular chromosome (2,777,134-bp), one circular megaplasmid (633,497-bp) and one circular replicon-type plasmid (57,656-bp). The average coverage was 761.6x and the GC content 40.2%. Ed-CK-24 showed 97.4% (JSpeciesWS) and 97.9% (OrthoANIu) similarity with the E. devriesei type strain DSM22802 (Bovine, Belgium, 2016; GenBank: GCA_001885905.1). 16S rRNA phylogenetic analyses revealed that Ed-CK-24 shared 100% homology with other deposited E. devriesei strains. In addition, core-genome analysis revealed that Ed-CK-24 was most closely-related to strain ERR9969252 (Human, Australia, 2010; GCA_963528665.1) and to strain CTOTU50461 (Environment, Germany, 2013; GCA_032102565.1). ASTs indicated resistance to cephalosporins (intrinsic resistance), clindamycin, fusidate, tiamulin and sulfamethoxazole. Tiamulin and clindamycin were associated with the presence of the lincosamide-pleuromutilin resistance gene lsa(A) on the megaplasmid. The virulence gene bpsC was identified on the chromosome.

Conclusion

This study provided the first complete genome of an E. devriesei isolated from Z. morio larvae and identified antimicrobial resistance and virulence genes. These findings contribute to expanding our knowledge regarding this rarely isolated Enterococcus species and suggest that the Ed-CK-24 genome may serve as a reference for future studies with this specie

O032

Whole genome sequence-based characterisation of Campylobacter isolated from broiler carcasses over a three-year period in a big poultry slaughterhouse reveals high genetic diversity and a recurring genomic lineage of Campylobacter jejuni

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Aims
Campylobacter is among the most frequent agents of bacterial gastroenteritis in Europe and is primarily linked to the consumption of contaminated food. The aim of this study was to assess genomic diversity and to identify antimicrobial resistance and virulence genes of 155 Campylobacter isolated from broiler carcasses (neck skin samples) in a large-scale Swiss poultry abattoir over a three-year period. Samples originated from broilers from three different types of farming systems (particularly animal-friendly stabling (PAFS), free-range farms, and organic farms).
Methods
Campylobacter jejuni (n=127) and Campylobacter coli (n=28) were analysed using a whole genome sequencing (WGS) approach (MiniSeq; Illumina). Sequence types (STs) were determined in silico from the WGS data and isolates were assigned into complex types (CTs) using the cgMLST SeqSphere+ scheme. Antimicrobial resistance genes were identified using the Resistance Gene Identifier (RGI), and virulence genes were identified using the virulence factor database (VFDB).
Results
A high degree of genetic diversity was observed. Many sequence types (C. jejuni ST19, ST21, ST48, ST50, ST122, ST262 and C. coli ST827) occurred more than once and were distributed throughout the study period, irrespective of the year of isolation and of the broiler farming type. Antimicrobial resistance determinants included blaOXA and tet(O) genes, as well as the T86I substitution within GyrA. Virulence genes known to play a role in human Campylobacter infection were identified such as the wlaN, cstIII, neuA1, neuB1, and neuC1. Subtyping of the Campylobacter isolates identified the occurrence of a highly clonal population of C. jejuni ST21 that was isolated throughout the three-year study period from carcasses from farms with geographically different locations and different farming systems.
Conclusion
The high rate of genetic diversity observed amongst broiler carcass isolates is consistent with previous studies. The identification of a persisting highly clonal C. jejuni ST21 subtype suggests that the slaughterhouse may represent an environment in which C. jejuni ST21 may survive, however, the ecological reservoir potentially maintaining this clone remains unknown

O033
Characterizing the mechanisms underlying the persistence of Chlamydiae

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CHUV

The life cycle of Chlamydiae includes a biphasic developmental sequence. Additionally, in response to stressors such as IFN-γ, temperature changes or iron deprivation, the bacteria can survive in an aberrant state. This persistent, non-proliferative state is reversible, however, the precise mechanisms leading to the transition from replicative, reticulate bodies (RBs) to aberrant bodies (ABs) have not been characterised. By comparing the transcriptomes of RBs and ABs, we have identified differentially expressed genes that were predicted to be part of a two-component regulatory system (TCS). TCSs are predominant approaches used by bacteria to sense and respond to their environment.

AB formation was induced in Chlamydia trachomatis and Waddlia chondrophila infected cells by depleting iron using the chelator 2,2'-bipyridyl (BPDL). To study reversion to RBs, the stressor was removed. RT-qPCR was performed to quantify RNA levels. Expression of the TCS candidate genes were compared between RBs and ABs. In parallel, immunofluorescence microscopy was carried out to compare gene expression to morphological changes associated with ABs. Similar experiments are currently being performed on other stress stimuli.

Results indicate that the two examined TCS predicted genes, are strongly down-regulated in ABs compared to RBs. After 8 hours following BPDL removal, expression levels returned to those found in control infections. Surprisingly, upregulation in gene expression was observed 16 hours prior to morphological changes by microscopy. Downregulation in gene expression was not observed in ABs induced by other stress stimuli, indicating a relationship between iron and the TCS in Chlamydiae.

Chlamydiae encode a limited number of TCSs, compared to other pathogenic bacteria. As TCSs normally detect environmental stress stimuli, our results suggest that these genes may regulate persistence during iron starvation. Further knowledge of the biological mechanisms triggering the development of persistent bacteria can give insights into mechanisms at play during chronic chlamydial infections.

O034

Interspecies peptide hijacks S. pneumoniae transporter to inhibit growth and colonization

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Aim:
To identify a previously unknown interspecies communication peptide from Klebsiella pneumoniae and analyze its effect on Streptococcus pneumoniae (pneumococcus) in vitro and in vivo with a view to investigating its potential as a therapeutic for pneumococcal diseases.
Methods:
The peptide was identified from the K. pneumoniae secretome by mass spectrometry and the effects on S. pneumoniae analyzed by optical density measurement, time-kill assays, transformation assays and microscopy. The effects on the transcriptome were determined by RNA-Seq and on colonization using primary human airway epithelial cells and an infant rat model. Peptide toxicity was assessed using primary human airway epithelial cells and zebrafish larvae.
Results:
The K. pneumoniae secretome contained a peptide that suppressed growth of genetically diverse clinical pneumococcal isolates, including antibiotic-resistant strains, in defined medium and human cerebrospinal fluid. Bacteriostatic growth inhibition was dependent on uptake via a functional Ami permease and caused downregulation of genes involved in amino acid and protein metabolism. Furthermore, the peptide caused irregular bacterial shapes, decreased chain length and decreased genetic transformation. Pneumococcal adherence to primary human airway epithelial cells and colonization of rat nasopharynx were also decreased. We did not detect toxicity of the peptide in vitro or in vivo.
Conclusion:
We identified a K. pneumoniae peptide which targets the pneumococcal Ami permease to inhibit pneumococcal growth and colonization. The peptide has potential as a therapeutic for pneumococcal diseases, including treatment of antibiotic resistant strains, while avoiding bacterial lysis and dysbiosis.

O035

Genetic dissection of the chemical defense of ink cap mushroom Coprinopsis cinerea against fungivorous nematodes

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The coprophile ink cap mushroom Coprinopsis cinerea is a well-established model organism for agaricomycetes. Fungivorous nematodes are ecologically significant predators of fungal mycelium. The chemical defense of C. cinerea against these predators includes both a constitutive (autonomous) and an inducible part. Latter part is manifested by the production of a series of nematotoxic intracellular proteins in the vegetative mycelium of this fungus upon attack by the stylet-harbouring fungivorous nematode Aphelenchus avenae1,2,3. Interestingly, this inducible chemical defense mechanism is not restricted to the sites of the predation but propagates along specific hyphae both acropetally and basipetally3. The signalling pathways that are responsible for triggering the inducible anti-nematode defense response in C. cinerea and the nature of signals mediating its propagation along hyphae remain unclear. Moreover, the physiological relevance of the constitutive and inducible chemical defense of C. cinerea against fungivorous nematodes has not been assessed so far.

Recently, we tested the vegetative mycelia of a series of monokaryotic and dikaryotic C. cinerea strains for their susceptibility to grazing by A. avenae. We found that on some strains, nematode populations thrive rapidly, while on others, nematodes struggle to propagate. We set out to unravel the genetic basis of the difference between permissive and prohibitive strains using forward genetics. To achieve this goal, we have crossed a permissive and a prohibitive strain and isolated 123 F1 progenies. Phenotyping revealed that 84 strains of the progenies were prohibitive while the rest was permissive. We will apply bulk segregant analysis (BSA) with and without backcrossing of single progenies to the parents to identify the gene loci associate with the permissive phenotype. To further validate the candidate genes and investigate their roles in the expression of nematotoxic effectors, we will apply reverse genetics i.e. create respective knout-out strains using CRISPR-Cas9. The results of this study will hopefully reveal the signalling pathways involved in the defense response of C. cinerea against A. avenae and demonstrate that this response is physiologically relevant for the fungus.

O036

Spatial and temporal distribution of Legionella pneumophila in the drinking water system of a large building over 25 years

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Aims

Legionella pneumophila, the causative agent of Legionnaires' disease, is often found in the plumbing systems of buildings from where it can be transmitted to humans via inhalation or aspiration of contaminated water drops. Routine water sampling from the potable water system of an occupational building in Basel over 25 years allowed analysis including whole genome sequencing (WGS) of the colonizing L. pneumophila isolates. This is the most detailed, long term building survey of L. pneumophila recorded.

Methods

Annual routine testing of the drinking water system at the building from 1994 to 2018, was performed in accordance with national guidelines. Isolates (n=113) were grown on Buffered Charcoal Yeast Extract Agar for 48-72 h. DNA was extracted using the Qiagen EZ1 with the DNA tissue kit. WGS used the NextSeq500 PE150 after Illumina DNA prep, resulting in >40x mean read depth per sample. Assembly with Unicycler v0.3.0b was used in core genome MLST (cgMLST) analysis in Ridom Seqsphere+ 9.0.10 according to the species scheme on https://www.cgmlst.org. Single nucleotide polymorphism (SNP) analysis was performed in CLC Genomics Workbench v22.0.2 and the resulting alignment analysed with Gubbins v3.3.0.

Results

Overall, 309 water samples were collected at 38 time points over the period of 25 years. L. pneumophila was recovered from 120 water samples (38.8%) and 113 L. pneumophila isolates from 26 time points were included in this study. After initial decontamination measures that were successful for approximately 12 years, an increase in the total number of Legionella as well as of L. pneumophila-positive site was noticed in 2008. WGS showed all L. pneumophila to be ST45 (SBT scheme). The isolates are very closely related, all clustering within cluster limits (4 cgMLST alleles). Recombination analysis showed that of >6000 identified SNPs between isolates, 94% fall within recombinations. SNP analysis of the remaining SNPs does not show temporal signal or clustering by location.

Conclusion

Over 25 years, a single ST lineage colonised the water system of this building. This ST is known from other publications to be able to cause disease; however, no infections with Legionella spp. have been reported as being associated with this building. The phylogeny of isolates can be interpreted as inferring good water circulation, possible recolonisation from a common source after cleaning, and possible evolutionary bottlenecks within the water system.

O037

Wastewater-based Infectious disease Surveillance of Respiratory Pathogens in Switzerland

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Wastewater-based infectious disease surveillance has helped to inform dynamics of SARS-CoV-2 in Switzerland since July 2020. Wastewater analysis complements traditional epidemiological indicators, such as reported cases, offering a unique perspective on disease dynamics. Since November 2022, wastewater sampling has been expanded to include monitoring of other priority respiratory diseases. Routine sampling is conducted with high temporal (5 days a week) and spatial (14 treatment plants across Switzerland, representing 26% of the population) resolution. Wastewater samples are received at a central laboratory weekly, total nucleic acids are extracted, and RNA concentrations of SARS-CoV-2, Influenza A and B, and Respiratory Syncytial Virus are quantified with a multiplex digital PCR assay offering absolute quantification. Digital PCR's accuracy is achieved by partitioning samples into thousands of individual reactions, enabling absolute quantification without the need for standard curves. Additionally, amplicon-based sequencing and associated bioinformatics pipelines facilitate the detection of variant signature mutations and estimation of SARS-CoV-2 variant proportions in wastewater. The wastewater data collected within the scope of the work has also been used to estimate the effective reproductive number of SARS-CoV-2 and Influenza over time, complementing clinical-based measures of disease dynamics. The work highlights the potential for low cost, environmental surveillance of populations to inform Swiss infectious diseases. Intended expansions include work on surveillance of other priority pathogens, including gastrointestinal diseases, seasonal coronaviruses, and antimicrobial resistance.

O038
The impact of zooplankton on solar inactivation of viruses

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The presence of enteric viruses in water is an ongoing global concern with negative human and ecosystem health impacts. Sunlight is an important inactivation mechanism for viruses, but inactivation rates can be impacted by interactions with abiotic and biotic factors. Filter-feeding organisms, like zooplankton, have been shown to remove viruses from water, but how this uptake impacts the efficacy of sunlight inactivation rates is not understood. Hence the aim of this research is to quantify the changes in sunlight inactivation in the presences of zooplankton. To achieve this aim, two model zooplankton species were evaluated Tetrahymena pyriformis (ciliated protozoa) and Brachionus calyciflorus (a rotifer) with two viruses MS2 phage and Echovirus-11 (EV-11). Batch experiments were conducted using a solar simulator (Sun 2000, Abet Technologies) in a temperature-controlled water bath (24°C), In addition batch experiments were conducted in the dark. Proper controls were employed to compare inactivation rates. Water samples were collected for up to 3-days and infective viral concentrations were quantified. Data will be presented showing comparison of dark and light removal rates for each zooplankton species. Data show that removal of MS2 and EV-11 exposed to sunlight is enhanced in the presence of ciliates (T. pyriformis), and protection effects are not observed. In contrast, sunlight inactivation of EV-11 is reduced in the presence of rotifers (B. calyciflorus), but the same protection is not observed for MS2 in the presence of rotifers. The results obtained to date highlight the complexity of zooplankton-viral interaction, with the potential for zooplankton to enhance as well as reduce viral inactivation. Further work is needed to elucidate the possible mechanisms that cause the differences in sunlight inactivation of viruses in the presence of zooplankton.

O039

Exploring the influence of substrate and pH on microbial community dynamics in anaerobic digestion

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The anaerobic food chain describes the sequential degradation and utilization of biopolymers by a community of microorganisms in the absence of dioxygen. The anaerobic digestion (AD) process utilizes such microbial degradation networks to reduce organic waste volume while producing biogas as a renewable energy source. More recent interest has developed in applying AD to produce fatty acids as useful precursors for repurposing organic wastes. Previous research has characterized the different trophic layers and microbial metabolic groups carrying out digestion and identified key biological and operational factors affecting AD dynamics. However, it is still challenging to design a stable process that continuously produces targeted fatty acids with high productivity and purity by finely tuning the AD microbiota. As an initial step towards understanding the interactions of different factors and the dynamic feedback between microbial activity and the environment, we carried out enrichment experiments of microbiota taken from three different AD reactors with varying feedstock, operational conditions, and geographical locations. Minimal media containing glucose, cellobiose, or no carbon substrates (as negative controls) at three different pH levels (5.5, 6.5, and 7.5) were used. The production of metabolites (short-chain fatty acids, H2, CO2, and CH4) was monitored. Despite the different inocula, we observed the enrichment community converging functionally depending on substrate and pH conditions. Through multidimensional analysis of metabolites and 16S sequencing results, we will evaluate the relevance of substrate and pH in selecting community function

O040
Targeting viral RNA as new approach for antivirals: from in silico to cells

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We are lacking antivirals for different viruses. In addition to targeting viral protein, focusing on viral RNA is a promising strategy to address this gap in antiviral therapeutics.

We previously showed geneticin's inhibition of various SARS-CoV-2 variants by targeting its -1 programmed ribosomal frameshift. This mechanism involves a pseudoknot, an RNA structure common in coronaviruses but uncommon in humans. To identify more potent inhibitors, we conducted virtual screening with a drug-like library on an RNA ensemble derived from molecular dynamics, using a refined SARS-CoV-2 pseudoknot structure. One of the hits demonstrated reduction of SARS-CoV-2 frameshift and exhibited antiviral activity in the micromolar range.

However, cellular studies revealed a different SARS-CoV-2 RNA folding, driving our focus on identifying molecules binding to alternative structures that could result in a conformational block, preventing the formation of the pseudoknot. Consequently, we are selecting potential inhibitors by virtual screening and using a refined dual luciferase construct in which the RNA can fold as identified in the cells.

This novel drug development strategy is applicable to various viruses. We are currently employing this approach on rhinoviruses by determining the predominant RNA cellular structure with DMS probing, selecting druggable pockets with antisense oligonucleotides, and using in silico screening to identify potential drug candidates. Overall, targeting viral RNA is an unexplored field with promising therapeutic opportunities.

O041
Inactivation mechanism of SARS-CoV-2 at acidic pH

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Respiratory aerosol particles have gained attention as a major transmission route for respiratory diseases in the last years. Our previous research suggests that small aerosol particles on a sub-micron scale acidify in seconds to minutes, reaching a pH of approximately 4. Gas phase adjustments, e.g. acidification, can further reduce the predicted aerosol pH. In this context, we set out to investigate the inactivation mechanism of SARS-CoV-2 at a pH below 3 with a focus on its surface glycoprotein Spike (S). S consists of the S1 subunit containing the receptor binding domain (RBD) binding to ACE2 and the S2 subunit responsible for fusion with the host membrane. Herein, we study SARS-CoV-2 after acidification and subsequent neutralization for infectivity and copy numbers. We use light and cryo-electron tomography (cryo-ET) to determine virion integrity as well as Immunofluorescence (IF) and biolayer interferometry (BLI) approaches to study binding on a molecular level.
The infectivity of SARS-CoV-2 particles decreases within minutes when the pH drops below 3, while genomic copy numbers remain stable. Cryo-ET showed virions were largely intact after acidification with signs of a disrupted inner structure. While we saw no decrease in the in vitro binding of recombinant S subunits (including the RBD, S1, the S1+S2 monomer and trimer) to ACE2 in BLI after acidic treatment, we found that the vast majority of SARS-CoV-2 virions lost the ability to bind to target cells after acidic treatment in an IF based assay. Additionally, we observed a reduction of RBD signal on SARS-CoV-2 virions treated with acidic pH in IF, but an increase of S2 signal of virions.
Overall, our data show a loss in binding capacity of SARS-CoV-2 virions to the target cell after encountering acidic pH below 3. Considering that, our in vitro BLI assays demonstrate that the RBD is still intact and capable of binding to its ACE2 receptor, our data suggest that the decrease in virion binding to cells is attributed to S1 shedding rather than conformational changes of S.

O042

Asymmetric expression of meiotic genes imposes distinct selective pressures on mating partners

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Anisogamous species, which produce gametes with significant morphological differences, are thought to have evolved from isogamous ancestors1. Lack of evolutionary records and experimental evidence has made it difficult to understand how selection could act on isogametes to select for increased asymmetries. I will show our recent data indicating that the morphologically indistinguishable fission yeast P- and M-gametes, which differentially express only a handful of genes, experience distinct selective pressures. We initially discovered that transcription of meiosis-specific genes, including the highly conserved meiotic cohesin rec8, occurs ahead of fertilization. Surprisingly, only P-gametes produce Rec8 protein during mating, whereas Rec8 encoded by the M-gamete genome becomes detectable only at the time of partner fusion. This asymmetry in Rec8 production is driven by distinct pheromone signaling between partners, and P-gamete-produced Rec8 is critical for meiotic chromosome segregation. Strikingly, early expression of Rec8 also places a fitness cost on P-gametes; P-gametes that engage partners but fail to fuse exhibit increased genomic instability when allowed to reproduce asexually, which is prevented by removal of the rec8 gene. Finally, we show that P-gametes are at a competitive disadvantage to M-gametes in evolving populations that undergo cycles of mating and asexual reproduction, and that the observed difference in fitness depends on the rec8 gene. Taken together, we demonstrate that distinct investments of P- and M-gametes in zygotic development impose different fitness costs. Our work provides the first example of how subtle molecular asymmetries can drive distinct selective pressures on isogametes during evolution.

O043

A high-throughput method for evaluating bacterial dispersal on hyphal networks

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In complex and heterogeneous environments, the hyphae of filamentous fungi and fungal-like Oomycetes are known to provide a support for the dispersal of other microorganisms. The use of these “fungal highways” is regulated by the interplay of both physical and biological constraints. Therefore, the ability of different species to establish fungal highways must be verified experimentally. Several experimental devices exist that can test specific pairings. However, these methods are time consuming and cannot be applied at a large scale and high-throughput format. Today, three-dimensional (3D) printing offer fast translation between digital design and a finished product. This allows testing intricate designs with greater reproducibility, and faster production times.
In this study, we used 3D printing to develop an experimental tool to allow fast and high-throughput evaluation of bacterial dispersal on hyphal networks in 96-well microplates. Different materials and designs were tested to produce a “crossing bridge” that is traversed by the fungus-bacteria partners. The design allows for the simultaneous testing of multiple species and the inclusion of any culturing media. By combining the transport with a redox active dye, the arrival of the partners to the target well can be quickly monitored. The devices were evaluated with several fungal and bacterial species and the performance of designs was compared. The optimal topology was selected to evaluate the effect of multi-trophic conditions on the effectiveness of the transport. This study provides an easy-to-implement approach for evaluating the effective transport of bacteria by fungi and fungi-like hyphal networks.

O044

UME6 controls aggregation, biofilm formation and pseudohyphae formation in Candida auris

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Aims :
The pathogenic yeast Candida auris has been classified as urgent threat by the Center for Disease Control and Prevention (CDC) because of its potential to cause outbreaks of candidemia. Some characteristics of C. auris include adherence to inert surfaces, biofilm formation and aggregation. C. auris can also form pseudo-filaments under specific conditions. The transcription factor Ume6 was shown to play a role in virulence and morphogenesis (e.g. filamentation) of Candida albicans. However, its role in C. auris remains unclear. The aim of this study was to investigate the role of Ume6 and its main downstream effectors (Als4498, Scf1, Hgc1) in C. auris morphogenesis and virulence.
Methods :
To assess the impact of UME6 hyperactivation and deletion, we constructed the UME6HA strain (in which UME6 was under the control of the ADH1 promoter and tagged at its C-terminal locus by a 3xHA tag) and the ume6Δ strain, respectively, in a non-aggregative C. auris strain from clade IV. Transcriptomic analysis was performed in the UME6HA strain and compared to its background strain. Genes that were overexpressed in the UME6HA strain were subsequently deleted in the UME6HA background. To study biofilm, we used crystal violet assay for quantification, SEM microscopy and confocal microscopy. Flow cytometry was used for adhesion assay and also Imaging flow cytometry for to quantify length of the fungal cells.
Results :
Deletion of UME6 did not result in any significant phenotype regarding morphogenesis. Hyperactivation of UME6 in the UME6HA strain induced aggregation, pseudohyphae formation and biofilm formation. Based on transcriptomic analysis, three genes (ALS4498, SCF1 and HGC1), which were highly overexpressed in UME6HA strain were subsequently deleted in UME6HA background. Deletion of ALS4498 (an adhesin) abolished aggregation and resulted in decreased biofilm formation. Deletion of SCF1 (adhesin known as surface colonization factor) only resulted in loss of aggregation, while deletion of HGC1 (transcription factor controlling morphogenesis) only resulted in loss of pseudohyphae formation.
Conclusion :
The transcription factor Ume6 plays a crucial role in morphogenesis of C. auris by controlling :
- Aggregation via Als4498 and Scf1
- Adhesion via Scf1
- Pseudofilamentation via Hgc1
- Biofilm formation via Als4498 (and Scf1, Hgc1)

O045

A novel approach to studying bacterial contractile injection system in natura

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Omics approaches employed to study biological molecules have revolutionized environmental microbiology and elevated our understanding of ecological processes in the biosphere. However, conventional omics applications often lead to the loss of information about macromolecular organization of the molecules within the cellular context. This can be a significant disadvantage since many proteins are functional only as macromolecular complexes in particular structural forms. A great example is bacterial contractile injection system (CIS), a syringe-like protein complex whose function is the translocation of molecules into a target cell to affect its state, often inducing lysis. Since the function of CIS is tightly linked to its intracellular localization, proteomics cannot confidently predict the function of novel uncharacterized CISs in natura. To overcome this challenge, we have developed a cryo-electron tomography workflow as a technique complementing metagenomics and proteomics. Additionally, we developed an immuno-electron microscopy protocol to identify and quantify CIS particles in environmental samples. Using this approach, we discovered a novel bacterial CIS in thermophilic multicellular Chloroflexota bacteria populating hot spring mats worldwide. We found that this system is similar phylogenetically and structurally to a recently described cytoplasmic CIS, which was found in multicellular Streptomyces and has been shown to be involved in cell cycle regulation. Interestingly, using our approaches, we have discovered that Chloroflexota cells produce different numbers of CIS particles depending on the mat micro-niches they occupy. In agreement with this, we observed that CIS was also non-constitutively expressed under laboratory conditions. Motivated by this discovery, we searched and analyzed similar CIS in extremophilic bacteria from other lineages. Overall, we have gained an understanding that bacterial cytoplasmic CIS is an overlooked cellular feature of the extremophilic bacteria, which is potentially involved in the cell fate control or intraspecies interaction within microbial community.

O046

Deciphering the Secretome of Brucella spp. during Infection using Bio-Orthogonal Non-Canonical Amino Acid Tagging (BONCAT)

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Brucellosis is an infectious disease spread around the world. It is a zoonosis transmitted to humans by animals they are working with closely, such as cattle, sheep and even dogs.
Brucella bacteria are host specific, but they can end up in humans as incidental hosts through the digestive or respiratory tract. Once in the host, Brucella are internalised into the Brucella containing vacuole (BCV). BCVs initially follow the canonical endocytic trafficking, fusing first with early, then late endosomes, followed by limited fusion with lysosomes. During this last step transient acidification triggers the T4SS leading to effector translocation and trafficking into the ER. Upon reaching the ER the BCV converts from its non-replicative state to the replicative state. During the endocytic path, Brucella escape host recognition and interact with the immune system of the host. Furthermore, they seem to be resistant to the complement system.
The major part of Brucella-host interaction is believed to be via proteins secreted into the host cells, so called effectors. Effectors known interact with parts of immune recognition. Due to several challenges with Brucella, not many effectors are known yet. Up until now, only biased approaches were used for the identification.
Bio-orthogonal non-canonical amino acid tagging (BONCAT) can be used as an unbiased approach to identify the secretome of Brucella using mass spectrometry. It allows identification of effectors of Brucella at different times during the infection process.
Due to the high genetic similarity of the Brucella species, BSL2+ classified species B. microti can be used as a surrogate to the BSL3 classified species B. abortus.

O047

The putative Type 4 secretion system effector BspD is involved in maintaining envelope integrity of the pathogen Brucella

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The Gram-negative, facultative intracellular zoonotic pathogen Brucella spp. cause the debilitating disease Brucellosis. Brucella, as an intracellular pathogen, encounters various stressful environments while being trafficked towards the replicative niche inside the host cell, which it either has to withstand or counteract. We aimed at understanding the contribution of the putative Type 4 secretion system effector BspD to intracellular survival and replication. To this end we used in silico analyses, classical growth curves and axenic stress assays, as well as host cell infection. We show that BspD is a conserved protein of the Rhizobiales, which does not show signs of co-evolution with the presence of a T4SS or a certain lifestyle. Further, using in vitro assays we show that BspD is critical for Brucella abortus envelope integrity in the stationary phase and in the presence of EDTA, a compound known to destabilize the outer membrane. Deletion of bspD resulted in abnormal bacterial morphologies, indicating its involvement in maintaining envelope integrity. In infection the absence of BspD led to the formation of fewer and smaller intracellular microcolonies in a macrophage infection model. From our observations, we propose that BspD of B. abortus is critical for preserving the integrity of the bacterial envelope, particularly under stressful conditions, which may enhance Brucella’s ability to survive within host cells independently of its putative status as a Type 4 secretion system effector.

O048

Ecological processes driving Erwinia tasmaniensis antagonism against Fire Blight (Erwinia amylovora) as revealed by genomic and metabolic analyses.

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Understanding the interactions between bacterial plant pathogens and their natural antagonists is essential for establishing sustainable agricultural practices. This study expands our knowledge about the mechanisms behind the antagonistic interaction between Erwinia tasmaniensis, a non-pathogenic member of the flower microbiome, and Erwinia amylovora, the causative agent of fire blight in rosaceous hosts (e.g apple, pear, quince). E. tasmaniensis is believed to interfere with the pathogen proliferation in the flower and thus with infection, so we characterized novel strains isolated from a fire blight-free orchard where it was previously shown to dominate (Gschwend 2021).

Comparative genomic analysis including our isolates and publicly available E. tasmaniensis and E. amylovora genomes, revealed a high degree of interspecies (83%) and intraspecies similarity (>99%), including a conserved core carbon metabolism. Notably, variable gene content was associated with functions like plasmid transfer, bacterial competition (T6SS, R-M systems, and toxin-antitoxin systems), and peripheral carbon utilization. To further explore these differences, a phenotypic microarray analysis using 191 metabolites was employed to characterize the carbon source utilization profiles of nine E. tasmaniensis strains and a representative E. amylovora strain (LMG1893). This analysis identified significant disparities in carbon source utilization and metabolic pathways between the two bacterial species, with substantial phenotypic variation observed within E. tasmaniensis itself.

Based on our integrated findings, we conclude that E. amylovora and E. tasmaniensis compete for nutrients present in flower stigma exudates, including simple sugars (glucose, fructose, sucrose) and complex carbohydrates (pectin, hemicellulose, arabinogalactan). The observed greater metabolic versatility of E. tasmaniensis in utilizing these floral metabolites may provide a competitive advantage over E. amylovora during flower colonization. This phenomenon holds promise for the development of novel biocontrol strategies to combat fire blight.

O049
Gut microbiota profiles of Salmonella in patients, a path toward protection

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While the majority of people exposed to Salmonella will likely develop gastroenteritis, a small proportion of the population (< 1%) will become asymptomatically colonised. We are interested in characterising the composition of the gut microbiota profile of asymptomatic Salmonella carriers as compared to symptomatic Salmonella patients and healthy controls, to understand the mechanisms which protect against a symptomatic infection.
The faecal samples from 9 non-typhoidal infected Salmonella patients, 23 asymptomatic Salmonella carriers, and 11 healthy individuals were collected. Their profiles were characterised by evaluating the 16S rRNA amplicon sequences, genomic variation, and functions predicted using 16S rRNA data.
The most common strain identified was serovar Typhimurium. Statistically, no significant difference in the presence of antimicrobial resistance genes, virulence genes, and plasmid replicons between the symptomatic and asymptomatic groups were observed. Both groups displayed similar levels of alpha diversity, which was statistically lower to healthy individuals. A significant PERMANOVA indicated that all profiles were statistically distinct from one another. Healthy and asymptomatic individuals possessed higher prevalence of bacteria belonging to the Lachnospiraceae, Ruminococcaeae, and Christensenellaceae family. Five species were identified as being differentially enriched in the asymptomatic group compared to the symptomatic group, of which four species belong to the Lachnospiraceae and Ruminococcaeae family. Compared to the symptomatic profile, the asymptomatic profile saw an increase in predicted functions associated with degradation and biosynthesis. Community profiling identified three clusters within our samples, where profiles resembled a eubiotic, dysbiotic, and intermediate profile. The eubiotic community saw an enrichment in functions associated with biosynthesis compared to the dysbiotic community.
The lack of significant differences among the virulence of the strains suggests that virulence may not play a role in influencing a symptomatic profile. Differential abundance analysis and bacterial prevalence imply symptomatic patients lack bacteria from the the Lachnospiraceae and Ruminococcaeae family, bacteria which have been reported to be positively associated with gut health. Future studies will focus on identifying a bacterial consortium and challenging Salmonella in mice to evaluate their protective potential.

O050

Metabolic Interplay Between the Honeybee Gut Microbiota and 1054 Agrochemicals: Combining High-Throughput Toxicity and LC-HRMS Biotransformation Screens

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Agrochemicals are key stressors affecting the health of bees and other pollinators. While incidences of bee mortality linked to acute pesticide exposure are well studied, sub-lethal pesticide exposure has now been shown to depress immunocompetence, pathogen resistance, and foraging behavior. These same traits are modulated by the specialized microbial communities in the gut of social bees. Therefore, investigating the mechanistic interplay between agrochemicals, bees and their microbiota is crucial to understand the full impact of chronic, sub-lethal exposure that is now common across diverse ecosystems. Limited findings already indicate that the honeybee gut microbiota is both perturbed by certain pesticides, while also biochemically altering others. Whether these microbial biotransformation's are beneficial or harmful to their bee host remains unknown. Unfortunately, the vast array of agrochemicals makes comprehensive experiments documenting interactions with microbes in the bee gut nearly impossible.

We propose high-throughput, in vitro screens as tools to identify agrochemicals that are either highly toxic to the gut microbiota, or that are biotransformed by the microbiota. The resulting positive hits can then be further validated using laboratory or field trials of managed and wild bees. As a first step in this effort, we report the toxicity of 1054 pesticides, antibiotics, and other agrochemicals towards 20 bacterial strains grown in isolation and in synthetic communities. These strains encapsulate the species level diversity within the gut of the western honeybee (Apis mellifera). We identified 126 compounds (12% of the panel) that were toxic to one or more strains at environmentally relevant concentrations (≤ 20 µM). Amongst the largest chemical classes in our screen, fungicides were substantially more likely to be toxic to the microbiota (6% of interactions) than herbicides or fungicides (1% of interactions). We further exposed the microbiota to a subset (656 agrochemicals) of the original panel and measured their degradation using high resolution LC-MS. Here we identified 55 chemicals (8% of the panel) that were significantly degraded by at least one strain.

Our results demonstrate the feasibility and utility of high-throughput screens to rapidly identify microbial interactions with agrochemicals, and they pave the way for further targeted experiments with high likelihood of finding microbiome mediated effects on the honeybee host.

O051
Explaining the risk posed by toxic cyanobacteria through service-learning activities

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Service-learning is a teaching and learning method that combines community service and education. It allows students to actively apply the knowledge acquired during their curriculum to address real-world societal issues. In the “Microbes go to school” program proposed by UniNE, students from bachelor and doctoral school programs visit classes to explain topics related to microbiology to children aged 6-12. The goal is to use alternative didactic approaches to teach a topic that is not covered in school. During the 2024 spring semester, a service-learning activity was implemented to explain the problems posed by the excessive development of toxigenic cyanobacteria in water bodies. This is linked to a real-world issue that has affected the Canton of Neuchâtel and other Cantons in recent years. Since 2020, in the region near the Areuse river, several dogs died after the ingestion of microbial mats composed mainly of benthic cyanobacteria. These events have created apprehension in residents about their security near recreational places along the river and shores of Lake Neuchâtel. As a result, misinformation about cyanobacteria emerged. It appeared therefore crucial to inform people about the real associated risks. For this, we created an activity to explain and present cyanobacteria to children of 4th grade (7-8 years old). The activity spreads over 4 days (2 hour/days) and was presented as a game in which children acted as detectives to discover the microorganism who had killed the dogs. At the beginning of the activity, four different suspects were presented to children (a virus, cyanobacteria, green algae, and bacteria). The activity included theory, a field excursion, and a visit of the University laboratory. During these sessions, the children had to collect clues to identify the microorganism responsible for the dogs' death. Some of the clues were given during theoretical lessons, while others were deduced from their observations in the field or during the laboratory session. With this activity, children acquired knowledge in microbiology including aspects of the ecology of the suspected microorganisms. To verify the knowledge acquired, a poster summarizing the activity was created by children. In addition to the impact on the public, the activity allowed the University students who set it up to gain knowledge about scientific communication and to develop effective communication tools to explain a societal issue in a pragmatic and entertaining manner.

O052

"Mission Antibiotix": A Virtual Escape Game to Promote Proper Antibiotic Use in Canton Vaud Hospitals

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Aims The inappropriate use of antibiotics in human medicine has exacerbated the threat of antimicrobial resistance (AMR). Urgent action is required to raise awareness among prescribers about the significance of judicious antibiotic use. Innovative educational tools tailored to accommodate their limited time availability for training could prove more effective in improving antibiotic prescribing practices.
Methods To mark the World Antibiotic Awareness Week 2023, the Infection Prevention and Control units (IPC) of the Canton of Vaud and CHUV developed a virtual game titled "Mission Antibiotix." This game was made freely accessible online and specifically aimed at hospital physicians. The educational materials were crafted by physicians from the two IPC units, with support from the AMR taskforce of CHUV. Game development was undertaken by the Continuing Education Center of the CHUV and a communication specialist from the cantonal IPC unit. Players were invited to immerse in a set of realistic clinical scenarios. They were tasked with making appropriate prescribing decisions by searching for clues in virtual patient rooms, all within a time limit of 20 minutes. Immediate feedback was provided following each decision-making moment. At the CHUV, the game was organized as interdepartmental championships in auditoriums or incorporated into internal postgraduate trainings. Meanwhile, the individual mode was used in other hospitals across Vaud. An evaluation form was accessible on the game's website for feedback.
Results Since its launch, the website has garnered over 850 visits. During the CHUV championship, 30 teams comprising over 135 players from 15 different wards participated over four days, engaging in four very lively meetings held either in auditoriums or during ward meetings. Feedback from the 82 championship participants was extremely positive, with many emphasizing the interactivity and realism of the chosen cases. 73% of players believed they have acquired knowledge useful to their practice.
Conclusion This serious game encouraged physicians' involvement, thanks to its playful approach and accurate representation of clinical reality. Its easy integration into their work schedule, along with its short duration, contributed to increased engagement. Additionally, playing as a team in a collaborative environment facilitated message anchoring. Finally, the platform's flexibility allows for future updates in content and target audience.

O053
To see, or not to see… pathogens in virtual reality hand hygiene training

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Background: VIRTUE, a virtual reality hand hygiene trainer, offers users the option of visualizing pathogen transfers during virtual patient care either in ‘real-time’ or at the end of a run as a ‘summary’ visualization. In this study, we aimed to evaluate the effect of different timings of pathogen visualisation (‘real-time’ vs. ‘summary’) on in-trainer performance and user’s immersion.
Methods: The study included first-year medical students undergoing hand hygiene training with VIRTUE, randomized to one of three visualization set-ups: set-up 1 (“on-off-off”, with ‘real-time’ visualisation at the first level only, and ‘summary’ visualization at level 2 and 3), set-up 2 (“off-on-off”), and set-up 3 (“off-off-off”). In-trainer performance was defined by number of pathogen transmission events (=contaminations) in level 3. The virtual experience of user’s (among others: immersion) was assessed with a questionnaire.
Results: 173 medical students participated in the study, with 58, 54, and 61 assigned to set-up 1, set-up 2, and set-up 3, respectively. Users assigned to set-up 3 with ‘summary’ visualization at all levels, performed best with 1.02 (SD +/- 1.86) contaminations, compared to 2.34 (SD +/- 3.09) and 2.07 (SD +/- 2.52) contaminations of users assigned to the other set-ups. ‘Summary’ visualization at all levels also resulted in higher immersion of users.
Conclusions: ‘Real-time’ visualization of pathogen transmission during virtual reality hand hygiene training with VIRTUE can affect in-trainer performance and user immersion. This emphasizes the importance of pilot testing the effect of VR-based trainings in order to understand the impact of the virtual reality on users.

O054
Call me Isolde!! Proof-of-concept for a Chatbot for standardized Isolation precautions

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Aims
Isolation precautions and their implementation are crucial aspects of an infection prevention team's daily responsibilities. This task is often repetitive and time-consuming, as the necessary information is scattered across various documents. We aim to develop a chatbot powered by generative AI that can efficiently answer standard questions about isolation precautions.

Methods
The core information source is derived from the comprehensive documents prepared by the infection prevention team. These documents encompass a detailed list of indications for isolation precautions and exhaustive guidance on how to implement various types of isolation measures effectively.
Using this information, a generative AI model is trained to address queries related to known pathogens only. In instances where a scenario is not covered in the existing documentation, the AI is designed to refer back to the infection prevention team for expert advice.
The model's responses are supported by direct links to the pertinent documents, ensuring accuracy and relevance.

Outcomes
We are evaluating the feasibility of deploying a chatbot tailored to provide guidance on isolation precautions. Our primary goal is to create a model that consistently offers precise and reproducible responses, each traceable back to its original document. The model is designed to eliminate any inaccuracies and halluzinations and will refer to human experts in infection prevention for queries concerning unknown pathogens. This project is a proof of concept; thus, results will be limited to those obtained from a controlled testing environment, as real-world data will not be available at the time of this abstract's presentation. Additionally, we explore the time required for development, necessary technical expertise, and associated costs.

Conclusions
We believe that generative AI holds immense potential in everyday clinical medicine. Most hospitals have internal guidelines on a variety of topics, and infection prevention measures, being highly standardized and consistent, are ideal for testing the capabilities of such artificial support systems. If the proof-of-concept proves successful, there is potential for expansion into numerous other areas.

O055

Diagnostic Stewardship Rules in Microbiology: Identification and Reduction of Unnecessary and Redundant Analyses are Feasible

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Background: Diagnostic stewardship (DS) in diagnostic microbiology (DM) aims at optimizing the ordering process for microbiological analyses. We present the first evaluation of DS rule algorithm incorporation in the digital order entry process (DOEP) for DM at our tertiary care hospital.

Methods: DS rule algorithms (RA), based on in-house guideline for DM, focus on frequent analyses with low pre-test probability or unnecessary repetitiveness. During a pilot phase of nine months (June 2023 to February 2024), we addressed 1.) blood cultures (BC), 2.) serostatus with prior positive finding and 3.) PCR for viruses in blood without prior positive serostatus.
Rule algorithms are based upon 1.) the priorly published Shapiro-Procalcitonin algorithm (SPA) for BC, and 2./3.) queries of prior positive serological findings. Background algorithms of the DOEP integrate clinical and laboratory findings within predefined time frames. The ordering person is guided through the algorithm during ordering process resulting in a recommendation for (positive) or against the test (negative). This recommendation can be overruled.
Rates of followed recommendations (positive or negative) and overruling are monitored according to professional state of the ordering person (physician vs. non-physician).

Results: 22'057 activated algorithms consisted of 19'289 (87.4%) followed positive, 1'109 (5.0%) followed negative and 1'659 (7.6%) overruled recommendations. For 2'541 BC algorithms, rates for negative recommendations and overruling were 25.8% and 24.8% respectively, for 18'170 serostatus 1.3% and 3.4% respectively and for 1'346 PCRs 16.7% and 30.2% respectively. Considering negative recommendations as prevented unnecessary analyses, 229 serologies, 225 PCRs and 655 BC could be saved.
Of all orders, 79.9% were done by physicians and 20.1% by non-physicians. For non-physicians, the odds ratios (OR) for both generating negative and overruled recommendations were increased (2.9-6.5). Especially Hepatitis B- and Hepatitis C-PCR RA showed high rates of overrulement by non- physicians belonging to distinctive departments and wards (53.0% and 53.4% of all overruled specific RA, respectively).

Conclusions: RA for DOEP can support and monitor evidence based ordering behaviour for microbiological analyses, reduce unnecessary analyses, identify critcal organisation units causing excessive redudancy and hence, potentially save resources and costs.

O056

Veränderung der Rate von lokalen Komplikationen nach Einführung der Desinfektion mit alkoholischem Chlorhexidin vor der Anlage eines peripheren Verweilkatethers.

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Für die Desinfektion der Haut vor der Einlage eines peripheren Venenverweilkatheters (PVK)können verschiedene Substanzen verwendet werden. In kontrollierten StudienA konnte gezeigt werden, dass die Hautdesinfektion mit 2% Chlorhexidine Gluconate - Alkohol 2% (CHG) gegenüber Isopropylalkohol 70% (IPA) hinsichtlich infektiösen Komplikationen von Vorteil ist. Wir wollten wissen, ob die Umstellung der routinemässigen Desinfektion der Einstichstellen vor der Einlage eines PVK) von IPA auf CHG mit einer Reduktion der lokalen Komplikationen assoziiert ist und damit die bisher gezeigten Vorteile aus Studien im klinischen Alltag eines Spitals reproduziert werden können.
Ziel: Der Vergleich der lokalen Komplikationsrate der PVK- Insertionsstellen vor und nach der Umstellung der Desinfektion von IPA auf CHG.
Methode: Eine Single-Center-Qualitätssicherungsstudie. Intervention war die Einführung von CHG auf allen Abteilungen. Outcome war das Auftreten einer lokalen Komplikation (eiternd, gerötet, Verhärtung der Vene) der Einstichstellen nach Einlage eines PVK. Sämtliche Patienten mit PVK wurden während der Beobachtungszeit nach internem Standard regelmässig monitorisiert, allfällige lokalen Komplikationen wurden systematisch dokumentiert. Ein Poisson Regressionsmodell wurde angewendet um das monatliche Inzidenzratenverhältnis vor und nach der Intervention zu vergleichen.
Ergebnisse: Wir haben total 35’681 Patienten nach Einlage eines PVKs überprüft. 24`369 in der IPA-Gruppe (Jan 2021 – Jan 2023), 11`312 in der CHG-Gruppe (Feb 2023 - Jan 2024). Frauen waren in der IPA Gruppe mit 53.8% vertreten, in der CHG-Gruppe mit 54.6%. Das mediane Alter betrug 67 (52-77, IPA) resp. 66 Jahre (52-77, CHG). Die Beobachtungsdauer betrug in beiden Gruppen im Median 2 Tage. Die Rate an beobachteten, kumulativen Veränderungen betrug 322 (1.3%) in der IPA Gruppe resp. 119 (1.1%) in der CHG Gruppe (p = 0.036). Die Verteilung der einzelnen Befunde in der IPA respektive CHG Gruppe war wie folgt: Eiternd: 4 (0.0%) versus 0 (0.0%) (p = 0.409), gerötet: 238 (1.0%) versus 95 (0.8%) (p = 0.233), verhärtet: 103 (0.4%) versus 29 (0,3%) (p = 0.021). Das Inzidenzratenverhältnis für alle Komplikationen nach der Einführung betrug 0.79 (95% CI, 0.64 - 0.97; p = 0.03).
Konklusion: Die Umstellung von IPA auf CHG als Desinfektionsmittel zur Vorbeugung von unerwünschten Ereignissen durch das Einsetzen von PVC war mit einer signifikanten Reduktion der auffälligen Katheter Einstichstellen assoziiert. Die a

O057

Perceptions and knowledge on antimicrobial resistance and antibiotic prescription habits: A survey among physicians in Valais.

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BACKGROUND AND AIM: Valais ranks second highest canton in antibiotic prescriptions for outpatient care in Switzerland (Swiss Health Observatory data). To tackle this public health concern, a cantonal plan aligning with the National Antibiotic Resistance Strategy (StAR) is underway. An evaluation phase is currently ongoing to tailor the plan to local needs.
METHODS: A 35-questions survey administered via RedCap® was emailed to all physicians practicing in Valais (~1300). Survey was available online from 22 November 2023 to 15 February 2024, with data collection conducted anonymously. Statistical analysis was performed in Stata. Ambulatory and in-hospital physicians answers were compared using Chi2 tests and Student-t tests.
RESULTS: 305 surveys were completed (response rate~23%), 53.1% (n=162) by women and 45.2% (n=138) by men. Participants working in outpatient settings (n=163) were significantly older than those working in hospitals (n=172) (49.3 vs 41.7 years on average, $p < 0.001$). Main reasons to prescribe antibiotics were. were urinary tract (reported by 70.9% of the physicians) and lower respiratory tract infections (63.7%). Major barriers to responsible antibiotic prescribing include patient pressure (47.5%), diagnostic difficulty (36.6%), and lack of continuing education (17.3%). Outpatient physicians reported more patient pressure (62.4% vs 31.0%), $p < 0.001$), while in-hospital physicians identified lack of ongoing education more frequently as a barrier to responsible antibiotic prescription (24.5% vs 11.5%, $p = 0.003$). In-hospital physicians consulted specialists more (48.1% vs 22.9%, $p < 0.001$), while outpatient practitioners relied on experience (54.2% vs 27.5%, $p < 0.001$). In-hospital physicians showed more interest in a smartphone application (49.7% vs 27.3%, $p < 0.001$). About half of the respondents (n=153) indicated missing resources to inform their patients about antibiotics. The majority of the respondents, 75.4% (n=230), felt that a public campaign would be relevant to increase population awareness and knowledge. Physicians highlighted the importance of utilizing social media (70.5%) and involving schools (45.5%).
CONCLUSION: Awareness of StAR resources for patient education seems limited. Tailoring interventions to meet the diverse needs of physician groups, addressing resource constraints and knowledge gaps, is crucial for effective implementation. A population survey is ongoing in Valais and both will guide implementation strategies

O058

Part of the crew, part of the ship: Investigating Bartonella Gene Transfer Agent - a domesticated phage

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Gene Transfer Agents (GTAs) are mobile genetic elements mediating horizontal gene transfer in bacteria and archaea. GTAs originate from bacteriophages that over time got “domesticated” by the cells and at present transfer random parts of the bacterial genome and are unable to self-propagate.
An interesting example is BaGTA (Bartonella GTA) present in bacteria Bartonella. BaGTA is highly conserved and emerged from two sequential domestications of phages which resulted in acquisition of the GTA locus forming the capsid and run-off replication (ROR) amplifying DNA prior to the packaging.
Both GTA and ROR are functionally linked and contribute to the gene transfer. However, little is known how both clusters cross-regulate. To address this, we assess how genes in the ROR (brrA, brrG, brrB) and orphan genes (bgtS, bgtTUV) are regulated and how they impact the GTA cluster transcription. For that, we harness methods of mass-spectrometry, flow cytometry and sequencing to investigate protein-protein interactions and unveil gene regulation.
GTAs are widespread in bacteria and archaea we believe it could be a fourth major mechanism of horizontal gene transfer that might drive evolution of their hosts.

O059

The completed BASEL phage collection: A tour into the fascinating diversity of bacteriophages

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Bacteriophages, the viruses infecting bacteria, represent the most abundant biological entities on Earth and hold great promise as alternative therapeutics for multidrug-resistant bacterial infections. Despite their potential, our understanding of the molecular mechanisms underlying phage-host interactions has been primarily confined to a few classical phage models. This not only restricts the immediate application of findings in phage therapy but also indicates that a vast reservoir of potent molecular biology within the natural diversity of bacteriophages remains untapped.
To address this gap, we have composed the BASEL (Bacteriophage Selection for your Laboratory) collection, a reference set of 105 newly isolated Escherichia coli phages extensively characterized through genomic and phenotypic analyses. Importantly, our upcoming expansion pack highlights previously overlooked phage groups, notably those that depend on LPS O-antigen glycans as host receptors absent in laboratory E. coli strains. Additionally, we isolated phages from rare groups susceptible to the resident bacterial immunity of E. coli K-12. Our findings demonstrate how the assorted diversity of phages can be leveraged to systematically uncover the molecular mechanisms underlying host recognition and resistance to bacterial immunity systems. These insights are directly informative for the selection of phages for clinical or biotechnological applications.
The BASEL collection has already been shared with multiple laboratories worldwide, serving as a valuable tool in exploring different aspects of phage-host interactions. Taken together, this work bridges the gap between model phages and the incredible diversity of phages in nature, offering crucial insights for both clinical applications and fundamental phage biology.

O060
Engineering a Minimal Pseudomonas Aeruginosa Bacteriophage

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Aims: Bacteriophages, or viruses that infect bacteria, are teeming with genes of unknown function. These accessory genes are often thought to code for diverse products, including toxins and virulence factors. These could pose problems when applying phages in a therapeutic setting. Therefore, we need a rapid and efficient method to delete these problematic phage accessory genes.

Methods: We focus on a modified Type I-C CRISPR-Cas3 system, also known as haCas3, to make small deletions in Pseudomonas aeruginosa phages. Together with a series of crRNAs, we can use the haCas3 system together with its wild-type counterpart to rapidly establish the essentiality of every hypothetical protein-coding gene in a phage genome, and isolate mutants lacking these genes.

Results: We were able to isolate mutants for the temperate virus JBD68 and therapeutic candidate Pb1. The haCas3 system produces random deletions within the range of around 10-3,000 bp without the need for a repair template. With this system we were able to map the essentiality of all the hypothetical protein coding genes in JBD68, and produce a minimal genome JBD68 by deleting more than 3,000 bp from its genome.

Conclusion: We demonstrated the efficacy of the wild-type Cas3 system at targeting and degrading diverse phages, as well as the ability of the haCas3 system in producing deletions in genes of interest that allow the phages to escape wild-type Cas3 selection. We also generated a genome-minimised phage based on JBD68. With its reduced genome, payloads such as bacteriocins can be easily introduced that would enhance its therapeutic potential without worrying about the effect on genomic space. Overall, we provide a new tool that can be used for the study of phage accessory genes and for easy and efficacious phage genetic engineering.

O061

Reviving an old enemy: prophage engineering to enhance virulence and lytic potential against multi-drug resistant Klebsiella pneumoniae.

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Context and Aim:
The global rapid spread of antimicrobial resistance calls for urgent action. Phage therapy is seen as a promising alternative to the antibiotic use. Until now, phage therapy has relied on the use of cocktails of natural lytic bacteriophages against susceptible bacteria. However, this approach requires extensive screenings of phage collections and might also trigger phage resistance in target bacteria. Most lysogenic phages or prophages, integrated in the genomes of most multi-drug resistant (MDR) bacteria, already possess all the genetic elements required to infect and lyse their bacterial hosts. However, they cannot be used as effective therapeutics due to their non-lytic profiles and to the presence of undesirable cargo on some of their genomes (i.e. antimicrobial resistance or toxin-encoding genes). To solve these problems, we aim to develop a synthetic genomics pipeline employing the yeast Saccharomyces cerevisiae as an engineering platform to turn prophages into tailor-made lytic phages to be used for the treatment of infections caused by MDR bacteria, focusing on Klebsiella pneumoniae.
Methods:
We first performed whole genome sequencing of a subset of clinical Klebsiella pneumoniae strains and explored the diversity of their prophage content in silico. Then, we induced functional prophages using Mitomycin C and determined phage host-range against clinical strains. We used the yeast genetic platform and TAR cloning to modify phage genomes by removing genetic elements necessary for establishment of lysogeny.
Results:
We successfully induced and characterized prophage Φ3_19KM57, which showed lytic activity against a clinical MDR strain K. pneumoniae 06KM907. Thus, Φ3_19KM57 was selected for phage engineering. We were able to modify the genome of this phage by removing the integrase (int) and major lysogeny regulator (C2) genes, which are essential for viral genome integration and establishment of lysogeny, respectively. The newly engineered phage versions showed enhanced virulence towards susceptible K. pneumoniae strain 06KM907 and increased lytic activity.
Conclusion:
We believe that other prophages like Φ3_19KM57 could be similarly modified and used safely as alternative therapies to antibiotics to treat bacterial infections. Moreover, the same pipeline could be used to enhance phage virulence towards other MDR bacteria.

O062

Cross-species transmission and evolution of Coronaviruses at the interface of camelids and humans

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The globally endemic low pathogenic human coronavirus (HCoV) 229E has been circulating in the human population for a long time with little sequence alterations. HCoV-229E-like viruses have been found in bat species (BatCoVs) as well as dromedary camels (DcCoVs) exemplary DcCoV-ACN4. For HCoV-229E and 229E-like DcCoVs no recent zoonotic spillovers are documented. Genome analysis propose that HCoV-229E evolved from bats via dromedary camels. This evolutionary scenario shows strong parallels to the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV is a highly pathogenic zoonotic virus that sporadically spills over from the dromedary camel reservoir to humans with a 36% fatality rate. Like HCoV-229E, the origins of MERS-CoV have been linked to ancestral BatCoVs. Consequently HCoV-229E and DcCoV-ACN4 can provide important insight into how emerging CoVs like MERS-CoV cross the species barrier and adapt to the human species.
To analyze potential species barriers, we first investigated the spike-receptor interaction of HCoV-229E and DcCoV-ACN4. We used vesicular stomatitis virus (VSV) CoV-S pseudotyped viral particles on HEK cells overexpressing the human or dromedary camel aminopeptidase N; receptor for HCoV-229E. Then we assessed permissiveness by performing replication kinetics on species-specific cell lines. To mimic in vivo conditions, we further performed the replication kinetics on human (h) and camelid (c) primary airway epithelial cell (AEC) cultures. Proceeding Immunofluorescence staining imply adverse cell tropism for HCoV-229E in hAEC, DcCoV-ACN4 in cAEC and MERS-CoV in hAEC and cAEC.
We show an entry-independent species-restricted replication of HCoV-229E and DcCoV-ACN4 in AEC culture that was not observed for MERS-CoV. Our findings indicate that the observed species barriers of HCoV-229E and DcCoV-ACN4 lie beyond the spike-receptor interaction and open the search for the viral and host factors influencing the different host specificities of HCoV-229E, DcCoV-ACN4 and MERS-CoV.

O063

The new in vivo model of intestinal colonization with Zophobas morio larvae: testing global ESBL- and carbapenemase-producing Escherichia coli clones

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Aims. Developing an in vivo model of intestinal colonization with multidrug-resistant E. coli (MDR-Ec). Moreover, testing bacteriophages as a possible decolonization strategy.

Methods. Zophobas morio larvae were colonized by implementing two different approaches. First, through contaminated food administration from T0 (day 0) to T7 with three MDR-Ec producing ESBLs and/or carbapenemases: Ec-4901.28 (ST131, CTX-M-15), Ec-042 (ST410, OXA-181) and Ec-050 (ST167, NDM-5). After T7, larvae received non-contaminated food until T28. In the second approach, force-feeding was performed to refine the model and expedite the experimental process using a single 5 µL 4901.28 at 108 CFU/mL per os injection on T0.
To detect MDR-Ec strains, larvae were homogenized at different time-points. Samples were plated on selective ChromID ESBL plates followed by 24 h incubation and colony count (CFU/mL). Decolonization using the INTESTI bacteriophage cocktail was performed by injecting 2 doses of 10 µL. Dynamic changes of Z. morio intestinal microbiota and resistome of challenged larvae were also investigated. Microbiota characterization was achieved through 16S rRNA amplicon sequencing and further diversity analyses were performed.
Results. With the first approach, contaminated food administration with the MDR-Ec strains induced rapid colonization with high bacterial load of ~106-7 CFU/mL at T7. Larvae remained colonized with Ec-4901.28 and Ec-042 until T28 (103-4 CFU/mL). Larvae receiving a force-feeding treatment with bacteriophages were decolonized by Ec-4901.28 (INTESTI-susceptible); however, Ec-042 and Ec-050 (INTESTI-resistant) did not. Natural microbiota of Z. morio larvae was very rich of bacterial genera (i.e., Lactococcus, Enterococcus, Spiroplasma, Latilactobacillus). Moreover, Escherichia-Shigella genera appeared in larvae only at T7-T10. Notably, even for the second approach, force-feeding resulted in a colonization effect with 105 CFU/mL at T14.

Conclusion. Larvae possess a rich microbiota and can be easily colonized with global clones of MDR-Ec. Moreover, with the second approach we optimized the time required to induce intestinal colonization and retain a high bacterial load. Therefore, the Z. morio model presents a high-throughput compromise to study novel gut decolonization strategies reducing the number of subsequent mammalian experiments in line with the 3Rs strategy.

O064
Study of a conserved chlamydial gene linked to aberrant bodies.

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The Chlamydiales order contains obligate intracellular bacteria (such as Waddlia chondrophila and Chlamydia trachomatis) sharing a common biphasic developmental cycle characterized by infectious, non-replicative elementary bodies and non-infectious, replicative reticulate bodies. When exposed to stress stimuli, reticulate bodies enter a persistence state called aberrant bodies (ABs). ABs are believed to play a role in chronicity and recurrence of chlamydial infections.

RNA-sequencing performed on Waddlia aberrant bodies allowed us to identify ispA (iron starvation protein A), an upregulated gene in the Chlamydiae phylum, and exclusively present in that phylum. W. chondrophila and C. trachomatis ABs were then obtained with other types of stresses to assess the behavior of this gene. Bioinformatic and wet-lab analysis were also performed to characterize the protein.

We confirmed that while ispA is upregulated upon iron-starvation in W. chondrophila, it wasn't the case for C. trachomatis. In fact, of all the stresses applied, heat-shock is the only tested condition where the upregulation was observed in both species. The secretion of IspA by the T3SS predicted bioinformatically was confirmed using Yersinia enterocolitica as heterologous system, but its localization within the host cell observed by immunofluorescence is unclear. Interestingly, IspA has a conserved C-terminal domain resembling that of a cystine-knot. Cystine-knots are described as a domain of protein interaction but was only described in eukaryotes. Regulation-wise, a conserved CIRCE sequence which allows repression by HrcA, a stress regulator, was identified upstream ispA.

Our preliminary results reveal IspA, a highly conserved, Chlamydiae-specific T3SS effector, with a conserved cystine-knot-like domain, and a conserved CIRCE regulatory sequence. Despite these conservations, ispA answers differently to various stresses across the phylum, but similarly to heat-shock for C. trachomatis and W. chondrophila. Further studies should aim at identifying IspA targets and other regulatory pathways to explain these discrepancies.

O065
Identification and characterization of Legionella effectors targeting the mitochondrial network

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Intracellular bacteria manipulate and subvert cellular processes in their eukaryotic hosts in a sophisticated manner. The facultative intracellular pathogen Legionella pneumophila naturally replicates in free-living amoeba. Inhalation of this pathogen can lead to a severe pneumonia, due to its ability to replicate within alveolar macrophages. To this end, L. pneumophila secretes over 300 “effector proteins” into host cells, which subvert phagosome maturation and other cellular processes, eventually leading to the formation of a specific Legionella-containing vacuole (LCV). While LCV formation represents an essential step for the intracellular survival and replication of L. pneumophila, virtually all host cell processes are targeted and subverted during infection.
Mitochondrial function and dynamics are essential for basically all cellular processes. Previous studies have shown that L. pneumophila specifically targets and modulates mitochondrial components and their function. Using bioinformatic tools and experimental approaches, we identified and characterized in this project novel L. pneumophila effectors localizing to and targeting the mitochondrial network. Deletion mutants lacking single effector proteins showed an intracellular replication defect, indicating that the mitochondria-targeting effectors have important functions during infection. Some of these effectors were found to affect host cell respiration. Current studies aim at identifying the eukaryotic targets of these effectors and elucidating their mode of action.

O066

Harnessing Synthetic Nanobodies for Rapid Diagnosis of Staphylococcus aureus Infections

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Aims

Staphylococcus aureus has been causing invasive, difficult-to-manage conditions such as endocarditis and osteomyelitis. Certain strains have developed resistance to antibiotics thus making them more difficult to treat. Current strategies to detect Staphylococcus aureus infections and their antibiotic susceptibility require a culturing step prior to diagnosis and characterization. We aim to eliminate the need for bacterial culturing and hence, reduce the time to diagnosis to a few hours. We present a single-domain-antibody-based strategy to capture S. aureus from solution. We aim to capture S. aureus from the site of infection, thus enriching the bacteria over the patient material, and hence, facilitating diagnosis.

Methods

We screened our synthetic library for single-domain antibodies that specifically bind to surface proteins of S. aureus. We chose Staphylococcal surface protein A as a target due to its high abundance and sequence conservation across species. After screening the library for single-domain antibodies we then coupled selected antibodies to a commercially available maleimide-PEG11-biotin linker construct via cysteine-maleimide coupling chemistry. This capture molecule would be able to bind to the surface protein on S. aureus, and further, streptavidin-magnetic beads could be used to fish out the bacterial cells via biotin-streptavidin chemistry.

Results

Using our strategy, we were able to demonstrate successful capture of S. aureus from buffer solutions and growth medium within an hour of incubation with the capture molecules. We have observed a capture efficiency of approximately 60-80% for various lab strains and clinical isolates. Our capture strategy can specifically enrich S. aureus even when the bacterial count is as low as 500-1000 CFUs, mimicking an early stage of infection.

Conclusion

The presented method reduces the detection time from a full day to a few hours. Importantly, the isolated bacterial cells are preserved in their most natural state facilitating characterization of the infection and antibiotic susceptibility testing. With some optimization, our capture strategy may be able to significantly enrich S. aureus over human material from patient samples, ensuring ease of diagnosis. The presented strategy is a proof of concept and should passage the advent of rapid and reliable diagnosis of S. aureus infections.

O067

Combination of Nanopore Sequencing with 16S Eubacterial PCR for rapid Pathogen Identification in Clinical Diagnostics

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Aims
The combination of IVD-R compliant 16S eubacterial PCR followed by Sanger sequencing enables a rapid, and cultivation-free identification of bacterial pathogens in clinical samples. With low biomass samples, Sanger sequencing is typically used given the expected lower diversity, lower cost, faster turnaround time, as well as simplified analysis, which usually outweigh the benefits of next-generation sequencing methods. Yet, Sanger sequencing often fails to resolve even low diversity microbial samples, spurring the need for cheap and more fine-grained technologies. Here, we present a novel approach coupling 16S eubacterial PCR with third-generation Oxford Nanopore Technologies (ONT) sequencing for clinical diagnostic applications.

Methods
To validate the specificity and sensitivity of our approach, we conducted comparative sequencing of 16S eubacterial PCR amplicons on both ONT and Illumina MiSeq platforms. Sequencing was performed on routine samples normally processed in our diagnostic department for eubacterial species presence. Reads obtained by both ONT and Illumina technologies underwent processing via the ONT-tailored LORCAN bioinformatic pipeline (1). To mitigate potential biases introduced by analyzing Illumina data with the LORCAN pipeline, Illumina read analysis was also validated with dada2 (2), a tool optimized for short-reads. Sensitivity was additionally assessed using dilution series of two bacterial species to confirm diagnostic performance.

Results
First results with a small sample set (about 40 samples) underscore the robustness and accuracy of ONT sequencing in capturing the diversity of clinical eubacterial samples. ONT sequencing followed by streamlined data analyses accelerates turnaround time to below one working day from samples to bioinformatic analyses, while maintaining cost efficiency, crucial for diagnostic applications. The higher resolution offered by ONT over Sanger sequencing provides a clear advantage for pathogen detection in complex or lowly concentrated microbial samples, as well as resolving bacterial background contamination.

Conclusion
The combination of 16S eubacterial PCR with ONT sequencing represents an important improvement in terms of sensitivity and specificity to Sanger sequencing, and leads to improved cost-effectiveness and turnaround time in clinical microbial diagnostics.

O068

Strongyloides stercoralis westernblot for serologic screening of solid organ transplant candidates

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Background/Aims
Strongyloides stercoralis affects >100 million individuals worldwide, especially immunocompromised patients are at increased risk for severe infection. Therefore, all solid transplant (SOT) candidates are screened for S.stercoralis infection before transplantation and prophylactic treatment is administered if ELISA test results are positive. ELISAs for S.stercoralis tend to cross-react if subjects are infected with other (non-strongyloides) helminths and central/northern Europe is a low-endemic area for S.stercoralis Therefore, a relevant proportion of pre-transplant S.stercoralis ELISA results may reflect false positive results and patients will receive unnecessary treatment. We evaluated a two-step testing approach using an ELISA for screening and a Westernblot (WB) for confirmation of S.stercoralis infection.
Methods
We recently developed and validated a WB for detection of anti-S.stercoralis specific antibodies using well-defined reference patient sera. Subsequently, we performed a pilot study for the technical validation of the WB. For this, we used frozen serum samples of SOT candidates who previously underwent S.stercoralis testing (ELISA) as part of their pre-transplant workup between 2018 and 2022.
Results
The novel WB specifically detects human IgG antibodies (two immunodominant antigenic compounds of 12 and 17kDa) against S.stercoralis with a diagnostic sensitivity of 91% and a diagnostic specificity of 100%.
We tested 310 serum samples of which 8.7% (27/310) showed positive (>10AE/mL)- and 6.5% (20/310) revealed borderline (1-9AE/mL) S.stercoralis ELISA results. Analysis of the 47 ELISA positive/borderline samples with the WB revealed that 87.2% (41/47) of these tests were false-positive. Half (3/6) of WB positive samples showed borderline results in the ELISA screening.
Conclusion
The novel WB specifically detects S.stercoralis antibodies in serum of SOT candidates. Our results indicate that also borderline ELISA test results may represent true-positive findings. Therefore, the level (AE/mL) of S.stercoralis antibodies measured by ELISA should not be used as a proxy for differentiation of false-positive from true-positive tests. As present guidelines recommend to screen for S.stercoralis infection in SOT candidates using serologic assays only, no stool samples are available for these patients. A future clinical study will assess the sensitivity and specificity of a two-step approach for serologic S.stercoralis testing.

O069

Improving tuberculosis diagnosis using a Hidden Markov Model-based decision model for multiple testing strategy

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Background
Molecular methods have significantly improved tuberculosis (TB) diagnosis. However, to enhance the detection rate, current diagnostic process involves multi-test strategy often utilizing microscopy, PCR, and culture in successive clinical specimens especially in patients with paucibacillary disease. While, evaluating the performance of individual test is manageable, assessing the overall performance, including sensitivity and specificity, of a multi-test strategy is complex and prone to over or undertesting. In this study we aimed to develop and validate a model specifically designed to assess the performance of multi-test strategies for TB diagnosis.
Methods
Using a database encompassing 34’429 specimens and 14’358 patients from our tertiary care teaching hospital (2008-2018), we first determined the diagnostic performance of individual microbiological TB tests using multiple variables including type of test, type of clinical specimen, patient age and gender. Subsequently, we applied a Hidden Markov Models (HMM) to derive a score indicating a patient's TB likelihood based on sequential molecular test results (1, 2, 3 or more). Finally, we determined the performance of the prediction model against the historical database.
Results
We calculated TB prevalence and individual tests sensitivity and specificity across diverse categories, including gender (male/female), age (categorized into five groups), four distinct types of microbiological tests (microscopy, Xpert MTB/RIF or in-house real-time PCR, and culture), and four clinical specimen types (sputum, induced sputum, bronchoalveolar lavage and bronchial aspirate). Utilizing the overall sequence of PCR tests for each patient in our cohort, this information was employed in a Hidden Markov Model (HMM) to generate a probability score (ranging from 0 to 1) indicating the likelihood of having TB. Using a gold standard based on culture and clinical data, we established an optimal score of 0.995 at which, the sensitivity and specificity for predicting TB of the HMM was 95,7% and 97,9% respectively.
Conclusions
In this study, we developed and validated a model that calculate the performance of TB multiple test strategies that integrates the comprehensive history of molecular test results alongside patient-specific factors. This model could further enhance diagnostic algorithms, offering valuable support for clinical decision-making across different stages of multiple test strategies.

O070

Listeria monocytogenes – Rocketing Towards Survival: The Thin Line Between Life and Death

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Listeria monocytogenes (Lmo), a Gram-positive foodborne pathogen, poses a significant threat to public health due to its ability to cause listeriosis, associated with high mortality rates among susceptible populations. Teichoic acids (TAs), including wall-teichoic acid (WTA) and lipoteichoic acid (LTA), are essential components of the cell wall, playing multifaceted roles in bacterial physiology and virulence. Previous studies suggest a potential link between Galactose (Gal)-deficient TAs and the virulence of Lmo, particularly through the modulation of ActA, the actin assembly-inducing protein, function.

Here, we aimed to dissect how Gal-TAs modulate ActA during the cell-to-cell spread process of Lmo. Through construction of in-frame deletion mutants lacking Gal-deficient decoration on WTA, LTA, or both TAs, we assessed their phenotypic traits during infection using immunofluorescence techniques. Furthermore, we purified WTA polymers and recombinant ActA for surface plasmon resonance analysis to explore the molecular interaction between WTA and ActA.

Our findings indicate that only Gal-WTA is responsible for ActA dysfunction, while Gal-LTA does not play a role therein. Additionally, we observed a weak binding affinity between ActA and WTA in the micromolar range, suggesting the involvement of additional host proteins in the ActA-WTA relationship.

Future investigation will focus on evaluating the contribution of various host proteins to the ActA-WTA interaction, providing insights into the structure-function relationship of cell wall-associated macromolecules in Gram-positive pathogens.

O071

Modular multi-step chemical derivatization of sialic acid for flagellin glycosylation in Caulobacter and a heterologous host for bio-conjugation

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Chemical diversification of sialic acids on surface structures including flagella, capsules or lipopolysaccharides is widespread across bacterial lineages. Despite the plethora of naturally occurring sialic acid derivatives, the genetic basis for derivatization remains unresolved. We discovered a tricistronic module, FlmEFX, that modifies the pseudaminic acid (Pse) moiety present on the six glycosylated flagellins of Caulobacter crescentus, a synchronizable α-proteobacterium assembling a polar flagellum.

The glycan modifications on Caulobacter flagellins from different strains (WT and mutants carrying in frame deletions of flmE, flmF and flmX alone or in combination) were identified by glycopeptide analysis. Mass spectrometry was used to dissect the interaction network of the flagellin glycosylation system. The conservation of the Pse biosynthesis pathway and specifically of the FlmEFX module was investigated by complementing Caulobacter mutant strains with orthologs from different species.

We found that FlmEFX controls the multi-step chemical derivatization of Pse and that balanced FlmEFX expression is important to prevent a block in Pse-dependent flagellin glycosylation, assembly and secretion. Transcription of the flmEFX module is integrated into the Caulobacter developmental regulatory circuit, as it is under control of the cell cycle master regulator CtrA.

Our results indicate that FlmE is a putative methyltransferase promoting a 28 Da modification of the basic Pse unit, whereas FlmX inhibits flagellin export and assembly in the absence of FlmE. Together the FlmEFX unit enhances heterologous bio-conjugation of flagellins with the terminal Pse-derivative in recombinant Escherichia coli producing Pse and the flagellin glycosyltransferase FlmG. FlmEFX co-occur mostly with FlmG-dependent flagellin glycosylation systems, yet orthologs are also encoded in O-antigen gene clusters of pathogens.

Our previously established heterologous flagellin bio-conjugation system in E. coli expressing the Pse biosynthetic pathway from Caulobacter allows the study of multi-step derivatization of Pse. In this work we identified a transferable genetic module, FlmEFX, that terminally modifies Pse. In contrast to the six Pse biosynthesis genes that are widespread, the flmEFX module is less common and primarily present in several clades of the Caulobacterales in the α-proteobacteria, suggesting that it confers diversification of flagellar glycans in these bacteria.

O072

Insights into the molecular mechanism for septal peptidoglycan reinforcement by PBP1b in E. coli

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E. coli encodes two class A penicillin-binding proteins (aPBPs), PBP1a and PBP1b. aPBPs were shown to play a critical role for cell wall reinforcement. Interestingly, the two aPBPs are functionally not redundant and PBP1b was found to display several synthetic lethal interactions with mutations perturbating cell division. While this overall illustrates the importance of PBP1b for cell division, it has remained largely illusive why this is the case.

Here we show using a combination of bacterial genetics, cryo-electron tomography, biophysics and advanced live-cell microscopy how PBP1b mediates the synthesis of the septal peptidoglycan (sPG) wedge to reinforce the division site. Cells lacking PBP1b, but not PBP1a, lack a discernable sPG wedge resulting in the softer division sites and lyse from their septa under osmotically stressed conditions. Moreover, we provide evidence that the intrinsically disordered N-terminal domain (N-IDD) plays a critical role in recruiting PBP1b to the division site through interactions with Z-ring component FtsA. Importantly, previous studies on PBP1b using N-terminal fluorescent fusion often omitted the expression of full-length IDD and thus didn’t find a specific septal localization. Moreover, FtsA lacks a N-IDD altogether.

Overall, our work highlights the critical importance for PBP1b-mediated septal cell wall reinforcement and demonstrates the importance of IDD for protein-protein interactions in the divisome.

O073

Identification of CMG2/ANTXR2 as the cellular receptor for Clostridium perfringens NetF toxin.

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Introduction:
Clostridium perfringens causes enteric diseases in different animal species and in humans. Its pathogenicity relies on the secretion of a large arsenal of virulence factors, many of them belonging to the family of hemolysin-like β-pore forming toxins (β-PFTs). Knowledge about role and action of many of these toxins is still limited. To understand their role in the pathogenesis of bacterial infections we need to determine the molecular and structural basis of their cell, tissue, and species specificity.

Aims:
We aimed to identify the cellular receptor and the pore-structure of Necrotizing Enteritis Toxin F (NetF), a hemolysin-like β-PFT associated with fatal hemorrhagic enteritis in dogs and foals caused by C. perfringens type A strains.

Methods:
Using recombinantly expressed NetF, we performed cell viability assays on 29 different mammalian cell lines. We compared gene expression profiles of susceptible and resistant cell lines. The best receptor candidate was confirmed using CRISPR/Cas9 single gene knockout and ectopic overexpression studies. Mutated and chimeric receptor proteins were expressed in HAP1 cells to determine the receptor specificity of NetF. We used cryo-EM to determine the pore structure of NetF .

Results:
We identified Capillary Morphogenesis Protein 2 (CMG2 or ANTXR2) to be important in NetF-mediated cytotoxicity. CMG2 is also one of the two known receptors for protective antigen (PA) of Bacillus anthracis anthrax toxin. We demonstrated that CMG2 expression on target cells is essential for NetF toxicity and that PA competitively inhibits NetF cytotoxicity. To further investigate the interaction of NetF with its putative membrane protein receptor, we engineered mutant versions of CMG2 lacking parts of its extracellular domain or presenting domains of similar proteins. Using this approach, we showed that the Ig-like domain of CMG2 is crucial for NetF toxicity and that the amino acid region 243-250 of CMG2 is part of NetF binding site. The oligomeric pore-structure of NetF shows cap-, rim-, and stem domains typical for hemolysin-beta-pore forming toxins.

Conclusions:
We identified the cellular receptor and determined the 3D structure of a central virulence factor of C. perfringens strains causing enteric disease in horses and dogs. Furthermore, our results highlight molecular mechanisms and structures that confer receptor-, cell-type and species specificity for clostridial hemolysin-like βPFTs.

O074

Comparison of MERS-CoV infection in human and camelid primary airway epithelial cells with single cell resolution

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MERS-CoV is a zoonotic coronavirus that can lead to severe respiratory illness in humans, but only causes mild respiratory illness in dromedary camels, which are a major reservoir host for MERS-CoV. To identify species-specific host factors that may influence disease outcomes in humans and camelids, airway epithelial cell (AEC) culture models were developed for C. bactrianus and L. glama.
Single-cell transcriptomics was performed on human, camel, and llama AEC cultures infected with MERS-CoV, HCoV-229E, and dcCoV-229E. HCoV-229E belongs to the common cold coronaviruses and causes mild symptoms in humans, while dcCoV-ACN4 is a phylogenetically related virus, which was isolated from dromedary camels. To compare the response to CoV infection among different host species, we established an extensive bioinformatics pipeline in R, that is compatible with camelid species and allowed functional analysis.
Using this workflow, we were able to annotate not only the human airway epithelial cells in our samples but also the main cell types of the camelid species. Additionally, we investigated how host cell tropism and viral entry receptor expression presented under uninfected and virus-infected conditions. Intriguingly, we found that viral entry receptor expression changes upon MERS-CoV and dcCoV-ACN4 infection and that these changes might play a major role in the manifestation of the host cell tropism for both viruses. Furthermore, we assessed the differential gene expression patterns between infected and uninfected cells in each species and airway epithelium cell type. We discovered that several genes involved in the unfolded protein response network (UPR), cilium organization and early anti-viral response were upregulated in MERS-CoV-infected secretory and ciliated cells.
Notably, this work represents the first steps in generating a comprehensive framework to analyze and identify the fundamental characteristics of virus-host interactions in the respiratory epithelium of well and less-studied species with single-cell resolution.

O075

Scoary2 and OpenGenomeBrowser: microbial GWAS for large phenotypic datasets

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Unraveling bacterial gene function drives progress in various areas, such as food production, pharmacology, and ecology. While omics technologies capture high-dimensional phenotypic data, linking them to genomic data is challenging, leaving 40-60% of bacterial genes undescribed. One approach to do this in high throughput is microbial GWAS (mGWAS), but existing solutions are made for analyzing very few traits only.

To overcome this bottleneck, we present Scoary2, an ultra-fast microbial mGWAS software. It automates post-mGWAS workflows using an interactive web-app which can be connected to external tools such as the comparative genomics software OpenGenomeBrowser, enabling fast and integrative data exploration.

Scoary2 is, to the best of our knowledge, the first software that makes it feasible to study large phenotypic multi-omics datasets using mGWAS. As proof of concept, we used Scoary2 to explore the metabolome of 44 yogurts, each produced by combining a different strain of the species Propionibacterium freudenreichii with starter culture and discovered two previously unknown genes which affect the carnitine metabolism.

O076

GPT-4 based AI agents – the new expert system for detection of antimicrobial resistance mechanisms?

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EUCAST advises screening for extended spectrum beta-lactamases (ESBL), plasmidic AmpC beta-lactamases, and carbapenemases in Enterobacteriaceae. GPT-4, a multi-modal large language model (LLM) by OpenAI, could potentially aid in this screening process.
Aim: We aimed to validate a customized GPT-agent to identify potential resistance mechanisms.
Methods: We customized a GPT-agent ("EUCAST-GPT-expert") using EUCAST guidelines, expert rules, and breakpoint table (v13.1). We uploaded disk diffusion images of 225 Gram-negative isolates that were analyzed for the following resistance mechanisms: "none", "ESBL", "AmpC", and "carbapenemase". We compared routine diagnostic output (reference standard) to (i) EUCAST-GPT-expert, (ii) medical microbiologists, and (iii) non-customized GPT-4, thereby evaluating sensitivities, specificities, negative and positive predictive values for detecting these resistance mechanisms.
Results: Three human readers showed concordance in 814/862 (94.4%) phenotypic categories and used in median eight words (IQR 4-11) for reasoning. Median sensitivity and specificity for ESBL, AmpC, and carbapenemase were 98% and 99.1%, 96.8% and 97.1%, and 95.5% and 98.5%, respectively. Three independent prompting rounds of the EUCAST-GPT-expert showed concordance in 706/862 (81.9%) categories but used in median 158 words (IQR 140-174) for reasoning. Median sensitivity and specificity for ESBL, AmpC, and carbapenemase prediction were 95.4% and 69.23%, 96.9% and 86.3%, and 100% and 98.8%, respectively. E. coli (n=132) showed lower median ESBL sensitivities, but higher specificities compared to Klebsiella pneumoniae and K. oxytoca (n=45) with 86.4% vs 100% and 76.9% vs. 61.9%, respectively. In the non-customized GPT-4, only 169/862 (19.6%) categories could be interpreted. Of these, 137/169 (81.1%) categories agreed with routine diagnostic. The non-customized GPT-4 used in median 85 words (IQR 72-105) for reasoning.
Conclusion: Human experts showed higher concordance (94.4% vs. 81.9%) and shorter argumentations (median 8 vs. 158 words) compared to EUCAST-GPT-expert. Human experts showed comparable median sensitivities and higher specificities compared to EUCAST-GPT-expert. GPT-agents more commonly showed unspecific flagging of ESBL and AmpC, potentially, resulting in additional testing and higher costs. GPT-agents are not IVDR/FDA-approved as diagnostic tools, thus in-depth validation of LLMs is critical and in silico datasets for benchmarking are needed.

O077

Multi-omics, multi assay analysis of evolved resistance to ceftazidime-avibactam in Pseudomonas aeruginosa , compared to meropenem resistance and co-resistance evolution

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Background: Knowledge on molecular mechanisms of Ceftazidime-Avibactam (CZA) resistance in Pseudomonas aeruginosa is scarce. In this multiomics study on laboratory-evolved CZA-resistant P. aeruginsa we unravel CZA resistance mechanisms and provide insights into its cross-resistance to meropenem (MEM).

Materials/methods: From six clinical P. aeruginosa, three representative clones of each isolate were anaylzed for resistance selection to sub-inhibitory concentrations (SICs) of CZA (n=3) or MEM (n=3). After three passages of being subjected to the same antibiotic concentration, minimum inhibitory concentrations (MIC) were determined. The final MIC was determined when at least one clone became resistant (EUCAST) and after a maximum of 54 days of consecutive incubation. Whole genome and transcriptome (Illumina platform) as well as proteome (Orbitrap Fusion Lumos Mass spectrometer) analysis of the parental isolates and their resistant derivatives (n=18) were then performed. In parallel, a transposon insertion mutant library of P. aeruginosa was screened, and CRISPR/Cas9 genome editing was performed to target specific mutations.

Results: Strains exposed to SICs of MEM exhibited a notably high resistance evolution rate, with only 22% (4/18) concurrently displaying cross-resistance to CZA. Conversely, resistance evolution to CZA was significantly slower in strains subjected to CZA. Addition of the efflux pump inhibitor phenyl arginine-β-naphthylamide (PaβN) resulted in a significantly higher CZA-MIC reduction in samples grown in SICs-CZA compared to MEM-MIC reduction in samples grown in SICs-MEM (p=0.0336). Principal components analysis of proteomics and transcriptomics data highlighted sample clustering based on strains. The multiomic approach (genomics, transcriptomics and proteomics) suggests multiple mechanisms contributing in CZA-resistance. CRISPR/Cas9 genome editing identified mutations in dacB directly associated with CZA resistance; ongoing work focuses on genome editing of ampC - which was upregulated in the majority of strains subjected to SICs of CZA - mexR/B, and arnA. .
Conclusions: This ongoing study contributes to a deeper comprehension of the intricate dynamics and molecular mechanisms that regulate CZA resistance in P. aeruginosa.

O078

Extensive phage invasion and bacterial host evolution in cheese starter cultures over an entire cheese-making season

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Phages and bacteria are engaged in a continuous arms race, illustrated by the recent discovery of numerous anti-phage and anti-defense systems. This diversity and prevalence highlight the strong selective pressure of phages on bacteria and vice versa. Yet, in nature, ecosystem functions mostly seem stable. We are largely unaware of how these defense mechanisms are distributed in natural communities and how they adapt. Specifically, do bacteria and their phages persist due to rapid co-evolution (evolutionary response), or is it mainly the selection of preexisting standing diversity (ecological response)? To answer these questions, we analysed mixed cheese starter communities that are continuously subcultured over an entire cheese-making season. In these communities, we have the selection for rapidly growing bacterial strains in milk with possible inflows and changes of microbes and phages. Daily metagenome sequencing and extensive metadata collection over several months at three cheese-making sites revealed the persistence of stable multi-strain bacterial communities. On the contrary, we observed hundreds of active lytic phages, prophages, and satellite phages. While many phages were occasional, others persisted without notable effect on the community function due to the presence of anti-defense systems. While the bacterial strain composition remained stable throughout the season, their genomes continuously acquired novel CRISPR spacers, anti-phage mechanisms and novel putative anti-phage genes. Altogether, these findings suggest that in nature the intricate dance between bacteria and phages is a complex mix of ecological forces (selection of pre-existing diversity) and rapid evolution (de novo) of both the phages and the bacterial hosts.

O079

Global soil prokaryotic communities' exposure to climate change: an ecological niche perspective

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In a previous study, we assessed the niche breadth and position of soil microorganisms within the Swiss Alps, revealing a narrowing of niche breadth towards environmental extremes. This observation underscored the prevalence of specialized taxa in more extreme environmental conditions. However, due to the localized nature of the study and partial coverage of environmental gradients, we were unable to discount the influence of niche truncation on the observed trends. Here, using a global database of soil prokaryotes, we present a comprehensive analysis of the environmental niche occupied by soil bacteria and archaea on a global scale, aiming to identify specialist and generalist microorganisms. Furthermore, using the niche marginality index in current and future climatic conditions, we evaluate their exposure to climate change. This study offers insights into the broader ecological implications of microbial responses to climatic perturbations.

O080

A meta-analysis of complete microbial genomes reveals differences in assembly quality, taxonomic coverage and serves as a resource to decipher the functions of microbiome isolates

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Aims:
Mapping functional traits to specific organisms is central to the understanding of ecosystem functioning, affecting natural environments, biotechnologically relevant consortia and synthetic communities alike. This heavily relies on the availability of complete, reliable reference genomes. However, despite their importance, complete genomes only account for a minor fraction of available assemblies.

Methods:
To bridge the gap from studying microbiome composition towards the function of key individual isolates, we analyzed the repeat structure and assembly complexity of 37’000 complete RefSeq and 46’500 Genbank genomes, and integrate additional analyses and meta-data to create a rich, publicly available resource for data mining. Nucmer was used to compute repeat pairs as a basis to calculate the overall repeat region content and assembly complexity, two metrics to identify genera with predominantly difficult to assemble genomes. By mining available meta-data, we devise a quality control step that identifies up to 7% of RefSeq assemblies that may contain assembly errors/miss sequence information.

Results:
Complete genomes represent an optimal basis for downstream functional genomics and comparative genomics studies [1,2]. Yet, our analyses revealed that up to 7% of RefSeq assemblies may contain assembly errors/miss sequence information. These mainly comprise cases that used reference-based assembly strategies or assemblies generated from short read datasets. Biologically relevant aspects were also analyzed including 16S rRNA, biosynthetic gene cluster content and their metabolic potential. Tracking the increasing taxonomic spread of complete genomes over time identifies new taxonomic groups, several of which harbor potential for biological applications. Our meta-analysis of complete prokaryotic genomes allows to identify potential assembly errors in RefSeq genomes, to devise optimal sequencing strategies for difficult to assemble genomes, to improve the resolution of 16S rRNA based amplicon sequencing, to track the increasing taxonomic spread of complete genomes and to link structural and functional traits to distinct taxonomic groups. In silico analysis and comparison of new isolates with this compendium can identify particular interesting strains for various applications in biocontrol, biotechnology and other fields.

O081

Automated image analysis for tracking host-endosymbiont interactions during Rhizopus microsporus germination

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The filamentous fungus Rhizopus microsporus engages in endosymbioses with bacteria from the family of Burkholderiaceae. The most prominent example is the obligate interaction with the endosymbiont Mycetohabitans rhizoxinica, which assists the host in defence against predators, facilitates carbon acquisition and is required for its asexual reproduction. Recently, we have introduced bacteria into a naturally endosymbiont-free strain of R. microsporus, which compromised host fitness. Adaptive laboratory evolution increased germination rates and stabilized the artificially induced endosymbiosis. To study the impact of bacteria on host fitness during germination, we used a high-throughput microscopy method with custom-trained U-Net deep learning models. The approach facilitated the segmentation and tracking of individual spores using multipositional time-lapse confocal imaging, allowing us to monitor changes in cell shape and to quantify fungal growth rates by analysing area fluctuations of segmented objects. We also found that the onset of germination can be identified by detection of a decrease in roundness. In addition, our method quantifies fluorescently labeled bacterial endosymbionts and allows for analysis of the relationship between endosymbiont and host growth under different conditions.

O082

An RNA replicon system to investigate promising inhibitors of feline coronavirus

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Question

Feline infectious peritonitis (FIP) is a fatal feline disease, caused by a feline coronavirus (FCoV), namely feline infectious peritonitis virus (FIPV). FIPV is of great significance in the cat population being a major cause of feline deaths in veterinary practices. As there are neither effective preventive measures nor approved treatment options available, there is an urgent need to identify antiviral drugs against FIPV.

Methods

We produced a baby hamster kidney 21 (BHK) cell line expressing a serotype I FCoV replicon RNA with a green fluorescent protein (GFP) reporter gene (BHK-F-Rep) and used it as an in vitro screening system to test different antiviral compounds.

Results

Two inhibitors of the FCoV main protease (Mpro), namely GC376 and Nirmatrelvir, as well as the nucleoside analog Remdesivir proved to be effective in inhibiting the replicon system. Different combinations of these compounds also proved to be potent inhibitors, having an additive effect when combined. Remdesivir, GC376, and Nirmatrelvir all have a 50% cytotoxic concentration (CC50) more than 200 times higher than their half-maximal inhibitory concentrations (IC50), making them important candidates for future in vivo studies as well as clinically implemented drug candidates. Additionally, results were acquired with a virus infection system, where Felis catus whole fetus 4 (Fcwf-4) cells were infected with a previously described recombinant GFP-expressing FIPV (based on the laboratory-adapted serotype I FIPV strain Black) and treated with the most promising compounds. Results acquired with the replicon system were comparable to the results acquired with the virus infection system, demonstrating that we successfully implemented the FCoV replicon system for antiviral screening.

Conclusions

We expect that this system will greatly facilitate future screens for anti-FIPV compounds and provide a non-infectious system to study and evaluate drug resistance mutations that may emerge in the FIPV genome.

O083

Suboptimal dosing of an antimicrobial peptide can induce cross-resistance to polymyxins in Acinetobacter baumannii

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Aim

Antibiotic resistance is an increasing threat for human health. Antimicrobial peptides (AMPs) have been proposed as an alternative to antibiotics against resistant bacteria. AMPs are naturally occurring peptides, part of the first immune defence of a wide variety of organisms. AMPs are assumed to cause low rate of resistance emergence. TAT-RasGAP317-326 is a peptide with both anticancer and antimicrobial activities. We performed in vitro resistance selection on Escherichia coli and could observe development of specific resistance to TAT-RasGAP317-326, without cross-resistance to other antimicrobial agents. We aimed here at determining whether this was also the case for other Gram-negative pathogens such as the important nosocomial pathogen Acinetobacter baumannii.

Methods

We performed in vitro resistance selections using A. baumannii strains and determined whether resistant strains became cross-resistant to other antimicrobial agents. We then used whole-genome sequencing and CRISPR-based targeted mutagenesis to identify mutations causing resistance.

Results

We observed, in some cases the emergence of cross-resistance to polymyxin B in strains that were selected with TAT-RasGAP317-326 only. Whole genome sequencing allowed us to determine that cross-resistant strains gained mutations in the pmrAB operon, mutations that were already described to cause resistance to polymyxin B. We could confirm by targeted mutagenesis that these mutations were indeed causing cross-resistance.

Conclusion

We thus show here that contact of A. baumannii with a peptide that is structurally very different from polymyxin B can induce the emergence of resistance to polymyxin B. This indicates that the clinical use of AMPs should be taken with caution, since unexpected cross-resistance could emerge.

O084

Trade-off between beta-lactam resistance and stability in Gram-positive class B1 penicillin binding proteins

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Introduction

Antimicrobial resistance is a serious threat to modern medicine. In Gram-positive bacteria, beta-lactam resistance is mainly driven by the production of low affinity penicillin binding protein (PBPs) belonging to the structural class B1 that includes among others PBP2a from Staphylococcus aureus and PBP5 from Enterococcus faecium conferring respectively resistance to methicillin and ampicillin. We aimed to decipher the biochemical and biophysical proprieties of class B1 PBPs.

Methods

Class B1 PBPs from S. aureus (PBP2a) and E. faecium (PBP5) were cloned in pET24a without their N-terminal membrane anchoring domain, recombinant proteins were expressed in E. coli and purified. Point mutation variants and chimeric proteins were created by overlap extension PCR and purified following the same protocol. Competition experiments using bocillin-FL were performed to determine the beta-lactam IC50. Protein stability was investigated by differential scanning fluorimetry using Sypro Orange.

Results

S. aureus PBP2a showed pronounced unfolding transition initiating at physiological temperatures that led to irreversible precipitation and complete loss of activity. Mutations responsible for ceftaroline resistance did not further exacerbate the instability. In E. faecium, stability of the ampicillin resistant variant PBP5 was dramatically decreased compared with WT (Δtm -13.5°C). Analysis of chimeric recombinant proteins showed that both resistance and stability are driven by the penicillin binding domain. In both PBP2a and PBP5 the loop gating the active site was key to explain the transition.

Conclusion

S. aureus PBP2a has an intrinsic thermal instability over a physiological temperature range. In E. faecium PBP5, acquisition of mutations reducing ampicillin affinity is associated major destabilization. In both species, the loop gating the active site played a key role in both the beta-lactam resistance and the thermal stability profile. Therefore, beta-lactam resistance is associated with a stability cost for class B1 PBPs that could be exploited to restore susceptibility.

O085

Escherichia coli acquires specific resistance to the antimicrobial peptide TAT-RasGAP317-326 via charge modification at the surface of the essential outer membrane insertase BamA

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Aims

Antimicrobial peptides (AMPs) are promising alternatives to classical antibiotics against multidrug resistant pathogens. TAT-RasGAP317-326 is an AMP with broad range antibacterial activity, but its mode of action is unknown. We recently determined that Escherichia coli can become partially resistant to TAT-RasGAP317-326 when passaged with increasing concentrations of this peptide in vitro via mutations of the two-component system EnvZ/OmpR. We aimed at understanding better how TAT-RasGAP317-326 interacts with E. coli by investigating further mechanisms of resistance to this peptide. To do this, we used an E. coli strain we isolated that is resistant to TAT-RasGAP317-326 but not to other antimicrobial peptides.

Methods

We used whole-genome sequencing to identify mutations involved in resistance. We then performed in silico modelling to predict how the discovered mutations may affect the efficacy of TAT-RasGAP317-326. Finally, we confirmed the role of these mutations using CRISPR-Cas9 based targeted mutagenesis.

Results

We found that the E. coli isolate specifically resistant to TAT-RasGAP317-326 had an additional mutation in bamA, an essential gene encoding an insertase involved in insertion of outer membrane proteins in Gram-negative bacteria. This mutation is predicted to modify the charge in a negatively charged loop (Q495-T505) at the surface of the BamA protein. In silico docking simulations predicted that binding affinity between TAT-RasGAP317-326 and BamA varies depending on the charge of the Q495-T505 loop. Using CRISPR-Cas9 based targeted mutagenesis, we showed that other mutations lowering the negative charge of the Q495-T505 loop decreased sensitivity of E. coli to TAT-RasGAP317-326 exposure. Interestingly, BamA activity was not affected by TAT-RasGAP317-326, indicating that BamA may be a potential specific receptor for the AMP TAT-RasGAP317-326.

Conclusion

Our study provides insight into mechanisms of AMP activity on Gram-negative bacteria. These types of studies are important as they contribute knowledge that is critical for the potential use of AMPs as alternatives to classical antibiotics for the treatment of bacterial pathogens.

O086
Subversion of IMPDH2 oncoprotein activity by Legionella effectors

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Subversion of IMPDH2 oncoprotein activity by Legionella effectors

Legionella pneumophila is a ubiquitous environmental bacterium and opportunistic human pathogen. Inhalation of contaminated aerosols facilitates infection of alveolar macrophages, and can cause Legionnaires' disease, a severe pneumonia. During infection, L. pneumophila delivers ca. 300 different "effector proteins" to the host cell using the Icm/Dot type IV secretion system. These effectors target various host cellular pathways to favor bacterial survival and growth, and to establish a specialized replication compartment, the "Legionella-containing vacuole" (LCV). Recently, we identified the oncoprotein inosine 5'-monophosphate dehydrogenase type 2 (IMPDH2) as a novel target of Legionella effectors. IMPDH2 is crucial for cell metabolism and catalyzes the conversion of inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP), the first and rate-limiting step of the de novo guanine nucleotide synthesis pathway. The homotetrameric IMPDH2 enzyme assembles into filaments under certain cellular conditions such as low GTP levels or treatment with inhibitors, affecting the regulation of its activity. We observed that L. pneumophila infection caused disassembly of IMPDH2 filaments induced in host cells with the IMPDH2 inhibitor mycophenolic acid (MPA). Intriguingly, this effect was dependent on a bacterial effector protein, which covalently modified IMPDH2 and caused disassembly of ATP-induced IMPDH2 filaments in vitro. Accordingly, covalent modification by the effector might alter IMPDH2 activity and/or its distribution in the host cell and thereby modulate local GTP levels. To assess the effects of the modification on IMPDH2 activity, we employ activity assays.
Current studies aim to clarify the cellular localization of IMPDH2 and bacterial effector proteins during L. pneumophila infection by analysing infected cells and isolated LCVs by confocal microscopy, western blot, and mass spectrometry. By studying this novel covalent modification of IMPDH2 and its functional consequences, we hope to improve our understanding of the complex regulation of this crucial enzyme, and its implications for infection biology, cell biology, and cancer biology.

O087
Moonlighting Ndk: Unveiling Pathogenic Roles in Chlamydiae

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Chlamydia trachomatis and Waddlia chondrophila are pathogenic intracellular bacteria, with complex life cycles composed of infectious elementary body (EB) and replicating reticulate body (RB). Nucleoside diphosphate Kinases (Ndks) exhibit intriguing moonlighting activities and multifunctionality, necessitating investigation in Chlamydiae. Ndks play a crucial role Chlamydia metabolism and its interactions with host cells. This study focuses on characterizing the chlamydial ndk and investigating its role in chlamydial development and pathogenicity.
In C. trachomatis, the ndk gene (Ctndk) exists as a single copy, while in W. chondrophila, two copies are present (Wcndk1 and Wcndk2). Notably, WcNdk2 in W. chondrophila is associated with a signal sequence at its N-terminal. WcNdk2 protein localization was observed in both the host cell nucleus and Golgi apparatus. The localization of WcNDK2 in the Golgi apparatus aligns with its secretion outside the host cell and its involvement in purinergic function. Additionally, we employed Azidothymidine (AZT) to inhibit Ndk function in C. trachomatis or W. chondrophila-infected cells. Since only W. chondrophila growth was affected by AZT treatment, we hypothesized that AZT inhibits the DNA-binding ability of WcNdk2. Indeed, Ndks has been reported to bind and regulate c-myc in other organisms.
The role of ndk genes in development and pathogenicity of Chlamydiae could be further investigated by generation of ndk null mutants and uncovering their target genes. By disrupting Ndk function, it becomes conceivable to target chlamydia development, therefore opening new avenues for drug development aimed at combating chlamydia infections.

O088

Proteogenomics identifies conserved and lineage-specific novel small proteins in clinical Mycobacterium tuberculosis reference strains

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Aims
Genome annotations frequently miss small protein-coding genes which play vital roles in cellular processes and hold potential for novel antibiotics development. Our study employs proteogenomics to uncover such overlooked genes in six clinical strains of Mycobacterium tuberculosis, one of the most severe infectious disease agents worldwide, comparing two lineages with different levels of virulence.

Methods
We de novo assembled complete genomes from PacBio long-read sequencing data, creating an optimal foundation for functional genomics. By hierarchically integrating reference genome annotations, ab initio gene predictions and a modified six-frame translation that considers alternative start codons, we created comprehensive but minimally redundant integrated proteogenomics search databases (iPtgxDBs). Total cell extracts were analyzed with state of the art parallel accumulation-serial fragmentation (PASEF) mass spectrometry (MS). We prioritized novel small protein candidates based on functional predictions and conservation and validated peptides by parallel reaction monitoring.

Results
Compared to our complete genomes, 1 to 4 percent of all annotated genes were partially or completely absent in pre-existing fragmented Illumina assemblies. Comparative genomics revealed a large core genome between the six strains, containing 91 percent of all annotated genes. PASEF MS allowed us to identify over two thirds of all annotated proteins per strain without requiring sub-cellular fractionation, and in combination with iPtgxDBs we were able to identify a total of 30 novel proteins shorter than 100 amino acids. These included proteins that were shared between all six strains as well as lineage and strain specific proteins, with a notable prevalence of toxin-antitoxin system proteins.

Conclusion
We illustrate the successful application of our proteogenomics framework for prokaryotes, which is available as a public web server (https://iptgxdb.expasy.org), to six clinical reference strains of a major human pathogen and leverage state of the art tandem MS to identify novel small proteins even from unfractionated samples.

O089

Detecting and characterizing ICEclc family of integrative and conjugative elements in Pseudomonas aeruginosa clinical isolates

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Integrative and conjugative elements (ICEs) are widespread autonomous mobile DNA occurring within bacterial chromosomes. ICEs contain the genes necessary for the excision of their own DNA from the chromosome, the consequent conjugative transfer to a new recipient cell and the chromosomal reintegration. The elements can also carry additional genes that are non-essential for their transfer, but that can confer adaptive phenotypes to the host. Examples of known adaptive genes include antibiotic or heavy metal resistance, or the ability to metabolize specific carbon sources. As such they are thought to provide important genetic variation among closely related strains. In this work, we characterized the presence, distribution, and variation of ICEs related to the well-described ICEclc among Pseudomonas aeruginosa clinical isolates within a geographically restrained environment to understand the factors contributing to their evolution. We examined a total of 181 P. aeruginosa genome sequences obtained from patient or hospital environment isolates, most of which were obtained from a single hospital during 20 years of sampling. More than 90% of the isolates carried one or more ICEclc-like elements, with different degrees of conservation to the known ICEclc lifestyle and transfer genes. ICE clones closely matched their host clonal phylogeny, but not exclusively, indicating that both clonal evolution and ICE horizontal transfer are occurring in the hospital environment. Variable gene regions among the clinical P. aeruginosa ICEclc-type elements were notably enriched for heavy metal resistance genes, toxin-anti-toxin systems, potential efflux systems and multidrug resistance proteins, a metalloprotease and for a variety of regulatory systems, but not for specific recognizable antibiotic-resistance cassettes. Clonal persistence suggests adaptive benefits of these functional categories, and micro-patterns of gene gain and loss indicate ongoing ICE evolution within the P. aeruginosa hosts. We next aimed at studying the activation and transfer process of a representative subset of the ICEclc-type elements identified in P. aeruginosa. We found that ICE excision could be induced in P. aeruginosa by ectopically expressing the homologs of the known master regulator of ICEclc activation, BisDC, pointing to a similar regulatory cascade. Some elements were successfully transferred to a P. putida host, in which they conferred increased tolerance to specific heavy metals.

O090

Behavioral split in motility endurance under carbon starvation reveals an ecological dichotomy among motile copiotrophs

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Many copiotrophic bacteria experience a feast-famine lifestyle in heterogeneous environments, where they experience starvation in between infrequent encounters with rich nutrient hotspots. Motility increases the encounters with such hotspots by 100-1000 fold, but is also energy demanding to a starving cell, giving rise to a risk-reward trade-off. Here, we used videomicroscopy and particle tracking to quantify the behavioral response of 26 strains from 18 different species of marine bacteria over two days of carbon starvation. Our results reveal a dichotomy in the motility behavior in response to carbon starvation: there are species that cease motility within hours (`limostatic'), whereas others retain motility for at least two days (`limokinetic'). The biomass during starvation remained constant for species that ceased motility but decreased approximately 10 % per day for the species that remained motile, of which several species accumulated energy storage compounds (PHB and polyphosphate) before starvation. This shows strains need to convert biomass into energy to fuel motility, but doing so extends their search behavior and thus increases the chance of future biomass gain. Using machine-learning classifiers we identified a genetic component associated with this dichotomy, sufficiently robust to predict the response of an additional set of marine strains with >80% accuracy. Overall, these results expand our understanding of foraging strategies in bacteria, revealing a dichotomy among copiotrophs: risk-prone foragers that retain motility during starvation, and risk-averse foragers that rapidly give up motility during starvation.

O091
Defining features of distributed metabolism in microbial communities

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Microbes live in complex communities, where they continuously engage in a range of interactions with other microbes. One class of interactions is the exchange of metabolites, with microbes performing different reactions that constitute a collective, distributed metabolism. While it is known that microbial communities from many environments exhibit distributed metabolism, the structure and features of these distributed metabolic networks is not well understood. To gain insights into the relationships between microbes in a community, we developed a consumer-resource model describing the evolution of metabolic dependencies and collective metabolism. Based on this model, we predicted conditions under which auxotrophy is most likely to evolve. To test these predictions, we are analysing the amino acid and vitamin auxotrophy profiles of genomes of isolates as well as of metagenome-assembled genomes (MAGs) collected across environments. Through this approach, we aim to describe the distribution of biosynthetic machinery in microbial communities, to understand the factors driving the evolution of distributed metabolism, and to identify any universal features of distributed metabolism that appear across environments.

O092
Antimicrobial activity of iron-depriving pyoverdines against human opportunistic pathogens

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Antibiotic resistance is a growing concern for global health, demanding the development of innovative and efficient strategies to counteract pathogenic bacteria. Iron is crucial for bacterial proliferation during pathogenesis. In polymicrobial infections and within the host, competition for iron is mediated by siderophores – secondary metabolites that scavenge iron with high affinity. Since siderophores are often species specific, they have two opposing effects: they facilitate iron availability for community members possessing compatible receptors while depriving iron from competitors lacking matching receptors. Here we exploited the iron-depriving properties of siderophores produced by non-pathogenic environmental bacteria as an antibacterial strategy to induce iron starvation and growth arrest in pathogens. In a screen involving 320 environmental Pseudomonas strains, we identified five chemically distinct siderophores (pyoverdines) with broad-spectrum antibacterial activity. Purified pyoverdines completely stalled Acinetobacter baumannii and Staphylococcus aureus growth, while showing intermediate activity against Escherichia coli and Klebsiella pneumoniae. Treating infected insect (Galleria mellonella) larvae with pyoverdine significantly increased host survival in the case of A. baumannii and K. pneumoniae infections. Furthermore, pyoverdines exhibited low toxicity against mammalian cells at effective concentrations. Resistance evolution was low and mutations involved in partial resistance were both species and strain specific. Overall, we established siderophore-induced iron starvation as a new strategy to combat bacterial pathogens, including members of the ESKAPE group that are often difficult to treat and resistant to antibiotics.

O093

Rational design of a two-phage cocktail against a contemporary A. baumannii strain recovered from a burned patient at CHUV

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Acinetobacter baumannii is currently a major threat to human health. With the spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, the development of complementary strategies is needed. A promising complimentary and realistic strategy could be phage therapy, which uses bacteriophages (phages), i.e viruses that specifically kill bacterial cells during their life cycle.
We designed a two-phage cocktail highly efficient against an XDR A. baumannii isolate collected from a patient with burn wound infection at CHUV (termed Ab125). A first in vitro screen of our collection of 50 different phages identified only phage vB_AbaM_3098 as capable of lysing Ab125. However, quick (ca. 2 h) selection of phage-resistant clones (termed Ab139) occurred. Comparative genomics between Ab125 and Ab139 revealed a Single Nucleotide Polymorphism (SNP) in an intergenic region, currently under investigation.
Very interestingly, we observed that Ab139 became susceptible to six different phages in the collection, otherwise inactive on Ab125. Phage-resistance was also selected when Ab139 was challenged with either of the six phages, with bacterial regrowth observed between 14 h and 16 h. However, combination of vB_AbaM_3098 and phage vB_AbaM_3014 led to a two-phage cocktail capable of totally inhibiting the growth of Ab125. Treatment with the phage cocktail led to 90% survival after 5 days in the in vivo Galleria Mellonella model of infectious diseases, compared to 0% in the non-treated group.
We show that the combination of a phage that only slightly shifted the in vitro bacterial growth curve with an “inactive phage” led to the formulation of a highly bactericidal phage cocktail against Ab125. The therapeutic potential of the assembled cocktail is currently tested in synergy with antibiotics. This work highlights the complexity sometimes involved in the assembly of potent phage cocktail.

O094

Bos taurus and Bos indicus cattle exhibit compelling different immune responses towards vector-borne viruses

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Cattle are the mammalian species with most global biomass associated with a huge impact on Earth. In line with the 3R principles, we developed an ex vivo platform using fresh primary bovine blood cells to characterize and dissect host-pathogen interactions. This procedure employs a novel multiparameter flow cytometry assay (measuring maturation / activation of most immune cell subsets) and a multiplex immunoassay (monitoring chemokine / cytokine secretions). We hypothesize that Bos taurus and Bos indicus genetic background are likely to show different immune responses and susceptibilities towards vector-borne diseases (VBDs) because of their different evolutionary trajectory and origin of domestication. Here we tested our hypothesis using two vector- borne viruses, namely Bluetongue virus (BTV) and Schmallenberg virus (SBV), both expected to trigger increasing numbers of outbreaks in Europe in the future due to climate change. When only considering Bos taurus, we found an ex vivo response towards SBV very moderate compared to BTV; this clearly indicates a fine-tuning of the bovine immune response depending on pathogen. The most striking finding was the genetic-borne differential response towards BTV. Bos taurus exhibited an enhanced activation of monocytes, dendritic cells, CD8 and gamma delta T cells, whereas Bos indicus relied mostly on CD4 T cells. Overall, we provide novel insights in the immune responses of cattle with markedly different genetic background to VBDs. Our platform can be applied to test immune responses of different cattle breeds and pinpoint immune responses that might confer protection and assist breeding programmes and vaccine development.

O095

Epidermal barrier impairment predisposes for excessive growth of the allergy-associated yeast Malassezia on murine skin

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Background
The skin barrier is vital for protection against environmental threats including insults caused by skin-resident microbes. Dysregulation of this barrier is a hallmark of atopic dermatitis (AD) and ichthyosis, with variable consequences for host immune control of colonizing commensals and opportunistic pathogens. While Malassezia is the most abundant commensal fungus of the skin, little is known about the host control of this fungus in inflammatory skin diseases.
Methods
In this experimental study, MC903-treated mice were colonized with Malassezia spp. to assess the host-fungal interactions in atopic dermatitis. Additional murine models of AD and ichthyosis, including tape stripping, K5-Nrf2 overexpression and flaky tail mice, were employed to confirm and expand the findings. Skin fungal counts were enumerated. High parameter flow cytometry was used to characterize the antifungal response in the AD-like skin. Structural and functional alterations in the skin barrier were determined by histology and transcriptomics of bulk skin. Finally, differential expression of metabolic genes in Malassezia in atopic and control skin was quantified.
Results
Malassezia grows excessively in AD-like skin. Fungal overgrowth could however not be explained by the altered immune status of the atopic skin. Instead, we found that by upregulating key metabolic genes in the altered cutaneous niche, Malassezia acquired enhanced fitness to efficiently colonise the impaired skin barrier.
Conclusions
This study provides evidence that structural and metabolic changes in the dysfunctional epidermal barrier environment provide increased accessibility and an altered lipid profile, to which the lipid-dependent yeast adapts for enhanced nutrient assimilation. Our findings reveal fundamental insights into the implication of the mycobiota in the pathogenesis of common skin barrier disorders.

O096

Enhancing Microbial Strain Typing Efficiency Through Nanopore Technology: A Solution for Rapid Outbreak Management

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Multi-locus strain typing (MLST) is a pivotal genomic typing method, utilizing 6-10 housekeeping genes to define strain types (ST) referenced in a specific database[1]. MLST offers high discriminatory power and concise information exchange among hospitals [2]. However, current methods suffer from high cost, workload, and turnaround time (TAT), limiting routine use in outbreaks and local surveillance [3]. To address these limitations, we propose leveraging nanopore technologies, which offer improved TAT and cost-efficiency while maintaining accuracy.
We use adaptive sampling to expedite sequence acquisition, enhancing efficiency [4]. The results will be compared to Whole Genome Sequencing (WGS) sequences acquired under similar conditions. Ultimately, the consensus sequences obtained are evaluated using the PubMLST database [5].
Benchmarking against WGS reveals that while WGS yields quality consensus sequences, adaptive sampling excels in sequencing higher sample volumes. Our findings suggest the scalability of this method across various settings, from clinical isolates to outbreak investigations. In less than two hours, consensus sequences can be obtained, highlighting the rapidity of the approach, in line with previous studies [4]. Future steps involve implementing live monitoring tools for real-time decision-making during sequencing. This innovation promises to revolutionize microbial typing, enabling swift and accurate outbreak management.

O097

Detecting the causative agents of infective endocarditis through metagenomic next-generation sequencing of excised valves

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Aims: Infective endocarditis (IE) is as a rare yet complex condition, with notable mortality rates and an increasing occurrence in Europe. Diagnosis is challenging, particularly when blood culture results are negative, making it difficult to precisely identify the causative agents. Our objective was to compare the diagnostic efficacy of metagenomic next-generation sequencing (mNGS) with conventional clinical laboratory tests using surgical samples from cardiac valves of 19 suspected IE patients.
Methods: We extracted DNA in duplicate from each resected cardiac valve, using two bacterial DNA enrichment protocols designed to remove host DNA. Metagenomic libraries underwent 2X250 sequencing on an Illumina MiSeq instrument, generating an average of 1.5 million quality-filtered reads per sample. Taxonomic assignments were carried out using read-based approaches.
Results: The consistency between the pathogens identified using mNGS and those from blood cultures was evident. Pathogens identified via valve mNGS but absent in blood cultures are likely genuine causative agents of IE rather than false positives. In three specific cases, the inability of mNGS to identify IE causative agents could be attributed to pathogen clearance, as the valves were resected more than two weeks after blood culture positivity. The prevalence of negative findings in valve cultures underscored the reduced diagnostic efficacy of this method compared to valve mNGS. Additionally, antibiotic-resistance genes were detected in six samples, and complete multi-locus sequence typing (MLST) profiles of suspected IE pathogens were obtained in three samples.
Conclusion: Our results underscore the significance of mNGS as a diagnostic resource for IE, showing its potential to complement blood culture testing.

O098

Pathogen rivalry: unraveling response diversity of Pseudomonas aeruginosa in interactions with other opportunistic human pathogens

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Bacterial infections often involve more than one pathogen. While it is known that polymicrobial infections can impact disease outcomes, we poorly understand how pathogens interact with each other, what molecular mechanisms are involved and whether these mechanisms are standard or tailored to specific opponents. Here, we explored how Pseudomonas aeruginosa reacts to six other opportunistic human pathogens that often co-occur in polymicrobial infections: Acinetobacter baumannii, Burkholderia cenocepacia, Escherichia coli, Enterococcus faecium, Klebsiella pneumoniae, and Staphylococcus aureus. We first used time-lapse fluorescence microscopy to follow interaction patterns and fitness of species in growing micro-colonies over time on agarose pads. We identified a broad spectrum of species-specific interactions including mutualism and antagonism. Second, we used a library of gene-expression reporters and deletion mutants to investigate the molecular mechanisms that P. aeruginosa deploys in interactions with the six other pathogens. Using a combination of flow cytometry and fluorescence microscopy, we observed both general and tailored responses of P. aeruginosa to specific pathogens but also environmental conditions. For example, a general response involved the upregulation of the production of the siderophore pyoverdine, an important agent for iron competition. We further observed differential and opponent-specific expression changes of various regulators including stress response, quorum sensing, and two-component systems. Overall, our insights improve our understanding of pathogen-pathogen interactions at both the ecological and molecular levels, highlighting that P. aeruginosa can distinguish between different opponents and mount tailored responses. This could help in predicting outcomes in polymicrobial infections.

O099
Phyllosphere microbiome-based biocontrol solution against grapevine diseases

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Viticulture requires intensive application of pesticides. In Switzerland, vineyards consume the highest amount of fungicides per unit of surface area in the country (23 kg/ha), while in France, vineyards represent 3% of cultivated areas and consume 20% of the total pesticides used in the country. Biocontrol solutions have been used for years, however, they have not been able to replace pesticides effectively. We believe that this lack of results comes from the almost systematic use of strains isolated from soils to treat the aerial parts of plants. Since these strains are not fully adapted to the phyllosphere conditions and cannot develop properly, they are unlikely to reach their full potential regarding their protective capacity. In this project, we used phyllosphere bacteria as biocontrol agents to limit the use of pesticides. We investigated the protective effect of bacteria isolated from grapevine leaves against Botrytis cinerea and Plasmopara viticola, two pathogens affecting the aerial parts of the plant. Such strains are expected to provide a higher level of protection in comparison to bacteria from other environments, since they are believed to successfully contribute to their host's resistance. Our results show that several strains were able to reduce the spore fitness of the two pathogens, to significantly reduce symptoms in planta and to trigger plant immunity through defense genes upregulation and stilbene production. Experimentations on whole plants have also shown that our strains could provide a systemic effect, since when only a part of the plant was inoculated with bacteria, even uninoculated leaves showed a significant reduction in symptoms after infection. We believe that our solution will provide a long-term and environmentally friendly protection against diseases and will help to enhance the sustainability of viticulture.

O100
Novel ΨSCCmecA-mecC hybrid element in methicillin-resistant Mammaliicoccus lentus isolated from sheep and dromedaries

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Aims: Mammaliicoccus species, which are members of Staphylococcaceae, are part of the natural skin microbiota of mammals, but they are able to acquire virulence and antibiotic resistance genes making some of them difficult to treat opportunistic pathogens. Sheep and dromedaries in Algeria were found to harbour methicillin-resistant Mammaliicoccus lentus. The aim of the study was to characterize the genetic element containing the methicillin-resistant genes in M. lentus from both animals using a WGS-based comparative analysis.
Methods: M. lentus strains were isolated from the nasal cavity of sheep and dromedaries using selective media. The species were identified using MALDI-TOF and the presence of mec genes was confirmed using PCR. The mec-positive M. lentus were sequenced using Illumina short-read and MinION long-read technology and hybrid assembled (Unicycler) to generate a fully resolved circular genome and a complete staphylococcal cassette chromosome mec (SCCmec). The mec containing element was identified by comparative analysis with known SCCmec using BLAST. The final visualization of the complete element was illustrated using Clinker v.0.0.28 81.
Results: A total of nineteen methicillin-resistant M. lentus were isolated from dromedaries (n=5) and sheep (n=14). They all harboured both mecA and mecC located two distinct fragments: the mecA containing fragment was situated downstream of the orfX gene and was most closely related to the class A mec complex (IS431-mecA-mecR1-mecI) of SCCmec type VII of S. pseudintermedius strain KM241; the mecC containing fragment was situated downstream of the mecA fragment and contains a class E mec complex (mecI, mecR, mecC, blaZ) of S. aureus LGA251. This hybrid element lacked the recombinase genes ccr gene complex classifying it as a pseudo-SCCmec hybrid element (ΨSCCmecA-mecC). At the 3' end, it contained an arsenical resistance operon (arsABD). Phylogenetic analysis showed that the ΨSCCmecA-mecC was present in genetically related strains indicating transfer of methicillin-resistant M. lentus between dromedaries and sheep.
Conclusion: A novel ΨSCCmecA-mecC element consisting of a hybrid of mecA and mecC fragments was present in M. lentus strains from different animals in Algeria. Absence of ccr genes and presence of ΨSCCmecA-mecC in genetically related strains suggest that a clone of methicillin-resistant M. lentus is disseminating among sheep and dromedaries.

O101

Dissemination of Antimicrobial Resistance through Pig Manure: A Long-Read Metagenomic Sequencing Approach

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Aims

Antimicrobial resistance (AMR) poses a significant global threat to public health, and livestock farming is recognized as a contributing factor to the emergence and spread of antibiotic-resistant bacteria. This study investigates the impact of antibiotic use in pigs on the microbial communities present in pig manure. Drug-resistant bacteria present in manure may reach the human food chain through the use of manure as fertilizer.

Methods

We collected 24 manure samples from 13 pig farms in Switzerland that differed in the amount of antibiotics used in the preceding months and years. DNA was extracted using the Zymo Quick-DNA HMW MagBead kit. Libraries were prepared with the SQK-LSK114 kit and sequenced on an R10.4 flow cell (Oxford Nanopore Technologies) with Guppy SUP basecalling.

Results

Nanopore long-read metagenomic sequencing revealed a significantly higher abundance of AMR genes in samples originating from farms with high antibiotic use. In all samples, resistance genes associated with antibiotic classes that inhibit protein synthesis were strongly overrepresented relative to other classes. Genes associated with resistance to important last-resort antibiotics in human medicine were rarely detected. In some samples, specific highly prevalent mobile genetic elements contributed substantially to the overall AMR gene abundance.

Conclusion

Our research offers insights into the dynamics of AMR in pig manure and provides guidance for promoting responsible antibiotic use in veterinary practices.

P001

Microbiological trends and outcomes in infective endocarditis: a nationwide cohort study

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Introduction: Infective endocarditis (IE) is a rare disease with significant morbidity and mortality, experiencing shifts in epidemiology due to an aging population and an increasing use of endovascular devices. This study evaluates changes in microbiological trends and outcomes in patients with IE over a decade in Switzerland.
Methods: In this nationwide cohort study, we analyzed in-hospital claims data from patients hospitalized with IE in Switzerland between January 2012 and December 2021. We assessed the monthly case numbers and incidence rates per 100,000 hospitalizations, categorizing them by common pathogens (Staphylococcus aureus, other Staphylococcus species (spp.), Enterococcus spp., Streptococcus spp., gram-negative bacilli, HACEK [Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, and Kingella], pathogens of culture-negative IE, unknown pathogens, and other pathogens). Odds ratios (OR) or log-transformed gamma regression coefficients with corresponding confidence intervals (CI) were calculated to estimate the association between pathogens and outcomes. Main outcomes included all-cause in-hospital mortality, all-cause 6-month mortality, intensive care unit (ICU) admissions and length of hospital stay (LOS).
Results: Among 15,255 hospitalizations with IE, the annual number of cases increased from 1,361 in 2012 to 1,636 in 2021. Predominantly diagnosed pathogens were S. aureus (increasing from 19.8% in 2012 to 30% in 2021, p for trend < 0.01) and Streptococcus spp. (from 17.6% to 24.4%, p for trend < 0.01). IE caused by S. aureus was associated with the highest in-hospital (19.9%) and 6-month (30.3%) mortality rates and showed no significant trend over the decade. When compared to S. aureus, IE caused by HACEK (OR 0.23, 95% CI 0.11 to 0.48) or unknown pathogens (OR 0.21, 95% CI 0.18 to 0.24) had the lowest in-hospital mortality rates. Patients suffering from IE caused by an unknown pathogen were less likely being admitted to an ICU. The longest LOS was observed in IE hospitalizations with S. aureus (mean 23.79 days) and the shortest LOS in IE with unknown pathogens (-31.19%, p < 0.01).
Conclusion: This nationwide cohort study showed an increase in IE hospitalizations from 2012 to 2021, mainly caused by S. aureus and streptococci, particularly noting that S. aureus was associated with adverse outcomes, which remained unchanged over time, when compared to other or undiagnosed pathogens.

P002

Emergence of toxigenic Corynebacterium diphtheriae infections in elderly patients in Switzerland - report of an ongoing outbreak investigation

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Aims
Since June 2022, an increase in Corynebacterium diphtheriae cases, mainly associated with migrant- related facilities, has been reported by the European Centre for Disease Prevention and Control. We present a cluster of genetically related strains of toxigenic C. diphtheriae causing infections in patients previously considered as being at low risk.
Methods
In October 2023, toxigenic C. diphtheriae was recovered from samples collected due to suspected infection from two patients hospitalized at two tertiary care centers within the city of Basel, Switzerland. Routinely performed whole genome sequencing revealed close genetic relatedness of the recovered strains, triggering a joint outbreak investigation involving the public health authorities.
Results
Patient A, a 78-year-old female was admitted due to acute respiratory failure related to suspected pneumonia. She died shortly after admission despite intensive care treatment. Toxigenic C. diphtheriae was cultured from the bronchial aspirate collected at admission. Her vaccination status remains unknown. Patient B, an 88-year-old male was admitted after biopsy of a chronic sacral wound showed growth of toxigenic C. diphtheriae. The patient improved after systemic antibiotic and local wound treatment and was discharged to a geriatric rehabilitation facility. He reportedly had not received any diphtheria vaccine since 1969 and the diphtheria antitoxin IgG level was < 0.1 IU/ml.
The two clinical isolates of C. diphtheriae were identified as ST574 and were genetically identical (zero allelic differences). Both isolates were closely related (one to five allelic differences) to a cluster of strains derived from five patients, all asylum seekers (AS) accommodated in a federal asylum center in Basel, from 2022.
The two current patients had no known interaction or epidemiologic link to each other or to the previously diagnosed cases and reported no travel history.
Conclusions
Toxigenic C. diphtheriae has emerged in Europe among populations typically not considered at risk – possibly facilitated by re-introduction of strains by migration and decreasing immunity due to lacking catch- up vaccination. This report calls for raised clinical and laboratory awareness for toxigenic C. diphtheriae infections and supports the promotion of respective vaccination regimens.

P003

Trained immunity drives neutrophil reprogramming providing protection against pneumococcal sepsis

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Aim. Trained immunity reflects the capacity of the innate immune system to adapt to an initial challenge and mount an improved response to a secondary challenge. The induction of trained immunity is associated with metabolic, epigenetic and functional reprogramming of stem cells, stromal cells and myeloid cells. We demonstrated that training protects mice from a wide range of bacterial infection (Ciarlo E. et al. J. Infect. Dis. 2020:222:1869). Surprisingly, while neutrophils play a key role in host defences, their role during training is poorly understood. We aimed to determine whether neutrophils are reprogrammed during training and to assess their role during streptococcal pneumoniae.

Methods. Mice were challenged with PBS (control) or β-glucan (training) given intraperitoneally. After one week, mice were sacrificed to collect organs and cells, or were challenged intranasally with Streptococcus pneumoniae with or without control or trained neutrophils. Samples were analysed by flow cytometry, scRNAseq and multiplex bead assay. Mouse morbidity and mortality were registered daily.

Results. Training increased bone marrow myeloid stem and progenitor cells, and immature and mature neutrophils in bone marrow, blood, spleen and lungs ($P < 0.001$). RNAseq demonstrated that the transcriptome of trained neutrophils was altered with gene pathways related to effector functions significantly enriched. In agreement, trained neutrophils showed increased chemotaxis, phagocytosis and S. pneumoniae-induced IL-1β, IL-6 and G-CSF production ($P < 0.05$). Training protected mice from lethal pneumococcal pneumonia. The protection was lost upon neutrophil depletion (0.0% versus 87.5% survival in neutrophil-depleted versus neutrophil-non-depleted trained mice, $P < 0.001$). The adoptive transfer of trained neutrophils to naive mice increased their resistance to S. pneumoniae infection.

Conclusions. Training triggered central and extramedullary hematopoiesis and elicited a marked rewiring of the neutrophil compartment. Neutrophils are altered upon training and play a key role in the protection against pneumococcal pneumonia. The molecular mechanisms underlying neutrophil reprogramming are under investigation.

P004
Enhancing care through digitalization: Sepsis quality of care as an example

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Aim
Sepsis remains a critical global health challenge. Digital advancements offer new opportunities to improve patient's management and outcome through enhanced data utilization and workflow automation. We thus aimed to deploy a digital pipeline to support and critically appraise a sepsis quality-of-care program.
Methods
We deployed in a tertiary care center (1500 beds) a fully automated pipeline extracting patient data from electronic health records as of November 2021. This digital pipeline feeds a dedicated registry for continuous monitoring of a sepsis care pathway (SCP). A Python-based pipeline extracts patient data from electronic health records within SCP-equipped units. Data goes through several processing scripts to compute clinical scores which are then stored in a dedicated registry within a data warehouse. The automatic generation of dynamically updated sepsis-specific indicators from the registry onto dashboards (e.g. time to antibiotics, in-hospital mortality) enables a continual assessment of the program's performance over tunable periods with resolution to hospital units.
Results
We monitored from January 2022 to December 2023 through our pipeline 26,000 stays and documented and analyzed 252 sepsis cases. Of these, 139 were treated within the pathway (SCP) and 113 without the pathway (SCWP). Patients' characteristics from SCP and SCWP groups were similar in age (median 67.545 vs. 66.547 p = 0.79), sex (74.8% vs. 65.5% males; p = 0.139). SCP had more chronic comorbidities than SCWP (median (IC 5 – 95%), Charlson comorbidity index 4.0 (0 – 12) vs. 2.0 (0 – 10), respectively p = 0.039). The fraction of sepsis patients receiving antibiotics within the recommended time frames according to the Surviving Sepsis Campaign was higher for SCPs than SCWP (antibiotics in < 1h, 23.4% vs. 11.6%, in 1 - 3h, 21.8% vs. 17.5%, and in > 3h 54.8% vs. 70.9% (p = 0.0281) for SCP vs. SCWP, respectively). Other indicators such as ICU transfers at 72 hours (36.7% vs. 45.1% for SCP vs. SCWP, respectively; p = 0.853) and in-hospital mortality (10.7% vs 16.8%) for SCP vs. SCWP, respectively; p = 0.228) trended lower in SCPs compared with SCWPs though not reaching statistical significance.
Conclusion
We have deployed a highly agile digital pipeline supporting the implementation and enabling the critical appraisal of a sepsis care pathway. Such an approach has the potential to be implemented – with suitable adaptations – to other institutions and/or acute pathologies.

P005

Machine Learning-Based Prediction of Active Tuberculosis in people with HIV using Clinical Data

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Aims

Coinfections of Mycobacterium tuberculosis (MTB) and human immunodeficiency virus (HIV) impose a substantial global health burden. Patients with MTB infection face a heightened risk of progression to active TB, which preventive therapy can mitigate. Current testing methods often fail to identify individuals who subsequently develop active TB, particularly among people with HIV (PWH), necessitating a new testing approach.

Methods

We developed artificial intelligence (AI) models to more accurately predict active TB progression using patients medical data at HIV-1 diagnosis. Training our model involved utilizing clinical data routinely collected at enrollment from the Swiss HIV Cohort Study (SHCS). This dataset encompassed 55 PWH who developed active TB six months post-enrollment and 1432 matched controls enrolled between 2000 and 2024.

Results

Implementing Random Forest modeling and Area under the Curve (AUC) metrics, we predicted active TB with an AUC of 0.78 (CI 0.76 - 0.8) within the SHCS. External validation utilized data from the Austrian HIV Cohort Study (AHIVCOS), comprising 43 incident TB cases and 1005 controls from the same time. Comparable AUC values of 0.71 for Swiss PWH and 0.68 for Austrian PWH were obtained after adjusting demographic parameters to enhance dataset comparability and re-fitting the model with a reduced parameter set. Comparative analysis against the standard of care for identifying patients at the highest risk demonstrated superior performance to current standard of care (Tuberculin skin test (TST) and Interferon gamma release assay (IGRA)) in terms of the Number Needed to Diagnose (NND) (2.3 vs. 4).

Conclusion

Summarized, the AI algorithm introduced in this study has the potential to diagnose PWH at high risk of active TB in low transmission settings, surpassing the current clinical standard. Because the algorithm relies on standard clinical assessment during HIV-1 diagnosis and does not require any additional laboratory tests, it holds promise as a highly effective screening method.

P006
Short Against Long Antibiotic Therapy for Infected Orthopaedic Sites (SALATIO trials) - 1st interim analysis at one year

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Aim
The optimal duration of postoperative antibiotic treatment in orthopedic bone and implant infections is unknown. We debride and randomize eligible adult patients since October 2022 into a short vs. a long postoperative antibiotic treatment arm. For this 1st. interim analysis, we evaluate the ITT (intention-to-treat)-population with uncensored follow-up times, emphasizing on failure and safety issues.

Methods
A 1:1 randomization allocates eligible episodes into a short or long postoperative antibiotic group. We treat the short group with 3 weeks of postoperative antibiotic therapy for bone infections and during 6 weeks in case of a retained implant. The long arm represents 6 weeks of therapy for implant-free osteomyelitis; and 12 weeks with residual implants. The outcomes are "clinical failure" (defined as revision surgery for wound problems, new infections or mechanical reasons), "microbiological recurrence" (infection recurrence with identical pathogens) and "serious adverse events" (SAE).

Results
As of the start of 2024, we included 170 infection episodes; 42 episodes (short arm; 3 weeks) vs. 48 cases (long arm; 6 weeks) for osteomyelitis; and 55 (short arm; 6 weeks) vs. 25 cases (long arm;12 weeks) for residual implant infections. The four frequent pathogen groups were Staphylococcus aureus (n=47; 28%), coagulase-negative staphylococci (28%), Gram-negative rods (18%), and various streptococci (9%). The median number of surgical debridement was 1 in all arms. For osteomyelitis, 5 (3/42; 7%) episodes yielded a “clinical failure” in the short and 9 (9/48; 19%) in the long arm (Pearson-χ2-test, p=0.32). The corresponding numbers for implant-related infections were 6/55 (11%) vs. 2/25 (8%), p=0.69, respectively. Overall, we detected 4 (4/170; 2%) "microbiological recurrence", with E. faecalis and P. aeruginosa in two long arms each, and with S. epidermidis and S. lugdunensis in two short arms each. Among the 38 SAEs (38/170; 22%), only 5 (3%) were antibiotic-related. The rest was panoply of various postoperative internist, anesthesiologic and uninfected wound problems. Both C. difficile colitis cases (2/170; 1%) occurred in the long treatment arms.

Conclusion
A shorter antibiotic therapy might be non-inferior when compared to the standard long treatment duration. The SALATIO trials continue and are open for multicenter recruitment.

P007
Cohort Profile: The Swiss Mother and Child HIV Cohort Study (MoCHiV)

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Background
The Swiss Mother and Child HIV Cohort Study (MoCHiV) is an ongoing prospective, multicentric observational study in Switzerland investigating measures to prevent mother-to-child-transmission in pregnant women with HIV (WWH) and assessing health and development of their exposed children as well as of children with HIV (CWH) in general. As part of the Swiss HIV Cohort study (SHCS, www.shcs.ch) its focus is on interdisciplinary clinical and translational research, epidemiological and social science and public health questions.
Methods
Information concerning pregnancy, delivery and the offspring of pregnant WWH is collected. CWH are followed semiannually until 18 years of age while HIV exposed uninfected children (HEU) are followed annually until 5 years of age. The dataset comprises socio-economic data and detailed information on the health status and therapy of pregnant WWH and their children. Information on pregnancy follow-up and delivery is also available. In addition, characteristics of CWH cover information on HIV-associated diseases, treatment and laboratory results. Blood samples from mothers and children are regularly taken and stored in a biobank.
Results
Between January 1986 and December 2022 a total of 1446 children were born from the 1041 WWH. Median age of women at birth of their first child was 30 years (IQR 25-35) and median age at HIV diagnosis was 26 years (IQR 23-31). Overall, 98 children turned out to be diagnosed with HIV resulting in a vertical transmission rate of 0.7% during the past 37 years. Additionally to the 1446 children mentioned above, data of 708 (521 HIV exposed children and 187 CWH) children without detailed information about the mother have been collected. Of the total 285 CWH enrolled, 95 (33.3 %) were lost to follow-up or voluntarily withdrew from the study (e.g. changed to a physician not participating in the SHCS), 61 (21.4 %) died and 16 (5.6%) are currently still being followed. 113 (39.6 %) children have reached adulthood and are now followed within the SHCS protocol.
Conclusions
MoCHiV provides access to a unique longitudinal data-collection on pregnant WWH, their offspring as well as on CWH. With its ability to answer clinical and translational research and public health questions this cohort has contributed to improve prevention of mother-to-child transmission (PMTCT) and secure optimal care of pregnant WWH and their exposed children, as well as of CWH over the last 30 years.

P008

Duration of viral shedding in immunocompromised patients with SARS-CoV-2 infection

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Aims
The duration of viral shedding in patients with SARS-CoV-2-infection may vary based on the type and extent of immunosuppression, with direct implications on infection prevention and control strategies. Consequently, systematic screening of immunocompromised patients was conducted at our institution before discontinuing isolation precautions. The objective was to evaluate the duration of viral shedding in immunocompromised individuals.
Methods
This single-center, retrospective cohort study was performed at the University Hospital Basel, a Swiss, tertiary referral centre. Immunocompromised patients with detection of SARS-CoV-2 by PCR between 03/2020 and 10/2022 were included. Solid organ transplant recipients, patients with haemato- oncological malignancies, or with neurological, rheumatological, dermatological, gastroenterological or renal disease requiring immunosuppressive treatment and patients on dialysis were considered. Patients with unknown infection onset or detection of a low viral load in line with past infection were excluded. Pertinent clinical data was extracted by medical chart review.
Results
Eligibility criteria were met by 163 patients whose duration of shedding could be estimated with follow- up testing. The main underlying conditions were haemato-oncological malignancies and solid-organ transplantation. Median duration of shedding was 17 days, ranging from 5 to 127 days. Viral shedding ≥28 days was observed in 50 patients (30.7%). Of these, 27 (54%) had an oncological malignancy (22.2% with lung-cancer), 15 patients (30%) had haematological malignancies and 10 (20.0%) were solid organ transplant recipients (50% kidney transplants, 40% lung transplants), with more than one immunocompromising condition being present in 16 patients (32%). 10% of patients with prolonged viral shedding ≥28 days were receiving anti-CD20 monoclonal antibodies.
Conclusion
Viral shedding, as detected by PCR, discontinues within four weeks for the majority of immunocompromised patients (69.3%). Prolonged shedding over four weeks was most commonly observed in patients with haemato-oncological malignancies or solid organ transplantation.

P009

β-lactam allergy: a still-neglected but fundamental aspect of the antimicrobial stewardship programmes

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Background
β-lactam antibiotic allergies are a major concern in antimicrobial stewardship programmes because most of patients who referred allergy are not truly allergic and this can lead to inappropriate antibiotic prescriptions. An adequate evaluation of allergy labelling is therefore mandatory to optimize antibiotic treatments in hospitalized individuals.

Aims and Methods
Aim of this study was to analyse information about antibiotic allergy labelling in the clinical records of patients hospitalized in all public hospitals in Ticino between July 1st and December 31st 2023 who received at least one antibiotic treatment during hospitalization. Allergy information analysed included the antibiotic to which the patient referred allergy, the severity of allergic reaction and the symptoms reported.

Results
A total of 437 allergies to β-lactam were reported in 398 patients (median age 73, 95% CI: 35-92 years), hospitalized for a median time of 8 days (95% CI: 2-31 days). 354 (81%) of the registered allergies concerned penicillins and derivatives, 71 (16.2%) cephalosporines, 10 (2.3%) carbapenems, and 2 (0.5%) unspecified antibiotics. In 299 cases (68.4%) no information about the reaction was recorded; 91 (20.8%) had a cutaneous reaction (e.g. rash or urticaria); 26 (5.9%) had anaphylactic reaction or DRESS syndrome. Only 8 cases of allergies (1.8%) were anamnestically confirmed by allergologic tests. The remaining reported symptoms (n=13, 3%) were unlikely being real allergic reactions but side effects of the treatments (as renal or hepatic failure, epilepsy). The allergy information was updated only in 154 (38.7%) patients during hospitalization and in 45 (11.3%) after discharge (median time 21 days, 95% CI: 0-125 days). In the others, the update dated a median of 440 days (95% CI: 6-1370 days) before admission.

Conclusion
Our analysis shows that allergy information in the electronic system is rarely updated during hospitalization; the information provided is usually of poor quality and missing data are predominant, leading to a suboptimal treatment of patients and to an increased use of second-line antibiotics. A proper anamnesis is mandatory to discriminate between real allergies or other symptoms unlikely related to antibiotics. In this context, the de-labelling of allergy becomes almost unfeasible. Efforts should be made by clinicians to ensure that allergy labels are promptly updated and pertinent for making safe antibiotic prescriptions.

P010

Concurrent detection of Swine Influenza A Virus (H1N1) in a farmer and pigs in Switzerland: Potential zoonotic event.

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Swine influenza A viruses (swIAV) are zoonotic pathogens that cause respiratory infections in pigs and can lead to mild or severe respiratory symptoms in humans. Pigs are susceptible to avian and mammalian IAVs, making them key contributors to the evolution of the virus through genetic shift and/or drift. These evolutionary mechanisms can lead to the emergence of novel strains with pandemic potential in humans. Active genomic surveillance of IAV diversity and transmission at the pig-human interface is relevant for efficient pandemic preparedness. Here, we report a suspected case of a zoonotic swIAV spillover at a pig farm in Switzerland.

On 23 November 2023, five pigs with Influenza-like illness (ILI) symptoms along with the pig farmer, who presented with fading ILI symptoms and tested positive for IAV via RT-qPCR. Notably, the farmer's son experienced severe ILI symptoms two weeks prior to the sampling day.

Whole genome sequence analysis identified swIAV subtype H1N1 in the human and pig samples. Pairwise nucleotide sequence analysis of all six samples showed > 99% similarity across all eight gene segments. HA sequences of all six samples formed a single phylogenetic cluster within the Eurasian-avian-like lineage in clade 1C.2.2. These sequences were closely related to previously isolated swIAV from Switzerland and other European countries.

Taken together, these findings suggest a likely scenario of a zoonotic spillover of swIAV at the pig-human interface. This is the first report of detection of swIAV in human and pig samples from the same farm in Switzerland. Early detection and further understanding the risk of zoonotic spillover events is essential for preventing the emergence of novel influenza strains with pandemic potential.

P011

Epidemiological trends and resistance patterns of Citrobacter spp. in Switzerland: a nationwide, retrospective surveillance study

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Aim: To analyze epidemiological trends in the incidence and resistance rates of Citrobacter spp. in Switzerland and its 3 main linguistic regions.
Methods: We conducted a retrospective, nationwide surveillance study on Citrobacter spp. clinical isolates reported by 71 hospitals that participated in ANRESIS from 2010 to 2022. Patient-level data (age, sex, type of specimen collected) and institution-level data (linguistic region of Switzerland, type of hospital) were extracted. We calculated incidence of Citrobacter spp. isolates per 100,000 inhabitants, stratified by the 3 linguistic regions. We evaluated the proportion of isolates with resistance to third and fourth generation cephalosporins, fluoroquinolones, and carbapenems.
Results: From 2010 to 2022, there were 60037 Citrobacter spp. isolates in the ANRESIS database. The median patient age was 65 (IQR: 50-80) years; 51% were female and 58% were outpatients. The incidence of Citrobacter spp. increased significantly from 35 to 80 cases per 100,000 inhabitants ($p < .001$), as well as in each of the three linguistic regions. C.koseri and C.freundii accounted for 57% and 29% of isolates, respectively. The most frequent source was the urinary tract (70%). An increase in C. koseri urinary tract isolates was observed, rising from 34% (908/2648) in 2010 to 47.5% (3425/7202) in 2022 ($p < .001$). There were 2137 cases of Citrobacter spp. bloodstream infections (3.6%), with a slowly increasing trend at the national level. This trend was more pronounced in the Italian-speaking region (from 3.9 in 2010 to 5.8 cases per 100,000 inhabitants in 2022; $p < .001$). Among isolates from intensive care units, between 42 to 55% of C.freundii complex isolates had resistance to third generation cephalosporins. C.freundii also demonstrated a higher proportion of resistance to carbapenems (1.5 to 12.5%) and cefepime (0 to 13%) compared to other species. Most of Citrobacter isolates remained susceptible to fluoroquinolones, between 95 to 98%.
Conclusion: Citrobacter spp. is an emerging pathogen of clinical importance in Switzerland. This observed increase emphasizes the value of monitoring trends of all enterobacterales.

P012

Cross-species transmission and evolution of Coronaviruses at the interface of camelids and humans

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The globally endemic low pathogenic human coronavirus (HCoV) 229E has been circulating in the human population for a long time with little sequence alterations. HCoV-229E-like viruses have been found in bat species (BatCoVs) as well as dromedary camels (DcCoVs) exemplary DcCoV-ACN4. For HCoV-229E and 229E-like DcCoVs no recent zoonotic spillovers are documented. Genome analysis propose that HCoV-229E evolved from bats via dromedary camels. This evolutionary scenario shows strong parallels to the recently emerged Middle East respiratory syndrome coronavirus (MERS-CoV). MERS-CoV is a highly pathogenic zoonotic virus that sporadically spills over from the dromedary camel reservoir to humans with a 36% fatality rate. Like HCoV-229E, the origins of MERS-CoV have been linked to ancestral BatCoVs. Consequently HCoV-229E and DcCoV-ACN4 can provide important insight into how emerging CoVs like MERS-CoV cross the species barrier and adapt to the human species.

To analyze potential species barriers, we first investigated the spike-receptor interaction of HCoV-229E and DcCoV-ACN4. We used vesicular stomatitis virus (VSV) CoV-S pseudotyped viral particles on HEK cells overexpressing the human or dromedary camel aminopeptidase N; receptor for HCoV-229E. Then we assessed permissiveness by performing replication kinetics on species-specific cell lines. To mimic in vivo conditions, we further performed the replication kinetics on human (h) and camelid (c) primary airway epithelial cell (AEC) cultures. Proceeding Immunofluorescence staining imply adverse cell tropism for HCoV-229E in hAEC, DcCoV-ACN4 in cAEC and MERS-CoV in hAEC and cAEC.

We show an entry-independent species-restricted replication of HCoV-229E and DcCoV-ACN4 in AEC culture that was not observed for MERS-CoV. Our findings indicate that the observed species barriers of HCoV-229E and DcCoV-ACN4 lie beyond the spike-receptor interaction and open the search for the viral and host factors influencing the different host specificities of HCoV-229E, DcCoV-ACN4 and MERS-CoV.

P013a

Phase 2 Study of Switch to Daily BIC + LEN in Individuals on a Multi-tablet HIV Treatment Regimen

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Background: While single-tablet regimens (STRs) are currently global standard for HIV treatment, some people with HIV (PWH) take multi-tablet regimens (MTR) due to treatment resistance, intolerance or drug interactions. The combination of bictegravir (BIC), an integrase strand transfer inhibitor, and lenacapavir (LEN), a first-in-class capsid inhibitor, could simplify treatment in virologically suppressed (VS) PWH for whom STRs are not indicated. We report Phase 2, 24-Week (W) primary outcomes for BIC + LEN versus stable baseline regimen (SBR) in VS PWH on complex regimens.

Methods: ARTISTRY-1 (NCT05502341) is an ongoing, randomized, open-label, multicenter Phase 2/3 study. In Phase 2, 128 participants on SBR (≥ 6 months prior to screening) were randomized 2:2:1 to receive once-daily oral BIC 75 mg + LEN 25 mg, oral BIC 75 mg + LEN 50 mg or continue SBR. All participants of the BIC + LEN arms received an oral loading dose of LEN 600 mg on Days 1 and 2 of treatment. The primary endpoint was the proportion of participants with HIV-1 RNA ≥ 50 copies/mL (FDA Snapshot) at W24. Secondary endpoints included the proportion of participants with HIV-1 RNA < 50 copies/mL, change from baseline in CD4 cell count and the proportion of participants with treatment emergent adverse events (TEAEs) up to W24.

Results: 51 and 52 participants received BIC 75 mg + LEN 25 mg or BIC 75 mg + LEN 50 mg, respectively, and 25 continued SBR. At baseline, 19% of participants were female, 31% were Black and 16% were Hispanic or Latinx; median (Q1, Q3) age was 60 (56, 65) years and participants were taking a median (range) of 3 (2–9) tablets per day. At W24 HIV-1 RNA was ≥ 50 copies/mL in 0/51 of participants in the BIC 75 mg + LEN 25 mg group, 1/52 (2%) in the BIC 75 mg + LEN 50 mg group (later suppressed to < 50 copies/mL without regimen change) and 0/25 in the SBR group. CD4 counts were comparable in all groups. Most common TEAEs in the two BIC + LEN treatment groups up to W24 were diarrhea (7%), COVID-19 (6%) and constipation (5%). Drug-related TEAEs occurred in 18%, 6% and 0% of participants, respectively.

Conclusions: BIC + LEN was highly effective in maintaining viral suppression in participants switching from MTR, with similar safety profiles observed in the two BIC + LEN treatment groups. These data support the use of BIC and LEN in combination to simplify treatment in VS PWH who are receiving complex regimens. A BIC/LEN STR will be tested in the Phase 3 part of the study.

P013b

Using weight loss to predict outcome and define a humane endpoint in preclinical sepsis studies

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Aims. Preclinical models are critical for understanding the pathophysiological response to infections and sepsis. In keeping with ethic values, researcher follow guidelines to monitor the health status and minimize suffering of mice. Weight loss is a criteria used as a humane end point, a cut-off value at which animals are euthanized. However, there is no official recommendation for a maximum weight loss leading to euthanasia. Our objective was to analyze the robustness of weight loss cutoff values used in preclinical models of sepsis.
Methods. Data were obtained from 2400 mice infected with Listeria monocytogenes, Streptococcus pneumoniae, Candida albicans and H1N1 influenza virus. Experiments were run over 10 years of research in the laboratory, without weight loss used as a humane endpoint or using a cutoff value of 30%. We performed statistical analyses applying different weight loss thresholds, and in-house data-based power calculation and simulation-based power calculation (more than 100’000 runs).
Results. The proportion of mice that did not survive infection was higher for L. monocytogenes (56.1%) and S. pneumoniae (57.3%) than for C. albicans (38.1%) and H1N1 (45.2%). In all models, independently of the conditions applied to the mice, weight loss segregated mice that survived from mice that did not survive. Statistical analyses indicated that fixing maximum weight loss thresholds at 25%, 20% and 10% of initial weight increased mortality rates ($p < 0.01$-0.001) by C. albicans and H1N1 IV, L. monocytogenes and S. pneumoniae infection, respectively. In-house data-based and simulation-based power calculations revealed great variability and/or reduction of power as weight loss thresholds approached 20% for S. pneumoniae and L. monocytogenes models.
Conclusion. To our knowledge, this represents the most extensive study exploring the relationship between weight loss threshold and outcome of sepsis. Our data indicate that weight loss is a valuable predictor of mortality. Yet, weight loss thresholds need to be adapted to each model of infection used in the laboratory to minimize mouse suffering without compromising statistical power and scientific objectives.
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P014
Beta-lactam antibiotics penetration in bone and soft tissues

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Background: Soft tissue infections, such as osteomyelitis, often require prolonged antibacterial therapy with initial intravenous administration. This approach is supported by the limited drug penetration in bone. However, data are lacking about the actual bioavailability of antibacterial agents at the site of infection in osteomyelitis and other soft tissue infections.
Methods: Patients who underwent debridement surgery for soft tissue infections and who were receiving intravenous antimicrobial therapy for ≥48h at the time of the intervention were prospectively enrolled. Drug concentration was measured by ultra-performance liquid chromatography-tandem mass spectrometry in bone or soft tissue samples (concentration in tissue, Ct) and in a plasma sample drawn at the time of the intervention (concentration in plasma, Cp). The Ct/Cp ratio was calculated.
Results: 59 samples (17 bones, 42 soft tissues) were collected from 17 patients with osteitis or oestomyelitis (N=6), infection of prosthesis or osteosynthesis material (N=5), necrotizing fasciitis (N=4), or cellulitis (N=2). Despite a wide range of results, all beta-lactams achieved median concentrations in bones that were above the epidemiological cut-off values for the most frequent pathogens of osteomyelitis (i.e. > 2 mg/l). The highest Ct/Cp ratios were obtained for ceftaroline and meropenem. Drug concentrations in soft tissues also displayed important variability. While penicillin and amoxicillin achieved the highest tissue concentrations and Ct/Cp ratios, carbapenems showed poor penetration in soft tissues.
Conclusions: This prospective analysis of beta-lactams penetration in bone and soft tissue after intravenous administration suggest that these drugs could achieve therapeutic concentrations in most cases. The highest Ct/Cp ratio was obtained with ceftaroline in bone and with amoxicillin and penicillin in soft tissues.

P015

Prospective identification of individuals at high risk of sexually transmitted infections: development of a machine learning based algorithm for screening

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Aims: Optimal screening approaches for asymptomatic sexually transmitted infections (STIs) among men who have sex with men (MSM) remain controversial due to the high costs, arguable epidemiological impact, and potential overuse of antibiotics. We use a machine learning approach to prospectively identify which HIV pre-exposure prophylaxis (PrEP) users are most at risk of infection with gonorrhea or chlamydia.

Methods: Data were extracted from the SwissPrEPared study, an ongoing, national, multicenter cohort of individuals interested in taking PrEP. Participants taking PrEP are recommended to test for STIs every three months, and were sent a digital survey questionnaire including questions on sexual behavior, substance use, mental health, and medical history prior to each visit. We tested the accuracy of prediction of STI diagnosis using random forest (split into training and testing dataset). Class imbalance was addressed by oversampling the minority class using the ROSE package in R.

Results: Of the 26,513 visits from 4,516 participants between July 12, 2019-October 30, 2023, 7% (n=1,865) resulted in a positive gonorrhea diagnosis and 8% (n=2,003) resulted in a positive chlamydia diagnosis. Random forest classification showed an area under the receiver operating characteristic (ROC) curve for gonorrhea of 63% (95% CI: 0.60, 0.65) and chlamydia of 61% (95% CI: 0.58, 0.63). For gonorrhea, the most important predictors were age, partner count, being on a daily PrEP scheme, nationality, relationship status, smoking status, previous STI diagnosis during the study, alcohol use, cannabis use, and popper use. For chlamydia, the most important predictors were age, partner count, relationship status, smoking status, nationality, previous STI diagnosis prior to enrollment in the study, alcohol use, previous STI diagnosis during the study, cannabis use, and being on a daily PrEP scheme.

Conclusion: Machine learning based algorithms show potential in identifying individuals at high risk of STIs, lessening the public health burden of frequent screening. However, additional improvements and external validation are needed before implementation in public health practice.

P016

Preliminary data of a multicenter retrospective analysis on invasive Group A Streptococcus (iGAS) infection in adults in Switzerland (iGASWISS) during winter 2022-23

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Aims
Streptococcus pyogenes causes infections ranging from mild localized to life-threatening (iGAS). Throughout the winter 2022-23, several countries reported an increase in iGAS in children and adults [1]. In Switzerland, pediatricians described a similar increase, whether little was known on the adult population. The aim of the iGASWISS study was therefore to collect iGAS infection cases in Switzerland from November 2022 to February 2023 in adults (>16 years old) to better understand clinical presentation as well as microbiological characteristics, to set a baseline for future comparison and to evaluate our practice throughout the country.

Methods
From November the 1st 2022 to February the 28th 2023, patients older than 16 years, hospitalized in one of the participating hospitals (University Hospitals of Geneva, Lausanne, Bern, Basel, Zurich; Cantonal Hospitals of St-Gallen, Valais, Fribourg and hospitals form the Hirslanden Group) with a diagnosis of iGAS (defined as either an isolation of GAS from a sterile site, necrotizing soft tissue infection or streptococcal toxic shock syndrome) were included in this study, according to ethical regulation (BASEC: 2023-00623). Patients baseline characteristics, severity of disease, treatment and outcome were collected. Emm typing and multiple-locus VNTR analysis (MLVA) were performed on the available strains.

Results
Among the 177 patients currently included in the study, 50.2% (n=89) were male and median age was 48 years (IQR: 37-69); 19 (10.7 %) had an immunosuppression and 37 (20.9 %) had a concomitant viral infection, of whom 17 (45%) had Influenza A. 60 (33.8%) required admission to ICU of whom 37 (61%) for mechanical ventilation and 7 (11.7%) had extracorporeal membrane oxygenation (ECMO). 92 (51.9 %) required at least one surgical intervention. Overall mortality at day 30 was 5% (n=9). Regarding microbiological investigations of the available strains (n=13) via emm typing and MLVA, emm-1 accounted for 69% (n=9) of cases and no sign of monoclonal strain dissemination was detected.

Conclusion

Among adults hospitalized for iGAS during winter 2022-23, the majority was immunocompetent with a median age of 48 years. Clinical severity was high considering that most patients required intensive care admission with an overall mortality rate of 5% at 30 days. Based on the available strains, iGAS cases appeared polyclonal with no change in previously described epidemiology.

P017

Uptake and Discontinuation of the Long-Acting Duo: 24-Month Preliminary Analysis on Cabotegravir Plus Rilpivirine in the Swiss HIV Cohort Study

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Aims
Routine clinical data on the uptake and discontinuation of cabotegravir plus rilpivirine long-acting (CAB+RPV-LA) is important for guiding clinical practice and shared decision making. We present data from the Swiss HIV Cohort Study (SHCS) for two years following market authorization (March 2022) in Switzerland.
Methods
We assessed sociodemographic, clinical, and behavioural baseline characteristics of all SHCS participants initiating the CAB+RPV LA regimen from March 2022 until the end of March 2024. Moreover, we describe reasons for CAB+RPV-LA discontinuation.
Results
From 9,818 active SHCS participants, 416 (4.3%) initiated CAB+RPV-LA, with a peak of 35 participants starting in June 2022. The most frequent reason for initiating CAB+RPV-LA reported by physicians was the availability of optimized treatment (n = 298; 73%). The median age was 47 years, and the median body mass index was 25.11 kg / m2. The majority of individuals were male (n = 326; 78%), of white ethnicity (n = 285 ; 69%), acquired HIV through men who have sex with men (MSM) sexual contacts (n = 247; 59%), had higher education (n = 333;81%), were in a steady partnership (n = 243;60%) and highly adherent (n = 315;78%) to the previous antiretroviral therapy regimen. Albeit 58% (n = 242) of participants were recruited in larger university hospitals, recruitment also took place in private practices (n = 110; 26 %) and affiliated hospitals (n = 64; 15%). Regarding prior regimens, 307 participants (74%) switched from INSTI-based regimens, with 123 (30%) switching away from bictegravir/ emtricitabin/ tenofovir alafenamide, 66 (16%) switching away from dolutegravir / lamivudine, and 17 (4%) from other dual regimens to CAB+RPV-LA.38 individuals (9.1%) discontinued the CAB+RPV-LA regimen. Reasons for discontinuation were confirmed virological failures (3 / 416; 0.7%, defined as two consecutive measurements of > 50 RNA copies/mL plasma), low-level-viremia (n = 2), and various other reasons (n = 33).
Conclusion
After two years of market authorization, only a small proportion of SHCS participants switched to the CAB+RPV-LA regimen, and most of them were white, highly educated MSM with previous optimal adherence to the oral regimen. Almost three quarters of participants switched from an integrase strand transfer inhibitor-based regimen. Approximately 9% discontinued the CAB+RPV-LA regimen but confirmed virological failure rate was low.

P018

Evaluation of the 2023 ISCVID and ESC Duke clinical criteria for the diagnosis of infective endocarditis among patients with positive blood cultures for new typical microorganisms

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Aims: The International Society for Cardiovascular Infectious Diseases (ISCVID) in 2023 introduced new pathogens as typical microorganisms for infective endocarditis (IE). We aimed to evaluate the performance of the Duke clinical criteria of the European Society of Cardiology (ESC; 2015 and 2023 versions) and the 2023 ISCVID in diagnosing IE among patients with bacteremia/candidemia by such microorganisms.
Methods: This retrospective study included adult patients with bacteremia/candidemia by such pathogens hospitalized at Lausanne University Hospital. Episodes were classified as IE by the Endocarditis Team.
Results: Among 933 episodes with bacteremia/candidemia by such pathogens, IE was diagnosed in 63 episodes (7%). IE prevalence was considered high (> 10%) among Abiotrophia spp. (17%), and S. lugdunensis (13%), with S. epidermidis showing a prevalence of 9%. Among 113 episodes with intracardiac prosthetic material, IE prevalence was considered high in episodes with S. lugdunensis (100%), C. acnes (67%), S. epidermidis (51%), Candida spp. (26%), and coagulase negative staphylococci other than S. lugdunensis and S. epidermidis (14%). Sensitivity for the 2015 Duke-ESC, 2023 Duke-ISCVID, and the 2023 Duke-ESC clinical criteria was calculated at 5% (95% CI: 1-13%), 57% (44-70%), and 8% (3-18%), respectively, with specificity at 100% (99-100%), 99% (99-100%), and 100% (99-100%), respectively.
Conclusion: Among the different versions of the Duke criteria, the 2023 ISCVID version performed better for the diagnosis of IE among new typical microorganisms.

P019

SARS-CoV-2 mRNA vaccination is associated with increase of Torque teno virus viral load – results from a prospective multicentre cohort study in healthcare workers

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Aims
Torque teno virus (TTV) as representative of the healthy human virome is increasingly used as marker of individual immune status, particularly in immunosuppressed patients. Experimental data has shown long-term transcriptional changes in immune cells after mRNA vaccination against SARS-CoV-2, indicating alterations of functional immunity. We investigated the association of SARS-CoV-2 vaccination and TTV viral load.
Methods
In a prospective multicentre cohort of Swiss healthcare workers (HCW), data on baseline characteristics, SARS-CoV-2 infection and vaccination were obtained and regularly updated since 08/2020. Additionally, sera were collected every 6 to 12 months. We identified 69 participants with available blood samples from July to October 2020 before SARS-CoV-2 vaccination became available, a documented consecutive SARS-CoV-2 Wild-type infection (before 02/2021) and corresponding samples from June 2022. At this later time point, 25 were unvaccinated, 22 were vaccinated with 1-2 doses and 22 with ≥3 doses. TTV viral load was determined in all samples using quantitative real-time PCR, viral loads before and after the vaccination period were compared. Proportions of participants with relevant change over time were calculated per vaccination group, and correlation of viral load with SARS-CoV-2 vaccination was calculated using Spearman rank test with additional permutation testing to account for tied data. Differences in viral load of factor 5 or more were considered relevant and a sensitivity analysis using factor 10 as definition for change was performed.
Results
We included 69 HCW (median age 45 years, range 26-63), 83.6% were female. Baseline characteristics were similar between groups, except that unvaccinated HCW were less likely to take any medication and those with ≥ 3 vaccinations included more males. Between 2020 and 2022, we observed an increase in TTV load in 8%, 14% and 27% in those with none, 1-2, and ≥ 3 vaccinations, respectively. A decrease was observed in 20%, 9%, and 5% of individuals in the respective groups. Changes in TTV viral load were significantly correlated with number of vaccinations, confirmed in a permutation test ($\varrho = 0.273$, $p = 0.01$). Sensitivity analysis confirmed our results.
Conclusion
The number of SARS-CoV-2 mRNA vaccine doses correlated with increasing TTV load in blood. This suggests functional changes in immune systems after SARS-CoV-2 vaccination. The clinical relevance of this finding remains unknown.

P020

A comprehensive overview and quantification of risk factors for invasive pneumococcal disease in adults: a systematic review and meta-analysis

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Background
Despite vaccination programs, the disease burden of invasive pneumococcal disease (IPD) in adults remains high. In addition to serotype replacement, this may be due to increased vulnerability of certain patients. It is important to better identify this population at risk to support effective intervention programs.
Methods
We conducted a systematic search in Pubmed and Embase in April 2023 and included all original studies describing risk factors for adult IPD, compared to the general population. Risk factors were grouped into “risk clusters”, for which meta-analyses were performed. If risk factors were substantiated by single studies or if determinants were too heterogeneously defined, results were reported separately.
Results
In the 56 studies that were included >50 different risk factors for IPD were examined. We formed 20 risk clusters. Meta-analysis on most prevalent conditions, showed for example an increased risk of IPD in adult patients aged ≥ 65 with an incidence rate ratio (IRR) of 3.86 (95% CI 2.27-6.58). IRRs for IPD in adults with haematological or solid malignancy were 17.06 (95% CI 7.91-36.80) and 4.24 (95% CI 1.76-10.19) respectively. In diabetes, IRR for adults aged < 65 was 6.24 (95% CI 3.16-12.33), versus 2.13 (95% CI 1.70-2.67) for those ≥ 65. The strength of evidence varied across risk factors, but was generally limited. Several studies indicated that patients with multiple risk factors can accumulate risk for IPD.
Conclusions
This meta-analysis provides the relative importance of all known risk factors for adult IPD. How risk factors interact in cases of multimorbidity remains largely unknown.

P021

Surgical site infection in Whipple Pancreatoduodenectomy – comparison of standard versus broad spectrum perioperative antimicrobial prophylaxis – a retrospective study

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AIM:
Pancreatoduodenectomy is a common treatment for both benign and malignant diseases of the head of the pancreas and periampullary region. Although there have been major improvements in perioperative mortality, morbidity remains high. The most common sources of severe perioperative morbidity are surgical site infection (SSI) and postoperative pancreatic fistula (according to the literature in up to 30%). Current guidelines recommend 1. or 2.generation cephalosporins as perioperative prophylaxis (1). Given the high rates of postoperative SSI, it was hypothesized that the use of broad-spectrum antibiotics for prophylaxis would decrease the rates of postoperative SSI (2).
To test this hypothesis, we performed a retrospective single center study comparing standard versus broad spectrum antibiotic prophylaxis focusing on the incidence of SSI.
METHODS:
We retrospectively analyzed 93 patients undergoing Whipple surgery at our tertiary care center between January 2022-2023. Bacterial cultures were obtained from a intraoperative choledochus duct sample as well as samples from the sites of SSI. We assessed time until the occurrence of infection, the impact of risk factors (e.g. biliary stent or cholestasis) and calculated the efficacy of standard perioperative prophylaxis versus broad spectrum prophylaxis with Piperacillin-Tazobactam.
RESULTS:
26 individuals (28%) developed postoperative SSI according to SwissNOSO definition. A microbiological choledochus duct sample was collected in 64 patients (69%). Susceptibility of the baseline choledochus bacterium to standard prophylaxis was found in 11% (7 patients). In 15 cases (23%), a polymicrobial choledochus colonization was identified, exhibiting only partial susceptibility to standard prophylaxis.
Among the 26 cases with SSI, perioperative standard prophylaxis showed an efficacy of 23% (6 pts) only. In contrast, broad spectrum perioperative prophylaxis with Piperacillin-Tazobactam would increase efficacy to 50% (13 pts).
CONCLUSION:
In elective pancreatoduodenectomy, broad spectrum perioperative antibiotic prophylaxis could significantly decrease the risk of SSI, based on our retrospective data. This is in line with recently published data from a multicenter open label randomized trial in the US and Canada (2).
We encourage to carry out further RCT comparing standard versus broader perioperative prophylaxis ideally in the setting of the widely established SwissNOSO surveillance system.

P022

Circovirus Hepatitis in an Immunocompromised Patient in Switzerland: A Case Report

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Aims

Cases of novel human circovirus (HCirV) infection were recently found in humans for the first time in France and China but have not yet been reported in other countries [1-3]. Their pathogenic relevance and distribution in humans remain obscure. Here we describe an immunocompromised 66-year-old female patient from Switzerland with a sudden onset of self-limiting hepatitis, mainly characterized by elevated liver enzymes.

Methods

Routine diagnostic work-up included serologic testing for Leishmania and hepatitis viruses A, B, C, and E. A liver biopsy was performed and analyzed histologically and with unbiased metagenomic Next-Generation Sequencing (mNGS). HCirV was quantified by an in-house real-time PCR in clinical samples from various body sites. Finally, mNGS was performed on longitudinal blood samples to observe changes in the viral sequence.

Results

No etiology of infection could be detected by routine diagnostics. Subsequent mNGS identified a new HCirV, tentatively named HCirV-1-CH. HCirV-1-CH showed 83.6% nucleotide identity with the closest known viral sequences (porcine circovirus 3) at the full genome level. HCirV-1 transcripts could be detected in hepatocytes, and the HCirV-1 genome was detectable in blood, stool, urine, and saliva samples of this patient for at least 20 months. The highest concentration was observed in the liver biopsy (3.63 x 10^9 genome copies/mL) and blood (2.83 x 10^5 genome copies/mL). Over the whole span of infection, no mutations were observed.

Conclusion

To our knowledge, this is the first report of HCirV-1 infection in Switzerland. Our findings strengthen the relevance of HCirV-1 as an emerging human pathogen that can replicate specifically in the liver and persist in susceptible patients over a prolonged period of time.

P023

BOrrelia BUrgdorferi Infections IN Children and Adolescents– a seroprevalence study (BOBUINCA)

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Lyme borreliosis is one of the most prevalent tick-borne diseases in Europe. Since studies on seroprevalence of anti-B. burgdorferi IgG antibodies in children are rare, the aim of this study was to determine the seroprevalence of B. burgdorferi IgG antibodies in children without symptoms of Lyme borreliosis residing in North-Western Switzerland and bordering regions of France and Germany.
This is a prospective cross-sectional observational single-centre study with further use of data and left-over plasma from clinical routine, based on general consent. IgG plasma antibodies against B. burgdorferi were determined according to a two-tier algorithm. All samples were screened by ELISA (Tecan, Switzerland) and positive or borderline result were confirmed by line blot (Virotech, Germany). Samples with positive or borderline ELISA results and positive line blot were considered as seropositive. Also, a subset of ELISA-negative specimens (matched with positive specimens by collection month, chronic disease, sex and age) was tested by line blot to determine the sensitivity and specificity of the ELISA analysis.
Specimens obtained from 962 children (average age 9.6 years, standard deviation 5.01 years, 54.5% males) between 29 June 2023 and 09 February 2024 were tested by ELISA; 135 (14%) were positive and 47 (4.9%) borderline positive for B. burgdorferi IgG. Of those, a total of 128 individuals (13.3%) were confirmed seropositive after lineblot. There was an equally high seroprevalence in both sexes and no higher seroprevalence could be demonstrated with increasing age. There is a trend towards more positive samples over the course of the year (7.5% in July vs. 21.8% in November). The sensitivity of the ELISA was 62.3% and the specificity 95.2%.
This study provides an estimate of IgG antibody seropositivity for B. burgdorferi s.l. in children and adolescents in North-Western Switzerland. The seroprevalence of 13.3% in this study is markedly higher than previously noted. These results confirm that B. burgdorferi infection is common in children and adolescents and may point towards an increasing risk of B. burgdorferi infection.

P024

Adherence to antimicrobial treatment guidelines for Legionnaires' disease – results from the SwissLEGIO multicentre study

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Aims:
Cases of Legionnaires' disease (LD) - as a cause for community-acquired pneumonia (CAP) - are increasing in Switzerland. Legionella spp. are intracellular pathogens that require treatment with antibiotics exhibiting intracellular penetration. Accordingly, upon confirmation of a Legionella infection, antimicrobial treatment guidelines suggest a discontinuation of beta-lactams and a switch to targeted monotherapy with quinolones, macrolides or tetracyclines. In this study, we assessed the choice, duration, and timing of the antimicrobial therapy for 203 LD patients.

Methods:
LD patients (median age 67 years, 33 % female) were enrolled from 5 university- and 15 cantonal hospitals across Switzerland between July 2022 and March 2024. Data on antimicrobial prescriptions were collected from electronic health records. The appropriateness of antimicrobial use was evaluated according to the Swiss national guidelines for CAP. Factors associated with non-alignment of antibiotic treatments with national guidelines were assessed using multivariable logistic regression models.

Results:
The initiation of Legionella-specific diagnostic testing and of Legionella-covering (empiric) antibiotics occurred with minimal delay upon hospital admission (median time for both was 0 days [IQR 0-1]). 85 % of patients further received a targeted monotherapy for LD within 24 hours after the Legionella infection was confirmed. Nonetheless, for 10.2 % of patients, treatment with beta-lactams was continued for more than 24 hours after the LD diagnostic test result was available and in the absence of any signs of bacterial co-infections. Finally, for 40 % of patients, the treatment duration with quinolones, macrolides, or tetracyclines was longer than the recommended 7-10 days (14-21 days for immunocompromised patients). ICU admission (OR = 5.88 [95 % CI 1.60–21.68]) and post-discharge antibiotic treatment (OR = 4.28 [95 % CI 1.27–14.43]) were independently associated with a prolonged treatment duration.

Conclusion:
Overall, effective antibiotic treatment is initiated with minimal delays after hospitalisation for LD and treatment choices are well aligned with guideline recommendations. However, antibiotic treatment duration exceeded guideline recommendations in 40 % of patients. Prolonged treatment is associated with the severity of the infection and the continuation of antibiotic treatment after discharge.

P025

The association between the delay of rifampicin introduction and clinical outcomes in orthopedic implant-related staphylococcal infections

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Aim
There is always discussion about the optimal postoperative timing of the introduction of rifampicin therapy in the immediate postoperative period of implant-related staphylococcal infections. We evaluate this timing in association to final outcomes and development of rifampicin-resistance.

Methods
We retrospectively included all patients with residual staphylococcal implant infections who consented to participate from January 2014 to October 2023; and with a minimal follow-up time of six months. We analyzed the delay of rifampicin use as continuous (in days) or as categorized variables. The “last Staphylococcus” represented any clinical sample at the end of the individual surveillance period.

Results
We included 96 independent episodes of staphylococcal infections with residual implants. The causative pathogens were S. aureus in 47 episodes (49%), of which two with methicillin-resistance (MRSA). The remaining were different coagulase-negative staphylococci. The median number of surgical interventions for infection was 1. The median duration of postsurgical systemic antibiotic treatment 81 days (IQR, 42-84 d). The median daily dose of oral rifampicin was 900 mg (range, 300 to 1800 mg), the median delay of its introduction 4 days (IQR, 2-8 d). During therapy, 16 patients (17%) witnessed an adverse event due to rifampicin (mostly gastrointestinal), requiring its stopping. The incidence of a microbiologically-identical recurrence with the same pathogen (S. epidermidis) was 1 (1%). The risk of rifampin-resistance among the “last Staphylococcus” isolates was 3%. In multivariate logistic regression analysis, the delays in rifampicin use (odds ratio 1.0, 95% confidence interval 0.96-1.04) failed to influence the risk for unplanned surgical revision.

Conclusion
In our small retrospective cohort of residual staphylococcal orthopedic implant infections, the timing of rifampicin failed to alter the need for surgical revision. Larger (multicenter) Swiss studies are required to provide more robust answers to this daily clinical question.

P026

Myeloid-derived suppressor-like cells as a prognostic marker in critically ill patients: insights from experimental endotoxemia and intensive care patients.

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Aims. Polymorphonuclear and monocytic myeloid-derived suppressor cells (PMN-MDSCs and M-MDSCs) are immunosuppressive immature myeloid cells expressed at low levels at homeostasis. Patients admitted to the intensive care unit (ICU) often experience endotoxemia, nosocomial infections and sepsis. MDSCs can have an important impact on the development of infectious diseases, but little is known about their potential predictive value in critically ill patients. Our objective was to characterize the dynamics of MDSCs in healthy subjects challenged with endotoxin and the clinical predictive value of MDSCs for patients admitted to intensive care unit (ICU).
Methods. Blood was sampled from eight healthy volunteers 0-168 hours after endotoxin challenge, and from critically ill patients at risk of developing infections sampled at ICU admission (n=32) and ICU discharge (n=17). Blood was analyzed by multivariate flow cytometry and unsupervised clustering, and by multiplex bead assay to quantify leukocyte subpopulations and cytokines.
Results. PMN-MDSCs and M-MDSCs increased 4-8 hours after endotoxin challenge and returned to baseline levels after 24 hours. PMN-MDSCs and M-MDSCs were elevated in patients at ICU admission and normalized at ICU discharge. A subpopulation of M-MDSC cells expressing intermediate levels of CD15 (CD15int M-MDSCs) negatively correlated with IL-31 concentrations ($r=-0.48$, $P=0.049$) and was associated with overall mortality ($P=0.02$). High abundance of PMN-MDSCs and CD15int M-MDSCs was a good predictor of mortality ($P=0.0046$ and 0.014), with area under the ROC curve for mortality of 0.70 (95% CI = 0.4-1.0) and 0.86 (0.62-1.0), respectively.
Conclusions. Elevated levels of PMN-MDSCs and CD15int M-MDSCs were independent predictors of mortality in ICU patients. Our observations support the idea that MDSCs represent biomarkers for sepsis, and that flow cytometry monitoring of MDSCs may be used to risk stratify ICU patients for targeted therapy.
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P027

SOLAR 12-Month European Results: Randomized Switch Trial of CAB+RPV LA vs. Oral BIC/FTC/TAF

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Aims: Cabotegravir+rilpivirine (CAB+RPV) administered Q2M is the only complete long-acting (LA) regimen for maintaining HIV-1 suppression and may address psychosocial challenges associated with daily oral therapy. In the Phase 3b SOLAR study, switching to CAB+RPV LA Q2M was noninferior to continuing daily oral bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) at Month (M) 12. In this post hoc analysis, we present M12 results for European participants.
Methods: SOLAR (NCT04542070) is the first randomized (2:1), open-label, multicenter, noninferiority study assessing switching virologically suppressed adults to CAB+RPV LA Q2M (with oral lead-in or starting with injections) vs. continuing BIC/FTC/TAF. The primary analysis (n=670) was based on the prespecified modified intention-to-treat exposed (mITT-E) population (n=11 from one non-European study site excluded from the ITT-E population for good clinical practice non-compliance) at M12. Endpoints included the proportion of participants with plasma HIV-1 RNA ≥ 50 copies/mL (primary endpoint) and < 50 copies/mL, incidence of confirmed virologic failure (CVF; two consecutive HIV-1 RNA ≥ 200 copies/mL), safety and tolerability, and treatment satisfaction (HIV Treatment Satisfaction Questionnaire status version [HIVTSQs]).
Results: Of 670 participants (mITT-E), 303 were from Europe; 67% (n=203/303) switched to LA and 33% (n=100/303) continued BIC/FTC/TAF. Baseline

characteristics were similar between arms . At M12, four participants (2%) in the LA arm and no participants in the BIC/FTC/TAF arm had HIV-1 RNA ≥50 copies/mL, of whom two had CVF (1%, n=2/203). Adverse events (AEs), excluding injection site reactions, were similar between the LA (79% [n=160/203]) and BIC/FTC/TAF arms (77% [n=77/100]). 1% of participants withdrew due to AEs in both arms (LA, n=3/203; BIC/FTC/TAF, n=1/100). Mean adjusted HIVTSQs scores improved significantly ($p < 0.001$) from baseline to M12 for LA (+3.64) vs. BIC/FTC/TAF participants (–2.19). Conclusion: Consistent with the overall SOLAR study population, switching to CAB+RPV LA from BIC/FTC/TAF was efficacious and well tolerated in European participants, with improvements in treatment satisfaction.

P028

Point-of-care lung ultrasound for the detection of pulmonary tuberculosis: the TrUST study

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Background and aim
Non-sputum triage tests are prioritised by the WHO to rule out tuberculosis (TB) and identify individuals that require further testing. We investigate the diagnostic performance of lung ultrasound (LUS) in a tertiary outpatient consultation in Benin.
Methods
This 2-year prospective cohort included adult patients presenting with a lower respiratory tract infection according to the treating physician (October 2021- August 2023). Standardized LUS images and videos were collected. All images were reviewed according to pre-specified categories. TB was defined as a positive GenXpert MTB/RIF® or Xpert Ultra® on sputum. All patients were screened for HIV (Alere Determine® HIV-1/2). We evaluated the association of these ultrasound categories with TB using univariate logistic and multivariate logistic regression.
Results
Out of 504 patients included, 192 (38%) were TB positive (TB+) and 312 (62%) TB negative (TB-). Overall, 78 (15%) patients had documented HIV (median CD4 count/mm3 of 92 [IQR43-358]). TB+ was associated with consolidations larger than 1 cm in any quadrant ($p < 0.001$, odds ratio [OR] 15 95%CI 9.7-23.8), apical consolidations larger than 1 cm ($p < 0.001$, OR 19 95%CI 11.2-32.8) and apical subpleural lesions less than 1cm ($p < 0.001$, OR 3.6 95%CI2.5-5.3). Multivariate logistic regression score showed an area under the receiver-operating curve of 0.88 to detect TB (sensitivity 90%, specificity 64%, negative predictive value 91% and positive predictive value 60%).
Conclusion and perspectives
LUS is a promising triage tool to exclude TB in the outpatient setting for symptomatic patients in a TB endemic region. Next steps are external validation and computer assisted diagnosis.

P029

Screening sites for detection of carbapenemase-producers– a retrospective cohort study

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Background
Colonization with carbapenemase-producing Enterobacterales (CPE) can be identified by rectal swabs in most patients. In contrast, knowledge regarding the optimal screening sites for detection of carbapenemase-producing non-fermenting bacteria (CPNF), such as Acinetobacter baumannii or Pseudomonas species, is limited. We evaluated different body sites for the detection of carbapenemase-producing bacteria (CPB) by comparing the proportions of positive sites between patients colonized with CPE and CPNF.

Methods
This retrospective, single-center cohort study was performed at the University Hospital Basel, Switzerland, a tertiary academic care center in a low CPB-endemicity setting. Consecutive patients with CPB-colonization detected between 01/2008 and 09/2023 were included. Results of clinical and screening samples were assessed to determine sites, most likely yielding growth of CPB. The following sites are systematically considered for screening at our institution: rectum, groin, throat, wounds, insertion sites of catheters and drainages and urine. All patients with known CPB-colonization are routinely screened on admission. Pertinent data was retrospectively extracted from patients' medical records. Comparisons between the proportions of positive sampling sites was performed by logistic regression analyses.

Results
We identified 119 eligible patients colonized with 158 CPB accounting for 115 cases of CPE and 43 cases of CPNF (co-colonization with both CPE and CPNF occurred in 11patients) (Table 1). Table 2 summarizes the results of both screening and clinical samples.
CPE were associated with a higher rate of positive rectal swabs (OR 4.62, 95%CI 1.93-11.07, $p < 0.001$) and urine samples (OR 4.36, 95%CI 1.40-13.59, $p = 0.011$) as compared to CPNF. Conversely, CPNF were more frequently found in respiratory samples (OR 0.11, 95%CI 0.04-0.29, $p < 0.001$), throat swabs (OR 0.23, 95%CI 0.07-0.79, $p = 0.020$) as well as chronic/acute wound swabs (OR 0.07, 95%CI 0.01-0.80, $p = 0.032$ / OR 0.17, 95%CI 0.05-0.63, $p = 0.008$).

Conclusion
While screening the rectal site and urine may be appropriate for detection of CPE, respiratory samples, throat and wound swabs may increase the sensitivity of screening protocols when aiming to detect CPNF colonization. Our results support the need for tailoring screening recommendations according to the bacterial species targeted.

P030

Evolution of serotype distribution and clinical syndromes of invasive pneumococcal disease before and during Covid-19 pandemic: A retrospective analysis in Switzerland 2012-2022

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Aims:
Anecdotally, we observed an increase of unusual non-respiratory manifestations of invasive pneumococcal diseases (IPD) in Eastern Switzerland after Covid-19 measures were loosened in spring 2021. Therefore, we aimed to analyse changes in pneumococcal clinical and serotype epidemiology.
Methods:
This was a retrospective analysis of the Swiss nationwide IPD database from 2012-2022 from the Federal Office of Public Health. We examined the prevalence of different serotype groups, including those covered by the different pneumococcal conjugate vaccines (PCV13, PCV15, PCV20) and non-vaccine serotypes (NVT, i.e. not covered by PCV20) and of organ manifestations of IPD (pneumonia, sepsis without pneumonia and other) for different age groups. The statistical analysis was performed by chi-squared test for trend in proportions.
Results:
In total, 8747 patients with a median age of 69 (IQR 25) were included in the analyses. Prevalence of infections with serotypes covered by vaccines decreased over the last decade significantly (PCV13, PCV15, PCV20: $p < 0.001$) whereas non-vaccine serotypes increased ($p = 0.01$). In 2012, 55.5%, 61.4%, 71.3% were covered by PCV13, PCV15 and PCV20, respectively, while the respective proportions were 26.3%, 32.8% and 55.4% in 2022. For the most frequent manifestation pneumonia, the same trend was detected (PCV13, PCV15, PCV20, NVT: $p < 0.007$). Proportions of pneumonia (2012: 73.6%, 2022: 63.3%, $p < 0.001$) and sepsis without pneumonia (2012: 15.2%, 2022: 8.9%, $p < 0.001$) decreased overall. In contrast the proportion of other organ manifestations increased (2012: 8.1%, 2022: 13.4%, $p = 0.001$).
Conclusion:
This study demonstrates an overall change in serotypes and organ manifestations in Switzerland which is specific by age.

P031

Performance of 18F-FDG PET/CT scanner in the diagnosis of endocarditis

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Introduction
Infectious endocarditis (IE) remains a diagnostic challenge that requires a combination of clinical, microbiology, and imaging clues. Usefulness of 18F-fluorodeoxyglucose positron emission tomography with computed tomography (PET/CT) in the diagnostic algorithms has been demonstrated. However, performances of PET/CT vary greatly between studies and types of IE. The purpose of this study was to define the performance of 18F-FDG PET/CT after echocardiography in the diagnosis of all types of IE in our institution.
Methods
This hybrid study was conducted at a university hospital from January 2014 to June 2022 (retrospective cohort: 2014-17 and prospective cohort: from 2018 onwards). Adult patients with a suspected valvular or CIED-IE were included. Realization of 18F¬FDG PET/CT was left at the discretion of the treating physician or by an endocarditis-team that was established in 2018. 18F-FDG PET/CT analysis were performed nuclear medicine physicians and radiologists (when the CT was contrast¬enhanced), based on visual interpretation. The final diagnosis of possible or definite IE was defined by the endocarditis team according to European Society of Cardiology 2015 modified Duke criteria.
Results
During the study period, 280 18F-FDG PET/CT with interpretable intracardiac results were performed for a clinical suspicion of IE in 247 patients. A final IE diagnosis was retained for 116 episodes (41%; 81 definite and 35 possible IE), among which 102 valvular (59 NVE and 44 PVE) and 20 CIED¬IE. 18F-FDG PET/CT showed radiologic signs of endocarditis in 60 episodes (21%) with an overall specificity, sensitivity, positive and negative predictive values (PPV, NPV) of 97.6%, 48.3%, 93.3%, and 72.7%, respectively. This led to an overall accuracy of 77.1% and between 80 to 85% when looking at specific types of IE. Addition of 18F-FDG PET/CT to the modified Duke criteria reclassified the diagnosis in 37 cases: from possible/rejected IE to definite IE in 25 cases (21.5%) and from rejected IE to possible IE in 12 cases (10.3%) (Figure 2).
Conclusion
Use of 18F-FDG PET/CT in a multimodal diagnostic approach led to an acceptable accuracy for all types of IE in our center. Signs of endocarditis on 18F-FDG PET/CT should be interpreted as confirmatory, while absence of sign do not efficiently rule out this diagnosis. In this cohort, 18F¬FDG PET/CT helped to reclassify 15.9% of possible or rejected IE.

P032

Antimicrobial prescribing in 3 public hospitals of rural South-Africa in 2023: results from the global point prevalence survey

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Aim
In low- and middle-income countries and especially in rural settings, antimicrobial stewardships (AMS) components such as local evidence-based guidelines and resistance patterns at the hospital level are often lacking, which is impairing the optimal use of antibiotics. We aimed at identifying targets for AMS activities in rural South Africa.

Methods
The Global-PPS, a standardized surveillance method to measure antimicrobial prescribing and resistance (www.global-pps.com), was conducted in February 2023 in 3 public hospitals of rural North Mpumalanga province. The survey included all inpatients receiving an antimicrobial on the day of the survey. Data collected included details on the antimicrobial agents, indications for treatment and quality indicators.

Results
Out of 289 inpatients, 159 (55.0%) were receiving at least one systemic antimicrobial on the day of survey. The highest prevalence was observed in pediatric medical wards (71.7%), adult surgical (67.5%), adult medical (50.8%) and neonates (38.7%). The most prescribed systemic antibiotics were ceftriaxone (23.8%), metronidazole (18.5%) and co-amoxicillin (14.1%) used to primarily treat skin and soft tissue infections (24.6%) and pneumonia (23.0%). Only 8 (3.2%) of the 248 systemic antibiotics prescriptions were targeted and biological fluid had been sent for culture for 27 (10.9%) patients. Antibiotics belonging to the WHO AwaRe classification Access group were prescribed in 76.1% of the patients, compared to 45.9% for those belonging to the Watch group. While 14.9% of the prescriptions were surgical prophylaxis, 94.6% of those were prescribed for more than one day. The reason for prescription and a stop/review date were respectively documented in 74.6% and 12.2% of the cases. A diagnosis was documented for 84.7% of the prescriptions and 53.1% of the prescriptions were made according to national guidelines.

Conclusion
Half of inpatients in this rural region were receiving antibiotics on the day of survey, which is higher than previous reports in South-Africa of 33.6% (1). We have identified three important AMS messages that are easy to convey through medical education and awareness: First, avoid the use of Watch antibiotics, second, avoid prolonged use of antibiotics for surgical prophylaxis and third, discourage metronidazole prescription. We faced challenges in assessing treatment appropriateness as documentation of diagnosis and treatment duration in patient records was often missing.

P033

Burden of schistosomiasis and strongyloidiasis in Sub-Sahara African participants in the Swiss HIV Cohort Study

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Background: With increasing migration from regions where schistosomiasis and strongyloidiasis are endemic and with no systematic screening in place, individuals from these regions are at potential health risk due to these neglected tropical diseases, which can persist in humans for decades. So far, no studies have assessed the burden of schistosomiasis and strongyloidiasis in people living with HIV who migrated from Sub Sahara Africa (SSA) to Switzerland.
Aims: The aim of this study was to determine the seroprevalence of schistosomiasis and strongyloidiasis in Swiss HIV Cohort Study (SHCS) participants originating from endemic countries in SSA. Additionally, we aimed to differentiate between active and past Schistosoma spp. infection, as screening is mostly done using serology (ELISA only), which does not differentiate between active infection and a serological scar.
Methods: Participants with origin from SSA (excluding South Africa and Namibia), registered in the SHCS after 01.01.2002 and in active follow-up at a cohort centre, were eligible for this retrospective study. 400 participants were randomly selected, with stored plasma samples used for laboratory testing. For schistosomiasis, screening was performed with an enzyme-linked immunosorbent assay (ELISA) and a rapid immune-chromatographic test (Schistosoma ICT IgG-IgM). For Strongyloides screening, ELISA was used. Both were confirmed by Westernblot. For activity measurement of schistosomiasis, the confirmed samples were tested with the ultra-sensitive and highly specific UCP-LF CAA assay and cell-free DNA PCR.
Results: Among 400 SSA participants, 123 (30%) had a positive ELISA screening test for schistosomiasis, with 70 (18%) confirmed by Westernblot. Of these, 31 (8% of all screened patients/ 44% of those with a positive Westernblot) yielded a positive cell-free DNA PCR result, indicating active disease. Results of the CAA assay are still pending.
For Strongyloides, 23 (6%) had a positive screening test, with 9 (2.3%) confirmed through Westernblot.

Conclusion: Screening of HIV-infected migrants from SSA showed a moderate to high rate of schistosomiasis and strongyloidiasis. Using only ELISA for screening overestimated the true burden. Many participants still have active schistosomiasis disease, reflecting the need for better systematic screening. In case of immunosuppression for rheumatic disease or transplantation, HIV-positive patients from SSA should always be screened for strongyloidiasis.

P034

Only a quarter of antibiotic prescriptions appropriate for nursing home residents with lower respiratory infections. What factors are associated with prescriptions?

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Aims

The challenges in identifying bacterial pneumonia in nursing home (NH) population with lower respiratory tract infections (LRTI) contribute to high rates of antibiotic prescriptions. We aimed to identify factors contributing to general and inappropriate antibiotic prescription among NH residents with LRTI, using for the first time a radiological reference standard based on lung ultrasound (LUS).

Methods

Prospective multicentric observational study. Residents with LRTI were recruited in 32 NH in Western Switzerland during winter 2022-2023. All residents underwent a lung ultrasound (LUS) within three days of LRTI onset, assessed independently by two LUS experts and serving as the pneumonia diagnosis reference standard. We used multivariable logistic regression with demographic, vital sign, diagnostic test, and LTCF characteristic variables to identify factors associated with (1) antibiotic prescription and (2) inappropriate prescription (defined as antibiotics administered in the absence of pneumonia on LUS), using backward selection with a p-value cutoff of < 0.1.

Results

Inclusion of 114 residents, 63% female, median age of 87 years and median Rockwood Clinical Frailty Scale (CFS) score of 7. Overall, 59 (52%) residents had diagnostic tests performed: 50 (44%) had a PCR for respiratory viruses (with 19 (17%) testing positive) and 16 (14%) had a blood test with CRP and/or blood count.

63 residents (55%) received antibiotics. Within 28 days, 2 residents were hospitalized (2%) and 13 died (11%).
Factors associated with antibiotic prescription were: CFS ≥ 7 (aOR 6.8, 95% CI 1.5-24.4), oxygen saturation < 92% (3.5, 1.4-8.8), performing a blood test (0.1, 0.0-0.6), rural NH (0.3, 0.1-0.7) and female physician (0.3, 0.1-0.8). Among residents receiving antibiotics, 48 (74%) had inappropriate prescriptions. Performing PCR for respiratory viruses was the only associated factor (0.1, 0.0-0.4), with lower odds of inappropriate prescription if performed.

Conclusion
While half of the residents with LRTI received antibiotics, falling within the lower range of European NH prescription rates (53-80%), most antibiotic prescriptions were inappropriate, highlighting the room for improvement. Utilization of diagnostic tests correlates with lower overall and inappropriate prescription. These results advocate for their use to optimize prescription practices in NHs and underscore the importance of developing antibiotic stewardship programs tailored to the NH setting.

P035

Opinions determining a conservative antibiotic treatment versus a direct surgical resection for diabetic foot osteomyelitis

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Aim
When confronted to a chronic osteomyelitis of the diabetic foot (DFO), the treating clinicians, patients and families have two basic options: A first-line conservative antibiotic treatment with minimal debridement (and surgery in case of immediate failure), or the direct surgical resection of all infected bone. Both approaches reveal similar successes in selected DFO patients. We ignore which minimal conditions predict a futility of any conservative attempt and should lead to direct amputation.

Methods
We performed an extensive narrative literature review in German and English languages. In a second step, we created and validated an online questionnaire in German and English versions, and sent it to 50 Swiss and 30 international DFO experts working in infectious diseases, vascular surgery, diabetology and orthopedic foot surgery.

Results
End of summer 2023, our literature review yielded 118 scientific publications with pertinent information on our study question. Almost all agree on the following minimal conditions in favor for direct amputation: In the Swiss opinion, the patient's wish has an important influence on the treatment decision with a prevalence of 93.75%. The wishes of the relatives would influence this decision to 68.25%. In the international evaluation, the specialists follow the patient's wishes to 100%. In the presence of a large/progredient soft tissue loss, both the Swiss and foreign specialists would primarily tend for direct amputation (75% vs. 60%). Equally, the past number of DFO episodes, or a rapidly spreading soft-tissue infection may favor resection. A multimorbid patient predicts a conservative approach in the Swiss opinion to 50%; but to 80% internationally. In Switzerland, local ischemia favors a direct amputation, whereas in the international opinion, unresolvable ischemia primarily leads to a conservative attempt. In clinical sepsis, 75% of Swiss experts would immediately amputate in contrast to 40% of the specialists internationally.

Conclusions
In Switzerland and other resource-rich countries, the patient’s choice is the most determining factor to decide the (first) approach in chronic DFO. Secondly, substantial soft tissue losses lead to direct amputation. Swiss experts (and patients) are less reluctant to amputate compared to foreign experts.

P036

Anti-HDV screening-uptake and prevalence in chronic hepatitis B patients and the potential benefit of an anti-HDV reflex testing in all HBsAg-positive patients

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Background/Aims:
Infections with the hepatitis D virus (HDV), requiring the presence of the hepatitis B virus (HBV) to propagate, are associated with a 2-3-fold higher risk of developing liver cirrhosis and hepatocellular carcinoma than HBV-infections alone. The estimated global anti-HDV prevalence among HBsAg carriers is 4.5% (3.0% in Europe, 6.0% in Africa) [1], with an HDV-RNA prevalence of 58.5% among anti-HDV-positive patients [1]. The EASL guidelines have recommended anti-HDV testing in all HBsAg-positive patients since 2017 [2]. All anti-HDV-positive patients should be HDV-RNA-tested and treated, if positive [3]. We aimed at estimating the number of undiagnosed anti-HDV-positive patients in our institution by extrapolating the anti-HDV-prevalence among tested patients to those not tested.

Methods:
From the laboratory database of our tertiary care hospital, all positive HBsAg-tests between 2013 and 2022 were extracted, together with all HBeAg-, anti-HBe-, HBV-DNA-, anti-HDV-, HDV-RNA-, anti-HIV-, anti-HCV- and anti-HAV-tests available for these patients in the same time frame. We determined the number and proportion of HBs-Ag-positive patients ever anti-HDV- and HDV-RNA-tested and calculated the anti-HDV- and HDV-RNA-prevalence. The potential benefit of an anti-HDV reflex testing was assessed.

Results:
Of 740 HBsAg-positive individuals (53.6% (397) male), 40.7% (301) had an anti-HDV test in our laboratory. Among these, 2.3% (7) were positive and 0.3% (1) borderline. Of the 7 anti-HDV-positive patients, 5 had an HDV RNA test. Four of them were HDV-RNA-positive. Extrapolating an anti-HDV prevalence of 2.3% (95% CI: 1.1-4.7%) to the 439 untested HBsAg-positive patients, another 10 (95% CI: 5-21) missed anti-HDV-positive patients can be expected. Thus, 58.8% (10/17) of the anti-HDV-positive patients might still be undiagnosed.

Conclusions:
In the database of our laboratory, less than 50% of the HBsAg-positive patients had an anti-HDV test. Thus, anti-HDV reflex testing in all HBs-Ag-positive patients has the potential to more than double the number of diagnosed anti-HDV-positive patients. Given that an effective treatment is now available, this might significantly reduce morbidity and mortality. Laboratory data base extracts might help to identify patients with rare diseases and link them to care.

P037

Persistent gaps in the HCV cascade and time trends in HCV testing-uptake and positivity rate in the laboratory of a tertiary care hospital

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Background/Aims:
In Switzerland, new HCV diagnoses must be reported to the cantonal physician / Federal Office of Public Health, but there is no HCV registry allowing follow-up. Since 2017, all patients with chronic hepatitis C can be treated with pangenotypic direct-acting antivirals irrespective of liver fibrosis stage and since 2022, all physicians can prescribe them. We evaluated whether laboratory data from a tertiary care hospital can help to identify still undiagnosed and untreated patients with active hepatitis C and investigated time trends in HCV testing-uptake and positivity rate.

Methods:
All HCV-related laboratory tests (antibody (ab), RNA, genotype (gt)) performed in the laboratory of the Cantonal Hospital Aarau between 2013 and 2022 were extracted. A patient was considered HCV-positive if he/she ever had either a positive HCV-ab-test, a positive HCV-RNA or an HCV-genotype result. We determined the number/proportion of HCV-positive patients never HCV-RNA-tested, the number/proportion of ever HCV-RNA-positive patients still HCV-RNA-positive, and the number of HCV-ab-, HCV-RNA- and HCV-gt-tests per year, including the positivity rate.

Results:
Between 2013 and 2022, 125'219 HCV-related tests were performed in 40'525 persons (51.0% men). Of 1’132 HCV-positive patients (66.5% men), 996 (88.0%) had an HCV-RNA-test, and 742 (74.5%) were ever positive. An HCV-gt was available in 62.4% (463/742). In their last HCV-RNA documented, 283 (38.1%) patients were still positive. Between 2013 and 2022, the number of HCV-ab-tests/year increased from 4'563 to 6'957 (+52.5%), while the positivity rate decreased from 2.8% (127) to 1.6% (112). The number of HCV-RNA-tests/year increased from 379 in 2013 to 712 in 2018 and went down again to 325 in 2022. The positivity rate declined from 40.6% (227/559) in 2015 to 16.6% (54/325) in 2022. HCV-gt-determinations decreased from 81 in 2017 to 11 in 2022.

Conclusions:
HCV-ab-testing has continuously been increasing, while HCV-RNA- and HCV-gt-testing have been decreasing since 2018. By the end of 2022, 283 (38%) patients were still viremic in their last HCV-RNA-test, and 136 (12%) HCV-positive patients have not undergone RNA-testing. Thus, laboratory data base extracts can help to identify still undiagnosed and untreated patients with chronic hepatitis C and link them to care. To ensure full work-up of HCV-ab-positive patients, HCV RNA reflex testing, as recommended by the EASL guidelines [1], should be implemented.

P038

Antiviral activity, safety, and pharmacokinetics of GS-1720, a novel weekly oral INSTI

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Background: Significant medical need exists for antiretroviral agents that can be administered less frequently. GS-1720 is an orally bioavailable integrase strand transfer inhibitor (INSTI) with potent antiviral activity and physiochemical properties well-suited for a long-acting formulation. We are investigating the antiviral activity, safety, and pharmacokinetics (PK) of GS-1720.

Methods: An open-label, multi-cohort Phase 1b study is being conducted in participants with HIV who are treatment-naïve or viremic and off antiretroviral therapy for at least 12 weeks. Based on safety and PK data from a Phase 1a study in healthy volunteers, participants are being administered GS-1720 on Day 1 and 2 and followed for a total of 10 days. The primary endpoint is plasma HIV-1 RNA (log10 copies/mL) change from baseline to Day 11. Secondary endpoints include plasma HIV-1 RNA change at Day 8 in addition to PK parameters and safety assessments. Genotypic and phenotypic sensitivity testing to drugs from the INSTI class is also being conducted from samples collected during screening and Day 11 visits.

Results: Preliminary PK from the Phase 1a study showed a median half-life of 9.4 days with a single GS-1720 dose of 450 mg. In the first Phase 1b cohort (n = 7; 6 males, 1 female and mean age 35) dosed daily on Day 1 and Day 2 with 450 mg, GS-1720 demonstrated an HIV-1 RNA mean log10 copies/mL reduction at Day 11 of 2.44 (95% confidence interval [CI] 2.04, 2.83) and at Day 8 of 2.04 (95% CI 1.72, 2.36). No participants experienced any serious adverse events (AEs), Grade 3 or higher treatment-emergent AEs, or AEs related to study drug. No treatment-emergent INSTI resistance was observed.

Conclusions: GS-1720 demonstrated potent antiviral activity and PK supportive of once weekly oral dosing while being well-tolerated. The observed > 2 log10 copies/mL decline in HIV-1 RNA and half-life > 1 week in this cohort demonstrates the potential of GS-1720 as part of an oral weekly INSTI-based regimen.

P039

Bacteriophage therapy plus fecal microbiota transplantation to treat recurrent urinary tract infection (rUTI): a case series

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Aims
Recurrent urinary tract infections (rUTIs) are a chronic and debilitating condition characterized by repeated treatment failures with antibiotics. Here, we present the attempt to decolonize intestinal and urinary reservoirs from the causative pathogen, Escherichia coli, using a combined approach of phage therapy followed by fecal microbiota transplantation (FMT).

Methods
In three female rUTI patients with a stable (same strain detected >2 visits) E. coli strain sensitive to lytic phages, a two-phage cocktail was administered orally (8 days) and intravesically (6 days) twice daily. This was followed by oral antibiotics in preparation for FMT in two of the three patients. Urine was collected prior to phage administration to monitor phage sensitivity.

Results
The microbiological response of patients during phage treatment varied. For two patients with bacteriuria at the start of treatment, urine E. coli titers decreased to low or below detection levels (10 colony forming units per mL) following intravesical administration. The strain of one patient developed resistance to both phages after five days of phage therapy. Phage was detected in urine and stool only for a short amount of time after administrations.

Both the phage administration and the FMTs were tolerated well by all patients with no related adverse events. Two patients have had markedly fewer episodes of UTI since the treatment 10 months prior and all indicated subjective clinical improvement. E. coli has been detected in the urine of all patients since the treatment.

Conclusion
Phage therapy initially showed a rapid reduction of bacteriuria. While both decolonization treatments were well tolerated, with no related adverse events, microbiological sterilization of E. coli was not achieved. However, a marked clinical improvement was observed. Further microbiological investigations into strain stability, immune response and concomitant changes in fecal and urinary microbiota compositions are ongoing.

P040

Public Knowledge, Views, Perceptions and Attitudes towards HIV and People Living with HIV in Switzerland – Results of a national survey

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Aims:
Limited public knowledge and awareness of HIV, and perceptions and attitudes towards people living with HIV (PLWH), may result in barriers to healthcare, impacting early diagnosis, access to specialist services, retention in care and quality of life. A multinational European survey was performed to gather insights into public opinion and knowledge of HIV, HIV transmission, PLWH and the U=U (undetectable = untransmittable) message to evaluate where focus is needed in future education campaigns. We report the results from Switzerland.

Methods:
A semi-structured HIV public opinion survey was conducted in November 2023 using a questionnaire with closed-ended questions to capture public stances on knowledge of HIV and PLWH. Participants aged ≥18 years were recruited by a panel institute providing population surveys by random selection and representative quota sampling across demographic variables such as age, gender, educational level and geographic location. An economic incentive was offered for survey completion.

Results:
A total of 1015 individuals took part in the survey (49.1% male, 50.7% female, 0.2% diverse). Median age was 51 years. Approximately 25% of respondents knew one or more PLWH and 45% had ever been HIV-tested. The majority of respondents rated themselves as very (13%) or quite well (70%) informed, most were aware of advances in HIV research in the past decade, and 75% agreed that HIV can be managed with antiretroviral therapy. Despite this, 60% believed that HIV can be transmitted through sex with PLWH on effective treatment. 22% believed that HIV transmission can occur through kissing. Only 20% of respondents agreed with the U=U message. Knowledge of the effect of modern HIV treatment on horizontal or vertical transmission was low (56% and 45%, respectively, responding “I don’t know”). The U=U awareness was higher among respondents who were younger, more highly educated and living in urban areas.

Conclusion:
In this representative national survey, considerable lack of knowledge towards HIV and PLWH appeared across different gender, age and education-level groups, which could enhance HIV-related stigma. Effective & continued awareness campaigns are needed to spread the U=U message, help foster a more informed public stance towards PLWH, and promote an evidence-based understanding of sexual health.

P041

Impact of antiviral treatment in patients hospitalized with influenza: a retrospective multicentre study in Switzerland over 5 consecutive influenza seasons (2018-2022)

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Aims: It remains unclear whether and for how long after symptom onset patients hospitalized with influenza benefit from oseltamivir treatment. We assessed the association between oseltamivir administration and the risk of death and development of critical disease in a Swiss cohort.

Methods: Our study focuses on adult patients admitted to general ward for influenza A/B infection over five consecutive influenza seasons. We extracted data collected between 2018-2022 in CH-SUR, a Swiss national multicentre hospital-based influenza and Covid-19 surveillance system. Patients presenting with symptoms ongoing for > 14 days or treated with an antiviral before admission, as well as pregnant women and nosocomial infections were excluded from the analysis. Primary outcome was the composite of death, intermediate or intensive care unit admission during hospitalization. Secondary outcomes included each component of the primary outcome and hospital length of stay (LoS). Associations with oseltamivir treatment were assessed using a multivariable logistic regression model with adjustment for age, sex, and pre-existing comorbidities. Subgroup analyses were performed in patients hospitalized early (< 48h after symptom onset) and late (≥ 48h).

Results: Among the 4310 registered admissions during the study period, 2835 adults (n = 1419 males, 50%) met the inclusion criteria. Median age was 74 (IQR 62 - 83), 2394 patients (84%) had comorbidities, 1971 patients (70%) received oseltamivir.

The composite outcome occurred in 58/864 untreated patients (7%) and in 151/1971 treated patients (8%) (OR = 1.15 [0.84 - 1.58], p = 0.374). Similar results were observed in the multivariable model (adjusted OR = 1.10 [0.80 - 1.50], p = 0.568). Mean LoS was 9 days (SD 13) in untreated and 10 days (SD 13) in treated individuals (mean difference = 0.9 [0.1 - 2.0], p = 0.084). In subgroup analyses, we found no association between antiviral treatment timing and the composite outcome (early hospitalization: OR 0.98 [0.62 - 1.55], p = 0.93; late hospitalization: OR 1.14 [0.73 - 1.78], p = 0.57). Our analysis was not adjusted to patient severity on admission due to lack of data, thus an impact of confounding by indication on these results cannot be excluded.

Conclusion: In patients hospitalized with acute influenza infection, we did not find a statistically significant association of oseltamivir treatment with the risk of death and critical disease development, neither with the LoS.

P042

Pertussis vaccination campaign among health care workers and validity of recall of previous adverse events following immunization

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Background: To minimize Bordetella pertussis transmission, health care workers (HCW) should be regularly vaccinated against pertussis, e.g. every 10 years. Following a vaccination campaign in our hospital 2012/2013, this was repeated in 2023. Here we describe our findings of the campaign and a nested study on the reliability of remembering experiences of adverse events 10 years earlier.

Methods: This was a prospective observational study of all HCW with patient contact. Those who had their last pertussis vaccination >10 years ago or with unknown date were offered a booster dose of Tdap (Adacel®) or Tdap-IPV (Adacel-Polio®) vaccine. Vaccinated HCW were asked to report reactogenicity for 7 days following vaccination. Those HCW who had participated in the 2012/2013 campaign, were asked to report recall of tolerability back then.

Results: Of 900 eligible HCW, 731 (81.2%) responded and 197 (26.9%) were not up-to-date. Of these, 156 (79.2%) were vaccinated (Tdap-IPV: N=91, 58.3%, Tdap: N=65; 41.7%). In summary, >690 (76.7%) of all HCW were up-to-date with their pertussis immunization status after the vaccination campaign. The most common adverse events in 134 (88.7%) of 156 HCW were pain (66.0%, day two), swelling (27.3%, day two) and exhaustion (21.5%, day two). Of 52 HCW who were vaccinated in both campaigns, 13 of 49 had had >1 adverse event back in 2012/2013, but only one remembered it. Recall of other previous adverse events such as swelling, redness and fever was also poor (Cohen's Kappa -0.11 for swelling, -0.03 for redness and 0 for fever).

Conclusion: We achieved an up-to-date pertussis vaccination status in the majority of HCW. Reaching all HCW remains challenging in the absence of mandatory immunization. The recall of previous adverse events following immunization was poor.

P043

Measles outbreak in a context of elimination and breakthrough cases: adapting the response to ensure monitoring of symptoms and testing of previously immunised people

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In 2024, Vaud experienced a measles virus (MeV) outbreak from 15th January to 5th of March including 51 cases. The majority were linked to a single imported case in a university campus. In this setting, using a conservative denominator which does not include visitors of the campus the overall attack rate was approximately 1% (n=37/3700). Most cases (55%; 28/51) were 18-26 years, 60.8% (n=31) vaccinated with two vaccine doses, 11.8% (n=6) with one dose, 21.6% (n=11) unvaccinated, and 5.9% (n=3) had an unknown vaccination status. Breakthrough MeV cases (considering at least one MCV or previous infection) was 72.5% (n=37). Most cases belonged to genotype B3 DSId 6418, the remainder to two 6418-derived mutants (4 cases with DSId 6495 and 1 with DSId 8778. To our knowledge, this is the first time variant 8778 has been identified globally. Control measures included rigorous case and contact tracing, isolation of identified cases, catchup vaccination and a campus-wide health communication strategy. Transmissions from vaccinated persons were observed. For example, 1 double vaccinated case visited a small medical centre, exposing sixteen people. Despite a universal masking policy in place in the facility at the time of the consultation, three subsequent cases were detected, resulting in an attack rate of 18.7% higher than the one observed for the university campus. Exposure in a closed environment may have played a role in transmission, regardless of vaccination status. Reasons explaining the high proportion of vaccinated cases were explored and include : early vaccination age, immunity evasion, exposure intensity and a statistical paradox. Considering the epidemiological and laboratory analyses we concluded that the statistical paradox is the most plausible explanation. In fact, in a highly vaccinated population with a highly effective vaccine, it is relatively common to expect an important proportion of cases among those fully vaccinated. When applying Orenstein's vaccine efficacy formula assuming 96% MCV coverage and 95% effectiveness of MCVs, one can predict that 55% of cases will be vaccinated, similar to what was observed during this outbreak (60.8%). In conclusion, response strategies to outbreaks in highly vaccinated populations should include rigorous monitoring of exposed individuals, prompt isolation, and testing irrespective of vaccination status. During outbreaks clinicians should test for MeV on suspected cases regardless of vaccination history.

P044

Post-pandemic SARS-CoV-2 vaccination and infection status and risk of influenza-like illnesses and work absenteeism in healthcare workers – a prospective cohort study

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Aims
We prospectively assessed whether the number of SARS-CoV-2 vaccinations was associated with influenza-like illness (ILI) episodes or work absenteeism in a cohort of healthcare workers (HCW).

Methods
In October 2023, we updated information on SARS-CoV-2 vaccination and infection within a prospective multi-centre HCW cohort. Anthropometric data and information on personal health, patient contact, and household structure were collected. Between 11/2023 and 04/2024 (i.e. follow-up), where community activity of SARS-CoV-2 and influenza were high, HCW filled in weekly questionnaires on acute viral symptoms and number of days absent from work. The main predictor was SARS-CoV-2 vaccination status grouped into unvaccinated, 1-2, 3, or≥ 4 vaccine doses. Outcomes were the number of ILI episodes and days absent from work. ILI was defined as acute-onset (≤ 7 days) of fever/feverish feeling and one respiratory symptom (runny nose, loss of smell, cough, sore throat). Using a negative binomial model, we calculated adjusted incidence rate ratios (aIRR) and 95% confidence intervals (CI) for SARS-CoV-2 vaccination status and the outcomes, adjusting for important confounders.

Results
We included 1’795 HCW (median 47 years, range 17-70), 178 (9.9%) were unvaccinated, 373 (20.8%) had 1-2 vaccinations, 916 (51.0%) 3 vaccinations and 328 (18.3%) ≥4 vaccinations. Overall, 760 (42.3%) experienced ≥ 1 ILI episode during follow-up, the mean number of days absent from work was 2. In multivariable analysis, number of SARS-CoV-2 vaccinations showed a positive association with number of ILI episodes (aIRR per dose 1.05, 95% CI 1.02-1.08), as did the number of positive SARS-CoV-2 tests (aIRR 1.13, 95% CI 1.11-1.16), smoking status (aIRR 1.18, 95% CI 1.13-1.22), comorbidity (aIRR 1.07, 95% CI 1.04-1.10) and male sex (aIRR 1.16, 95% CI 1.12-1.21).Seasonal influenza vaccination (aIRR 0.80, 95% CI 0.78-0.83) was associated with decreased risk. Patient contact and number of household contacts were not associated with the number of ILI. SARS-CoV-2 vaccination was also associated with days absent from work (aIRR 1.07, 95% CI 1.02-1.11).

Conclusion
The number of vaccinations against SARS-CoV-2 is not associated with decreased ILI episodes and absenteeism in HCW during high community transmission level of SARS-CoV-2 and influenza. In contrast, smoking and male sex are associated with increased and influenza vaccination with decreased risk.

P045

Predictors of antibiotic combination therapy in community-acquired pneumonia: A retrospective cohort study

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Aims:
The duration of antibiotic combination therapy for community-acquired pneumonia (CAP) is an insufficiently researched topic. We aimed to examine the current use and duration of antibiotic combination therapy and to identify predictors for prolonged courses of antibiotic combination therapy.
Methods:
The study was a retrospective analysis of 2017-2021 data from the prospective international multicenter cohort study CAPNETZ. Antibiotic combination therapy referred to beta-lactam antibiotics paired with macrolides, tetracyclines, or fluoroquinolones, with a minimum overlap of one day. The statistical analysis was performed by means of linear, logistic, and negative binomial regression analyses and Benjamini-Hochberg procedure (false discovery rate correction) using R.
Results:
1380 patients were included in the analysis, 65.4% of which were male. The median age was 66 (interquartile range [IQR] 24) years. 48.2% received an antibiotic combination treatment with a median duration of 3 (IQR 2) days. 68.4% of the combination therapy regimens consisted of a penicillin with a beta-lactamase inhibitor and a macrolide. Hospitalisation (OR: 7.450, 95% CI: 3.157-21.942, $p < 0.001$), increased CRP levels (OR: 1.002, 95% CI: 1.001-1.003, $p = 0.003$) and detection of atypical pathogens increased the chances of receiving combination treatment, while immunocompromise conferred a lower risk (OR: 0.492, 95% CI: 0.296-0.795, $p = 0.004$). The most important predictors for prolonged duration of antibiotic combination therapy were underlying bronchiectasis (OR: 1.963, 95% CI: 1.443-2.653, $p < 0.001$), new onset invasive ventilation (OR: 1.568, 95% CI: 1.189-2.051, $p = 0.001$), and age (OR: 1.003, 95% CI: 1.000-1.006, $p = 0.039$).
Conclusion:
This study identified predictors for combination therapy status and duration, which were related to respiratory comorbidities, severity of CAP and presence of atypical pathogens.

P046

A distinct lymphocyte distribution in routine hematology scattergrams predicts Covid-19 infection

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Background: The COVID-19 pandemic has emerged as the most substantial health crisis since the influenza pandemic in 1918. Due to its aerosol transmission, rapid diagnosis and isolation of contagious patients are key. In the absence of a specific clinical case definition, repetitive testing with PCR or antigen rapid tests has resulted in enormous costs. Osman et al. demonstrated in a pioneering study, that a specific white blood cell distribution with presence of plasmacytoid lymphocytes on scattergrams of routine hemograms are suggestive of SARS-CoV-2 infection.
Objective: This retrospective cohort study aimed at investigating the diagnostic accuracy of this newly described plasmocytoid lymphocyte scattergram (PLS) provided by our laboratory as a routine analysis.
Methods: We collected 201 patients with a positive SARS-CoV-2 PCR and a concomitant scattergram admitted to our hospital between March and December 2020 in comparison with 205 random controls admitted in the same period with a negative SARS-CoV-2 PCR and a concomitant hemogram. We analyzed comorbidities, signs, symptoms and laboratory values including the PLS as predictive markers for Covid-19 infection in univariate and multivariate analyses. The presence or absence of PLS was independently assessed by two blinded reviewers.
Results: We included 406 patients (33% females, median age 69 (IQR 59-79) years). Comparing COVID+ with COVID- patients, upper/lower respiratory tract symptoms were present in 19%/78% and 12%/54%, respectively. Fever occurred in 45% in both groups. PLS was present in 148/201 COVID+ (74%) and 55/205 COVID- (27%) patients, resulting in a sensitivity and specificity of 73% each as well as a PPV and NPV of 73% and 74%, respectively. Restricting multivariate analysis to factors associated with at minimal change by factor 2 in univariate analysis, obesity (OR 3.3, 95% CI 1.8-6.1), neoplasia (OR 0.4, 0.2-0.8), coronary artery disease (OR 1.9, 1.0-3.4), myalgias (OR 2.5, 1.1-5.8), rales (OR 2.7, 1.6-4.4), diarrhea (OR 2.6, 1.2-5.4) and PLS (OR 6.1, 3.6-10.2) were significantly associated with COVID-19 positivity.
Conclusions: With a PPV of 73%, PLS, which is readily available in a routine setting, can be used to substantiate clinical suspicion of COVID-19.

P047

Does a long preoperative antibiotic use in diabetic foot infections reduce the risk for treatment failures after surgical debridement?

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Aim
Many patients with community-acquired diabetic foot infections (DFI) are under systemic (empirical) antibiotic treatments before surgery; e.g. to reduce the presurgical inoculum of infection. We explore the impact of this presurgical antibiotic use on postoperative outcomes.

Methods
We retrospectively analyze the influence of pre-surgical antibiotic therapy (as binary (yes/no) or continuous (in days) variables) on failures after a combined surgical and medical treatment.

Results
Among 1,235 mostly moderate to severe DFI cases (22% females, 53% revascularized), 912 episodes (74%) had a presurgical antibiotic prescription within 2 weeks before surgery. The median number of surgical interventions was 1, the median duration of presurgical antibiotic treatment was 13 days (IQR, 5-27 d) and the median duration of postsurgical treatment 21 days (IQR, 12-41 d). The presence of a presurgical antibiotic therapy was not associated with postsurgical infection recurrences (856/1168 vs 49/60, p=0.15). In separate multivariate logistic regression analyses, both, the duration of presurgical treatment (odds ratio 1.0, 95% confidence interval 0.99-1.01), and the duration of postoperative antimicrobial therapy (OR 1.0, 95%CI 1.0-1.0) were unrelated to “clinical failure” or “microbiological recurrence” (OR 1.0, 0.99-1.01 and OR 1.0, 1.0-1.0), respectively.

Conclusion
In our single-center database, the duration of preoperative systemic antibiotic therapy does not seem to alter the fate of postoperative failures.

P048

Acceptance of doxycycline post-exposure prophylaxis and four-component meningococcal B vaccine for the prevention of bacterial sexually transmitted diseases in men who have sex with men and transgender women living with HIV in Switzerland

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Background:
Bacterial sexually transmitted diseases (STDs) cause a substantial disease burden and stigma among men who have sex with men (MSM) and transgender women (TGW) living with HIV. There is growing evidence that event-driven doxycycline post-exposure prophylaxis (dPEP) and the four-component serogroup B meningococcal vaccine (4CMenB) can reduce the incidence of the major bacterial STDs (i.e. syphilis, gonorrhea, and Chlamydia trachomatis).

Methods:
From May 15th to December 15th, 2023, we conducted a survey exploring the acceptance of the off-label use of dPEP and 4CMenB among participants of the Swiss HIV cohort study at highest risk for STDs, i.e. MSM and TGW diagnosed with ≥ 1 STI within the preceding 3 years and/or reporting condomless sex with ≥ 1 occasional partner within the preceding 6 months. The participants were asked if they had already heard about dPEP and 4CMenB as STD prevention strategies and if they consider their use.

Results:
637 out of 2129 eligible persons answered the questionnaire of whom 122 (19.2%) and 49 (7.7%) had already heard about dPEP and 4CMenB used for STD prevention, respectively. 340 (53.4%) and 305 (47.9%) consider the off-label use of dPEP or 4CMenB, respectively. Proportions of acceptance were highest in individuals younger than 35 years of age (58.7% and 53.3% for dPEP and 4CMenB, respectively). Acceptance for both interventions was highest in patients who had an STD in the previous year as compared to people with the last STD episode > 1 year ago or no STD history (dPEP 65.1% vs. 49.1%; 4CMenB 61.5% vs. 42.9%; $p < 0.001$). In centers of the French and Italian speaking parts of Switzerland, acceptance for dPEP was higher than in German speaking regions (64.7% vs. 50.5%; $p = 0.001$), whereas 4CMenB was more accepted in Swiss German centers (45.1% vs. 49.0%; $p = 0.03$).

Conclusions:
People living with HIV in Switzerland at highest risk for bacterial STDs show high acceptance for the off-label use of dPEP and 4CMenB to prevent bacterial STDs. Clinicians should become familiar with these interventions and consider their use as an adjunct to standard screening, counseling, and treatment efforts in persons at highest risk for STDs.

P050

Efficacy of Bictegravir/Emtricitabine/Tenofovir Alafenamide (B/F/TAF) Versus Dolutegravir (DTG)-Based 3-Drug Regimens in Adults With HIV Who Have Suboptimal Antiretroviral Adherence

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BACKGROUND: Adherence to daily oral antiretroviral therapy is important for sustaining HIV suppression. B/F/TAF Studies 1489, 1490, 4458, 1844 and 4030 demonstrated the noninferior efficacy of B/F/TAF versus DTG + 2 nucleoside reverse transcriptase inhibitors (NRTIs). We retrospectively assessed drug adherence and effect on virologic outcomes.

METHODS: All studies were double-blind, placebo-controlled, and enrolled treatment-naïve (1489, 1490, 4458) or virologically suppressed (1844, 4030) adults. Participants were randomized 1:1 to receive B/F/TAF or DTG + 2 NRTIs plus placebo; as a result, all received multiple tablets. Participants with ≥ 1 returned pill bottle and ≥ 1 on-treatment HIV-1 RNA measurement were included in the analysis. Adherence was calculated by pill count; virologic outcome was assessed by last on-treatment HIV-1 RNA.

RESULTS: Altogether, 2'622 participants were included (B/F/TAF: 1'306; DTG + 2 NRTIs: 1'316). The proportions of participants with high (≥ 95%), intermediate (≥ 85% – < 95%) or low (< 85%) adherence were similar between the 2 groups; few had low adherence (B/F/TAF: 46 [3.5%]; DTG + 2 NRTIs: 69 [5.2%] through Week [W] 48). Overall, 98.5% (n = 1,287) in the B/F/TAF group and 98.2% (n = 1,292) in the DTG + 2 NRTI group had virologic suppression (HIV-1 RNA < 50 copies/mL) at last on-treatment visit through W48. In the B/F/TAF group, virologic suppression was similar in those with high and intermediate adherence versus those with low adherence; however, in the DTG + 2 NRTI group, virologic suppression was significantly higher in those with high and intermediate adherence compared with low adherence (P ≤ 0.002). Similar results were observed at W144 in 2 studies (1489, 1490) with additional follow-up data. Nine participants with low adherence had HIV-1 RNA ≥ 50 copies/mL at their last visit through W48: 3 subsequently resuppressed (B/F/TAF: 1; DTG + 2 NRTIs: 2), 5 discontinued (all DTG + 2 NRTIs) and 1 was lost to follow-up (B/F/TAF).

CONCLUSIONS: Overall, most participants receiving either placebo-controlled B/F/TAF or DTG + 2 NRTIs demonstrated ≥ 85% adherence. In those with suboptimal adherence, B/F/TAF treatment maintained high levels of virologic suppression, while those with suboptimal DTG + 2 NRTI adherence had reduced virologic suppression.

P051
Ureaplasma associated peritonitis in a patient with anti-CD20 induced hypogammaglobinaemia – a Case Report

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Aims: To showcase that in patients with humoral immunodeficiencies, such as anti-CD-20 induced hypogammaglobinaemia, and signs of abdominal infection, especially in the presence of abscesses, invasive infection with Ureaplasma sp. is a possible cause, which should be considered and searched for.

Case: A 44-year-old female with multiple sclerosis on ocrelizumab presented to the emergency room with fever, abdominal pain and diarrhoea. The initial CT showed signs of an enteritis. Her symptoms persisted after 4 days on empirical treatment with ciprofloxacin and metronidazole. An exploratory laparoscopy showed an abscess in the region of the appendix and cecum and ileocecal resection was performed. After initial improvement, she again developed fever after 6 days. The antibiotic therapy was changed to piperacillin/tazobactam, and in the re-laparotomy the right salpinx and ovary showed signs of severe inflammation and were removed. By this time, a culture from the first operation showed growth of Ureaplasma sp. No other pathogens were found. Thus, we added doxycycline to her antibiotic regimen. Concurrent anti-CD20 therapy induced hypogammaglobulinaemia was substituted with intravenous immunoglobulins. Her fever ceased, but her inflammatory markers increased again 5 days later. A repeat CT showed multiple intra-abdominal fluid collections, which were percutaneously drained. The drainage again showed only growth of Ureaplasma sp. and the polymerase chain reaction was positive for Ureaplasma urealyticum. We continued the antibiotic treatment with doxycycline, on which her symptoms, abscesses and inflammatory markers gradually decreased.

Conclusion: Ureaplasma spp. are frequent colonizers of the genitourinary tract. Invasive infections, especially abdominal infections, are rare. However, humoral immunodeficiencies, especially hypogammaglobulinemia, have been recognized as a risk factor for invasive disease [1-3]. Thus, it is important to consider Ureaplasma spp. infections in patients with humoral immunodeficiencies, such as anti-CD20 therapy [4]. This case report also highlights, like earlier reports [1-3, 5] that Ureaplasma tends to form recurrent abscesses in this patient population. The microbiological diagnostic may have been flawed by the use of antibiotics at time of sample collections, but given their microbiological spectrum and the clinical course after tailoring the treatment to Ureaplasma, we consider it to have been the main cause of the peritonitis.

P052

Increased abundance of ESBL producing Enterobacterales in municipal wastewater receiving hospital effluent during and after the COVID-19 pandemic

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Aims: Extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) contribute significantly to the global burden of antimicrobial resistance. The COVID-19 pandemic and its travel restrictions may provide a good opportunity to study the impact on the local epidemiology of ESBL-PE. Wastewater surveillance may be useful to evaluate the extent of circulating ESBL-PE. We applied an established wastewater surveillance system to measure potential changes in the abundance of presumptive (prESBL-P) E. coli and Klebsiella, Enterobacter, Serratia and Citrobacter (KESC) group in municipal wastewater prior, during and after the pandemic in the City of Basel, Switzerland.
Methods: Municipal wastewater samples were collected across Basel during the same time-period (April to June) in 2019 (n = 63) prior the pandemic, 2021 (n = 62) during the pandemic and 2023 (n = 63) after the pandemic. Samples were taken at 21 sampling points (4 of which containing untreated effluent from hospitals) representing all postcodes of Basel. PrESBL-P E. coli and KESC group colonies counted on selective, chromogenic agar [1]. Colony counts were compared between study years, stratified by species (E. coli and KESC) and sampling sites with and without hospital effluent.
Results: Median values of prESBL-PE were lower prior to the pandemic and differed between the study years when analyzing all species combined or stratified for E. coli and KESC ($p < 0.001$). While median counts of prESBL-P E. coli and KESC were similar between sampling sites collecting urban mixed with hospital effluent as compared to sites receiving only urban wastewater, higher counts of both species were recorded in wastewater including hospital effluent during and after the pandemic (p-value = 0.002).
Conclusions: Our findings reveal a significant increase in prESBL-P E. coli and KESC during and after the pandemic, particularly in samples containing hospital wastewater. This suggests a disproportionate increase of ESBL-PE within healthcare settings as compared to the community. Social distancing and travel restriction measures, together with reduced antibiotic use in the community during the pandemic may have prevented a further increase in community settings. Wastewater ESBL-PE surveillance may serve as a non-invasive, sensitive, rapid and cost-effective strategy for early detection and monitoring the local epidemiology of ESBL-PE.

P053

Association of duration of antibiotic combination therapy in community-acquired pneumonia with clinical outcomes: A retrospective cohort study

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Aims:
It is unknown how long antibiotic combination therapy should be given for community-acquired pneumonia (CAP). We aimed to determine the impact of the duration of combination therapy and other predictors on clinical outcomes.
Methods:
This was a retrospective analysis of 2017-2021 data from the prospective international cohort study CAPNETZ. Antibiotic combination therapy was defined as beta-lactam antibiotics paired with macrolides, tetracyclines, or fluoroquinolones, with a minimum overlap of one day. Endpoints included poor outcome (composite of 30-day mortality, secondary ICU admission, treatment failure), complications, and length of stay (LOS). Linear, logistic, and negative binomial regression analyses, as well as the Benjamini-Hochberg procedure (false discovery rate correction), were performed as applicable using R.
Results:
The analysis included 1380 patients with a median age of 66 (interquartile range [IQR 24]) years; 65.4% were male. Longer duration of combination therapy was associated with higher risk of complications (OR: 1.157, 95% CI: 1.026-1.303, p = 0.016) but was not associated with poor outcome, 30-day mortality, secondary ICU admission, treatment failure, or LOS in multivariable analyses. The most important risk factors were coalescent infiltrate for poor outcome (OR: 4.371, 95% CI: 2.304-8.241, p < 0.001), viral respiratory tract infection for secondary ICU admission (OR: 2.642, 95% CI: 1.614-4.299, p < 0.001), new onset invasive ventilation for 30-day mortality (OR: 28.876, 95% CI: 9.558-86.897, p < 0.001) and complications (OR: 7.177, 95% CI: 1.298-26.565, p = 0.008) and new onset non-invasive ventilation for LOS (OR: 1.932, 95% CI: 1.620-2.317, p < 0.001) and treatment failure (OR: 14.078, 95% CI: 7.002-30.012, p < 0.001).
Conclusion:
Our study found no beneficial effect of prolonged combination therapy on clinical outcomes. Characteristics and markers of severity of pneumonia were strong predictors of poor outcome. The observed associations are hypothesis-generating but do not prove causality.

P054

HCV screening and treatment of opioid agonist therapy (OAT) patients in the canton Aargau directly in the OAT-providing pharmacy – first results

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Background/Aims:
HCV screening- and treatment-uptake can be increased by point-of-care tests (POCT), dried blood spot (DBS) testing, intregrated care1, decentralisation and task-shifting to non-specialists2. In the canton Aargau, the OAT provider seeing the patient at least once a week is in ~80% a pharmacy, making it an ideal setting for HCV screening and treatment.
Methods:
Instructed pharmacists enroll patients into the Argovian OAT-cohort and perform CE-marked rapid POCTs with capillary blood (OraQuick® HCV antibody test (5µl, 20min), Determine® HIV Early Detect (50µl, 20min), OnSite® HAV IgG/IgM (5µl, 15min)) and DBS sampling for HCV-RNA-quantification (2x 100µl). Waiting for the rapid test results, OAT patients are interviewed (baseline/follow-up questionnaire of the cohort study). Pharmacies are reimbursed per patient tested.
Results:
Of 44 OAT patients registered in the first pharmacy in July 2023, 5 had already left (one death), when testing started in October 2023. Of the remaining 39 patients, 9 already had adequate HCV management (last HCV antibody test ≤1 year ago if HCV-antibody-negative or last HCV RNA test ≤1 year ago if HCV-antibody-positive-RNA-negative), two of them successfully treated for HCV in close collaboration with the pharmacy in 2023. Of 30 OAT patients approached, 18 (60%) agreed to participate. During ~12 weeks, 18 HIV rapid tests (all negative), 10 HCV antibody rapid tests (all negative), 8 HCV-RNA-tests in DBS (all negative) and 17 HAV rapid tests (5 (29%) positive) were performed. 10 patients were newly enrolled into the Argovian OAT-cohort, while 8 patients had a follow-up. The proportion with adequate HCV management could be tripled (20% (9/44) → 61% (27/44)). The proportion of HCV-antibody-negative patients with an HCV antibody test ≤1 year ago increased from 23% (3/13) to 81% (13/16) and the proportion of HCV-antibody-positive-RNA-negative patients with an HCV RNA test ≤1 year ago from 30% (6/20) to 67% (14/21). A presumably still HCV-RNA-positive patient initially refusing participation could be enrolled later (currently under HCV treatment in a rehabilitation clinic).
Conclusions:
Swiss pharmacies can test OAT patients for viral hepatitis and HIV with capillary rapid POCTs and do DBS sampling for viremia measurements. Since the OAT provider in Switzerland is a pharmacy in >50%, HCV/HIV screening-uptake could massively be improved, if capillary HCV/HIV rapid testing and DBS sampling in pharmacies would be reimbursed.

P055

Impact of discontinuation of contact precautions on nosocomial cases of ESBL-producing Escherichia coli in a large tertiary care hospital in Switzerland

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Aim
On 1 January 2019, contact precautions for patients with extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli were discontinued at a large tertiary hospital in Switzerland. We sought to determine the impact of this change in practice on the incidence of nosocomial cases of ESBL-E.coli in this institution.
Methods
We conducted a retrospective analysis of routine microbiological and epidemiological surveillance data from 2015 to 2023 of all nosocomial ESBL-E.coli isolates identified by the bacteriology laboratory. Isolates were considered as nosocomial when collected at least 48 hours after admission to hospital. We considered four time periods: 1. pre-intervention (from 1 January 2015 to 31 December 2018); 2. post-intervention and pre-pandemic COVID-19 (1 January 2019 to 31 December 2019); 3. COVID-19 pandemic before Omicron variant (1 January 2020 to 31 December 2021); and 4. COVID-19 pandemic after the appearance of the Omicron variant (1 January 2022 to 31 December 2023). We performed an interrupted time series analysis and Poisson regression of positive nosocomial clinical samples (excluding screening samples) and calculated the incidence rate ratio (IRR) of nosocomial ESBL-E.coli for each time period. Incidence was expressed as ESBL-E.coli per month per 1000 patient days. For the time series analysis, data were pre-processed to remove random noise and seasonality.
Results
Between 1 January 2015 and 31 December 2023, we detected 1643 incident cases of ESBL-E.coli from clinical isolates (excluding screening samples) detected after 48 hours from admission. We detected a declining trend in the incidence of nosocomial ESBL-E.coli from clinical isolates across the first time period; followed by an increase prior to the COVID-19 pandemic, and a further gradual increase in the incidence of nosocomial ESBL-E.coli during the two COVID-19 pandemic time periods. As compared to the first time period, the IRR for each of the post-intervention periods was 0.08 (95% CI, 0.63-0.94) for the pre-COVID-19 period; 0.65 (95% CI, 0.55-0.76) for the first COVID-19 period; and 0.68 (95% CI, 0.58-0.79) for the second COVID-19 period.
Conclusion
The discontinuation of contact precautions for patients with ESBL-E. coli has not led to a substantial increase in the number of nosocomial clinical infections. Nevertheless, a potentially upward trend has been identified in the last period, underlining the importance of renewing this analysis in the coming years.

P056
A Rare Complication of Anti-CD20-Therapy: A Case Report

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Learning Objectives / Aims
• Newly developing liver lesions in haemato-oncologic patients may have unrelated aetiologies, in particular in immunsosuppressed patients.
• Rare cases of active alveolar echinococcosis (AE) associated with specific immunosuppressive therapies have been reported; AE on rituximab-therapy, even if extremely rare, is possible
• Serologies may be false-negative under immunosuppression, especially with regard to Anti-CD20-therapies, thus hampering diagnosis as well as subsequent treatment surveillance

Case Description
A 76-year-old patient presented for rituximab maintenance therapy for mantle cell lymphoma in remission, reporting lumbar back pain without neurologic deficits. Bone metastases were ruled out by a spine CT scan. Compared to a previous scan two months earlier two new liver lesions were discovered. Considering the patient's history, metastases were suspected. A CT-guided puncture revealed no malignant cells but PAS-positive vesicular laminated structures with parasite/helminth parts evidenced by histology. Polymerase chain reaction (PCR) with subsequent sequencing of the COX-1 gene yielded Echinococcus multilocularis as causative agent. Specific serologies (ELISA and Westernblot) were negative, most likely due to Rituximab treatment.
Two months later a follow-up scan indicated significant progression of the liver lesions. Consequently, we initiated long-term treatment with albendazole. The patient remained asymptomatic in respect of his diagnosis.

Conclusion
This case demonstrates an unusual initial manifestation of progressive AE emerging during rituximab therapy without concurrent steroid use.
Immunosuppressive therapies after solid organ transplantation (SOT) and tumour necrosis factor alpha (TNF-alpha) blockers, typically administered alongside steroids, are linked to E. multilocularis re-activation or progression [1]. In contrast, cases with progressive de novo AE during rituximab treatment seem to be rare. When they do occur, steroids are usually co-administered.
While studies regarding the likelihood of progression under rituximab are not firmly established, the current case warrants attention due to the development of initial lesions within three months and significant progression within two months. Further difficulties may arise, since therapy surveillance by serology will not be possible in this case due to subdued antibodies with anti-CD20-therapy [1]. PET-CT as an alternative follow-up may be discussed.

P057

HIV-1 RNA Blips and Low-Level Viral Replication: SOLAR (CAB+RPV LA vs. BIC/FTC/TAF)

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Aims: Cabotegravir plus rilpivirine long-acting (CAB+RPV LA) administered every 2 months (Q2M) is the first and only complete LA regimen recommended for virologically suppressed people living with HIV-1. Here, we report HIV-1 RNA viral blips and target virus not detected (TND), as well as the impact of HIV-1 RNA blips on viral load measurements at Month 12 and confirmed virologic failure (CVF), in participants switching to CAB+RPV LA vs. continuing daily oral bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) through Month 12 in the SOLAR study.
Methods: SOLAR (NCT04542070) is a Phase 3b, randomized (2:1), open-label, multicenter, noninferiority study assessing switching virologically suppressed adults to CAB+RPV LA Q2M vs. continuing BIC/FTC/TAF. The analysis was based on the modified intention-to-treat exposed (mITT-E) population (exclusion of one trial site for non-compliance to protocol entry criteria). HIV-1 RNA viral blips were defined as a single HIV-1 RNA value between 50 and < 200 c/mL with adjacent values < 50 c/mL. CVF was defined as two consecutive HIV-1 RNA ≥ 200 c/mL values. Plasma samples were analyzed for HIV-1 RNA viral load using the Abbott RealTime HIV-1 assay, and TND outcomes were provided for HIV-1 RNA < 40 c/mL.
Results: Of 670 participants (mITT-E), 447 (67%) switched to LA and 223 (33%) continued BIC/FTC/TAF. The proportion of participants with HIV-1 viral blips through Month 12 was 4% (n=19/447) in the CAB+RPV LA arm and 4% (n=9/223) in the BIC/FTC/TAF arm. Of participants with viral blips, 5% (n=1/19) and 11% (n=1/9) in the CAB+RPV LA and BIC/FTC/TAF arms, respectively, had HIV-1 RNA ≥ 50 c/mL at Month 12; no participants with HIV-1 RNA viral blips developed CVF. The proportions of participants with viral blips were consistently ≤1% of participants with available data across both treatment arms at any time point. TND outcomes at individual study visits were similar between study arms (CAB+RPV LA, 85–88%; BIC/FTC/TAF, 80–86%), and the proportions of participants with HIV-1 RNA < 40 c/mL (CAB+RPV LA, 90–97%; BIC/FTC/TAF, 90–97%) were comparable between treatment arms through Month 12.
Conclusion: The proportions of study participants with HIV-1 RNA viral blips, TND, and HIV-1 RNA < 40 c/mL were similar between CAB+RPV LA and BIC/FTC/TAF through Month 12. HIV-1 viral blips with CAB+RPV LA did not appear to be associated with CVF, consistent with prior CAB+RPV LA Phase 3 clinical study data.

P058

3-Year Outcomes for Dolutegravir (DTG) + Lamivudine (3TC) in ART-Naive and Pre-treated People Living With HIV-1 in Germany: Real-world Data From the German URBAN Cohort

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Aims: Although clinical trials have assessed DTG + 3TC for first-line therapy and maintenance of virologic suppression, clinical practice observations can complement these data in more diverse populations. The URBAN study provides real-world data on effectiveness, tolerability, metabolic parameters, and patient-reported outcomes (PROs) in people living with HIV-1 using DTG + 3TC. Here we present Year 3 results.
Methods: URBAN is a prospective, non-interventional, multi-center, 3-year German cohort study in ART-naive and pre-treated individuals receiving DTG + 3TC. The primary endpoint was proportion with virologic suppression (viral load [VL] < 50 or 50-200 c/mL with subsequent VL < 50 c/mL within 120 days; discontinuation = failure) at 3-year follow-up. Lipid and liver parameter changes were assessed. PROs were assessed via HIV Treatment Satisfaction Questionnaire, status version (HIV-TSQs) and HIV Symptom Distress Module (HIV-SDM).
Results: Of 366 individuals, median baseline age was 47 years; 93.2% were male. Overall, 332/366 (90.7%) individuals were eligible for the primary analysis; those with missing data (n = 8) or lost to follow-up (n = 26) were excluded. In pre-treated individuals, median time on ART before switch to DTG + 3TC was 7 years (IQR, 4-13; n = 303), and 32.8% had a history of ≥ 3 ART switches. Year 3 virologic suppression rates were 83.0% for pre-treated and 77.8% for ART-naive individuals. Overall, 6/332 (1.8%) individuals discontinued DTG + 3TC for virologic reasons at investigator's discretion with VL ≥ 50 c/mL (n = 5 pre-treated, n = 1 ART-naive); no emergent resistance was reported. Median (IQR) weight change from baseline at Year 3 was 2.0 kg (−1.0, 6.0; n = 131) in pre-treated and 5.0 kg (1.0-10.0; n = 13) in ART-naive individuals. Lipid and liver parameter changes from baseline were minimal. Pre-treated individuals who completed baseline and Year 3 questionnaires had statistically significantly increased HIV-TSQs scores: baseline mean (SD), 54.2 (7.5); Year 3 mean (SD), 56.4 (6.5); Year 3 change from baseline mean (SD), 2.2 (8.9); P < 0.0001. HIV-SDM scores remained stable.
Conclusion: At Year 3, high virologic suppression rates and few discontinuations for virologic reasons were observed with DTG + 3TC; no emergent resistance was reported. Treatment was well tolerated, with minimal lipid and liver parameter changes. Pre-treated individuals reported statistically significant improvements in treatment satisfaction.

P059
Multidose Oritavancin in clinical practice

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Objectives: Oritavancin is licenced for acute bacterial skin and skin structures infections with gram positive infections, as a single dose 1200 mg. With a low rate of MRSA, there are rarely indications in Switzerland for this situation. However, in patients with chronic infection due to difficult to treat organism where no oral therapy is possible, treatment may be improved and simplified by a treatment which can be given every 7 to 14 days.
Results: To date, 3 patients had S. epidermidis, 1 patients E. faecalis with allergy to amoxicillin (anaphylaxia) were included, two (2/4) still in the very early treatment phase. All patients treated so far tolerated the treatment well, no side effects were documented. Patients with completed treatment, renal function remained stable. Through levels of oritavancin were always in the therapeutic range and well above MIC, also for the estimated non-protein bound fraction. With therapeutic drug monitoring, interval between doses could be extended to up to 14 days.
Discussion: Multidose treatment in the outpatient setting with oritavancin is feasible and well tolerated. With therapeutic drug monitoring, treatment can be individualized and intervals safely extended, with significant decreases of costs and improvement of life quality for patients. Results on clinical outcomes are pending.

P061
A Pain in the A*s: A Case Report of Unusual Gonorrhea Presentation in a Young MSM

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Introduction:
Infections with Neisseria gonorrhoeae are a common sexually transmitted infection (STI) with global incidence experiencing a rise in recent years. It typically presents with urogenital symptoms [1]. However, extragenital manifestations including perianal abscesses can occur [2].

Case Presentation:
We report a case of a 20-year old male patient with no prior medical history who presented to the emergency department with severe anal pain. The clinical examination revealed a perianal abscess that was drained. The patient, who identified as an MSM, denied any urethral discharge, dysuria or other urogenital symptoms and was afebrile. Laboratory assessment showed a slightly elevated c-reactive protein at 9.1 mg/L (normal range < 5 mg/L) as well as normal number of leukocytes (11’030/µL, normal range 3’700 – 11’200/µL). Initially, no further testing for STIs was performed. However, microbiological evaluation of the drained pus revealed Neisseria gonorrhoeae. This prompted a comprehensive STI evaluation, including a pooled PCR test for chlamydia and gonorrhea using swabs from oral, rectal and urethral sites. All subsequent tests including the pooled PCR returned negative, along with tests for HIV, Syphilis, Hepatitis B and C. The patient received a single dose of intravenous ceftriaxone 2g and made a full recovery without further complications. His partner was also treated for gonorrhea.

Conclusion: This case underscores the potential for a rare extragenital manifestation of gonorrhea even in the absence of classical urogenital symptoms. While the prevalence of extragenital gonorrhea remains relatively low, MSM populations exhibit a significantly higher incidence of rectal gonorrhea (0.2–24%), compared to the general population. Pharyngeal gonorrhea also presents a noteworthy extragenital manifestation in MSM with a reported incidence of 0.5–16.5%. Furthermore, MSM with extragenital gonorrhea are frequently asymptomatic, with only around 11.9% of rectal gonorrhea cases experiencing symptoms [3]. This highlights the importance of a high index of suspicion and appropriate testing for gonorrhea in MSM, particularly when evaluating perianal abscesses, despite lacking typical genitourinary complaints. It is also important to acknowledge the significantly lower prevalence of rectal gonorrhea in women who have sex with men compared to MSM [3].

P062

Once HIV knowledge is addressed: HIV-stigma from the perspective of healthcare professionals working in HIV facilities in French-speaking Switzerland

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Aims
Stigmatising behaviour towards people living with HIV (PLWH) by healthcare professionals (HCPs) has been observed when HIV knowledge is lacking. We conducted a qualitative study among HCPs working in HIV healthcare facilities to identify what stigma drivers remain when HIV knowledge is good.
Methods
Semi-structured interviews were conducted among HCPs working in French-speaking Switzerland. Lexicographic computer-assisted software (IRaMuTeQ) performed content analysis to identify themes and sub-themes.
Results
Ten HCPs were interviewed: four nurses and two reception staff working at Lausanne University Hospital and four physicians working in university hospitals or private practice. Three main themes were identified: clinic reception, care provision for PLWH, and HIV knowledge, gathering six sub-themes. Reception staff described a complex workload generated by maintaining different patient approaches to confidentiality. This included enabling patients to wait outside the clinic rather than the waiting room and ensuring different clinic dates for PLWH who might know each other socially but who have never shared their HIV status. Nurses and physicians emphasised the importance of a holistic approach to care and, as with other chronic conditions, the advantage of HIV care provision by the same team long-term. Physicians described insufficient time during medical consultations to both fulfil their medical role and discuss stigma. Lack of HIV knowledge in the general population and among non-HIV-specialist HCPs was described as perpetuating HIV-stigma.
Conclusions
Even among HCPs with good HIV knowledge, HIV-stigma challenges remain. Well-intentioned efforts to encourage PLWH to avoid sharing their HIV status may ultimately reinforce rather than address HIV-stigma. The different roles of HCPs suggest that stigma reduction interventions in healthcare settings need to be tailored to different professional groups. Ensuring continuity of care in HIV facilities requires prioritisation as HIV is a chronic condition. Finally, improving public and HCP knowledge will help to tackle HIV-stigma encountered outside specialist centres.

P063

Villains of Antibiotics: A Multi-faceted Antibiotic Stewardship Intervention with Educational Feedback

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Aims

Inappropriate antibiotic use is a major driver of antimicrobial resistance, a growing global threat impacting patient outcomes and healthcare costs [1]. This study aims to evaluate a multifaceted approach to improve antibiotic stewardship among internal medicine physicians. We hypothesize that by combining baseline assessment of physician knowledge with anonymized, monthly feedback reports, we can achieve a measurable improvement in overall antibiotic prescribing patterns, promoting prudent use and ultimately reducing resistance.

Methods

This prospective observational study will enroll all physicians from department of internal medicine of our hospital, which includes emergency room, intermediate care, cardiology, respiratory medicine, neurology, geriatrics and outpatient clinics. The study will involve three phases:

Baseline Assessment: A questionnaire will be distributed to all physicians assessing their knowledge of antibiotic therapy, covering topics such as indications for switching from intravenous to oral therapy, optimal treatment durations and principles of antibiotic escalation and de-escalation.

Intervention: Monthly reports will be issued highlighting the top 10 antibiotic prescribers among the physicians. These reports will be anonymized and detail the most frequently prescribed antibiotics and include educational pearls on appropriate antibiotic use and stewardship.

Follow-up Assessments: The same questionnaire will be administered to all physicians at 3, 6, and 12 months following the initiation of the intervention.

Outcomes

The primary outcome of this study will be a comparative analysis of overall antibiotic prescribing patterns at the hospital, focusing parenteral usage and prescription of watch- and reserve-antibiotics. This may include adherence to established treatment guidelines. Secondary outcomes will be a comparison of baseline physician knowledge scores with scores obtained at follow-up assessments.

Conclusions

This study will evaluate the effectiveness of a multifaceted approach combining baseline assessment and anonymized feedback reports to improve antibiotic stewardship among internal medicine physicians. We anticipate a positive impact on prescribing patterns, ultimately contributing to the fight against antimicrobial resistance. The study design allows for the identification of potential knowledge gaps or persistent prescribing patterns requiring further tailored strategies.

P064

Hospitalized patients with Covid 19 in the situation of high seroprotection rate. Analysis of 403 patients from Oct to December 2023

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Aims: due to generally high seroprotection rate in the swiss population, either as results of vaccination and/or previous infection, and a lower virulence of the current Covid19 strain, the disease is generally seen as minor problem, with low numbers of severe infection and mortality. Some patients may be hospitalized due to Covid, other with Covid, but not as primary diagnosis.
Methods we retrospectively analysed charts of all patients admitted to the Kantonsspital Olten and Bürgerspital Solothurn between 15.10. and 31.12 2023. Focus of the investigation was the reason for hospitalisation (Covid 19 as primary or non-primary cause), duration of symptoms before admittance, duration of hospitalisation and mortality. Secondary endpoint was the analysis of comorbidities on the outcome.
Results 403 patients were hospitalized during the 11-week period. 203/403 (50.3%) were male. 180/403 (44.6 %, CI95 39.8.-49.5%) had symptoms less than 5 days, 192/403(47.6%, CI95 42.8-52.5%) patients had COVID 19 as primary cause of admittance. 34/403 (8.4 %, CI95 6.1-11.5%) had a nosocomial covid infection. Inhouse mortality was 14/192 (7.3%, CI 3.8-15.2%) in patients with Covid 19 as primary diagnosis and 13/177 (7.3 %CI95 4.4-12.2%) in patients with COVID19 as concomitant disease, and 4/34 (11.7% CI95 4.8-26.7%) in nosocomial Covid19 infection. Mean age death in patients with primary diagnosis was 84±5.0 years and 83±4.4 years with patients with Covid19 as concomitant diagnosis. Age > 75 years (14.3%), immunosuppression(16.7 %) and male gender (16.9%) were factors for higher mortality. Hospitalisation was 8.2 days in patients with Covid 19 as primary diagnosis and 10.7 days in those with other primary diagnosis. 35/403 (8.6% (CI 6.3-11.8 %) patients received nirmatrelvir/ritonavir, 26 with early infection, 7 with late infection and two with nosocomial infection. Nirmatrelvir/ritonavir had no benefit neither in reducing mortality nor for duration of hospitalization.
Conculsion: Mortality remains high in patients hospitalized with Covid19, higher than for influenca. Almost half of the patients are admitted early during infection, still in viremic stage, potentially candidates for antiviral treatment. However, use of antiviral treatment was not associated with lower mortality. 53% of patients are hospitalized due to Covid19, while in 47% of the patients Covid 19 was only concomitant diagnosis

P066

Macozinone Revealed: Nanomotion-Based Rapid Phenotypic Evaluation of New Drug Candidate for Mycobacterium tuberculosis Treatment.

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The surge in global Mycobacterium tuberculosis (MTB) infections, compounded by the post-COVID-19 landscape, underscores the urgency for swift diagnostics, especially given the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Conventional antimicrobial susceptibility testing (AST), notorious for its time-intensive nature, impedes the prompt identification of drug-resistant cases, posing a challenge in managing escalating global MTB infections. This study delves into nanomotion-based AST, lauded for its ultra-rapid phenotypical capabilities in the 2023 Tuberculosis Diagnostics Pipeline Report (2) for its high performance on established antitubercular agents (RIF, INH) (1). Our focus lies in evaluating the adaptability of this method for detecting third-line drugs, such as Macozinone (MCZ).

Employing the Phenotech AST device, we assessed its efficacy in distinguishing Macozinone profiles, utilizing MTB susceptible (H37Rv) and resistant (NTB1 with DprE1 mutations) strains (3). Changes in bacterial metabolism patterns were correlated with drug susceptibility profiles, and a machine learning approach gauged the device's accuracy, sensitivity, and specificity in predicting strain phenotypes.

Susceptible MTB strains exhibited a notable decline in cantilever oscillations, signifying reduced bacterial metabolism and eventual inactivation. Conversely, resistant strains remained unaffected by the drug in their environment. Classification models for the DprE1 inhibitor Macozinone demonstrated high training accuracy, surpassing 95%.

The nanomotion-based rapid AST protocol was effectively applied to the developmental drug MCZ. Crucially, the approach validated Macozinone's novel effects in vitro. The Phenotech AST device exhibits promise for direct deployment in endemic countries, facilitating timely, accurate treatment decisions for patients with results delivered in under a day (21 hours).

P067
What Corynebacterium amycolatum has to do with bones?

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EOC Lugano

Introduction
Orthopaedic infections are associated with a substantial risk of recurrence due to the difficult penetration of antibiotics in bone tissue. Though the most often cause are Gram positive bacteria, in some cases, unusual pathogens can cause bone infections due to underlying clinical conditions.

Case report
A 56-year-old woman went to her general practitioner because of a haematoma in the right-side gluteus secondary to a fall. She had in anamnesis an autoimmune hepatitis treated with mycophenolic acid (1g per day) and steroids (prednisone 12.5 mg per day) since 2004, and a previous diagnosis of chronic osteomyelitis by Citrobacter Koserii and Proteus vulgaris. At that point, she underwent a complex surgery and a long course of antibiotic treatment. No relapse was observed during the post-surgery follow-up.
For the management of the haematoma, her general practitioner performed three evacuations, before referring her to the hospital. She never reported fever or other systemic symptoms.
At the hospital, blood check showed a mild increase of the C-reactive protein and of the leucocytes.
A CT scan of the pelvis showed an abscess of the right gluteus (41 x 68 mm x 7.5 cm) and an exophytic part of the bone close to a minor bleeding arteriole. The haematoma was surgical evacuated and a drainage was placed; a biopsy of the exophytic mass was also performed. The biopsy showed emphysematous chronic osteomyelitis.
Both the microbiological examination of the bone and the drainage performed before antibiotic treatment were positive for Corynebacterium amylicatum, in absence of other pathogens. At first, she was treated empirically with 2 days of vancomycin (1g/12h) and piperacillin/tazobactam (4.5g/8h). After identification of C. amycolatum, the antibiotic was changed according to the antibiogram and she started clindamycin (600mg/8h) that was continued for a total of 6 weeks. No clinical or laboratory recurrences were observed after two months of follow-up.
The patient local infection improved after surgical procedure and long-course antibiotic treatment, the inflammation markers improved and she attended our outpatient clinic for follow up. After 3 months of follow-up, no recurrence was observed.

Conclusion
In immunosuppressed patients, even if unusual, C. amycolatum can cause severe infections in multiple sites (ear, corneal liquid, peritonitis, endocarditis). However, bone involvement with osteomyelitis had never been described before.

P068
Melioidosis, a tropical disease on the rise: A Case Report

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Introduction:
Melioidosis is an infectious disease caused by the gram-negative bacterium Burkholderia pseudomallei, an environmental germ that occurs in the soil and water of highly endemic areas such as Southeast Asia or Australia. In Europe, the disease is mainly travel-related.
Case presentation: We report the case of a 67-year-old male patient with diabetes, who presented with a chronic cough for six months. Regarding his travel history, he reports spending his holidays in Thailand several times a year. Imaging of the lungs showed a mass in the right lung apex that was suspicious for malignancy. A bronchoscopy only revealed necrotic material. Due to rapid radiomorphological dynamics and the persistency of the slightly impaired general condition, a second bronchoscopy was performed. Finally, Burkholderia pseudomallei/mallei was detected from the cultures of the bronchoalveolar lavage. Identification was carried out using mass-spectrometry. The exact differentiation of a B. pseudomallei and the resistance testing took place externally at the national reference center for highly pathogenic bacteria. The chronic respiratory symptoms, the appropriate travel history and the radiomorphology with cavitating-inflammatory lung infiltrates and splenic abscess as well as the detected pathogen led us to confirm pulmonary melioidosis. Antibiotic therapy with Ceftazidime was administered for 14 days. We switched the antibiotic therapy to high-dose Sulfamethoxazole/Trimethoprim after hospital discharge and stopped the therapy after a total of 12 weeks.

Conclusions:
Melioiodosis is an underdiagnosed disease that has worldwide implications and that was called to be included in the WHO list of neglected tropical diseases. The lack of laboratory resources in rural and remote endemic areas makes the fight particularly difficult. To date, most cases have occurred in Southeast Asia and Australia, but data suggests that the affected areas are expanding to the Pacific, Africa, and Central/South America. It is predicted that the incidence will increase with climate change due to known associations between cases of illness and severe weather events.

P069

Elucidating the molecular determinants underlying the anti-Phytophthora activity of potato-associated Pseudomonas.

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Pseudomonas strains are well known for their ability to inhibit plant pathogens through a variety of mechanisms, including the production of siderophores and antimicrobial compounds. A single bacterial strain can express several of those biocontrol traits, often depending on environmental conditions, such as the presence of other microorganisms.
Pseudomonas donghuensis strain R32 is known to inhibit the growth and development of Phytophthora infestans, the causing agent of potato late blight, which severely impacts potato production worldwide. By analyzing R3's genome, it was found to produce pyoverdine, a fluorescent siderophore produced by many Pseudomonas strains, and HCN, a highly toxic compound. We could already confirm the relevance of HCN in R32's anti-Phytophthora activity, and the goal of our current work is to investigate the contribution of pyoverdine to this activity.
In order to better understand the importance of those two known biocontrol traits, we created single and double knock-out mutants. While characterizing those mutants, we found that pyoverdine mutants were still secreting some other type of siderophore, thus indicating the presence of an additional, yet unknown biocontrol trait. We are currently investigating which type of siderophore this molecule could be and how it is involved in anti-Phytophthora activity.
In parallel, to identify yet unknown biocontrol traits in R32, a Tn5 transposon mutant bank was created and screened for loss of anti-Phytophthora activity in a high throughput screening procedure monitoring zoospore germination. This led to the identification of 26 candidates impaired in their inhibition of P. infestans zoospore germination. The affected genes in these candidate mutants are now being identified. This may reveal new mechanisms of action or help to identify important regulatory pathways.
Our ultimate goal is to use the better understanding of the genetic and regulatory elements underlying the antagonistic behavior of plant-associated Pseudomonas to improve the performance of these biocontrol strains in the field.

P070

The structural and functional characterization of the septal pore apparatus in the agaricomycete Coprinopsis cinerea

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Introduction

The vegetative mycelium of multicellular fungi is a complex network of branching and fusing filaments, referred to as hyphae. Vegetative hyphae of mushroom-forming fungi (agaricomycetes) exhibit regular septation, which allows the differentiation of hyphal compartments within the mycelium. These septa contain pores for maintaining the cytoplasmic bulk flow and thereby the exchange of nutrients, signals, vesicles and organelles within the mycelium. Agaricomycetes have a barrel-shaped swelling around the pore, referred to as dolipore, which is usually associated with a membranous structure surrounding it. Latter structure is called the septal pore cap (SPC) and the entire structure, dolipore together with SPC; is termed septal pore apparatus.

Aims

We set out to characterize the structure and the protein composition of the septal pore apparatus in the model agaricomycete Coprinopsis cinerea using a combination of microscopic, genetic and biochemical approaches.

Methods

We will investigate the dynamics of the structure and function of the apparatus during normal vegetative growth and upon environmental changes, such as sudden changes in osmotic conditions, physical damage or predation applied to the hyphae.

Conclusion

This study will contribute to our understanding of fungal cell biology, but also provide broader insights into intracellular dynamics of coenocytic organisms in general.

P071

Galleria mellonella – a mini in-vivo infection model to assess the inhibition potential of antagonistic bacteria against Aspergillus spp.

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Aims
Over the past decades, the frequency of fungal infections has increased dramatically, particularly in immunocompromised patients. This is particularly the case of Aspergillus spp. which produce large quantities of airborne conidia (up to 10^8 conidia per m3). Aspergillus causes a wide range of diseases, from non-lethal hypersensitivity responses to lethal invasive infections, called aspergillosis. Given the unprecedented rise of resistance to current antifungal drugs, alternative treatments to manage these infections are imperatively needed. The aim of this study was to develop a mini in-vivo infection model using the larvae of the greater wax moth, Galleria mellonella, to assess the biocontrol potential of antagonistic bacterial strains against Aspergillus spp.

Methods
To achieve this goal, we used A. niger as model opportunistic fungal pathogen, and the soil bacterium Cupriavidus oxalaticus as biocontrol agent. We injected them both alone or in combination in Galleria larvae, and we monitored survival twice per day for 3 days.

Results
The administration of 10^7 A. niger conidia was found to reduce Galleria larvae survival by 50%. Moreover, the injection of as few as 100 C. oxalaticus cells proved fatal to the larvae, whether alone or when co-injected with A. niger conidia.

Conclusions
Further experiments are ongoing to determine the possible causes of larvae death due to C. oxalaticus, which is crucial for validating this model for a first screening step before moving to pre-clinical models such as mice and understanding the interactions in this tripartite system.

P072

A novel mechanism for archeological iron stabilization using dead biomass of the yeast Meyerozyma spp.

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The use of an environmental strain of the yeast Meyerozyma spp. to remove chloride from archeological iron was the subject of this research. The choice of this specific strain was predicted by its remarkable ability to withstand extreme conditions such as presence of heavy metals and high salinity. Preliminary investigation uncovered unique properties of dead biomass of Meyerozyma spp. that enable two pathways of interacting with corroded iron in an aqueous environment. The first mechanism is biosorption of iron and chloride ions onto the cell wall while the second shows the ability of dead biomass to initiate redox reactions allowing conversion of reactive corrosion products into more thermodynamically stable compounds. The latter was verified through Raman spectroscopy and X- ray diffraction (XRD) conducted both before and after treatment.

The extraordinary corrosion conversion behavior observed with Meyerozyma sp. may be linked to the presence of capping or chelating agents within the biomass matrix. In order to better understand this transformation, our current experiments aim to study the dynamics of this electrochemical system and investigate the surface properties of biomass. Zeta potential and point of zero charge analyses have provided insight into the reactivity of the biomass surface under different pH conditions, shedding light on its potential electrostatic interaction with corroded iron plates. This result combined with Fourier Transform Infrared spectroscopy (FTIR) analysis of Meyerozyma spp. biomass, helped to narrow down a number of functional groups situated on the cell walls surface that play role in the corrosion conversion process. Additionally, membrane dialysis has been used to separate and characterize molecules present in the aqueous solution that might also be active during redox reaction. All the results suggest a novel redox active mechanism that can be used for the conservation and protection of iron objects.

P073

Rapid detection of HSV1, HSV2 and VZV in cutaneous or mucocutaneous lesions by isothermal amplification in a routine laboratory of dermatology

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Aims:
After a positive evaluation of the Solana HSV 1+2/VZV assay (Quidel, San Diego, CA) performance compared a to a real time PCR assay, a prospective study was conducted in the laboratory of dermatology at the University Hospital of Geneva. All vesicular skin and genital lesions submitted to the laboratory for a viral detection were processed and the presence of the three virus was analysed as well as their distribution in the different part of the body.
Methods:
The statistical analysis was performed from the data extracted from the laboratory information system that were collected during seven months. Cutaneous or mucocutaneous specimen were collected essentially by dermatologists from vesicular skin and genital lesions. A total of 103 clinical specimens from various part of the body were received in our laboratory and immediately processed for analysis as recommended by the manufacturer. All 3 viruses could be detected in a single reaction tube with a qualitative interpretation.
Results:
Out of 103 specimens, 60 were negative (58.3%) and 43 were positive for a virus (41.7%). HSV-1 was present in 9 specimens (8.7%), HSV-2 in 6 specimens (5.8%) and VZV in 28 specimens (27.2%). As expected HSV1 was rather recovered near or in the oral sphere and HSV-2 in the genital sphere. Regarding VZV, it was predominantly detected in other parts of the body, particularly in herpetic lesions of thorax or abdomen. Sometimes, HSV-1, HSV-2 or VZV were identified in non-predictable location such as VZV in a penis smear or HSV2 in a back smear. Interestingly, a VZV positive result was also detected in a skin sample from a one-month-old newborn. No coinfection with any of these viruses have been found.
Conclusion:
The Solana HSV 1+2/VZV assay is very helpful to quickly and accurately diagnose HSV and VZV infections. In patient with primo-infection or immunosuppression virus identification HSV or VZV is mandatory to initiate the treatment promptly. This test is slightly less sensitive than real time PCR but provide results in a short turnaround time (about an hour). Samples can be processed one by one leading to provide result immediately to the clinician without a 24-48h delay when performing test in large series. The recovery of unsuspected HSV and VZV from clinical specimens supports the implementation of this combined HSV/VZV assay in dermatology diagnostic.

P074

Relative inhibitory activities of the broad-spectrum ß-lactamase inhibitor xeruborbactam in comparison with taniborbactam against metallo-ß-lactamases produced in Escherichia coli and Pseudomonas aeruginosa

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Aims. Metallo-ß-lactamases (MBLs) are problematic carbapenemases, conferring resistance to all ß-lactams except monobactams, and being resistant to all clinically-available ß-lactamase inhibitors (BLI). By contrast to other newly-developed BLI, taniborbactam (TAN) possesses the ability to inhibit all MBL hydrolytic activity, but not IMP-type enzymes, as well as NDM-9 and VIM-83. Here, we assessed the relative inhibitory activity of xeruborbactam (XER), the latest BLI with inhibitory activity against MBL, in comparison with TAN, against a wide range of acquired MBLs.
Methods. Genes encoding a series of subclass B1 MBLs (11 NDM variants, 8 VIM variants, 10 IMP variants, together with DIM-1, GIM-1, SIM-1, SPM-1), B2 (PFM-1) or B3 (AIM-1) were amplified by PCR and corresponding amplicons cloned into plasmid pUCp24, and transformed in E. coli and P. aeruginosa. Susceptibility testing was performed for meropenem (MER), cefepime (FEP), ceftazidime (CAZ) and their combinations with XER and TAN at a fixed concentration of 4 mg/L. Fifty percent inhibitory concentrations (IC50) of XER and TAN were also determined for all MBLs.
Results. The combination of XER or TAN with ß-lactams led to significantly decreased MIC values for B1-type MBL-producing E. coli, including most recombinant strains producing NDM, VIM, IMP, GIM-1 and DIM-1 enzymes. While TAN-based combinations significantly reduced MIC values of ß-lactams for MBL-producing P. aeruginosa recombinant strains, those with XER were much less effective, related to the MexAB-OprM efflux pump significantly impacting MIC values when testing XER-based combinations in P. aeruginosa. IC50 values were similar for XER and TAN against NDM and VIM enzymes, but XER was effective against NDM-9, NDM-30, VIM-83, and most of IMP enzymes, those latter enzymes being resistant to TAN. However, no significant inhibition was observed with XER against IMP-10, SIM-1, SPM-1, SIM-1 as well as the subclass B2 and B3 enzymes, PFM-1 and AIM-1.
Conclusion. This research revealed that XER possessed a remarkable broad activity against a large panel of MBL enzymes, including IMP-like ß-lactamases (except for IMP-10), and specific MBL variants previously identified to be resistant to TAN. However, and by contrast to TAN, the activity of XER against the hydrolytic impact of MBLs appeared to be very moderate when testing P. aeruginosa recombinant strains, due to the influence of the MexAB-OprM multidrug efflux pump system in this species.

P075

Chlamydia vaughanii: the biology and pathogenic role of a newly discovered intracellular bacteria

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Background and aims:
Bacteria belonging to the Chlamydiales order are strict intracellular, Gram-negative organisms which are characterized by a two-stage developmental cycle.
Several new species of Chlamydia-related bacteria such as Piscichlamydia salmonis or Clavochlamydia salmonicida were recently discovered in fish farms. These fish pathogens can cause epitheliocystis, a respiratory system disease which drives important economic loss.
Recently, our group has isolated a new species belonging to the Chlamydia genus, Chlamydia vaughanii, from a tropical fish. This discovery came about due to the major pathogenic role of this bacterium, where a higher than 95% mortality was observed among fish in a tropical aquarium.

Methods
Using a specific, real-time PCR which targets the mutS gene, we investigated the permissivity of different cell lines to C. vaughanii.

Results
Bacteria were able to grow inside McCoy and Vero cells, and like other species of the Chlamydia genus, could not infect amoebae nor insect cells. Surprisingly, it could not multiply in a fish cell line (EPC175) at 25°C. However, growth could be detected in the same cell line at 30°C.
We will now test several other fish cell lines and amoebal species to define their permissivity towards this novel Chlamydia. In addition, to understand the zoonotic potential of C. vaughanii, infection potential in human macrophages, pneumocytes and endometrial cells will be assessed.

Conclusion
C. vaughanii is the first Chlamydia isolated from fish and cultivated in mammalian cells. By clarifying its permissivity towards animal and human cell lines, a better understanding of the pathogenesis and zoonotic potential of this novel species is developed.

P076
Characterization of Waddlia chondrophila T3SS effectors

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Background and aim.
Waddlia chondrophila is an emerging pathogen which causes adverse pregnancy outcomes in humans and abortion in ruminants. It is a strict intracellular bacterium that alternates between infectious (EBs) and replicative (RBs) forms, that can turn into a persistent stage (ABs) when the bacteria encounter stressful conditions.
To escape host defense mechanisms and establish a replicative niche, W. chondrophila closely interacts with its host cell through the direct secretion of T3SS effectors in the host cytoplasm.
The aim of this work is to better understand the transition between the three developmental forms by characterizing stage specific T3SS effectors.
Methods
Based on RNAseq data, we selected three proteins, Wcw_0260, Wcw_0429 and Wcw_1046, whose genes are differentially expressed between the developmental forms, and which are predicted to be T3SS effectors.
We analyzed their gene expression during the bacterial life cycle by RT-qPCR and by using heterologous systems, their T3SS-dependent secretion and intracellular localization were assessed.
Results
The three genes were mainly transcribed in the early stage of the bacterial life cycle. Moreover, Wcw_0260 and Wcw_1046 were shown to be secreted by the T3SS in Yersinia enterocolitica.
Finally, in transfected cells, Wcw_0260 and Wcw_1046 localized at the plasma membrane, while Wcw_0429 associated with uncharacterized vesicles in the cytoplasm.
Conclusion
Our results suggest that Wcw_0260 and Wcw_1046 could decorate the nascent inclusion and play a role in the early events leading to the establishment of an efficient replication niche. Wcw_0429 could be implicated in the subversion of host cell vesicles to the benefit of the rapidly growing inclusion. The exact role of these T3SS effectors would undoubtfully benefit from further investigation.

P077

Impact of the behavioral changes induced by the SARS-COV-2 pandemic on Microbes Prevalence (the IOMP study): 11-year view on respiratory infections.

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Aims: Due to the significant healthcare burden of acute COVID-19, numerous behavioral interventions were implemented at the pandemic's onset to mitigate infection spread. These interventions likely impacted also other respiratory pathogens. Recognizing shifts in endemicity is vital for infection control measures, vaccine requirements, and clinical management. This study aimed to evaluate respiratory pathogen changes in response to behavioral changes triggered by the SARS-CoV-2 pandemic, enhancing understanding of associated epidemiology and informing clinical guidelines.
Methods: We analyzed PCR results for respiratory pathogens achieved at the Institute of Microbiology, CHUV, Lausanne, between January 1, 2013, and December 31, 2023. Pathogens assessed included SARS-CoV-2, Influenza (A and B), RSV, Picornavirus, Adenovirus, Coronaviruses (E229, OC43, HKU1, NL63), Parainfluenza (1-4), HMPV, Chlamydia pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis and B. parapertussis, Legionella pneumophila, Coxiella burnetii, Chlamydia psittaci, Measles, Mycobacterium tuberculosis, and Pneumocystis jirovecii. We compared the proportions of positive results among three periods: January 1, 2013–March 31, 2020; April 1, 2020–June 30, 2022; and July 1, 2022–December 31, 2023.
Results: A statistically significant reduction in viral ($p < 0.001$) and bacterial ($p < 0.001$) infections occurred from period 1 to period 2. No significant increase was observed between period 2 and period 3 for viruses ($p = 0.8$) or bacteria ($p = 0.01$). However, a significant increase in bacterial infection among children was noted between period 2 and 3 ($p = 0.002$). Most pathogens exhibited a decrease in cases between period 2 and 3, including Influenza (A+B), Picornavirus, Mycoplasma pneumoniae, and Bordetella pertussis showing significant reductions (all $p < 0.001$). Notably, Mycoplasma pneumoniae showed a significant rate increase between period 2 and 3 ($p = 0.005$).
Conclusion: Behavioral changes likely played a pivotal role in reducing community circulation of non-SARS-CoV-2 respiratory pathogens. Reductions in infection rates during these changes were confirmed, as well as increases following the relaxation of restrictions. However, only a few pathogens exhibited significant reductions during the COVID-19 peak. The notable rebound in Mycoplasma pneumoniae cases post-peak underscores the need to adapt therapeutic options for patients with acute respiratory symptoms suggestive of pneumonia.

P078

Recombinant depolymerases broaden the host range of phages against Klebsiella pneumoniae

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Background
Phage therapy has been gaining interest as a promising alternative therapeutic option against Klebsiella pneumoniae. K. pneumoniae capsule plays key roles in bacterial virulence but also form a protective barrier against phages. Some phages are equipped with specific enzymes, named depolymerases, that help them to overcome the capsule barrier by degrading capsular polysaccharides. A major hurdle in the development of phage therapy is the large capsule diversity of K. pneumoniae that strongly restrict phage host range. We aimed to evaluate whether recombinant phage depolymerases can enlarge phage host range.

Methods
Depolymerases were identified in phage genomes, cloned, expressed in Escherichia coli Tuner(λDE3), and purified on nickel-affinity column. The anti-capsule activity of the recombinant proteins was confirmed by spot assay on double layered agar, Percoll density gradient and fluorescence exclusion microscopy. Synergy with phages from the Geneva Phage (GENPH) Collection was assessed by spot host range and confirmed in one-step infection growth curves. Structures of the depolymerases were predicted with alphafold2. Monomer were separated from homotrimers by size-exclusion chromatography.

Results
Capsule-specific phages presented a characteristic depolymerization halo surrounding its lysis plaques, signing for the presence of a depolymerase able to degrade the pathogen's thick polysaccharide capsule. We successfully identified and purified depolymerases against capsule types K1, K2, KL51, KL107. The recombinant depolymerases showed highly effective and KL-specific capsule removal activity. Some depolymerases required trimerization for proper activity adding constraint to the purification process. When combined with depolymerases phage host range was enlarged.

Conclusion
Recombinant depolymerases targeting clinically relevant capsule types are an attractive tool to improve anti-Klebsiella phage activity and broaden their host range.

P079

Fritschea bemisiae, endosymbiont of Bemisia tabaci: a potential chlamydial benefactor?

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Bemisia tabaci, of the Aleyrodiadae family, is a sap-feeding insect found worldwide that feeds on various types of plants such as tobacco, cotton, potato or tomato plants. They thus represent an agricultural menace causing tremendous amount of damage to crops through virus transmission during feeding. Plant sap represents a poor nutritional diet that is deficient in vitamins and amino acids among other nutrients. To compensate this, sap-feeding insects have developed symbiotic relationships with intracellular bacteria that will help synthetize missing nutrients and provide sap-feeding insects with a constant balanced diet. In the case of Bemisia tabaci, many different bacterial species can co-habit in the bacteriocytes, including an obligate one: Portiera aleyrodidarum.

Through metagenomic analysis of public sequencing data of Bemisia tabaci, we identified and extracted the full genome of Fritschea bemisiae, a Chlamydiae member of a Simkaniaceae family which was identified and described in 2005 but solely on a 16.6kb fragment that held the 16S and rRNA operon sequences. Chlamydial species have always been described as detrimental to their hosts. Genome analysis of Fritschea bemisiae, as compared to its phylum relatives shows horizontal gene transfers might have happened in a way that benefits its whitefly host. Notably, we found the presence of genes responsible for the synthesis of B6 vitamin. Not only these genes are absent in other members of the Simkaniaceae family, but it has been also shown that B6 vitamin synthetized by endosymbionts enhances fecundity and proline homeostasis in tsetse flies.

Previous research also suggested that bacterial communities within the bacteriocytes can go through metabolic cooperation to provide Fritschea bemisiae with lysine, which is required in high levels for vitellogenin synthesis. Vitellogenin accumulates in the oocytes and is an important factor of reproduction and fitness. In the case of Fritschea bemisiae, despite a low genome size that is typical for intracellular bacteria, it encodes an almost full lysine synthesis pathway. While this feature is also shared by several other chlamydiae, it might be the context of co-habitation with other intracellular bacteria within the bacteriocytes that might turn the feature beneficial to Bemisia tabaci.

P080
Lithium bioextraction from geothermal brines

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To achieve the European objective of climate neutrality by 2050, the evolution of the energy and transport sectors is key. This will, however, lead to changes in the current demand for raw materials. Europe has created a list of critical raw materials (CRM) whose demand is set to rise significantly, far exceeding current resources (Carrara et al. 2023). Among them lithium (Li) and rare earths will soon become more important than oil and gas. Some geothermal brines contain significant quantities of these CRM, and the CRM-geothermal project has been funded to explore their extraction coupled to the exploitation of geothermal heat. Within the project, we are exploring a biological extraction method using a naturally occurring mechanism: the oxalate-carbonate pathway (Cailleau et al. 2011). In the OCP, two microbial metabolisms, fungal oxalogenesis and bacterial oxalotrophy, allow to mobilize and immobilize minerals, enabling the separation and specific precipitation of Li.
The aim of this study is to adapt this natural mechanism to an industrial biotechnological process for Li bioextraction. Besides Li geothermal brines contain many ion species in higher concentrations, so an initial purification step is necessary. For this, the properties of oxalate produced by fungi can be used to precipitate most of these impurities, without precipitating Li. In a second step, oxalate can be converted to carbonate by oxalotrophic bacteria, and this carbonate combined to a pH increase can be used to precipitate Li-carbonates.
The key to an economically viable bioextraction process is to optimize the production and consumption of oxalate by microorganisms. First, to select the best oxalic acid producers and production conditions, we compared two species of fungi as a function of carbon sources and pH. The production criteria considered were production rate, product/substrate ratio, and survival. Likewise, we assessed optimal oxalotrophic activity as a function of starting pH, salinity, and Li concentrations. To date, the best strains are the filamentous fungus Aspergillus niger, the yeast Meyerozyma sp. and the oxalotrophic bacteria Pandoraea sp. To set production objectives, oxalate and carbonate precipitation tests are conducted on real geothermal brines. The quantities required for a complete precipitation are expected to be 3.57 gC2O4/gimpurities and 4.29 gCO3/gLi. It should be noted, however, that these ratios will depend on the composition of the brine.

P081

Survey of carbapenemase-producing Enterobacteriaceae in a private laboratory in Switzerland

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AIMS
In Switzerland, the spread of Carbapenemase-Producing Enterobacteriacae (CPE) has become a growing concern, not only in hospitals but also in the community. It is important to detect all CPE in a routine laboratory, including those with a weak carbapenemase activity. Our objective is to present our results of detection of CPE since 2015.
METHODS
CPE were detected in routine using the disk diffusion method. Detection of carriage was performed using the ChromID CARBA SMART medium (bioMerieux). Ertapenem-resistant Enterobacteriaceae strains detected by disk diffusion (D < 25mm) were subjected to 2nd intention methods to confirm carbapenemase production. Confirmation was provided by either immunochromatography (Coris Resist-5 O.O.K.N.V), molecular diagnosis (Xpert-Carba-R), biochemical test (Rapidec Carba NP) and combined disk-tests of meropenem with inhibitors. Definitive CPE typisation was performed by the NARA.
RESULTS
From 2015 to April 2024, a total of 74 isolates of CPE were retrieved from 68 patients. 21 CPE were isolated from anorectal or stool specimens and 53 from clinical specimens. Overall, Escherichia coli (55.3%) and Klebsiella sp. (32.9%) were the most represented CPE.
Clinical CPE were mostly detected in urine (90.6%). Two CPE were isolated from blood cultures: the first one was detected in 2022 and the second in 2023, in two patients without recent history of travel. We noted an increasing trend of CPE over time, between 2015 and 2023, from 3 strains/year to 20 strains/year. In 2024, 12 CPE were already isolated between January and April 2024, which suggests an accelerating dissemination of CPE. Different types of carbapenemases were identified, with a majority of OXA-48-like carbapenemases.
Specifically, OXA-244 (OXA-48-like) expressing carbapenemase was first detected in 2020 and it represented 40% of CPE isolates in 2023. All OXA-244 CPE were E. coli. One of E.coli producing OXA-244 was retrieved in one out of two CPE positive blood cultures and was susceptible in vitro to all carbapenems. Two interhospital patients transfers from abroad displayed isolates producing 2 different carbapenemases (NDM-1 and either OXA-48 or OXA-181).

CONCLUSION
Our 2015-2024 monitoring displays an increasing incidence of CPE, and in particular of OXA-244, a difficult to detect enzyme. The rise of the proportion of community-acquired strains suggests ongoing dissemination in the population and therefore advocates for close monitoring.

P082
Refined algorithm for detection of OXA-48-like carbapenemase producing Enterobacterales

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Background
OXA-48-like carbapenemases are challenging to detect due to their potential susceptibility to third generation cephalosporins (3GC) and carbapenems. Based on analysis of a molecular screening of our positive blood cultures, we refined the carbapenemase screening algorithm for our routine microbiology laboratory.

Methods
Between March 2021 and October 2023, primary positive blood cultures were analysed with the BioFire® Blood Culture Identification 2 (BCID2) panel.
During September and October 2023, piperacillin/tazobactam (PT) resistant Enterobacterales were screened using disc E of the MASTDISCS® Combi Carba plus, consisting of temocillin + metallo-β-lactamase inhibitor (TEM/MβLI). Suspect colonies were confirmed with eazyplex® SuperBug CRE and whole genome sequencing (WGS).

Results
In 1055 (31.3%) of 3366 positive blood cultures, Enterobacterales were detected by BCID2. Five (0.5%) of them had a positive result for OXA-48-like genes. Of those, one Klebsiella pneumoniae isolate was also NDM and CTX-M positive and showed resistance to all tested carbapenems and cephalosporins. Three Escherichia coli isolates were additionally CTX-M positive and resistant to 3GC with variable susceptibility to carbapenems. One E. coli isolate had no other β-lactam resistance markers and was fully susceptible to all measured carbapenems and 3GCs. Notably, all five isolates were resistant to PT. This prompted us to validate a refined screening algorithm for carbapenemases to detect additional OXA-48-like-producers.

Of 112 PT-resistant Enterobacterales, four (3.4%) were TEM/MβLI screening positive, including one OXA-48-like E. coli susceptible to meropenem, imipenem and ertapenem. WGS revealed this strain to harbour blaOXA-244 as well as blaCTX-M-15. All TEM/MβLI screening positive strains were also resistant to 3GCs.

Conclusions
Introducing TEM/MβLI screening for PT and 3GC-resistant Enterobacterales enabled identification of OXA-48-like carbapenemase producers fully susceptible to carbapenems. Yet, this method might miss 3GC-susceptible strains. Routine BCID2 panel testing is a powerful tool to monitor the prevalence of these strains as well as to ensure their detection in severe infections.

P083
Characterization of the Host Cell Entry Mechanism of Marburg Virus

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Marburg virus (MARV) is a non-segmented, negative polarity, single-stranded RNA virus belonging to the Filoviridae family and the Marburgvirus genus, causing outbreaks with a high fatality rate. All outbreaks are from animal origins. MARV infection causes severe haemorrhagic fever, leading to organ dysfunction and death. The modes of human-to-human transmission of MARV are similar to those of the better characterised Ebolaviruses, such as Ebola virus (EBOV) and Sudan virus (SUDV). However, liver failure induced by MARV is more severe than that of EBOV, and studies on fatal human cases and non-human primate models have revealed that the liver is a crucial replication site for MARV. To date, no approved vaccine or therapy exists to treat Marburg virus disease, despite the urgent need of an appropriate therapy. Various attachment factors, as well as cellular receptors, have been suggested as potential mediators of filovirus entry. Potential routes of entry into cells include clathrin-mediated endocytosis, macropinocytosis and glycoprotein-facilitated receptor binding. These mechanisms are not mutually exclusive, and our study aims to characterise their contribution to viral entry into the host cell.

We generated vesicular stomatitis-based pseudoviruses for Ebola Zaire virus (EBOV), Sudan Ebola virus (SUDV) and Marburg virus as validated biosafety level 2 (BSL2) surrogates to study the mechanisms of entry into the host cell. To identify the cellular factors involved in viral entry into established cell lines, we used well-characterized inhibitors, most of them coming from pharmaceutical repositioning.

We measured neutralizing antibody titers for KZ52, 16F6 and MR78 and found that pseudovirus-based neutralization assays were highly sensitive and specific for EBOV, SUDV and MARV, respectively. Using African green monkey-derived Vero cells (Vero E6), susceptible to a range of different viruses including filoviruses, as a reference, we showed that MARV exhibits a strong tropism for human hepatocellular carcinoma, compared to EBOV and SUDV. We further characterised receptor usage and host cell entry mechanisms for EBOV, SUDV and MARV, demonstrating a predominant role for macropinocytosis.

Our studies provide a better understanding of this fundamental step in viral entry, and the cellular factors will be evaluated as potential targets for therapeutic intervention, opening up new avenues for the development of effective antiviral drugs.

P084

Rapid IPR Pseudomonas NP test for rapid detection of imipenem/relebactam susceptibility/resistance in Pseudomonas aeruginosa

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Aims: Carbapenem-resistant Pseudomonas aeruginosa (CRPA) are one of the important multidrug resistant bacteria worldwide. Imipenem-relebactam (IPR) has been recently introduced for the treatment of severe urinary tract infections, pyelonephritis, and complicated intra-abdominal infections in adults. IPR has been reported to show a good activity against non-metallo-ß-lactamase CRPAThe Rapid IPR Pseudomonas NP test was therefore developed for the identification of IPR suscepitbility/resistance for multidrug-resistant Pseudomonas aeruginosa.
Methods: The principle of the Rapid IPR Pseudomonas NP test is based on the visual detection of glucose metabolization by a yellow to red or orange color change of red phenol, a pH indicator, caused by bacterial growth in the presence of imipenem at 2 mg/L and relebactam at 4 mg/L (after testing several concentrations) using cation-adjusted Mueller-Hinton broth. A total of 80 clinical Pseudomonas spp. isolates from various clinical sources and of worldwide origin were analyzed, among which 42 isolates were IPR resistant according to EUCAST guidelines (MICs, susceptible ≤ 2 mg/L, resistant > 2 mg/L). The results obtained with the Rapid IPR NP test have been compared with the reference broth microdilution (BMD), considered as reference. All experiments were repeated in triplicate in separate experiments.
Results: The sensitivity, specificity and accuracy of the test were found to be 100%, 86.9% and 93.8%, respectively using the BMD reference method as a comparator. Moreover, 5 out of the IPR susceptible isolates (n=38) with an MIC close to the breakpoint (MIC = 1 mg/L, 2 isolates; MIC=2 mg/L, 3 isolates) displayed a major error, giving a positive result with the rapid IPR NP test. Interestingly, the IPR-resistant isolates (n=42) were all correctly categorized. Results were obtained within a 4 h incubation time at 35°C ± 2°C which is a gain of time of at least 16 h compared to currently used antimicrobial susceptibility testing including BMD.
Conclusions: The Rapid IPR Pseudomonas NP test is sensitive, specific, and easy to perform and interpret. It is therefore suitable for implementation in clinical microbiology routine laboratories. The use of such test may rapidly and accurately provide the information needed for the implementation of an adequate treatment.

P085
Analytical performance evaluation of a new homogenization system for tissue biopsies

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Fondation ADMED

Aims

Diagnostics of infections from solid tissues require particular attention on the pre-processing step of homogenization, shredding and breaking up of tissues.
A new homogenization instrument (SpinAX) in conjunction with a new sampling and processing tube (ProbeAX Evolution) has recently come on the market. The aim of this study was to compare the new system with a well-known, trusted homogenization system, the Ultra-Turrax (UT) tube drive and ProbeAX tubes.

Methods

Two types of biopsies were compared: soft tissue (fibrous and connective tissue) and hard tissue (tendon, cartilage, bone). Patient biopsies were split into two parts to be processed with either SpinAX or UT system. Gram stained cell counting and semi-quantitative 4-quadrant measurement of bacterial growth on four types of media plates (chocolate-, chromogenic-, blood- and Schaedler agar) and on Thioglycolate (THIO) enrichment broth were analyzed. Growth was ranked from 0 (no growth) to 4 (growth in the 4th quadrant). Growth was defined as superior/inferior if growth rank of the two systems differed by > 1 or when there was no growth with one method and growth with the other.

Results

A total of 96 clinical samples biopsies, 31 hard and 65 soft tissue, from 58 patients were analyzed.
The Gram staining cell count was similar for 73 % (154/210) of the observed cell types (Gram-positive cocci (GPC), Gram-negative bacilli (GNB), erythrocytes, leucocytes, epithelial cells) in all specimen. The remaining observations were possible with SpinAX only in 20 % (42/210) or UT only in 7 % (14/210). Microorganisms or host cells were not detected in 10 samples following UT homogenization, with GPC not observed in five samples and GNB in two samples. Bacterial growth was detected in 42 / 96 samples (43.8 %) from 26 patients (44.8 %). Overall 94 bacterial and yeast species were recovered. Among those, 84 (89.4 %) exhibited a similar growth with the two methods, 4 (4.3 %) a better growth with the SpinAX and 6 (6.4 %) with the UT system. Ten (10.6 %) and 11 (11.7%) clinical isolates were not recovered with the SpinAX and the UT, respectively. Among those, clinical isolates of Staphylococcus aureus from three samples were recovered with the SpinAX but not with the UT system.

Conclusion
The SpinAX homogenization is thus providing an overall similar performance than UT with a possible better homogenization and/or ultra-structure preservation for Gram staining microscopy.

P086

Bacterial genomic epidemiology in the clinical setting: Performance evaluation of the latest transposase-based Oxford Nanopore Technologies workflows for typing and AMR prediction

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- Aims -
Rapid identification of pathogens and the reconstruction of potential transmission chains are key factors in efficient outbreak control. In clinical settings this typically involves Illumina short-read sequencing followed by typing approaches and prediction of antimicrobial resistance factors. Long read sequencing technologies, especially Oxford Nanopore Technologies (ONT), have gained considerable attention, due to their fast and cost-efficient sequencing abilities. However, it is unclear whether the latter also provide the accuracy needed for performing accurate clinical genomic epidemiology.
Here we examined whether the latest generation of ONT kits and software provide sufficient accuracy as compared to the Illumina gold standard. We evaluated genotyping (MLST, cgMLST) accuracy, antimicrobial resistance prediction, sequencing costs per isolate and turnaround time.
- Methods -
We used 21 Corynebacterium diphtheriae (CDIP; Kofler et al. Eurosurveillance 2022:27:44), and 21 vancomycin resistant Enterococcus (VRE; unpublished) strains, which were previously Illumina sequenced and genomically and phenotypically characterized. These strains were sequenced with ONT Nanopore V14 transposase-based library preparation kits (RBK and the new RPB). The resulting data was basecalled with different software and model versions, resulting in the comparison of 366 genomic assemblies in total.
- Results -
A perfect to near-perfect replication of Illumina results was observed for MLST in both tested species, and for cgMLST in CDIP, for all ONT kits and software used. For VRE, cgMLST result accuracy varied between different strains, library preparation kits, and analysis parameters, likely due to the presence of difficult-to-resolve base methylations. The latest software versions (MinKNOW, Dorado) combined with the PCR based kit (RPB) yet led to consistent replication of the Illumina results for all tested VRE strains. In all cases, AMR predictions matched perfectly between Illumina and the ONT workflows. The latest ONT kits were more competitive in terms of cost, and turnaround time than their Illumina counterparts.
- Conclusion -
Single base accuracy obtained with the latest ONT products is sufficient to perform accurate genomic epidemiology of the tested clinical bacterial species. Our results also show that previously published results based on older ONT kits and software versions may require close attention.

P087

In-vitro activity of dimercapto-succinicid acid in combination with carbapenems against carbapenem-resistant clinical isolates strains and isogenic strains carbapenemases producing Pseudomonas aeruginosa

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Aims: Metallo-beta-lactamase-producing Pseudomonas aeruginosa (MBL-PA) have emerged as an urgent threat in the healthcare setting. Meso-dimercaptosuccinic acid (DMSA) is a heavy metal chelator used for over 30 years for the treatment of heavy metals intoxication. Since MBLs harbour zinc ions, DMSA was hypothesised to show activity against MBL-PA. We performed an in-vitro analysis of the effect of DMSA in combination with carbapenems against carbapenemase-producing P. aeruginosa.
Methods: A total of fifty-nine clinical P. aeruginosa strains from the Swiss National reference Center and of worldwide origin were analysed, including MBL producers (n=42), isolates producing Ambler class A enzymes (n=2), natural AmpC overproducers (n=1), isolates with decreased permeability (n=7), and wild-type isolates (n=7). Recombinant P. aeruginosa PA14 and PA14 ΔoprD strains harbouring genes (GES-5, KPC-2, IMP-1, NDM-1, VIM-2, AIM-1 and SPM-1) cloned in the pUCP24 shuttle vector were tested under the same conditions. Minimal inhibitory concentrations (MICs) of imipenem (IPM) and meropenem (MEM) alone, and in combination with DMSA 3 mM, were determined by broth microdilution. A time-kill assay was performed against recombinant strain PA14/pUCP24-VIM-2, with MEM at 4 mg/Land 8 mg/L, alone or in combination with 3 mM DMSA.
Results: For the recombinant strains, MIC results showed that the addition of 3 mM DMSA to both IPM and MEM resulted in significant fold decreases of 4-32 against MBL-producers. However, this effect was largely negated in PA14ΔoprD against IPM/DMSA. A significant growth inhibition was observed using MEM/DMSA at both half (4 mg/L) of and the MIC (8 mg/L) value when compared to MEM alone, indicating the inhibitory efficacy of DMSA. Within the clinical MBL-PA (n=42), the addition of 3 mM DMSA resulted in significant decreases in MICs to IPM and MEM in 24/42 (57%) and 27/42 (64%) of isolates. Most isolates that did not exhibit significant MIC decreases were OprD deficient (17/18 for IPM and 13/15 for MEM).
Conclusion: DMSA addition to MEM lead to a significant decrease in MIC, regardless of the OprD status . This study shows the potential of DMSA)-like molecules to be developed for use in treating infections caused by MBL-producing bacteria.

P088

MultiRapid ATB NP test for detecting concomitantly susceptibility and resistance to last-resort novel antibiotics available to treat multidrug-resistant Enterobacterales infections

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With the urgent need to develop new antibiotics for treating carbapenem-resistant Enterobacterales (CRE) infections, a significant concern for global public health, the pharmaceutical industries have introduced novel antibiotics in recent offering promising therapeutical options. Among these are the β-lactam-β-lactamase inhibitor combinations ceftazidime-avibactam (CZA), meropenem-vaborbactam (MEV), and imipenem-relebatam (IPR), as well as the siderophore cephalosporin cefiderocol (FDC). However, resistance to these novel antibiotics has already been extensively reported. This study aimed to develop a rapid test, called the MultiRapid ATB NP test, for the rapid identification of susceptibility/resistance to CZA, MEV, IPR, and FDC in Enterobacterales in a single test aiding rapid clinical decision-making. The MultiRapid ATB NP test detects glucose metabolization occurring after bacterial growth in presence of defined concentrations of CZA, MEV, IPR, and FDC, followed by visual detection of color change of the pH indicator red phenol (red to yellow) generated by the acidification of the medium upon bacterial growth. The test is performed in 96-well microplates. The Multi Rapid ATB NP test was evaluated with 78 Enterobacterales isolates, and its performance was compared to the reference method, broth microdilution. The MultiRapid ATB NP test showed 97.0% sensitivity (97.0%, CI 92.6 - 98.8%), 97.7% specificity (97.7%, CI 94.3 – 99.1%), and 97.4% accuracy (97.4%, CI 95.0% - 98.7%). Results were obtained after 3 hours of incubation at 35°C ± 2°C, representing at least a 15 hours time-saving compared with currently used antimicrobial susceptibility testing methods. The MultiRapid ATB NP test accurately detected susceptibility/resistance to CZA, MEV, IPR, and FDC in Enterobacterales and may be suitable for implementation in any microbiology routine laboratory.

P089

Immune responses elicited by Janus-faced Mycoplasma mycoides - glycans make the difference.

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Mycoplasmas are minute cell wall less bacteria that encompass a number of livestock pathogens. Mycoplasma vaccines, if available at all, have low efficacy and often induce only short-term protection. The development of rationale vaccines would benefit from a better understanding of the interplay between livestock mycoplasmas and host cells. Mycoplasma mycoides subsp. capri GM12 (GM12) is a highly virulent strain, causing severe septicemia in goats. This strain is amendable to novel genome editing techniques, making it a good model to study host-pathogen interactions. According to 3R principles, we developed a blood-based ex vivo platform to compare the response of most immune cell subsets towards the highly virulent wild-type (GM12), a control strain carrying an antibiotic marker (GM12::YCpMmyc1.1) and several mutants, including a CPS-deficient strain (GM12::YCpMmyc1.1-Δglf) and the fully attenuated mutant GM12::YCpMmyc1.1-Δ68. For this purpose, ruminant peripheral blood mononuclear cells (PBMCs) were stimulated with GM12 and its different isogenic mutant strains. GM12, GM12::YCpMmyc1.1 and GM12::YCpMmyc1.1-Δ68 had only moderate effects on PBMC apoptosis and surface marker expression, while stimulation with the mutant strain GM12::YCpMmyc1.1-Δglf, which exposes surface proteins including lipoproteins due to the lack of CPS, led to high apoptosis and strongly suppressed MHC expression on antigen-presenting cells, suggesting immunosuppressive effect. Moreover, exposure to GM12::YCpMmyc1.1-Δglf produced a variety of pro-inflammatory cytokines/chemokines that could promote a robust T-cell mediated and inflammatory response.
Since macrophages stand as one of the primary immune defense of the host against mycoplasma infection, we generated monocyte-derived macrophages and investigated their interactions with GM12. Interestingly, GM12 showed the capacity to replicate and grow inside the macrophage.
In conclusion, we showed that a CPS-deficient strain induces cell apoptosis, pro-inflammatory cytokine induction and immunosuppression, while the presence of CPS does not. We provided evidence that GM12 can survive and persist within macrophages in vitro.

P090

Comparison of the qualitative and quantitative results of the BioFire FilmArray RP2.1 and in-house TaqMan Realtime PCR for the Rapid Detection of 22 Respiratory Pathogens

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Aim:
The SARS-CoV-2 pandemics established the importance of reporting viral loads when a respiratory pathogen is detected [1]. The BioFire FilmArray Respiratory panel RP2.1, a point-of-care syndromic panels based on multiplexed nested PCR for the qualitative analysis of 22 respiratory organisms, now offers a quantitative analysis. This study aims at evaluating the clinical performance of the BioFire RP2.1 in detecting respiratory pathogens and determining the viral loads from the cycle threshold (Ct) values.

Methods:
A retrospective selection of 70 patient samples and external quality control was conducted. A total of 100 samples were collected and stored at -20°C. Each sample was tested on the BioFire RP2.1, the Xpert, Xpress Cov-2/Flu/RSV plus [2] (SARS-CoV-2, Influenza and Respiratory Syncytial Virus) and the in-house TaqMan PCR assay [3]. The agreement between the assays was calculated with qualitative (kappa coefficient) and quantitative (Deming regression) analyses.

Results:
92/100 samples had a qualitative result concordant between the different methodologies. 6 samples (2x Bordetella pertussis, Coronavirus NL63, Influenza A and 2x Influenza B) were not detected by BioFire RP 2.1 and positives on the reference methods (in-house TaqMan or Xpert, Ct > 37). 2 samples were negatives by the in-house TaqMan PCR assay but detected by BioFire RP 2.1. The BioFire RP2.1 and the reference methods (Xpert and TaqMan PCR assay) demonstrated a positive percent agreement of 92% and a kappa coefficient of 0.9. Positives samples with Ct values within the linear range of the different techniques were highly correlated across the detection range with correlation coefficients $R^2 > 0.85$.

Conclusions:
This study demonstrated that the BioFire RP2.1 accurately detects a range of respiratory pathogens with a high correlation and agreement across the detection range. The number of discrepant results is low and is related to samples with high Ct values. Both platforms are effective in detecting the different respiratory pathogens qualitatively. This study suggests also that in the future, an algorithm used to quantify the viral loads (copies per milliliter) of the in-house TaqMan PCR assay could be applied on the BioFire RP2.1 to support patient management, assess contagiousness and disease progression.

References:
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P091

Endolysin PlyV12 and antibiotic Tunicamycin: A synergistic combination against pathogenic enterococci

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Antibiotic resistant bacteria are on the rise and pose a great threat for humankind. Future infection of Gram-positive bacteria such as Enterococcus faecalis may become increasingly difficult to cure due to limited treatment options. As few new classes of antibiotics have come onto the market in recent years, alternatives are strongly needed.

One promising approach involves the utilization of endolysins. Endolysins are bacteriophage-derived peptidoglycan hydrolases capable of targeting and cleaving specific bonds in the conserved peptidoglycan. Specific cleavage leads to disruption of the bacterial cell wall, resulting in osmolysis and bacterial cell death. The peptidoglycan of Gram-positive bacteria is not protected by an outer membrane rendering it susceptible to endolysins applied exogenously.

The enterococcal endolysin PlyV12 has shown high killing efficacy making it a promising drug candidate. It consists of an enzymatically active domain (EAD) with amidase activity and a cell wall binding domain (CBD). The EAD of PlyV12 cleaves the bond between the sugar backbone and peptide moiety in the peptidoglycan. Wall teichoic acids (WTAs) extending out of the peptidoglycan can shield the bacterium and sterically hinder the action of endolysins. To overcome this obstacle, we explored the synergistic potential of PlyV12 combined with the antibiotic tunicamycin. Tunicamycin inhibits TagO, a pivotal enzyme involved in WTA biosynthesis. The lack of WTA enhances the enzymatic activity of PlyV12. However, a general synergy between tunicamycin and endolysins was not detected, indicating specificity in the interaction. This synergy appears to be linked to the amidase cutting site of PlyV12, as other endolysins with endopeptidase activity did not benefit from the absence of WTAs.

These results underscore the promise of integrating endolysins with conventional antibiotics as a viable strategy to combat antibiotic-resistant bacteria effectively. Further research in this area holds potential for the development of innovative and sustainable approaches to address the escalating threat of antimicrobial resistance.

P092

Assessing Lectin Inhibitors in Pseudomonas aeruginosa Infections: A Tissue Model Approach

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Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen which is responsible for 10% of all nosocomial infections in patients with impaired immunity. LecA and LecB are two virulence factors of Pseudomonas aeruginosa contributing to host cell infection. In vitro, LecA interacts with a specific glycosphingolipid, Gb3, resulting in complete membrane engulfment of the bacterium. Meanwhile, LecB binds strongly to glycosylated moieties of β1-integrins and other integrins on the basolateral side of epithelial cell membranes, triggering rapid endocytosis of these integrins and impairing wound healing processes.
We want to assess the effectiveness of lectin inhibitors in Pseudomonas aeruginosa PAO1 infections. To do so, we first investigate tissue infection with PAO1 lecA, lecB and lecAB double mutants using time-lapse confocal microscopy. Our model comprises expanded bronchial epithelial progenitor cells cultivated on a PET membrane within Transwell cell culture inserts to establish the lower airway tissue air-liquid interface (ALI) model. By comparing PAO1 mutants with the wildtype PAO1 alongside their respective lectin inhibitors, we aim to determine the inhibitors' effectiveness. In addition, we plan on exploring the role of LecA in the internalization of P. aeruginosa in goblet cells and its consequence on drug susceptibility.
The success of this endeavor could mark a significant step in validating drug screening methods using ALI transwell lung models and exploring new paths in alternative drug research.

P093
Isolating and evaluating thermophilic bacteria for PHA production

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In a world where plastics are omnipresent, biopolymers are one renewable, bio-based, and biodegradable solution. In particular, the polyester poly(3-hydroxyalkanoate) (PHA) materials present thermoplastic to elastomeric properties depending on their carbon chain length [1]. PHAs are biosynthesized as intracellular granules in microorganisms such as bacteria, yeast, or archaea. However, producing these biopolymers can be costly especially for longer chain lengths [2]. One research focus is to reduce production cost for instance by mitigating the cooling power needs, operating at higher temperatures. Less energy is required to maintain the temperature at 60°C than at 30 or 37°C. The goal of this work is thus to isolate new thermophilic microorganisms that are PHAs producers from Swiss hot springs and a composting unit from a wastewater plant. Thermophilic microorganisms also present the advantage of limiting the risk for contamination during cultivation.

A specific isolation protocol was set up: samples were first harvested from Swiss hot springs and a composting unit to inoculate 2 types of petri dishes: LB-Agar and Thermus agar plates (DSMZ medium 1033). Second, to verify if there was PHA in the growing microorganisms, they were transferred on specific selection plates, that combined the same media with an addition of 0.5 g/l of dodecanoic acid and 0.5 g/l of Nile red. This stain will color specifically any intracellular lipids and show PHA inclusion bodies. Dodecanoic acid was a selecting agent as it can be toxic for some microorganisms but can also be polymerized into poly(3-hydroxydodecanoate) through the β-oxidation pathway in some bacteria [3]. Isolation was furthermore conducted under different conditions: temperature for low thermophile (50°C) or medium thermophile (60°C), as well as in aerobic and anaerobic conditions.

In presence of Nile red, a red coloration was measured on colonies with lipids inclusion bodies and fluorescence was observed for 8 colonies under blue light emission (ongoing study). Selected microorganisms were cultivated in both LB and/or Thermus agar liquid media for their growth, and their PHAs content was determined using a gas chromatography method. This also enabled to identify the class of PHA: short chain length PHA (2 to 5 carbon atoms monomers) or medium chain length PHA (6 to 14 carbon atoms monomers. The microorganisms will also be identified externally by MALDI-TOF MS or 16S RNA sequencing technology.

P094

Intestinal colonization with multidrug-resistant Enterobacterales (MDR-Ent) in expatriates returning to Switzerland

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Aims:
Colonization at the intestinal level with MDR-Ent associated with ESBL production (ESBL-Ent) in healthy people (HP) has increased worldwide. In particular, it is well-known that travelers to endemic regions are at risk of acquiring ESBL-Ent. In contrast, data regarding the epidemiology and effects on intestinal colonization in expats living long-term in endemic countries are lacking.

Methods:
Swiss HP living abroad for ≥ 3 months and aged ≥ 18 years or older were eligible for this study. Volunteers provided written consent and completed epidemiologic questionnaires. Stool samples were collected and screened for third-generation cephalosporin-resistant- (3GC-R-), carbapenem-resistant- (CR-), and colistin-resistant- (COL-R-) Ent using pre-enriched culture-based methods. Species identification (ID) of colonies was performed by MALDI-TOF-MS, while phenotypic testing by the Sensititre broth microdilution method. Selected strains were further characterized by Illumina whole-genome sequencing to confirm species ID and to determine antimicrobial resistance genes (ARGs). Risk factors were analyzed in R using the epidemiologic questionnaires completed by the volunteers.

Results:
During 2021-2023, a total of 195 Swiss expatriates living in 57 different countries on 4 continents (Europe, Africa, Asia, Americas) were enrolled in this study. An overall intestinal colonization prevalence of 42.56%, 95% CI [35.53%-49.83%] was identified. Living in countries based in Asia [adjusted (a)OR = 5.98, 95% CI (2.43-15.81), P = 0.00017] and Africa [aOR = 3.40, 95% CI (1.39-8.81), P = 0.0088)] were risk factors significantly associated with colonization with MDR-Ent. Furthermore, E. coli (Ec, n = 107 of 119 strains) was the main MDR-Ent (n = 104 of 107 Ec) displaying resistance primarily to 3GC-R (85.6%), trimethoprim-sulfamethoxazole (56.7%) and ciprofloxacin (34.6%). No CR Ec were observed, whereas 14.4% were COL-R only. Three non-MDR-Ec were only COL-R. Notably, the main resistance mechanisms against β-lactams were ESBLs (CTX-M-types; 83.2%), while mcr-1.1 (7.5%) was identified as the main mobilized mechanism against COL.

Conclusion:
Our findings suggest that HP living abroad for extended periods in endemic regions such as African and Asian countries are at increased risk of intestinal colonization with superbugs carrying dangerous ARGs. The impact of the importation of MDR-Ent in low-prevalence countries should be investigated.

P095
The Gram-stain poorly predicts the pathogens of operated diabetic foot infections

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Aim
In resource-poor setting with limited access and costs to microbiological cultures and antimicrobial agents, many experienced clinicians rely on the inexpensive Gram-stain to tailor the pre-emptive antibiotic agent for diabetic foot infections (DFI). We evaluate this possibility for Switzerland.

Methods
We retrospectively analyze the performance of the routine Gram-staining (by professional microbiologists) in different strata of (operated) adult operated DFI patients in a single center. We exclude episodes with uncertain stain results, which we might interpret as contaminations, and focus on clear microscopical visualizations of organisms in (several) intraoperative samples.

Results
Among 1,235 moderate to severe DFI cases, the four most frequently-cultured primary pathogen (groups) were coagulase-negative staphylococci (n=258), S. aureus (224), enterococci (60), and Pseudomonas spp. (52). Among the 172 episodes, for which the intraoperative Gram stain clearly yield a pathogen in (several) samples, 101 (8%) were “Gram-positive”, 32 (3%) “Gram-negative”, and 39 (3%) witnessed both, Gram-positive and Gram-negative germs. Overall, sensitivity, specificity, positive and negative predictive values of the Gram-stain regarding Gram-positive pathogens was 56%, 93%, 97%, and 38%. The corresponding values for Gram-negative germs were 61%, 97%, 50%, and 82%. When stratified for episodes under current (preoperative) antibiotic therapy, the combined values were 67%, 96%, 97%, 49%. When stratified for strict monomicrobial infections, these performances were 51%, 92%, 93%, and 48% for Gram-positive cultures, and 52%, 98%, 88%, and 90% for monobacterial Gram-negative cultures, respectively.

Conclusion
In our urban setting of a large Swiss city, the routine Gram-stain results stemming from intraoperative DFI samples revealed an insufficient sensitivity throughout all investigated strata in terms of the prediction of pathogens. The overall negative predictive values were miserable, and only acceptable for the subgroup of Gram-negative rods.

P096
An innovative human bladder microtissue model to study UPEC infections

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Urinary tract infections (UTIs) are a major cause of morbidity, hospitalization and prescription of antibiotics worldwide. The primary source of UTIs are uropathogenic Escherichia coli (UPEC) that are responsible for approximately 80% of cases. The high recurrence rates show that even the gold-standard treatments are suboptimal, and antimicrobial resistance is on the rise.
Research in the UTI field has been dominated by studies using cancer cell lines or animal models that do not fully recapitulate ultrastructure and physiology found in humans. Complex human bladder models are needed to advance our knowledge and we have therefore adapted the recently described 3D-UHU microtissue model (Jafari et al. 2023 , PMID: 37051302) to expand its potential and make it amenable to live cell imaging and to the addition of a urine flow.
Human epithelial bladder (HBLAK) cells are stratified into tissue of ~7 cell layers, embodying the three main urothelial subtypes (basal, intermediate and umbrella). The result is a Transwell® platform with the tissue in inverted orientation that successfully mimics the key features of the human bladder, with tissue differentiation (confirmed by UPIII and CK20 markers), barrier function (measured with TEER and visualized with ZO-1 staining in tight junctions) and urine tolerance.

We are currently using this model to assess UPEC infection spatial-temporal dynamics, both static and under flow conditions. Using a microfluidics device that we have developed as Transwell® platform, the study of UTI is possible at single cell resolution under human urine flow in a live cell setup. UPEC-associated physiological events of both host and bacteria are monitored in real time.

This model provides useful insights at both cell and tissue levels. Moving forward, we aim to use it to unravel the mechanics of UTI recurrence and investigate how antibiotic treatment influence clearance and relapse.

P097

Population genomic structure and antibiotic resistance pattern of APEC in South Africa

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Aims

Avian pathogenic Escherichia coli (APEC) is causing serious economic losses in the poultry industry worldwide requiring wide antibiotic usage due to the lack of efficient vaccine protection. The aim of this study was to determine the epidemiological picture of APEC in South Africa by analyzing the WGS-based phylogenesis of serological characteristics, antibiotic resistance, and virulence profiles.

Methods

A total of 109 E. coli were isolated from samples taken from airsacculitis lesions during post mortem examinations of broilers from 21 different farms in South Africa between June 2021 to January 2022. Species were identified by MALDI-TOF MS and all isolates were sequenced using Illumina technology. Molecular serotyping, sequencing typing, cgMLST, and antibiotic resistance genes detection were performed using SeqSphere+; antibiotic resistance genes and mutations were also searched using ResFinder. Pangenome analysis was performed by Roary. Phylogrouping and virulence gene identification were conducted by ClermonTyping and ABRicate. A cgMLST-based phylogenetic tree was constructed using iTOL. The MICs of 15 antibiotics were tested according to EUCAST recommendations.

Results

The 109 APEC belonged to 21 serotypes and 18 sequence types (ST) with the most predominant being O78:H4 (36.7%; ST117, ST15549), O36:H5 (9.2%; ST5764), O5:H10 (6.4%; ST93), and a currently unknown serogroup U:H5 (7.3%; ST140). The cgMLST-based phylogenetic analysis confirmed the presence of clones within the same farm as well as in different farms. Of 168 virulence genes associated with important virulence factors were identified, 35 genes were shared by all strains. All strains were susceptible to meropenem, azithromycin, tigecycline, and colistin. Resistance to ampicillin, ciprofloxacin, nalidixic acid, tetracycline, and trimethoprim were the most frequent and were found in more than 50% of the strains. Multidrug resistance to 3-9 antibiotics was found in 56.9% of the strains. Resistance phenotype was associated with the presence of acquired resistance genes and mutations.

Conclusion

This study reveals the population genomic structure with predominant serogroups, as well as a high number of different virulence genes including a substantial set of genes shared by all strains, and multidrug resistance in APEC in South Africa. The presence of the same clones in different farms suggests either a common source of contamination or establishment of successful APEC strains.

P098

Comparison of MALDI-TOF identification methods for Mycobacterium other than tuberculosis (MOTT)

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Backgrounds:
Mycobacteria other than tuberculosis (MOTT) infection are increasing worldwide affecting both immunosuppressed and immunocompetent patients posing diagnostic and treatment challenges. Mass spectrometry technology for microorganisms' identification, such as MALDI-TOF/MS, as significantly advanced laboratories practices offering simplicity and time-saving benefits in identification processes. Over years improvement of protocols and database helped in bacterial, fungal but also MOTT identification.

Aim:
The gold standard for MOTT identification involves gene amplification associated with sequencing (e.g. 16S rRNA) but it is time-consuming and labor-intensive. Our objective is to establish and implement a protocol with a high rate of successful and accurate MOTT identification directly from positive liquid cultures in mycobacterial growth indicator tubes (MGIT).
Method:
We compared the Brucker extraction procedure with an in-house procedure designed to enhance MOTT identification rate in primoculture and subcultures. Samples underwent MALDI-TOF/MS analysis, and scores obtained were compared based on group or species-level of identification.

Results:
A total of 82 samples were extracted following both methods, including 39 primocultures and 43 subcultures. The Brucker procedure identified 43 subcultures to species level (86%) or group level (26%) with a MALDI-TOF score mean of 2. However, it faced challenge in identifying primocultures with a MALDI-TOF score mean of 1.66 with 50% identification to species level and 6% to group level only, requiring an adaptation of this procedure. Our in-house protocol yielded a better rate of MALDI-TOF identification with a mean of 2.12 with 92% identification to species level and 8% group level identification. When applied to primocultures (n=10), both protocols exhibited good species level identification with the higher score achieving higher scores (mean of 2.06 vs. 1.68 for Brucker). Furthermore, analysis of MALDI-TOF/MS scores relative to different group of MOTT helped us to establish cutoff for species and group level identification.

Conclusion:
Although further refinement is needed, this analysis The in-house protocol demonstrated a higher rate of MALDI-TOF identification for MOTT directly from positive MGIT compared to Brucker's and could improve our time of results which could help in rapid growth MOTT identification.

P099

Epidemiological changes in Chlamydia pneumoniae molecular detections before, during and after the SARS-CoV-2 pandemics: results of a global survey

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Aims

During the SARS-CoV-2 pandemic, the incidence of most respiratory pathogens notably decreased, as exemplified by Mycoplasma pneumoniae, and this was followed by a clear rebound during the second half of 2023 (1,2). In Lausanne, Switzerland, there was an increase in Chlamydia pneumoniae detections between October 2023 and January 2024 (3). To assess the global epidemiology, a survey conducted by the ESGMAC (see additional information) was performed.

Method

We collected data on C. pneumoniae PCR detections from laboratories and surveillance systems globally, with a focus on Europe. The study period spanned from January 2018 to December 31, 2023, and included monthly case data. Additionally, when available, yearly case data from 2014 to 2017 were also recorded.

Results

Twenty-eight sites participated in the survey and provided data for 2023. During the study period from 2018 to 2023, seventeen sites fully responded, while ten sites contributed data for the earlier period from 2014 to 2018. Nine different European countries were represented, and one site was from outside Europe (Taiwan). Of the sites, 17.2% used in-house PCR assays (singleplex or multiplex), whereas 82.8 % utilized commercial PCR assays. In total, 964,800 tests were recorded throughout the study. Notably, monthly positivity rates spiked to 0.77% in November 2023, a significant increase compared to the pandemic and post-pandemic period from April 2020 to August 2023, which exhibited rates of 0.07% ± 0.18% (2 SD). There were important discrepancies between countries. Spain, Switzerland, and Slovenia reported the highest percentages of positivity peaking at 7.8 % (Dec 2023), 3.2 % (Nov 2023), and 3.1 % (Sep 2023), respectively. In the other sites, positivity rates stayed below 1% since April 2020. Interestingly, pre-pandemic (2014 to 2019) C. pneumoniae detection rates ranged from 0.77 % to 2.08%.

Conclusion

Among ten countries, a resurgence of C. pneumoniae detection has been noted in Switzerland, Spain, and Slovenia, with Spain experiencing the highest rates. This increase could be attributed to diminished immunity from reduced exposure during the 2020-2021 lockdowns and other anti-COVID-19 measures. In contrast, the other seven countries have maintained lower detection levels than those seen pre-pandemic. This study underscores the need for clinicians and clinical microbiology labs to be vigilant, as similar increases are expected in other countries due to likely declines in immunity.

P100
A novel species of the Actinomycetaceae associated with pyothorax in cats

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Aims
The taxonomy of the actinobacteria has recently been revised (Nouioui C. et al. Front Microbiol 2018: 9: 2007) and several species previously assigned to the genus Actinomyces have been grouped into new genera. Actinomycetaceae are Gram-positive rods found on the mucous membranes of many animals, where they can act as opportunistic pathogens causing pyogranulomatous infections. One of these infections is pyothorax, regularly found in cats after penetrating trauma such as bite wounds, where actinomycetes are usually isolated together with mixed anaerobes. Here we propose a novel species of the genus Buchananella (syn. Actinomyces) isolated from four cases of pyothorax in cats.
Methods
Clinical actinomyces isolates from our veterinary diagnostic laboratory not identifiable by MALDI-TOF (Bruker, 11897 MSP library, 2022) were collected and subjected to 16S rRNA gene sequencing. Four matching isolates from cases of pyothorax in cats (22MD1512, 14KM1171, 17KM0085 and 22MD1345) that could not be assigned to any known species were further analyzed by PacBio whole genome sequencing. To determine the phylogenetic position of these strains, digital DNA-DNA hybridization (dDDH) was calculated by submitting the sequences to TYGS (https://tygs.dsmz.de/) using formula d6, which is most consistent with other phylogenetic analyses. Average nucleotide identity (ANI) with the most closely related species was calculated using the OAT software version 0.93.1.
Results
Based on 16S rRNA the most closely related species was Buchananella hordeovulneris (NR_026225.1) with 96.9% identity clearly below the accepted species threshold of 98.65% (Kim Oh et al. Int J Syst Evol Microbiol 2014:64(Pt 2): 346). dDDH among the four analyzed isolates was 96-97.5%, which is well above the 70% threshold of species delineation indicating that they belong to the same species, while the closest known species (Buchananella hordeovulneris) only showed 15% dDDH. Results for ANI, where the species threshold is commonly set at 95%, were similar with 98.3-98.5% identity among the four cat isolates and 73.6% with Buchananella hordeovulneris.
Conclusion
Our results indicate that the four cat isolates belong to the same novel species. They are most closely related to Buchananella hordeovulneris, indicating that they belong to a second species of this genus for which we propose the name Buchananella felis sp. nov..

P101

Quantitative Assessment of Mycoplasma hominis and Ureaplasma urealyticum: Enhancing Diagnosis and Treatment Decision-Making with Allplex™ STI Essential Assay Q

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Aims
Sexually transmitted infections (STIs) remain a worldwide problem and a burden on public health. Cases of infections with Chlamydia trachomatis (CT), Mycoplasma genitalium (MG), Neisseria gonorrhoeae (NG) or Trichomonas vaginalis (TV) have been rising steadily over the past 20 years in Switzerland. Commercial STI multiplex PCR assays also cover Mycoplasma hominis (MH), Ureaplasma urealyticum (UU) and Ureaplasma parvum (UP) which is controversially discussed because of their significance. To obtain a better interpretation of STI multiplex PCR, it is recommended to report UU and MH as quantitative results. UP will not be further discussed in this study.
Methods
We compared the new quantitative Allplex™ STI Essential Assay Q to our current standard method (Allplex™ STI Essential Assay, Seegene, South Korea) by testing 73 previously tested patient samples, primarily consisting of vaginal swabs (48.0%), primary urine (34.7%) and urethral swabs (13.3%) with the new quantitative assay. Both assays test for NG, MG, CT, TV, UU, MH and UP. We chose 12 negative samples and 61 samples with at least one positive target in the standard method. Additionally, a 10-fold dilution series ranging from 1:4 to 1:40000 was prepared from patient samples to examine the linearity of quantification for MH and UU.
Results
Concordant results were achieved in 100% of the samples for UU, NG, MH, MG, CT, and TV and in 98.6% for UP. Ct-values between methods did not differ significantly for all targets except for TV, where the quantitative assay would frequently measure higher Ct-values than the reference method ($p = 0.0137$, Wilcoxon test). Quantification was found to be linear for UU ($R2 = 0.9998$, range: 0.66 – 795.6 cp/µl) and MH ($R2 = 0.9935$, range: 0.80 – 1196.5 cp/µl). Results ranged from 1.05 x 10-1 to 2.14 x 105 cp/µl (Ct 34.69 to 14.56) for UU and from 5.50 x 10-1 to 2.63 x 106 cp/µl (Ct 33.26 to 10.13) for MH.
Conclusion
These findings demonstrated consistent results between the two methods, although small discrepancies were observed for TV that were highly dependent on sample age. The possibility to quantify MH and UU offers an additional tool to differentiate between infection and colonization, but further investigation is needed to define cutoffs that aid clinicians in making informed treatment decisions. However, asymptomatic carriage is frequent and positive findings in multiplex STI-screenings can lead to unnecessary administration of antibiotics.

P102
Characterization of clinical Mycobacterium kansasii complex isolates – epidemiology and resistance profile

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Aims:
Mycobacterium kansasii can cause tuberculosis-like lung infections in both immunocompetent and immunocompromised individuals. The M. kansasii complex is currently divided into 5 (or 6) species, i.e., M. kansasii sensu stricto, M. persicum, M. pseudokansassii, M. innocens, M. attenuatum and eventually 'M. ostraviense' (Tagini, 2019). Some of these were only recently elevated from subtype to species level based on phylogenomic analyses. Species level identification of non-tuberculous mycobacteria from clinical origin is recommended to elucidate their clinical and epidemiological relevance. Within this study, we investigate the capacity of the currently applied routine identification methods (sequencing of the 16S rRNA gene and MALDI-TOF MS) to differentiate M. kansasii complex isolates to species level. Furthermore, we analyse drug susceptibility patterns of the isolates.

Methods:
For 54 clinical M. kansasii complex isolates collected consecutively between 2022 and 2023 at our laboratory identification by 16S sequence analysis, MALDI-TOF MS (Bruker MALDI Biotyper, Bruker Daltonics, Bremen, Germany) and whole genome sequencing (WGS) using the Illumina technology (San Diego, CA) was compared. Phenotypic drug susceptibility testing (DST) was performed using microdilution (Sensititre SLOMYCOI plate, Thermo Fisher, Waltham, MA).

Results:
M. kansasii sensu stricto (70 %), M. persicum (15%) and M. pseudokansasii (13%) were the most common species isolated from clinical specimens in our laboratory as determined by WGS. One isolate of 'M. ostraviense' was identified. Reliable identification at species level by MALDI-TOF MS and 16S sequence analysis, respectively, was only achieved for a subset of species. Low MIC values were generally measured for the therapy-relevant drugs. Interestingly, two isolates were resistant to streptomycin (MIC > 64 mg/L). Phenotypic resistance was confirmed at the molecular level by identification of corresponding rpsL resistance mutations.

Conclusion:
Species differentiation within the M. kansasii complex is currently not possible with routine diagnostic methods. There is an urgent need to establish routine diagnostic tools to differentiate M. kansasii isolates to species level.

P103

Evolution of microbiological aetiology for tinea capitis in western Switzerland: a retrospective bicentric study

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Background
Tinea capitis is a superficial infection of the scalp by Arthrodermataceae called dermatophytes and occuring predominantly in children. The epidemiology of tinea capitis varies widely from one region to another.

Aims
To characterize the microbiological aetiology of tinea capitis and evaluate epidemiological evolution over time

Methods
A retrospective observational study was carried out in the two main dermato-mycology units of French-speaking Switzerland. This study was based on the results of positive dermatophyte cultures.

Results
1215 scalp or hair samples had positive culture for a dermatophyte between 01.01.2007 and 31.12.2022. While the incidence was stable over time, different genus appeared and replaced the others. Over time, genus Trichophyton has taken over Nannizzia and Microsporum spp. Zoophilic strains are currently less prevalent while antropophilic strains have become largely predominant. T. violaceum is now the first aetiological agent for tinea capitis in French-speaking Switzerland.

Conclusions
We report on local evolution of microbiological aetiology for tinea capitis. Human migrations have to be accounted for to understand epidemiological shifts. Microbiological monitoring by dermatophyte identification is an important tool to provide a scientific basis for local care and treatment protocols.

P104
HIV viral load determination on fully automated molecular systems: Comparison of NeuMoDx 96 and Cobas 5800

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1 Viollier AG; 2 KSA Aarau; 3 QIAGEN

Background: According to WHO, HIV-infected patients under treatment should be monitored every 6 to 12 month and should show viral load (VL) suppression of below 50 copies/ml1. Therefore, reliable detection of the VL with molecular diagnostic platforms is essential for monitoring and treatment2.
The aim of the study is to compare the VL measured with the NeuMoDx HIV-1 Quant Assay on the Neu-MoDx 96 Molecular System with the Cobas® HIV-1 on Cobas 5800.

Methods: 68 positive HIV samples were collected and stored at -20°C for several months. Before testing, all samples were diluted 1:1 with fetal bovine serum (FBS) and re-analysed in parallel on both systems . Correlation of positive samples between the assays was determined by linear regression and Bland-Altman Plot.

Results: Out of 68 samples, 53 (78%) were concordant (32/60% positive, 21/40% negative). Fifteen samples (22%) showed discrepant results: 9 were negative on Cobas but positive on NeuMoDx with a wide range of 10 - 861 copies/ml and 6 were negative on NeuMoDx but positive on Cobas 5800 with viral loads < 75 copies/ml. . Comparison of the concordant positive results showed a correlation of R2 0.84. The mean difference be-tween the two the assays was 0.29 log copies/ml , with higher VL for the NeuMoDx.

Conclusion: Our study could confirm that the NeuMoDx can accurately detect and quantify HIV-1 viremia and shows satisfying correlation. Nevertheless, the low VL of patients under treatment are a challenge in reaching consensus on the results. The three negative samples on the Cobas with copies/ml > 200 on the NeuMoDx, raise questions and require further investigation. A previous clinical evaluation of the NeuMoDx HIV-1 Quant Assay showed very similar results (R2: 0.89; mean difference: 0.23 log copies/ml).

P105
Resolving host-uropathogenic interactions at single-cell level on a human bladder model

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Urinary tract infections are among the most common bacterial infections in humans. With a high recurrence rate of approximately 30% within six months, antibiotic treatment and prophylaxis are often suboptimal, exacerbating the current antimicrobial resistance crisis. Researchers commonly rely on in vivo (murine) or in vitro cancer cell line-based models to study host-uropathogen interactions. However, the urothelium – the tissue covering the lower urinary tract – significantly differs from mice to humans, in terms of ultrastructure, physiology and innate response. Moreover, the few complex in vitro models reported usually struggle to capture key human features such as stratification, differentiation and/or urine tolerance, which affect uropathogenesis. In this project, we want to employ the recently published 3D-UHU model, a novel in vitro human urothelium microtissue that comprehends the key features abovementioned, to investigate host-pathogen interactions on a single-cell level. Using single-cell transcriptomics, we want to identify the molecular signatures among different cell subpopulations of the human urothelium. Subsequently, we want to unveil critical molecular players/pathways among the different populations, which are involved in host responses to uropathogen infection, mainly regarding the pathogen intracellular lifecycle. We expect that our findings will provide deeper insights into the tissue distribution of the human urothelial cell populations and will reveal novel molecular players in host-uropathogen interactions, uncovering innovative therapeutic venues and a better understanding of urinary tract infections.

P106

Design and validation of genus-specific semi-nested primers for an easy and accurate identification of Enterobacter strains

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Aims
Enterobacter is a genus of the family Enterobacteriaceae with a high clinical and environmental relevance. The genus has undergone frequent taxonomic changes, making it challenging to identify them even at genus level. The primary aim of this study was to design Enterobacter genus-specific primers that can be used for simple PCR identification of large sets of putative Enterobacter isolates.
Methods
A comparative genomics approach was used to identify homologous genes that are abundant in a set of 4276 refseq genomes of Enterobacter but lacking in other Enterobacteriaceae. Primers were designed based on a multiple sequence alignment of the target gene with flanking regions of 1069 Enterobacter strains and 85 other Enterobacteriaceae and tested on a collection of strains that were supplied as Enterobacter spp. All tested strains were additionally identified with MALDI-TOF MS using the MABRITEC database.
Results
Comparative genomics indicated the absence of an Enterobacter-specific gene that covers all species and is not abundant in all other Enterobacteriaceae. Therefore, the hpaB gene was selected as target gene as it is absent in the closest neighbors Huaxiibacter and Lelliotia, although present in other Enterobacteriaceae. The novel designed semi-nested primer set was tested in single-tube reactions on 122 strains. All positive reactions matched with the identification as belonging to the genus Enterobacter by MALDI-TOF MS. Enterobacter strains positive in the PCR yielded two bands at 110 bp and at 370 bp, while strains only displaying the 110 bp band were classified as Leclercia pneumoniae. Strains showing no or only the large band did not belong to the genus Enterobacter. The PCR was additionally validated on 120 strains from the NRRL culture collection that were originally accessioned as Enterobacter sp. revealing that one third of the strains had an incorrect genus assignment.
Conclusion
We found that Enterobacter strain names in culture collections may not be in accordance with the current taxonomy as many of these strains were deposited prior to broad use of DNA-based identification methods. As the primer design was based on a large-scale genomic comparison, covering also currently undescribed species clades, it can even remain valid after possible taxonomic changes within the genus. This assay represents a quick and cost-effective method for culture collections to assess the accuracy of Enterobacter strains in their holding.

P107

Nanopore long-reads sequencing for epidemiological surveillance of Klebsiella pneumoniae using cgMLST

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Aim

Epidemiological surveillance of nosocomial pathogens is crucial for understanding transmission routes and mitigating hospital outbreaks. The advent of Next Generation Sequencing has led to the development of techniques using whole genome sequences, such as core genome Multi Locus Sequence Typing (cgMLST). While this method has been successfully applied by epidemiologists, it has predominantly utilized short-read sequencing. With the advancements in long-read sequencing, there is a growing interest in employing this technology in various fields, including epidemiology. This study focuses on comparing the performance to identify transmission chains of Nanopore long-read sequencing and Illumina short-read sequencing for cgMLST of Klebsiella pneumoniae strains.

Methods

Twenty-one carbapenem-resistant K. pneumoniae strains composed of sporadic cases and identified clusters were selected. These isolates underwent sequencing using both short-read technology (Illumina) and long-read technology (Nanopore, R10.4 chemistry). Genomes were assembled using either short or long reads, and hybrid assemblies were performed using both read sets, serving as reference in subsequent analyses. Nanopore read sets were downsampled to assess the impact of coverage. cgMLST was conducted for all assemblies utilizing the K. pneumoniae core genome scheme from Ridom®. The distance (loci number) between Nanopore or Illumina assemblies and hybrid assemblies was evaluated. Minimum Spanning Trees (MST) were calculated for each method.

Results

The difference between long reads and reference assemblies was 0-47 loci, with 8 samples showing a difference of 14-47 loci, and 13 samples demonstrating better convergence with a difference of 0-8 loci. This discrepancy was notably influenced by sequencing coverage, with a minimum of 30x-40x required for optimal convergence. Conversely, the difference between short reads and reference assemblies was only 0-2 loci. MST structures were similar between short and long reads, although the MST of long reads generally displayed higher distances between samples. Clusters were clearly identified using either short or long reads cgMLST.

Conclusion

Long-read technology appears promising for K. pneumoniae cgMLST studies and in epidemiological surveillance. Further investigations of the low convergence obtained with some samples are required in order to make Nanopore sequencing fully suitable for epidemiological investigations.

P108

In-vitro activity of the novel ß-lactam/ ß-lactamase inhibitor combinations and cefiderocol against carbapenem-resistant Pseudomonas spp. clinical isolates collected in Switzerland in 2022

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Aims. To evaluate the in-vitro activity of the novel commercially-available drugs, including meropenem-vaborbactam (MEV), ceftazidime-avibactam (CZA), ceftolozane-tazobactam (C/T) and imipenem-relebactam (IPR) as well as the novel siderophore cephalosporin cefiderocol (FDC), against carbapenem-resistant P. aeruginosa (CRPA) isolates.
Methods. All carbapenem-resistant Pseudomonas spp. (CRP) isolates collected at the Swiss National Reference Laboratory (NARA) over the year 2022 (n=170) have been included in the study. Most of these isolates (n=121) were non-carbapenemase producers, but exhibited a combination of AmpC overproduction, efflux system modifications, and porin deficiency. Among the 49 carbapenemase producers, 47 isolates produced metallo-ß-lactamases (MBL) and two isolates produced the class A carbapenemase GES-5. Susceptibility testing was determined by broth microdilution method (BMD), or disk diffusion test following EUCAST guidelines. Both techniques were interpreted according to EUCAST breakpoints.
Results. The susceptibility rates for MEV, CZA, C/T and IPR were found to be 41%, 45%, 59% and 58%, respectively, for the whole set of isolates tested. Excluding carbapenemase producers, susceptibility rates for these ß-lactam/ß-lactamase inhibitors combinations were significantly higher, at 55%, 61%, 83%, and 82%, respectively. Overall, 91% of isolates from the whole collection were susceptible to FDC. A total of 10 isolates (6%) were found to be resistant to all drugs tested, all these isolates being MBL producers.
Conclusion. Overall, FDC exhibited an excellent in-vitro activity against this collection of CRP recovered from Switzerland in 2022, including MBL producers. On the other hand, the new ß-lactam/ß-lactamase inhibitor combinations displayed significant activity against non-carbapenemase CRP, with IPR and C/T showing the highest susceptibility rates.

P109

Characterisation of a novel AmpC beta-lactamase, DHA-33, resistant to inhibition by cloxacillin

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Aims
Plasmid-encoded DHA-type AmpCs have been extensively reported in Enterobacterales and the identification of infections caused by AmpC-producing bacteria is a necessity, both for infection control purposes and to inform treatment choices. A common testing method for AmpC production in the clinical laboratory setting is to supplement Mueller-Hinton agar plates used for antibiotic disk diffusion with cloxacillin, a potent inhibitor of AmpC enzymes. Here we describe a novel DHA variant, produced by a clinical Escherichia coli isolate, which is resistant to cloxacillin inhibition.
Methods
A cephalosporin-resistant E. coli isolate, FR23, was sent to the Swiss National Reference Centre for Emerging Antibiotic Resistance for further investigation. Susceptibility testing to beta-lactam antibiotics was performed by disc diffusion in the presence/absence of 250 mg/L cloxacillin, and MICs were determined by broth microdilution. blaDHA alleles were amplified and cloned into pCR-Blunt II-TOPO, before transformation into E. coli Top10. Whole genome sequencing (WGS) was performed using both Illumina and Oxford Nanopore platforms. IC50 measurements using cloxacillin were performed for DHA-1 and DHA-33.
Results
Susceptibility testing showed that the isolate was resistant to all tested penicillins, including combinations, cephalosporins, and aztreonam, but was sensitive to carbapenems, and no inhibition was observed in the presence of 250 mg/L cloxacillin. WGS identified this isolate as belonging to ST9748, a single locus variant of ST131, and harboured a novel DHA variant, DHA-33. Susceptibility testing of recombinant strains showed that, compared to DHA-1, DHA-33 exhibited less activity towards amoxicillin, piperacillin, and cephalothin, but conversely, had higher MICs to ticarcillin, aztreonam and cefiderocol. Enzymatic assays showed that the inhibitory activity of cloxacillin was considerably decreased against DHA-33 compared to DHA-1, with a >4,000-fold increase in IC50.
Conclusions
In this study we described a novel DHA variant, namely DHA-33, that exhibits increased activity against aztreonam and cefiderocol relative to DHA-1. DHA-33 was resistant to inhibition by cloxacillin allowing the enzyme to initially evade identification by routine methods for AmpC activity. The dissemination of such inducible cloxacillin-resistant AmpC variants is concerning and could be problematic for both epidemiological surveillance and infection management.

P110

Relative inhibitory activities of newly-developed diazabicyclooctanes, boronic acid derivatives, and penicillin-based sulfone ß-lactamase inhibitors, against broad-spectrum ampC ß-lactamases

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Aims. Broad-spectrum AmpC ß-lactamases are highly disseminated in gram-negative pathogens. These enzymes can hydrolyse penicillins, monobactams, cephalosporins, and even, for some of them, cefepime or carbapenems although at low level. AmpC enzymes are poorly inactivated by the old ß-lactamase inhibitors (BLIs) clavulanic acid and tazobactam. The recent development of new BLI including (i) diazabicyclooctanes (DBO) represented by avibactam (AVI), relebactam (REL), zidebactam (ZID), nacubactam (NAC) and durlobactam (DUR), (ii) boronic acid derivatives (BAD) such as vaborbactam (VAB), taniborbactam (TAN) and xeruborbactam (XER), and (iii) a penicillin-based sulfone such as enmetazobactam (ENM) (derivative of tazobactam), has contributed to the development of new therapeutic possibilities. However, little is known about the spectrum of action and relative potency of these new inhibitors toward AmpC-type beta-lactamases, which was evaluated in the present study.

Materials. Genes encoding a series of class C ß-lactamases (ACC-1, FOX-1, FOX-5, CMY-2, CMY-42, DHA-1, ACT-7, ACT-17, LAT-1, MIR-17, PDC-1, PDC-5, YRC-1) were amplified by PCR and corresponding amplicons cloned into plasmid pUCp24, and then transformed in Escherichia coli. Susceptibility testing was performed for ceftazidime (CAZ) and their combinations with CLA (CAZ-CLA), TZB (CAZ-TZB), AVI (CAZ-AVI), REL (CAZ-REL), TAN (CAZ-TAN), XER (CAZ-XER), NAC (CAZ-NAC), ZID (CAZ-ZID) and DUR (CAZ-DUR) at a fixed concentration of 4 mg/L, while VAB (CAZ-VAB) and ENM (CAZ-ENM) were fixed at 8 mg/L. Fifty percent inhibitory concentrations (IC50) of all ß-lactamase inhibitors were also determined for all cephalosporinases.

Results. All AmpC enzymes conferred resistance to CAZ, and by combining CAZ with a DBO (AVI, REL, NAC, ZID, DUR) or BAD (XER, TAN), all MICs values were reduced by more than 8-fold dilution. However, CAZ-VAB was less effective than others combination including CAZ with a DBO or others BAD, and poorly effective against FOX-like, CMY-42, and PDC-like producers. Interestingly, CAZ-ENM was as poorly effective as CAZ-TZB against these AmpC producers. In line with these results, IC50 values of DBOs, XER and TAN were 10-fold lower than those obtained with VAB and ENM for all AmpC enzymes.

Conclusions. This study highlighted the excellent performance of all newly developed DBOs, as well as XER and TAN against AmpC enzymes, and conversely that ENM and VAB were poor inhibitors against class C ß-lactamase.

P111

Impact of extended-spectrum chromosomal AmpC (ESAC) of Escherichia coli on susceptibility to cefiderocol

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Escherichia coli is the most common pathogen intrinsically susceptible to most antibiotics, particularly ß-lactams. Although acquired resistance to broad-spectrum cephalosporins in that species may be related to the acquisition of expanded-spectrum ß-lactamases, it may also be related to the production of the intrinsic chromosomally-encoded AmpC (cAmpC). Some variants of those cAmpCs possess the capacity to confer reduced susceptibility to 3rd but also 4th generation cephalosporins once produced at high level. These specific variants have been defined as extended-spectrum AmpC β-lactamases (ESAC), and they possess increased catalytic activity against oxyminocephalosporins, including cefepime (FEP) and cefpirome. The objective of the present study was to evaluate whether ESAC produced by some E. coli isolates might interfere with the activity of the newly developed siderophore cephalosporin cefiderocol (FDC), considering the structural similarities between FDC and ceftazidime (CAZ), but also to some extent FEP and cefpirome. Seven clinical E. coli Isolates encoding different cAmpC were obtained from the Medical and Molecular Microbiology (Fribourg, Switzerland) and included in the study. Six isolates producing ESACs, namely AmpC-EC13, -EC14, -EC15, -EC16, -EC17, -EC18, and a single isolate producing a narrow-spectrum AmpC, AmpC-EC5. All cAmpCs were transformed into five different E. coli backgrounds, E. coli TOP10, E. coli MG1655 and its counterpart E. coli MG1655 exhibiting modifications in its PBP3 penicillin-binding protein, corresponding to either amino acid insertions YRIN (Tyr-Arg-Ile-Asn) or YRIK (Tyr-Arg-Ile-Lys), and the porin-deficient E. coli HB4 (lacking both OmpC and OmpF porins). Broth microdilution was performed to evaluate any changes in MICs of CAZ, FEP, and FDC. A significant increase of the MICs was observed for all the recombinant strains carrying an ESAC compared with their respective parental strain and with the cAmpC-EC5 when testing all the antibiotics. The range of MICs for FDC in the parental strains and AmpC-EC15 was ≤ 0.06 – 0.5 mg/L, while for the ESACs it increased to a range of 1 – 8 mg/L. Our results showed that ESACs indeed exhibited increased activity toward FDC and potentially constitute sources of reduced susceptibility to that novel cephalosporin in E. coli. Our data therefore identify an additional pathway potentially affecting susceptibility to FDC in E. coli.

P112
Pitfalls and challenges in molecular diagnostics of Corynebacterium diphtheriae - toxin or no toxin?

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Background
Due to comprehensive vaccination programs diphtheria became rare in Europe. Yet, during Summer 2022 various European countries experienced an outbreak of diphtheria amongst asylum seekers caused by tox+ and tox- strains of Corynebacterium diphtheriae. In June 2022, a pharyngeal swab from an outbreak-unrelated patient admitted to a tertiary-care hospital due to diagnosed diphtheria was sent to our lab (IMM). PCR analysis on previous samples sent to another microbiological laboratory (Lab A) by the patient's general practitioner revealed an infection with a tox+ C. diphtheriae.

Methods
At IMM C. diphtheriae was isolated from pharyngeal swabs using CTBA plates. The tox gene RT-PCR was performed using oligos described by Sing et al. (2011) (IMM) or by Schuhegger et al. (2008) (Lab A). The Elek test was performed according to the recommendations of WHO. WGS of the isolated strain was performed using Illumina MiSeq and ONT GridION.

Results
PCR analysis of the strain isolated from the patient’s swabs was tox- negative. To verify our result, the isolate was sent to Lab A which had tested a previous patient isolate as tox+ and that routinely uses different oligos. In addition, we performed an Elek test. The external PCR analysis was again tox+, however the Elek test was repeatedly negative. To clarify these discrepancies, the isolate was subjected WGS. Analysis of the tox gene revealed the presence of an insertion sequence (IS1132) at its 5’ end and a deletion of the first 339 bp, rendering the gene unfunctional and abolishing the binding site of the forward primer used in our lab. The positive PCR result in Lab A were due to the use of oligos addressing a tox gene region located further downstream that remained unaffected by the insertion and the deletion.

Conclusion
In most laboratories, microbiological diagnostics of diphtheria comprises cultural and molecular approaches for pathogen isolation and tox gene identification. In contrast, the Elek test is nowadays only performed in a few specialised institutions and reference centres. Based on our observations, molecular results regarding C. diphtheriae tox gene PCR i) should be interpreted with caution and ii) positive results should be confirmed by an Elek test to ensure adequate patient management.

P113

Investigating the potential of Human Milk Oligosaccharides (HMOs) for the inhibition of pneumococcal adherence

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The human pathogen S. pneumoniae (the pneumococcus) is a leading cause of death in children under 5 years of age globally. Human Milk Oligosaccharides (HMOs) have antibacterial effects against neonatal pathogens and can be taken up by infants during breastfeeding, subsequently residing in the mucus to prevent infections. The main sugar in human milk is Lactose, a disaccharide of Glucose and Galactose, followed by the most prevalent HMO 2'-Fucosyllactose (2FL).

The growth of S. pneumoniae strains was investigated in different media with the addition of various HMOs (2FL, 3'-Fucosyllactose, Lacto-N-tetraose, Lacto-N-neotetraose (LNnT), 6'-Sialyllactose, and 3'-Sialyllactose) as sole Carbon-source (C-source). The potential of HMOs to inhibit pneumococcal adherence was also investigated using Detroit cells. Furthermore, we established an adherence assay with an air-liquid interface culturing system with nasal primary cells obtained from healthy donors.

Our research showed that S. pneumoniae can grow with specific HMOs as a sole C-source, but only after growing in Galactose, a common saccharide in the nasopharynx where the bacterium primarily resides as a commensal. We found that Galactose increases the expression of certain genes (bgaA and nanA), aiding in the breakdown of specific HMOs. 2FL was the only HMO, that couldn't be used as a sole C-source by different pneumococcal strains. Furthermore, the addition of 10mM Lactose resulted in an approximately 50% decrease in adherence on Detroit cells. Additionally, preliminary data indicates that 2FL as well as LNnT have the potential to reduce adherence. Preliminary data for primary cells demonstrates that after 24 hours there is decreased adherence with mucus compared to no mucus condition.

Following these promising results, we will continue investigating if HMOs can inhibit pneumococcal adherence. 2FL may be a promising agent in the fight against this pathogen, as it can not be metabolized but has the potential to reduce adherence and therefore infection.

P114
Characterization of Chlamydia trachomatis proteins upregulated in elementary bodies

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The aim of this project is to characterize proteins which are potentially implicated in processes specific to the developmental forms of Chlamydia trachomatis L2 (Ctr), the elementary body (EB) and the reticulate body (RB). The infectious EB and replicative RB have of Ctr must undergo differentiation to complete their lifecycle, but a precise understanding of the triggers in which the bacteria transform between the two forms is not fully explained. Therefore, characterization of these proteins will lead to a better understanding of the lifecycle of Ctr and the mechanisms in which it is able to thrive during infection.
In order to initially identify proteins which may be implicated in the differentiation of Ctr, RNA sequencing was performed. RNA transcripts were differentially evaluated between RBs (24 hpi) and EBs (72 hpi) to detect those proteins which were either up- or down-regulated between the populations. Following RNA sequencing, five proteins were selected for further evaluation due to their higher transcript expression in EBs compared to RBs. All proteins were first cloned into eukaryotic expression vectors for initial visualization of localization by immunofluorescence. Cloning for transformation into Ctr L2 for overexpression of the proteins was also performed. Characterization of the localization and effects of protein overexpression were evaluated by immunofluorescence microscopy and inclusion forming units were determined.
RNA sequencing allowed for the selection of five proteins, which are differentially expressed between EBs and RBs. CTL0163, CTL0164, CTL0290, CTL0291, and CTL0473 each displayed transcript levels at least 2.4-fold higher in EBs than in RBs with CTL0163 at the highest upregulation of over 20-fold higher in EBs. Eukaryotic expression of the proteins within HeLa cells revealed distinct localization patterns to different membranous structures within the cell, indicating that these proteins may be transported to those specific membranes also during infection. Further evaluation of effects of protein overexpression during Ctr infection is ongoing.
This study identified five candidate proteins which may be involved in Ctr differentiation due to their specific, high expression during the EB stage of development. This high expression indicates that they each have a utility particular to EB development. As the role of each of these proteins have not yet been characterized in Ctr, continued work will attempt to uncover their function.

P115
Evaluation of the ELITech multiplex GI parasitic Plus PCR

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Aims: Parasites diagnosis is based on microscopy on fresh and formalin fixed stools. However, microscopy is fastidious, time consuming, requires a solid expertise and lacks sensitivity. Several nucleic acid and amplification assays are commercialized for intestinal parasites detection. We conducted a retrospective evaluation of the performance of the multiplex GI parasitic Plus PCR assay from ELITech on the BeGenius system.
Methods:
A total of 34 native samples (29 positive and 5 negative) and 18 positive formalin fixed stool samples were tested with the GI parasitic Plus ELITe MGB PCR Kit (ELITech). This assay allows the detection of 5 protozoa: G. lamblia, E. histolytica, Cryptosporidium spp, E. bieneusi and Encephalitozoon spp. The BeGenius system (ELITech) was used for nucleic acid extraction and amplification. Results were compared to the Novodiag Stool Parasites PCR assay (Mobidiag) and conventional microscopy, and a qualitative evaluation was determined by the Kappa coefficient for both native and formalin fixed samples.
Results: Among the 52 samples tested, 29 positive and 5 negative were concordant with the comparison methods. Eighteen positive samples were non-concordant. Concordant results were all from native samples, showing a Kappa coefficient of 1. Non concordant results came all from formalin fixed samples, with a Kappa coefficient of 0.
Conclusion: The GI parasitic Plus ELITe MGB PCR Kit (ELITech) on the BeGenius system can detect the most prevalent protozoan intestinal parasites in native stool samples. However, the ELITech extraction and PCR protocols are not able to highlight parasite DNA in formalin fixed samples. This assay is interesting for the identification of protozoa, as these parasites can not be detected (microsporidia) or identified (E. histolytica versus E. dispar) by routine microscopy.

P116
Recharge drives microbial heterogeneity in a karst aquifer of the northern United States

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Aims

Despite the importance of subsurface microbial communities to groundwater quality, little is known about how land use and other surface influences impact groundwater microbes. Our study aimed to better understand the microbial diversity and dynamics within a karst aquifer used for private drinking water, yet subject to domestic and agricultural contamination.

Methods

Large-volume, dead-end ultrafiltration was used to collect water samples (n=138) from private wells in a rural region of the United States. Wells (n = 22 to 30) were randomly selected from a pool of participants in each of 5 sampling events over the course of one year. Following ultrafilter backwashing, concentration, and DNA extraction, samples was analyzed using 16S (V4) Illumina sequencing. Amplicon sequence variants (ASVs) were generated from the demultiplexed reads and decontaminated using pre-amplified sample dsDNA concentrations. To relate microbial community composition to environmental and land use factors, sample communities were clustered by Bray-Curtis dissimilarity using a DBSCAN algorithm. Cluster assignment was evaluated relative to land use, geology, groundwater recharge, pathogen and antibiotic resistance gene (ARG) occurrence, and microbial source tracking (MST) markers for human and bovine fecal contamination. Random forest classification was used to select the most important attributes for predicting microbial clusters. Logistic regression was used to select only significant variables from this subset of attributes.

Results

Groundwater communities from across the county were highly homogenous. Core taxa, defined as microbial orders appearing in over 95% of samples, included representatives of Patescibacteria, Proteobacteria, Firmicutes, Verrucomicrobiota, Crenarchaeota, Dependentiae, and Nanoarchaeota. The median sample relative abundance of these core taxa was 94%, though some samples included as little as 19% core taxa. Marked change in community composition was particularly observed during the January sampling event. Multiple attributes related to water infiltration from the surface (snowmelt, recharge, and precipitation) were associated with clustering.

Conclusion

Recharge and infiltration were the main source of microbial variation in an otherwise homogeneous aquifer. Well water primarily represents the planktonic subsurface community, which may be more variable than the attached community.

P117
Antimicrobial activity of AMPs encapsulated in lipid-based formulations

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Aims. Antimicrobial peptides (AMPs), such as LL-37, are promising therapeutics with broad-spectrum activity and low induction of resistance. The self-assembly of LL-37 in lipid-based nanostructures is an emerging strategy to overcome the limitations of AMPs' stability and enhance drug delivery. This study investigates how the LL-37's encapsulation in glyceryl monooleate (GMO) nanocomplexes impacts its antimicrobial efficacy.

Methods. Various nanocomplexes were made by varying the GMO content relative to LL-37 concentrations. The nanocomplexes' antibacterial activity against planktonic cells was evaluated using Pseudomonas aeruginosa and Staphylococcus aureus through colony-forming units (CFU) counting. The ability of the nanocomplexes to inhibit biofilm formation and eradicate mature biofilms was assessed using crystal violet staining.

Results. Our assays demonstrate that the antimicrobial efficacy of LL-37/GMO nanocomplexes significantly decreases as the GMO content increases. While 64 µg/mL of LL-37 in its free-form reduced P. aeruginosa planktonic populations by 3 logs, its association with an equal amount of GMO resulted in no killing. Similar results were obtained for the nanocomplexes' antibiofilm activity. Overall, the results suggest that the GMO content relative to LL-37 should not exceed 25% to preserve LL-37's intrinsic activity. The physicochemical and biological characteristics of the nanocomplexes were thoroughly investigated to understand the factors altering antimicrobial efficacy.

Conclusion. This study highlights the limitations of lipid-based nanocarriers on the encapsulated AMPs' activity when inadequately prepared, offering a novel perspective on the design of lipid-based antimicrobial therapeutics.

P118

Implementation of AI-assisted digital microscopy in a routine hospital mycobacteria laboratory

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Aims
We aimed to transition from manual microscopy (MM) of acid-fast bacilli (AFB) to an automated, AI-assisted digital microscopy (DM) system, and highlight crucial steps involved in its validation.

Methods
For DM, images of auramine-rhodamine-stained samples were scanned and analyzed by Metasystems automated AFB microscopy scanning and deep-leaning-based image analysis system. First, a new neuronal network was trained with 20,000 negative and 7,000 positive images. During November to December 2023, 283 samples from routine mycobacteria workflow (microscopy and culture) were scanned and analyzed by DM. Automated proposed results and automated proposed results reviewed by a technician (machine assisted results) were compared to the standard MM. Thirty-four MM positive samples were used to compare CDC-based grading of MM and machine-assisted results.
For 28 series of 9-14 samples, time from start of reading (MM) or scan (DM) respectively, to final interpretation was measured and average time to result (TTR) per sample calculated.
To assess the reproducibility, the same slide was interpreted by MM by14 trained technicians as well as five times by DM.
Results
Of 15 culture positive samples, 6 (40% sensitivity) could be detected by MM, 14 (93% sensitivity) by automated proposed results and 8 (53% sensitivity) by machine assisted results.
268 cultures were negative. Of those, 265 (99% specificity) were assessed negative by MM, 71 (27% specificity) by automated proposed results and 259 (97% specificity) by machine assisted results.
For 20 (59%) of AFB positive smears, there was a categorical grading agreement, for 13 (38%) there was one category difference and one sample differed by 2 categories.
TTR for MM took 3.8 min on average per slide, DM took 13.5 min consisting of 1.7 min of hands-on time plus 11.7 min scan and analysis time.
While manual grading varies even within trained staff, DM showed excellent reproducibility.

Conclusion
The implementation of DM in routine practice requires significant investment in hardware and personnel resources for training and local adaptation. Automatic proposed results of DM still need to be reviewed by a trained technician to achieve results with acceptable specificity, and TTR is prolonged due to computing-power-intensive analysis.
Once implemented, machine-assisted microscopy shows a higher sensitivity, reduced hands-on time and improved standardization compared to MM.

P119

Data comparability for Microbial Genotyping in a Medical Microbiology Laboratory across three different Illumina platforms

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Aims: For whole genome sequencing (WGS) in medical microbiology, accuracy, reliability, backup systems, and turn-around time are critical. As part of the ISO accreditation process, we compared sequencing results of a diverse set of bacteria on three different Illumina sequencing platforms: MiSeq, MiniSeq and NextSeq1000.
Methods: A spectrum of Gram-negative and -positive strains was selected: a) 11 bacterial species with diverse genome sizes (Mbp) and %G+C: Fusobacterium nucleatum (21 |27%), Campylobacter jejuni (1.7|30%), Staphylococcus aureus (2.8|32%), Streptococcus pyogenes (1.8|38%), Streptococcus pneumoniae (2.1|39%), Prevotella melaninogenica (3.1|41%), Burkholderia stabilis (8.5|64%), Mycobacterium tuberculosis (4.4|65%), Mycobacterium abscessus (5.1|66%), Mycobacterium chimerae (6.3|66%), Micrococcus luteus (2.5|74%).
and b) outbreak isolates from 4 species: Acinetobacter baumannii, Staphylococcus aureus, Enterococcus faecium, and Klebsiella pneumoniae.
Acceptance criteria were: for a) max 10 SNP differences or b) max 1 cgMLST allele difference between MiniSeq and NextSeq libraries compared to the MiSeq (already ISO accredited).
QiaSeq FX kit (QIAGEN) libraries were prepared and sequenced on 3 platforms: MiSeq, MiniSeq, and NextSeq. Data QC was performed with an in-house developed bioinformatic pipeline (IMMense). This was followed by SNP analysis in CLC Genomics Workbench (QIAGEN) for the diverse strains and core genome multi locus sequencing typing (cgMLST) analysis in SeqSphere+ (Ridom) for the outbreak strains in comparison to the accredited MiSeq data, the reference dataset for all comparisons.
Results: We observed maximum 1 SNP between the devices, being well below our acceptance value and affirming the high-quality of the sequencing outputs. cgMLST showed concordance across all devices for the libraries sequenced, with a max. of 1 allele difference observed within clusters, suggesting high reproducibility and reliability across all three platforms. QC proved to be critical, allowing the exclusion of contaminated strains which would otherwise have biased the findings.
Conclusion: The findings confirm that NextSeq and MiniSeq provide consistent and reliable genotyping results compared to MiSeq data. This enables flexible and cost-effective sequencing options for our routine laboratory. The NextSeq offers significant cost benefits and higher throughput, thus can be recommended for broader implementation in diagnostic medical microbiology.

P121
Impact of nitrogen source variation on the biosynthesis of the capsule of S. pneumoniae

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Streptococcus pneumoniae is a major causative agent of severe diseases such as meningitis and pneumonia. There are so far >100 serotypes known, some of them having or not having amino sugars in their capsule. Auxotrophic for arginine, it possesses a variable set of arginine metabolism genes, which are regulated by Catabolite Control Protein A (CcpA) and ArgR1/AhrC complex [1]. The arginine deiminase system (ADS) encoded by arcABCD is important for energy production and arginine metabolization. It was observed that the deletion of arginine transporter arcD led to the reduction of capsule thickness and attenuated virulence [2].
Aim: We aim to determine whether different carbon sources affect arginine consumption and if there is a correlation with capsule thickness. More specifically, we investigate if pneumococcal strains with amino sugars in their capsules are more affected by varied arginine concentration compared to other strains.

Methods: The pneumococcal strains (106.66 (serotype 6B), 208.41 (7F; amino sugar containing serotype) and D39 (serotype 2)) and their arcD deletion mutants were cultured in chemically defined media (CDM) with various carbohydrate sources and arginine concentrations. Capsule thickness was evaluated by fluorescence microscopy with FITC dextran.

Results: The growth for different arginine concentration did not differ among of three strains. Additionally, the growth analysis revealed that arcD deletion did not affect the growth pattern, implying the presence of at least one other alternative arginine transporter. Interestingly, both the wild-type and mutant strains exhibited growth inhibition when cultured in 10mM of arginine. In addition, despite its theoretical role in upregulating ADS genes, the use of galactose did not induce changes in growth patterns. Furthermore, upon glucose depletion the expected CcpA increased growth due to upregulation of arginine deiminase operon did not occur. No difference in capsule thickness was observed between wild-type strains and their mutants suggesting that arginine is not a major nitrogen source for the biosynthesis of the capsule with amino sugars.

Conclusion/Outlook: The precise mechanism behind the inhibition under 10mM arginine is so far unclear. Further usage of GC/MS aims to further explore arginine transport in WT and arcD knock out strains. In addition, we aim to investigate the nitrogen metabolism in connection to S. pneumoniae having amino sugars in the capsule.

P122
Real time PCR for resistance testing of Mycoplasma pneumoniae and Chlamydophila pneumoniae

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Dr Risch

Aims: During the last months, an unusual high number of positive clinical specimens of the respiratory pathogens Mycoplasma pneumoniae (Mp) and Chlamydophila pneumoniae (Cp) was detected in Switzerland. Macrolide antibiotics are commonly used for treatment of respiratory bacterial infections. Therefore, it is important to know if resistance occurs. The aim of the study is the evaluation of 3 real time PCR for macrolide resistance of Mp and Cp as well as quinolone resistance of Mp. The analyzed target genes were 23SrDNA (macrolides) and parC (quinolones). parC gene of Mp is investigated since there is a high percentage (>10%) of mutated parC gene in Mycoplasma genitalium (Mg) and it is suspected that resistance mechanisms are very similar of both species of the same genus.

Methods: Two cost effective time kits from TibMolbiol were used for the detection of macrolide resistance. Additionally, primers were designed and used in order to amplify and sequence the relevant parts of 23SrDNA and parC genes of Mp either to confirm commercial test or since there is no commercial test available. DNA extraction was mainly performed with BDMax or NeuMoDx. PCR was performed on LC480. 70 positive clinical specimens of Mp and 12 positive clinical specimens of Cp were analyzed with realtime PCR. The kit for Mp can be used also for Mg and the kit for Cp can be used also for Chlamydia trachomatis: combined tests are useful in (routine) laboratory.

Results: Both realtime kits are able to detect wildtype and relevant mutations. 8% of the clinical specimens positive for Mp were with a mutation of the 23SrDNA. 10 clinical specimens for 23SrDNA (with wildtype or mutated result in commercial test) were sequenced and these results confirmed the commercial test. Therefore, it is recommended to routinely test for macrolide resistance of Mp. For parC, no mutation was observed within the same region as the QRDR of Mg. The study is ongoing to have more data for Cp.

Conclusions: The workflow (DNA extraction & realtime PCR) was optimized to have a fast, sensitive and cost effective procedure for resistance testing of positive specimens. It remains interesting why Mg shows a much higher percentage of mutations of 23SrDNA as well as parC gene (data from Dr Risch laboratory).

P122a

Exploring the mechanisms of Mycobacteria mediated membrane damage: Small or catastrophic, two types of damages leading to different bacterial fates

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During Mycobacterium tuberculosis (Mtb) infection, the establishment of a permissive replicative vacuole is driven by cycles of membrane damage and repair events occurring at the Mycobacterium-containing vacuole. The autophagy and ESCRT repair machineries are key host players, while the bacterial pore-forming peptide EsxA, secreted thought the ESX-1, and the methyl-branched cell-wall lipids PDIMs participate in damage induction. Despite intense study of this critical step, detailed mechanisms and functions of the damage/repair cycles are not well understood. Using a set of Mycobacterium marinum (Mm) mutants, a close relative of Mtb sharing most virulence mechanisms but experimentally easier, we distinguished for the first-time two types of membrane damage during mycobacteria infection. We generated single and double Mm mutants in genes encoding the type VII secretion system ESX-1, EsxA and/or TesA, an enzyme synthesizing the PDIM/PGL lipids, PGL being the glycosylated form of PDIM mainly absent in Mtb except in hypervirulent strains, to evaluate their complex contributions to damage. Our results showed that EsxA produces small damage that recruit the ESCRT repair machinery, whereas PDIMs are necessary for the progression to catastrophic damage, allowing cytosolic access to the bacteria. The in vitro damage activity measured by hemolysis identified only EsxA as the dominant damage factor, while a membrane partitioning assay showed that PDIMs facilitate EsxA activity. Our infection model system with the amoeba Dictyostelium discoideum showed that each type of damage generates a different outcome for Mm infection. By monitoring the intracellular growth of the Mm mutants in D. discoideum cells knocked-out for membrane microdomains or the repair pathways, we underlined the role of autophagy and ESCRT machinery to control the EsxA damage. The conservation of the mechanism will be tested in mammalian cells, using mouse microglial BV-2 cells expressing membrane damage reporters. Altogether, our results strengthen the understanding of damage/repair cycles occurring during Mycobacterium infection, demonstrating in cellulo the hypothesis proposed in recent years.

P123

Investigation of the clonal spread of VRE vanA ST612 with reduced daptomycin susceptibility in Switzerland – preliminary results

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Aims: In early 2024, an increase in vancomycin-resistant Enterococcus faecium (VRE) ST612 was documented in various parts of Switzerland. We aimed at identifying epidemiological links and determining risk factors for acquisition of this emerging strain.
Methods: Laboratories were asked to share their sequence data or send their VRE strains to the National Reference Center for Antimicrobial Resistance. Of all VRE vanA ST612 cases identified since 2018 we contacted concerned hospitals to collect clinical and demographic data of sporadic cases and local clusters along with information on outbreak investigations. Results are descriptively summarized.
Results: Until April 2024, 106 VRE ST612 cases from 23 institutions have been identified, with predominant occurrence (93%) in the German-speaking part of Switzerland. Detailed data were available for 86 (81%) cases, and revealed 38 (44%) sporadic cases, and 7 epidemiological clusters comprising 48 (56%) cases. Most cases were detected by rectal swab (n=61; 71%) or urine cultures (13; 15%). Most sporadic cases were detected in nephrology (6; 16%), gastroenterology (5; 13%), and general surgery (5; 13%), while attribution of clusters was more scattered. Preceding antibiotic exposure was common in both groups (74%). VRE infections were documented in 2 sporadic cases (5%) and 8 (17%) cluster-associated cases. While the most probable source of acquisition remained unknown in sporadic cases (71%) as well as index cases of clusters (71%), many sporadic cases were previously in contact with the Swiss healthcare system (76%). However, a previous hospitalization abroad was more frequent in the index cases of clusters (29% vs. 3%). The definition of contact patients was heterogeneous, leading to limited data on contact patients beyond immediate roommate screening.
Conclusion: The high proportion of sporadic VRE ST612 cases points to wide-spread dissemination of this clone. This could be due to hidden transmission chains within the Swiss healthcare system, suggesting that more effective control measures including intensified screening cultures of transferred patients and patients with frequent health care exposure are urgently required. Inconsistent tracing of contact patients limits the in-depth investigation of epidemiological links. A unified case report form and a centralized database could leverage identification of epidemiolocal links and early detection of interfacility spread.

P124
From Isolation to Innovation: Establishing "Standard Precautions+" for Advanced Infection Prevention Techniques

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Background: Contact, droplet and airborne isolation are terms used in German-speaking countries to describe measures to prevent the transmission of multidrug-resistant organisms (MDRO) or communicable diseases. The term “isolation” should no longer be used for the following reasons: The strict differentiation between droplet and airborne transmission overlooks the continuum of respiratory particles. Additionally, “isolation” practices are associated with impaired patient care, depression, anxiety and with adverse outcomes. This project aimed to replace the term “isolation” with an easy-to-understand terminology that builds on the most important measures, the standard precautions, and integrates new insights into infection prevention practices. The ultimate goal should be to mitigate stigma and adverse outcomes for patients.

Methods: A comprehensive approach was chosen to revise the nomenclature and practices surrounding infection prevention measures. This involved the engagement of infection prevention specialists and other healthcare workers to discuss and redefine terms and procedures based on the latest scientific evidence regarding respiratory particles and practical considerations.

Results: The focus moved from terminology based on transmission routes to a more inclusive and descriptive framework. The initiative led to the replacement of the term "isolation" with "Standard Precautions+," where the “+" signifies additional required actions tailored to specific pathogens or clinical contexts. The new implementation categorizes measures into more intuitive categories, such as "Standard Precautions+ Disinfection Single Room" (formerly contact isolation) or "Standard Precautions+ Surgical Mask Single Room (formerly droplet isolation). This shift simplifies understanding required precautions across all professionals in contact with these patients.

Conclusion: Introducing "Standard Precautions+" represents a significant step forward in infection prevention, offering a more nuanced and adaptable framework that reflects the complexity of pathogen transmission. Early feedback suggests high acceptance and appreciation for the more precise, actionable guidance. This initiative underscores the importance of evolving infection prevention practices in line with scientific advancements and operational realities, potentially setting a new standard for hospitals and healthcare settings worldwide. A evaluation of the measures is planned to close possible gaps.

P125

Development and validation of selection algorithms for a non-ventilator-associated hospital-acquired pneumonia (nvHAP) semi-automated surveillance system

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Objectives: Semi-automated surveillance systems are a time-saving alternative to traditional manual surveillance (1, 2). This is especially true for non-ventilator-associated hospital-acquired pneumonia (nvHAP), an infection all non-intubated patients are at risk for (3). In this study, we aimed to evaluate the performance characteristics of single indicators and algorithms that pre-select patients for manual nvHAP chart review.
Methods: Single indicators were identified based on literature, expert opinion and data availability. They were then combined in simple and complex algorithms. All single indicators and algorithms were applied on a patient cohort of 157,902 patients from a 4-year period. This cohort included 947 patients with nvHAP identified by a validated semi-automated nvHAP surveillance system plus the manual surveillance of patients with ICD-10 discharge diagnostic codes (gold standard). The performance characteristics assessed were sensitivity, specificity, workload reduction, overall performance, and number needed to screen.
Results: Compared to the gold standard, single indicators had a sensitivity from 35.1% (worsening oxygenation) to 99.7% (radiologic procedure). The workload reduction varied from 57.3% (length of hospital stay >5d) to 98.4% (ICD-10 discharge diagnostic code). The single indicator radiologic procedure of the chest was highly sensitive (99.7%) and reduced manual review by 82.8%, or by 90.3% when considering the full text of radiologic reports (sensitivity 99.3%). The highest workload reduction was found in complex algorithms, e.g. the combination "radiologic procedure including full text AND temporally related abnormal WBC or fever AND anti-infectives AND CRP AND decreased oxygenation AND stay ≥5 days AND no intubation" had a workload reduction of 96.2% by maintaining a sensitivity of 92%.
Conclusions: We identified several single indicators and algorithms with a high workload reduction and a sensitivity above the PRAISE-network defined sensitivity threshold of 90% (4). Our results could assist hospitals in developing algorithms customized to their specific local conditions, consequently enabling semi-automatic surveillance of nvHAP.

P126

Screening for latent tuberculosis among healthcare workers in a low incidence setting : A retrospective observational study in a large cantonal Swiss hospital.

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Background: The cost-effectiveness of systematic latent tuberculosis infection (LTBI) screening for healthcare workers (HCWs) in low-incidence Tuberculosis (TB) countries remains unclear. Our study aims to estimate LTBI incidence and management of cases among healthcare employees screened at recruitment in a large Swiss cantonal hospital, as well as lab costs of the screening strategy.

Methods: This retrospective observational study involved all employees in contact with patients newly hired at Valais Hospital from 1 January 2019 to 31 December 2022. LTBI screening was performed using an Interferon Gamma Releasing Assay (IGRA) (QuantiFERON-TB Gold Plus ®). Positive cases were assessed for TB preventive treatment based on specific criteria, including age and additional risk factors, after ruling out TB disease. Data on screening outcomes, treatment acceptance, and associated lab costs were collected and analyzed. LTBI screening strategies in HCWs of several Swiss hospitals through were also collected through a rapid survey among occupational health divisions.

Results: Out of 1 696 IGRA tests performed, 53 (3.1%) returned positive. A third of the positive tests (17/53; 32.0%) were in HCWs that had already been diagnosed with LTBI in the past. These individuals were not offered a TB preventive treatment. A significant proportion of HCWs with positive IGRA (22/53, 41.5%) either did not meet preventive treatment criteria or were lost to follow-up. Only 14/53 (26.4%) individuals were offered a preventive treatment, and only 5 of them (35.7%) started it. One employee who refused the preventive treatment subsequently developed a TB disease. The estimated lab cost of IGRA testing for the 4-year period was CHF 207 929. Five of the seven hospitals surveyed had a LTBI screening policy on recruitment based on the risk of subsequent occupational exposure (employees in contact with patients, laboratory or environment).

Discussion: The low positivity rate of LTBI screening among new employees and the even lower treatment uptake highlight the challenges in managing LTBI in a low incidence setting. Despite rigorous screening policies, ensuring adherence to preventive treatment remains problematic. These findings question the cost-effectiveness and practical value of routine LTBI screening at recruitment in low TB incidence environments, especially considering the strict infection control measures in case of suspected or TB case.

P128

Administration of Cephalosporine Surgical Antimicrobial Prophylaxis in Low-risk Cholecystectomy and Its Association with Surgical Site Infections

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Aims
Current guidelines do not recommend routine surgical antimicrobial prophylaxis (SAP) administration prior to low-risk cholecystectomy (LR-CCE). However, data from large real-life cohorts supporting this recommendation is limited.
Our Aim was to assess whether administration of cephalosporine ± metronidazole SAP versus absence of SAP is associated with a decreased risk of surgical site infections (SSI) in LR-CCE.
Methods
This cohort study included adult patients who underwent LR-CCE, documented by the Swissnoso SSI surveillance system between 1/2009 - 12/2020 at 66 Swiss hospitals, with a completed 30-day follow-up. LR-CCE was defined as elective endoscopic surgery, age < 70, no active cholecystitis, ASA score < 3, operating time < 120 minutes without implantation of foreign material.
Exposure was defined as administration of cefuroxime or cefazoline ± metronidazole within 120 minutes prior incision versus no SAP administration.
Main Outcomes and Measures were the occurrence of SSI until day 30 according to CDC definitions. Logistic regression models were used to adjust for institutional, patient, and perioperative variables.
Results
Of 44 682 surveilled adult cholecystectomy patients, 12 521 (8 726 women [69.7%]; median [IQR] age, 49.0 [38.1 - 58.2] years), fulfilled inclusion criteria. SSI was identified in 143 patients (1.1%): 106 (0.8%) with a superficial incisional, 12 (0.1%) with a deep incisional and 25 (0.2%) with an organ-space SSI. SAP was administered in 9 269 patients (74.0%); mostly Cefuroxime (6 403, 69.1%). Metronidazole was added in 466 patients with SAP (5.0%). SAP was significantly associated with a lower SSI rate (adjusted odds ratio [aOR], 0.49; 95% confidence interval [CI], 0.35 - 0.70; p < .001). Additional analyses showed that SAP administration was associated with a lower superficial (aOR, 0.45; 95% CI, 0.30 - 0.70; p = 0.01) and deep incisional SSI rate (aOR, 0.22; 95% CI, 0.06 - 0.67; p < .001), but not with a lower organ-space SSI rate (aOR, 1.37; 95% CI, 0.54 - 4.18; p = 0.53).
Conclusions and Relevance
Overall LR-CCE SSI rate was low. However, cephalosporine SAP administration was associated with significantly lower risk of LR-CCE SSI, mainly due to lower odds of superficial and deep incisional SSI. Our results suggest that cephalosporine SAP should also be administered in LR-CCE.

P129

Contact! – Retrospective Analysis of MDR-Contact-Tracing in a low endemic setting

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Introduction:
Multi-drug resistance (MDR) is a significant public health concern because it complicates the management of infections, leading to higher morbidity, mortality, and healthcare costs.
Inadequate infection control measures or lack of awareness of colonization can lead to exposure with MDR in the inpatient setting. Guidelines do recommend to actively screen these contact-patient to identify possible transmission. We examine transmission rates in our own cohort of contact-patients.

Methods:
This retrospective study was conducted at a medium-sized 400-bed hospital in Switzerland. We maintain a systematic database of all patients colonized with multi-drug resistant (MDR) organisms since January 2022. Patient whose MDR-colonization was initially unknown were identified. Then contact-patients were searched using the electronic-medical record database. The criteria for defining a contact and the subsequent procedures followed are in alignment with national guidelines published in 2021. Statistical analysis was conducted to describe the population of index-patients as well as contacts-patients and possible transmissions.

Results:
We identified 112 index patients, of which 47 were either outpatients or had no contacts. The majority of the index cases were colonized with MRSA (86), followed by CPE (13 cases) and VRE (7 cases). In total, we found 117 contact patients. The mean number of contacts was as follows: CPE: 1.13 contacts, MRSA: 2.15 contacts, and VRE: 1.75 contacts, respectively. CPE had a mean contact time of 3.31 days, MRSA had a mean contact time of 2.27 days, and VRE had a mean contact time of 2.0 days. All contacts of CPE were screened and were negative. Only 27% of MRSA contacts were screened. One MRSA contact was positive, but genotyping did not show a correlation to the index case. None of the VRE contacts were screened.

Conclusions:
In our retrospective analysis, we observe that active follow-up of MDR contacts succeeds in only a limited portion of the population. The reasons for this may be multifaceted. Primarily, many contact patients have already been discharged by the time we became aware of the indication for screening.
Nevertheless, the yield from the contact screenings that were conducted was very low. Given the expenses and costs this incurs, we are questioning the current approach.
In need of further data, we plan to conduct a prospective analysis of contact patients.

P130
Defining Criteria for the Discontinuation of Isolation in Patients with Carbapenemase-Producing Enterobacteriaceae – A Real-World Experience

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Background: Swissnoso emphasizes the critical role of enhanced infection prevention measures, including isolation and tailored assessments by the infection prevention team, for discontinuing isolation in the context of managing carbapenemase-producing Enterobacteriaceae (CPE). This study evaluates the duration until de-isolation in a real-world setting.

Methods: This analysis included inpatients and outpatients at the Cantonal Hospital of St. Gallen with an initial CPE detection between February 2012 and February 2023, who underwent at least one follow-up screening. We examined patient and isolate baseline characteristics, the number of follow-up screenings, and the time from initial detection to de-isolation or the last control screening. The study also evaluated the incidence of screening failures among de-isolated patients.

Results: Among 121 patients diagnosed with CPE, 55 received at least one follow-up screening. Of these 55, 40 were male (73%). The median age at initial detection was 60 years (range: 15-84 years). Acinetobacter spp. (n=21; 38%) and Klebsiella pneumoniae (n=13; 24%) were the predominant pathogens identified. The initial detection was most commonly gastrointestinal (n=28; 51%). Among 40 patients (76%) without de-isolation, median follow-up was 11 months (range: 0-81 months) with a median of 1 screenings (range: 1-13). Among 15 patients (27%) with de-isolation, median follow-up was 22 months (range: 10-85 months) with a median of 3 screenings (range: 1-9). Post-de-isolation screenings in 7 patients (47%) revealed no failures.

Conclusion: The median time to de-isolate CPE carriers in this real-world cohort exceeded two years, highlighting a reluctance to end isolation after initial detection, underscored by an extremely broad range. In this small sample, no re-detection of CPE was observed after de-isolation. Further investigation and systematic follow-up are necessary to assess the safety of reducing isolation duration and to define criteria for discontinuing isolation.

P131
Hygienebeauftragte Personen: Wie kommt Schwung in die Rolle?

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Ziele
Nosokomiale Infektionen stellen im Spitalalltag eine grosse Herausforderung dar. Um die Umsetzung der Hygienevorschriften auf den Abteilungen zu verbessern, arbeitet die Inselgruppe mit hygienebeauftragten Personen (HP). Dies sind Personen die auf Pflegeabteilungen tätig sind aber vertieft in Infektionsprävention geschult wurden. Um diese in ihrer Rolle besser unterstützen zu können, und den Informationsfluss zu verbessern, wurde ein Merkblatt mit den möglichen Aufgaben einer HP sowie ein Newsletter eingeführt. Da dies als sehr positiv rückgemeldet wurde, stellte sich die Frage: Wie kann die Spitalhygiene eine hygienebeauftragte Person befähigen, ihre Rolle im Alltag umzusetzen und welche konkreten Massnahmen zur Unterstützung können dafür geplant und implementiert werden?
Methode
Es wurde eine Literaturrecherche, sowie qualitative Interviews mit sechs hygienebeauftragten Personen durchgeführt. In den Interviews konnten die hygienebeauftragten Personen ihre Wünsche und Bedürfnisse äussern, welche für die Ausübung ihrer Rolle unterstützend wären. Die Interviews erfolgten im Einzelgespräch während maximal 45 Minuten.
Resultate
In den Interviews zeigt sich: Zeit und ein klarer Rahmen sind zentrale Faktoren, damit die HP sich in ihrer Rolle ausleben können. Um ihre Tätigkeit sicher ausüben zu können, benötigen sie das Wissen und die Unterstützung durch die Spitalhygiene. Alle interviewten HP zeigten sich sehr motiviert. Die hohe Arbeitsbelastung aufgrund der Personalmangellage wird als Limitation in der Umsetzung der Funktionserfüllung wahrgenommen ist. Eine stärkere Gewichtung des Themas Infektionsprävention im Alltag wäre ebenfalls unterstützend. HPs wünschen sich mehr Informationen, Materialien, Unterlagen oder Arbeitsinstrumente von der Spitalhygiene, um ihre Aufgaben besser umsetzen zu können.
Schlussfolgerung
Der wiederholt erwähnte Wunsch nach mehr Arbeitsinstrumenten wurde aufgenommen: Es wurde im Hygieneordner ein neuer Bereich (in Form einer Kachel) implementiert, welcher für die hygienebeauftragten Personen gedacht ist. Darin erhalten die HP Informationen zu Schulungs-/Weiterbildungsangeboten, Checklisten um Überprüfungen oder Einführungen strukturiert durchführen zu können, Informationsblätter welche sie zu Schulungen oder für Themenwochen benutzen können und eine Ablage von Newslettern, damit auch neue HP an ältere Informationen kommen. Damit sollen HP Informationen niederschwellig und ohne grossen zeitlichen Aufwand abholen können.

P132
Identifying patients at high risk for multidrug-resistant organisms after hospitalization abroad

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Aims: The number of patients with multidrug-resistant organisms (MDRO) is rising worldwide. Low-prevalence regions attempt to prevent hospital introduction of MDRO by patients repatriated from high-prevalence healthcare settings by screening and isolation. We aimed to quantify the percentage of MDRO carriers among repatriated patients, identify factors associated with MDRO carriage, and evaluate the yield of MDRO detection per screened body site.

Methods: This retrospective cohort study was conducted at a tertiary care center in Switzerland and included adult patients who had spent more than 24 hours in a healthcare institution abroad. These patients underwent screening for MDRO carriage, with samples taken from standard sites such as the nose and throat, groins, and, starting from mid-2018, the rectum. Additionally, samples were collected from risk-based sites including wounds, urine, and tracheal secretions. MDROs were defined as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), extended-spectrum β-lactamase (ESBL)- and carbapenemase- producing Enterobacterales (CPE), multidrug-resistant (MDR) Enterobacterales, and MDR nonfermenting gram-negative rods. Risk factors for MDRO carriage were assessed using multivariate logistic regression.

Results: Between May 2017 and April 2019, 438 patients were screened and 107 (24.4 %) tested positive for an MDRO, primarily for ESBL-producing and MDR Enterobacterales. Risk factors for MDRO colonization were the length of stay in a hospital abroad, region of hospitalization abroad and antibiotic use categorized by the WHO as 'Watch' and 'Reserve'. Rectal swabs had the highest yield for detecting patients with MDR intestinal bacteria, while nose/throat and groins, or wound samples showed higher sensitivity for MRSA or nonfermenting gram-negative organisms, respectively.

Conclusions: We identified risk factors for MDRO carriage and body sites with the highest yield for a specific MDRO, which might help to target screening and isolation and reduce screening costs.

P133
Antibiotic Stewardship Interventions in a Geriatric Hospital by Clinical Pharmacists

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Background and Aim
The national strategy to combat antimicrobial resistance 3 is currently focusing on the implementation of antibiotic stewardship in Swiss Hospitals and is supported by associations of various professional groups (Swissnoso 2023).
The aim of this project was to assess the potential of antibiotic stewardship interventions by clinical pharmacists in geriatric medicine.

Methods
Over a period of three months (01/2024 - 03/2024), each antibiotic prescription was assessed by a clinical pharmacist. Interventions were suggested to the physician and the implementation rate was calculated. Indication and choice of substance were described.

Results
In total, 409 antibiotic prescriptions were assessed. In 21 % of the prescriptions, the clinical pharmacist had suggestions for changes in therapy. For 16 % (66/409) of antibiotic prescriptions, recommendations were communicated. 66 % of the recommendations were implemented or lead to other therapy adjustments.
32 % of antibiotics were prescribed for pneumonia, 27 % for urinary tract infections and 10 % for bacteraemia including (uro)-sepsis. Ceftriaxone was the substance used the most (26 %), followed by amoxcillin/clavulanic acid (24 %) and piperacillin/tazobactam (17 %).

Conclusion
Clinical pharmacist can contribute to optimize antibiotic therapy, especially in wards with mainly uncomplicated infections such as pneumonia and urinary tract infections. The high implementation rate highlights the acceptance of this interprofessional treatment approach. This indicates that existing structures of various professional groups should be expanded for antimicrobial stewardship activities.

P134

Update of the guidelines of prevention and treatment of infection in nursing homes: A paradigm of intercantonal collaboration for antimicrobial stewardship in long-term care

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Aims: The frequency and non-specific presentation of infections in the elderly together with the lack of direct medical access in most Swiss nursing homes (NHs) impose tailored guidelines for infection prevention and treatment in this context. The pocket guide of infection prevention and treatment in NHs was created in 2013 under the coordination of the infection prevention and control unit of Vaud (HPCi Vaud) with an update in 2018. A new update was undertaken in 2024.

Methods: HPCi Vaud coordinated the 2024 update. A redaction group comprising of 5 geriatric, 1 infectious disease (ID) specialist and 1 pharmacist, was responsible for reviewing the previous recommendations and adapting them to new evidence considering the particularities and constrains of NHs. All proposed changes were validated by a second independent group of 22 professionals with diverse profiles that included doctors and infection prevention and control nurses in NHs, ID, geriatric, and palliative care specialists. The groups included professionals from 7 cantons with a representation of the 3 major Swiss linguistic regions.

Results: The main updates of the 2024 version were: restriction of the geriatric syndromes that should lead to infection investigations to delirium, introduction of an algorithm for antimicrobial treatment documentation and reevaluation, reduction of treatment duration of urinary tract infections and bacterial pneumonias and incorporation of SARS-CoV-2 in the respiratory infection chapter. Modifications in the prevention chapter included introduction of new vaccination recommendations for zoster, pneumococcal pneumonia, and COVID-19.

Conclusions: The update of guidelines is currently finalized, and next steps include issuing of the updated pocket guide and its dissemination in NHs of the participating cantons. HPCi Vaud is also exploring ways of giving access to the guidelines via a mobile application. This exceptional intercantonal collaboration underlines the commitment of participating cantons towards antimicrobial stewardship implementation in long-term care.

P135

Long-term, silent MRSA ST4110 outbreak in outpatient wound care revealed by cgMLST

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Aim
Community- and healthcare-associated methicillin-resistant Staphylococcus aureus (MRSA) case numbers have been declining in Switzerland and other European countries. An increase in MRSA cases in May 2023 at two acute care hospitals with no apparent epidemiological link prompted in-depths investigations.

Methods
In a case study and retrospective chart review of recent MRSA cases three of 35 patients exhibited chronic wounds raising the suspicion of transmission through wound care. Whole genome sequencing (WGS) and core genome multi-locus sequence typing (cgMLST) showed all belonging to ST 4110, a strain that has previously been described in small numbers in Germany and England. Concerned about a larger reservoir in outpatient wound care, we extended the chart review to the preceding six months, identifying 75 patients with same day treatment as the index cases. Screenings targeted at patients with chronic wounds and multiple interactions with an index case within this time. Further measures involved observations of wound care managers and environmental sampling.

Results
Of 76 patients screened, eleven (14.5%) tested positive for MRSA, with seven (73.3%) showing the ST4110 strain in their wound. Pooled screenings of nose, throat, axilla, and groin remained largely negative. Four of 19 environmental samples (21.1%) were also positive for MRSA ST-4110. All isolates clustered within 24 allelic differences. Observations of wound managers revealed frequent and prolonged glove use combined with interruptions during wound care through phone calls resulting in repeated breaches of standard hygiene measures. In response, training in hand hygiene and environmental cleaning and disinfection wasoffered. Additionally, organizational adjustments, such as call diversion during treatment and clear separation between clean and dirty zone, were undertaken. Subsequent weekly surveillance cultures revealed no further cases. Healthcare personnel screened negative for MRSA.

Conclusion
High level of suspicion and a careful case study unveiled silent transmission of this rarely occurring ST4110 MRSA in patients with chronic wounds. Insufficient human resources coupled with substantial lapses in standard precautions likely facilitated prolonged circulation of this strain among patients. WGS and cgMLST allowed us to confirm relationships between isolates, pinpoint the source, and prompted targeted control measures.

P136

Success rates of methicillin-resistant Staphylococcus aureus (MRSA) decolonization and factors associated with failure over a 16 year-period

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Aims
To evaluate the success rate of methicillin-resistant Staphylococcus aureus (MRSA) decolonization and to identify risk factors for decolonization failure.

Methods
In our tertiary care centre, we prospectively registered patients with MRSA colonization since 2007 and collected anthropometric data, comorbidities including presence of wounds or catheters, and data on colonization such as date and circumstances of detection (screening vs. clinical), colonized sites and date of decolonization as well as results of control swabs. Decolonization consists of a 5 day regimen with daily chlorhexidine body wash, mupirocin nasal ointment, chlorhexidine mouth wash, and systemic antibiotic treatment for selected cases. Control swabs are scheduled at 1, 3, 6 and 12 months post-decolonization. In case of decolonization failure (defined as a MRSA detection in one of the control swabs) patients can undergo further decolonization cycles.
We assessed the success rate for decolonization cycles, including all decolonizations with ≥2 control swabs with a minimal follow-up of 6 months. We used multivariable logistic regression to assess risk factors for failure (random effects model to account for multiple episodes within the same patient), accounting for the above-mentioned co-variables including period of decolonization (i.e. 2007-2012, 2013-2017, 2018-2023); adjusted odds ratios (aOR) and corresponding confidence intervals (CI) were calculated.

Results
From 2007 to 2023, we registered 728 decolonization cycles in 1196 patients with a success rate of 50.3%. For 105 cycles, systemic antibiotics were used.
Success rate was 56.9% for the first decolonization cycle (n=275), 38.5% for the second cycle (n=58), and 36.3% for cycles three or higher (n=33).
In multivariable analysis, throat colonization (aOR 2.03, 95% CI 1.2 – 3.4, p < 0.01) and number of previous decolonization cycles (aOR 1.3 per cycle, 95% CI 1.03 – 1.6, p 0.02) were risk factors for failure. Period of decolonization (aOR 0.8, 95% CI 0.53-1.3, p 0.4) and use of systemic antibiotics (aOR 1.0. 95% CI 0.6 - 1.7, p 0.99) were not associated with the outcome.

Conclusion
Over a 16-year period, only 50.6% of all decolonizations were successful.
Decolonization failure is associated with MRSA throat colonization; also, the failure rate increases with every additional decolonization cycle, irrespective of the use of systemic antibiotics.

P137

Evaluating and Enhancing the Continuing Education of Infection Prevention Specialists in the German-speaking Part of Switzerland: Bridging the Gap for Effective Healthcare-Associated Infection Prevention

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Background: Healthcare-associated infections (HAI) pose significant challenges, requiring comprehensive efforts. Infection prevention (IP) specialists are crucial, as their education and continuous professional development are vital for implementing preventive measures, educating healthcare staff, and conducting research. This study examines the continuing education and core competencies of these specialists in Switzerland, assessing their readiness and the educational system's ability to meet evolving healthcare demands. It aims to identify gaps in the current educational framework and proposes recommendations for enhancing their education.

Methods: In this study, a qualitative study design was chosen using semi-structured expert interviews to gain detailed insights into the perceptions of current and desired continuing education and core competencies of IP specialists in Switzerland. A purposive sampling strategy was used to select participants to ensure the different perspectives of eight infection prevention leaders from different universities and cantonal hospitals in German-speaking Switzerland.

Results: The study identified deficiencies in the current continuing education for IP specialists in Switzerland. The current education system falls short of the expectations and requirements of leaders in infection prevention, with substantial critiques regarding the content of courses and their lack of practical applicability. A need for standardization of the roles and competencies expected of IP specialists was expressed. These findings suggest that significant revisions of existing continuing education programs would be necessary to better meet contemporary healthcare needs.

Conclusion: The identified gaps in the continuing education of IP specialists draw attention to the need for revision and updating of existing programs. Standardization and clarification of roles and competencies are crucial to align with the expectations of leaders in infection prevention. However, this study recognizes its limitations in specifying the exact contents and structures for such education. Consequently, it suggests further comprehensive research with broader stakeholders and settings. This additional research could provide a more detailed depiction of the subject, facilitating the development of an educational system that effectively equips infection prevention specialists to tackle the challenges of HAI.

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Automated surveillance of non-ventilator-associated hospital-acquired pneumonia (nvHAP): a systematic literature review

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Background: Hospital-acquired pneumonia (HAP) and its specific subset, non-ventilator hospital-acquired pneumonia (nvHAP) are significant contributors to patient morbidity and mortality. Automated surveillance systems for these healthcare-associated infections have emerged as a potentially beneficial replacement for manual surveillance. This systematic review aims to synthesise the existing literature on the characteristics and performance of automated nvHAP and HAP surveillance systems.

Methods: We conducted a systematic search of publications describing automated surveillance of nvHAP and HAP. Our inclusion criteria covered articles that described fully and semi-automated systems without limitations on patient demographics or healthcare settings. We detailed the algorithms in each study and reported the performance characteristics of automated systems that were validated against specific reference methods. Two published metrics were employed to assess the quality of the included studies.

Results: Our review identified 12 eligible studies that collectively describe 24 distinct candidate definitions, 23 for fully automated systems and one for a semi-automated system. These systems were employed exclusively in high-income countries and the majority were published after 2018. The algorithms commonly included radiology, leukocyte counts, temperature, antibiotic administration, and microbiology results. Validated surveillance systems' performance varied, with sensitivities for fully automated systems ranging from 40 to 99%, specificities from 58 and 98%, and positive predictive values from 8 to 71%. Validation was often carried out on small, pre-selected patient populations.

Conclusions: Recent years have seen a steep increase in publications on automated surveillance systems for nvHAP and HAP, which increase efficiency and reduce manual workload. However, the performance of fully automated surveillance remains moderate when compared to manual surveillance. The considerable heterogeneity in candidate surveillance definitions and reference standards, as well as validation on small or pre-selected samples, limits the generalisability of the findings. Further research, involving larger and broader patient populations is required to better understand the performance and applicability of automated nvHAP surveillance.

P139

Effect of masking policy on influenza vaccine uptake among personnel of long-term care facilities of Vaud

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Aims: Vaccination of personnel in long-term care facilities (LTCF) against seasonal influenza is crucial for the protection of residents. Since September 2015, canton Vaud mandates face masks for influenza-unvaccinated LTCF personnel during influenza seasons. We aimed to investigate the impact of this policy on influenza vaccine uptake among personnel. Additionally, we investigate the impact of universal masking policies during the COVID-19 pandemic.

Methods: We conducted a retrospective before-after study. The effects of Intervention 1: vaccinate against influenza or mask (onset in 2015-16 season), and Intervention 2: universal masking independently of vaccination status (onset in 2020-21 season), were assessed using a segmented multilevel binomial regression model accounting for the clustering of data within institutions. Only LTCFs with complete data from 2011-12 through 2022-23 seasons were included.

Results: Ninety-six (60 %) of the 160 LTCFs of Vaud were included. Increasing trends of vaccine uptake were identified in 48 (50 %) LTCFs before Intervention 1, compared to 62 (66 %) between Intervention 1 and 2 ($p = 0.04$) and 10 (10 %) after Intervention 2 ($p < 0.01$). The model showed that before Intervention 1 the odds of vaccination uptake was growing at a rate of 3% per year (95 % Confidence interval (CI): 1 – 5 %, $p < 0.01$). Intervention 1 led to an immediate increase of the odds of vaccination by 12% (95 % CI: 5 – 19 %, $p < 0.01$) and a supplementary subsequent sustained increase of 5% per year (95 % CI: 2 – 7 %, $p < 0.01$) up to Intervention 2. Intervention 2 had no significant immediate effect but was associated with a sustained decrease of the vaccination odds of -36% (95 % CI: -38 – -34 %, $p < 0.01$).

Conclusions: Our results suggest that masking policy can significantly impact influenza vaccination uptake. Intervention 1 had significant immediate and sustained effects over time while the arrival of COVID-19-related Intervention 2, practically cancelling Intervention 1, led to striking decreases in vaccination uptake. Follow-up is necessary to evaluate if return to previous masking policy standard will restore pre-pandemic vaccination rates in LTCFs.

P140

Survey of excreta management in nursing homes of Vaud: a first step towards the development of targeted actions

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Aims: Safe excreta management in healthcare facilities is crucial for the protection of residents and healthcare workers (HCW) and decreases the risk of outbreaks and the spread of multidrug resistant organisms. Data on excreta management in nursing homes (NH) are scarce. We aimed to investigate multiple aspects related to excreta management in the nursing homes of Vaud.
Methods: The cantonal Infection Prevention and Control unit of Vaud (HPCi Vaud) developed a questionnaire examining internal protocols, NH architecture, architecture and equipment of sluice rooms, practices of transport of excreta-related medical devices (MD), elimination of excreta, MD washing and disinfection, and use of washer-disinfectors (WD). HPCi Vaud invited infection prevention and control link nurses and chief nurses of the 120 NHs of the canton to answer the questionnaire.
Results: Response rate was 73 % (87 / 120 NHs). The median of individual room percentage was 86% and of rooms with toilet 43 %. Only 33 % of NHs had protocols for excreta management. Separation of clean and contaminated compartments in sluice rooms was present in 43 % of institutions. Presence of complete materials for hand hygiene and personal protection were present in 73 % and 24 % of cases respectively. All NHs had washer-disinfectors while 89 % of them had the recommended number. DM transport was always done with a cover in 33 % and gloves were used for the transport in 98 % of NHs. Only in 32 % NHs elimination of excreta was done exclusively in the WD. Manual washing of MD was exceptional (2%). WD were validated and maintained in the recommended frequency in 92% and 99% of NHs, respectively.
Conclusions: This extensive survey highlighted that protocols, hand hygiene and use of personal protective equipment constitute actionable priorities. Additionally, it underscored the need for regular training of HCWs on appropriate practices regarding the transport of MD. Following the survey, each participating NH received a personalized consultation pinpointing areas for improvement. Additionally, HPCi Vaud initiated training sessions on safe excreta management tailored for NH professionals and is finalizing the creation of excreta management guidelines for NHs.

P141
Sensibilisierungs-Kampagne zur Händehygiene an einer Universitätsklinik "Geringer Aufwand, grosse Wirkung" - Analyse vor, während und nach Intervention

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Ziel: Multimodale, multidisziplinäre Händehygiene-Kampagne zur Sensibilisierung aller Berufsgruppen mit Patientenkontakt.

Methode: Multimodale, repetitive Intervention der Spitalhygiene und Unternehmenskommunikation von August 2023 bis Januar 2024 mit aktiver Unterstützung durch Medizinische Direktion, Pflegedienst, Hauswirtschaft, 20 Link Nurses und Stationsleitungen; basierend auf Informationen, Reduktion des nicht-indizierten Handschuhgebrauchs, immediate Restitution, Role Modeling und konstantem Monitoring. Mit folgenden Massnahmen: wissenschaftliche Beiträge im Mitarbeitermagazin, Intranet und Kolloquien, Videobotschaften mit Testimonials, Awareness-Stickers auf Boden in zielgruppenrelevanten Räumen, klinikweit auf Büromaterial und Desinfektionsmittel-Spendern; immediate Restitution der Beobachtungsergebnisse, und repetitives Verteilen von Desinfektionsmitteln und Infokarten. Zudem punktuelle Interventionen bei spezifischen Problemen bezüglich der Spitalhygiene. Kampagne war für Projektbeteiligte mit zusätzlichem Arbeitsaufwand verbunden. Für kommunikative Massnahmen und Materialien wurde ein Projektbudget bereitgestellt.

Ergebnisse: Wir beobachteten 2059 Handlungen. Die Compliance der Pflegefachpersonen vor Patientenkontakt stieg von n=56 (71%) auf n=210 (78%,) nach Patientenkontakt von n=64 (80%) auf n=280 (83%), nach Körperflüssigkeiten von n=46 (75%) auf n=108 (87%), vor invasiven Tätigkeiten von n=28 (62%) auf n=80 (66%) und nach der Patientenumgebung von n=42 (82%) auf n=132 (92%) an. Bei der (chirurgischen) Ärzteschaft verbesserte sich die Compliance vor Patientenkontakt von n=37 (77%) auf n=203 (78%), nach Patientenkontakt von n=64 (80%) auf n=283 (94%), nach Körperflüssigkeiten von n=17 (74%) auf n=94 (92%), vor invasiven Tätigkeiten von n=15 (60%) auf n=66 (72%) und nach der Patientenumgebung von n=26 (84%) auf n=117 (86%). Insgesamt verbesserte sich die Händehygiene über alle Berufsgruppen hinweg von 79% auf 84%. Konsum der Händedesinfektionsmittel nahm kampagnenbedingt zu, Anzahl der frühen wund-assoziierten Infektionen tendenziell ab, wobei beim letzteren die Surveillance-Periode noch weiterläuft. Die Gesamtinzidenz aller nosokomialen Infektionen blieb tendenziell gleich.

Schlussfolgerungen: Die Händehygiene-Kampagne war erfolgreich. Die Herausforderung liegt jetzt in der Nachhaltigkeit. Repetitive (multimodale) Interventionen sind geplant.

P142
"They are from another planet …": a Social Network Perspective on Infection Prevention in Surgery

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Aims
Healthcare-associated infections (HAIs), including surgical site infections (SSIs), are a global challenge, significantly impacting patient outcomes and increasing healthcare costs. Surgical environments are characterised by complex settings, interdisciplinary teams, and diverse risk factors, making it difficult to consistently apply infection prevention and control (IPC) measures. This first study used a social network approach to elucidate surgical and IPC teams' beliefs, collaboration, and information flow for SSI prevention.

Methods
Semi-structured interviews were conducted with healthcare professionals involved in surgery and IPC across multiple Swiss hospitals. The interviews were recorded and transcribed verbatim, followed by iterative inductive coding to extract pertinent themes. The analysis focused on the nuances of social dynamics and interactions, underpinning views, and the application of IPC measures.

Results
We interviewed 11 healthcare professionals; nurses (5), surgeons (2), and physicians (4) across neurology, visceral surgery, anaesthesia, and IPC in 5 Swiss hospitals of different size categories. Three major themes emerged: 1. 'Different Planets': This quote points to a significant gap between the belief systems of surgical staff and IPC teams and a disconnect between guidelines and the clinical context. 2. Boundary-Spanners: The ease of collaboration and information flow through boundary-spanners. IPC experts with surgical frontline experience or link-nurses with supervising positions act as horizontal or vertical boundary-spanners. 3. Time Spent Together: Informal relations, often based on common personal histories, scientific interests, or collaboration in targeted IPC interventions, can bridge such 'interplanetary gaps'.

Conclusions
Bridging the gap between surgical practice and IPC necessitates harnessing the power of boundary-spanners, engaging personalities and nurturing informal networks. Involving IPC experts with direct surgical experience through interventions or joint-collaboration projects promotes closer collaboration and informal interactions across team members. The insights gained will help design a larger quantitative social network study across Swiss hospitals and to further explore the effects of social networks on IPC measures.

P143

Performance evaluation of an automated surveillance algorithm for central line-associated bloodstream infection (CLABSI)

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Aims: Surveillance of central line-associated bloodstream infection (CLABSI) is part of healthcare-associated infection prevention programs. Since 2004, an automated hospital-wide surveillance algorithm (Episcope) categorizing as CLABSI episodes with coagulase-negative staphylococci bacteraemia or those with the same organism cultured from a catheter tip within 72 hours of first positive blood cultures; its sensitivity and specificity was initially calculated at 80%. Our aim was to evaluate and enhance the performance of the actual automated surveillance algorithm for CLABSI.
Methods: This retrospective study was conducted at the Lausanne University Hospital, Switzerland, from 2015 to 2021. All episodes with positive blood cultures with staphylococci, streptococci, Pseudomonas aeruginosa or Candida spp among adult patients were reviewed. Episodes were categorized as CLABSI based on clinical and microbiological data. Episcope performance (sensitivity, specificity, accuracy) was evaluated with the current algorithm then after improvement.
Results: Among the 2191 included bacteraemia/candidaemia episodes, the distribution was as follows: 846 (39%) S. aureus, 238 (11%) coagulase negative staphylococci, 707 (32%) streptococci, 265 (12%) P. aeruginosa, and 209 (10%) Candida spp, with 360 (16%) being polymicrobial. A central venous catheter was present in 895(41%) episodes, of which 533 (60%) had a catheter tip culture performed; among these, 195 (37%) exhibited a positive catheter tip culture with the same organism. A differential time to positivity was present in 159 (17%) episodes of patients with central venous catheter. A total of 474 (22%) episodes were classified as CLABSI. The actual Episcope algorithm categorized 343 (16%) episodes as CLABSI. Sensitivity, specificity and accuracy were 55%, 95%, and 86%, respectively. By incorporating into a new algorithm, the differential time to positivity and the presence of catheter tip culture (irrespectively of the result of the culture) from 72 hours before to 96 hours after the first positive blood culture, 625 episodes were classified as CLABSI. Sensitivity, specificity and accuracy were 93%, 89%, and 90%, respectively.
Conclusion: The sensitivity of the actual Episcope algorithm was lower than initially calculated. The updated algorithm, demonstrated improved sensitivity in detecting CLABSI.

P144

A prolonged methicillin-resistant Staphylococcus aureus outbreak in an ICU detected through regular screening.

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Background/Aim: Despite infection control efforts, methicillin-resistant Staphylococcus aureus (MRSA) infections remain a burden in hospitals, requiring ongoing surveillance and intervention strategies. We aim to report an MRSA outbreak in an adult intensive care unit (ICU) that was detected through regular patient screening.

Methods: Following the detection of a few patients with MRSA infections or colonisation with isolates showing identical double-locus sequence typing (DLST) genotype in the ICU of a large cantonal hospital in Switzerland, an investigation was conducted by the infection control team. A 3-year retrospective evaluation of all MRSA cases detected in the ICU was performed. Patient medical charts were reviewed, and descriptive analyses including demographics and infection characteristics were performed. Whole genome sequencing of MRSA strains with similar DLST was performed.

Results: The outbreak included 16 MRSA cases from April 2021 to August 2023, with patients predominantly male (13/16) and with a median age of 66 years (IQR 61-75). Half of the cases (8/16) were identified through routine MRSA screening (groin and nasal swabs). Most patients developed an MRSA infection during their ICU stay (11/16, 68.7%), mainly ventilator-associated pneumonia (9/16, 56.2%) and/or bacteraemia (3/16, 18.7%). Seven (43.7%) patients died in the ICU with an active MRSA infection. Whole genome sequencing revealed two distinct clusters of 14 and 2 strains respectively, suggesting clonal spread within the ICU. Infection control measures, such as increased hand hygiene proper glove use and proper disinfection of reusable medical devices, were reinforced. MRSA screening of patients was intensified to weekly screening at the time of the outbreak detection for two months, but no additional cases were detected. Healthcare workers were not screened for MRSA carriage, given the resolution of the outbreak.

Conclusion: This prolonged outbreak, in which half of the cases were detected by active MRSA screening, highlights the importance of this strategy as part of infection control measures in high-risk settings. DLST allow rapid assessment of potential clonality and likely cross-transmission events, which should be confirmed by whole genome sequencing. Some cases of MRSA colonisation may have been missed due to the colonisation/infection ratio. Carriage of MRSA by healthcare workers could be suspected given the sometimes long intervals between cases.

P145

Ventilator-associated lower respiratory tract infections in intensive care unit patients at a tertiary care centre after implementation of a multifaceted intervention – a before-after study

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Background: Due to high rates of ventilator-associated lower respiratory tract infections (VA-LRTI) in our intensive care unit (ICU), we implemented a multifaceted intervention bundle.
Methods: We performed a before-after study in the medical ICU of our tertiary care. We enrolled ICU patients with a length of stay > 2 days and performed prospective surveillance. The study was divided into pre-intervention (06/2022 - 10/2022), intervention (11/2022 - 05/2023), and post-intervention period (06/2023 - 10/2023). The intervention bundle consisted of minimisation of sedation, head-of-bed elevation, replacement of chlorhexidine with toothbrushing for oral hygiene, education of health-care workers, and hand hygiene promotion. Outcomes were VA-LRTI per 1000 ventilation days and healthcare-associated infections (HAI) per 1000 patient days, calculated as incidence rate ratio (IRR) between the post- and pre-intervention period. Also, patient characteristics, device days and adherence to bundle elements were assessed.

Results:
We included 386 patients, 102 in the pre-intervention, 166 in the intervention, and 118 in the post-intervention period. Patient characteristics differed between the pre- and post-intervention period, with patients being less severely ill (Simplified Acute Physiology Score 51 vs 44.5, $p = 0.02$) and less likely ventilated (0.62 ventilation days per patient vs 0.42, $p < 0.001$) in the post-intervention period (Table).
IRR for VA-LRTI remained unchanged (1.07, 95% CI 0.31 - 3.47), although all VA-LRTI in the post-intervention period occurred in the first month (Figure); IRR for HAI showed a non-significant decrease (0.68, 95% CI 0.31 - 1.41).
Chlorhexidine usage plummeted from 100% to 0% ($p < 0.001$), while toothbrushing surged from 0% to 100% ($p < 0.001$). Overall hand hygiene adherence remained unchanged (78% vs. 80%), except for the indication “before aseptic procedures” (increase from 39% to 62%, $p = 0.063$). No improvement was observed for head-of-bed positioning (median angle of the bed 20% in both periods).
Conclusion: We could not observe a reduction in VA-LRTI rates after the implementation of a multifaceted bundle, although our observation period might have been too short. Replacement of chlorhexidine to toothbrushing for oral hygiene was the most prominent change, whereas head-of-bed elevation was not successfully implemented.

P146

Multimodal strategies for the implementation of infection prevention and control activities in healthcare facilities: an update and systematic review of the WHO core component five

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Background: The use of multimodal strategies is one of the eight World Health Organization (WHO) core components for effective infection prevention and control (IPC). The objective of this study was to update the evidence on this core component and estimate the effectiveness of multimodal IPC strategies to reduce health-care associated infections (HAI), infections due to antimicrobial resistant (AMR) pathogens and on hand hygiene compliance in acute healthcare facilities.

Methods: Medline (via PubMed), EMBASE, CINAHL, and the Cochrane library were systematically searched for articles indexed from 24 November 2015 to 30 June 2023. Randomized controlled studies, interrupted time series (ITS) and before-after studies were eligible for inclusion. Risk of bias was assessed using the EPOC criteria. Data synthesis was descriptive, summarizing relevant information from the extracted data.

Results: A total of 5678 publications were identified, of which 32 were eligible for data extraction and analysis. Of these, fourteen reported on the effect of multimodal strategies to reduce device-associated healthcare-associated infections, four on surgical site infections, nine on infections due to AMR and six on hand hygiene compliance. We included eleven controlled studies, eleven ITS and 10 non-controlled before after studies. Twenty-three of the studies originate from high-income countries and the overall quality of the individual studies was medium to low. A majority of the studies showed either a significant reduction of HAI or significant improvement in hand hygiene.

Conclusion: Most of the studies in this updated systematic review demonstrate a positive effect of multimodal strategies on HH, HAI and AMR in acute healthcare settings. These strategies are important for effective IPC programs. Although many studies have been published since the last review on this topic, the quality of evidence remains limited, and only a minority of studies are from low- or middle-income countries.

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Characterisation of the plasmid population in hospital associated vancomycin resistant Enterococcus faecium

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Aims
Despite all surveillance efforts, the management of nosocomial vancomycin-resistant Enterococcus faecium (VREfm) transmissions poses a major challenge to hospitals. While modern sequencing methods have been used to investigate antimicrobial resistance gene carrying plasmids in gram-negative bacteria, little is known about the role of plasmids or mobile genetic elements for the transmission of VREfm in hospitals.

Methods
All VREfm isolates were collected as part of routine surveillance at a tertiary hospital in Germany during a one-year period (2022). Samples were sequenced with a PacBio® Sequel IIe system. After de novo assembly, the WGS datasets were analysed using the Ridom SeqSphere+ v.9.0.1 software. Here we utilized MOB-Suite v.3.1.8 and NCBI AMRFinder Plus v.3.11.26 to characterize plasmids and determine resistance genes. Genetically similar plasmid clusters were defined by mash distance (≤ 0.001). Possible nosocomial transmissions were identified by infection control staff evaluation and genetic relatedness of isolates.

Results
In total, we analysed 232 VREfm isolates. van genotypes were almost equally distributed:122 vanA and 111 vanB VREfm. While vanB was exclusively located within the chromosome, vanA was always plasmid encoded. Matching the epidemiology in German hospitals, the most frequent sequence types (ST) were ST80 (47%) and ST117 (47%).
Based on contact tracing, 7 possible clonal nosocomial transmission were identified. In 2 of these possible transmissions, VREfm isolates also shared similar vanA plasmids within the cluster. Additionally, we analysed vanA plasmids across all isolates (n=150). Using a mash-based approach, these were classified into 9 plasmid clusters. By far the largest cluster contained 97 plasmids ranging from 22 to 39 kb in size. Interestingly, this cluster was detected in different ST (ST80, ST117, ST2566). The replication type of this plasmid was characterized as rep_889 alone or a combination with rep_1763, predicting it as non mobilizable. However, in all plasmids vanA genes were associated with transposable elements such as IS1216E.

Conclusion
Our study offers a first overview of vanA plasmids in the hospital setting. Sequencing data analyses indicates a strong role of mobile genetic elements in the dissemination of vancomycin resistance. Larger scale investigation of plasmid clusters in the hospital setting could help us to improve our understanding of VREfm transmission and epidemiology.

P148

Antibiotic-resistant bacteria colonisation patterns in different neonatal populations across European neonatal units

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Aims

Colonization by antibiotic-resistant bacteria (ARB) is a risk factor for severe hospital-acquired infection/sepsis, especially for infants born < 32 weeks' gestation (high-risk). However, the burden of ARB colonization in European high-technology neonatal units remains mostly unknown. NeoDeco, part of the Horizon 2020-funded NeoIPC project (n. 965328), is a cluster randomised hybrid effectiveness-implementation study, starting in 2024. It aims at assessing if the implementation of an optimised skin-to-skin contact reduces neonatal severe infection among infants born < 32 weeks' gestation, defined as high-risk, in neonatal intensive care units (NICUs). A 4-week feasibility study assessing unit-level resistant colonisation preceded NeoDeco in 14 European sites in 2022-2023.

Methods

During the feasibility study, stool samples (N= 754) were collected from admitted infants during four point-prevalence surveys (PPSs) at variable timepoints in 14 European neonatal units over six countries (Estonia, Germany, Greece, Italy, Spain and Switzerland). DNA extraction (NucliSENS easyMAG, bioMérieux) was followed by RT-qPCR detection of carbapenemases and extended-spectrum-beta-lactamases (CBPs, ESBLs) and vancomycin-resistant enterococci (VREs). Collected anonymized data were analyzed per sample per PPS, comparing high and low-risk infants.

Results

26% (217/754) of stool samples showed ESBL, CBP, or VRE positivity overall. ESBLs (19%) were most prevalent, followed by CBPs (10%), and VREs (7.2%). Resistant colonization varied significantly by country and site ($p < 0.001$, range 0.0% - 94% for individual sites), with the highest proportion in Greece. Colonization rate stability over time was found to be ARB-dependent; only VRE colonization increased from 3.8% to 13% over the study period ($p=0.002$), while the CBP and ESBL positivity remained stable over time ($p > 0.05$). 40% of high-risk infants were colonized, compared to 60% of low-risk infants (Pearson's $\chi 2$, $p = 0.3$). However, high-risk infants were more frequently ESBL-colonized (21% vs. 16%, $p = 0.08$).

Conclusion

ARB prevalence differed considerably across countries and neonatal units, being highest in southern Europe. Stability of colonization rate/PPS varied for different ARBs. The proportion of colonized low-risk infants was higher, though not significantly. In NeoDeco, the same pragmatic approaches will be applied to data collection and monitoring, with room for implementation and use for wider applications.

P149
Bacterial contamination of used healthcare workers clothing

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Aim: This study aimed to explore bacterial contamination on healthcare workers' (HCWs) used clothing at the University Hospital of Bern and identify factors associated with clothing change frequency and contamination level.

Methods: One hundred HCWs were randomly selected and surveyed, with microbiological samples collected from their work attire upon return at the automated delivery system. A standardized survey gathered participant characteristics, workplace details, clothing usage, and patient contact information. Microbiological examination targeted the outermost layer of clothing, which included scrubs, coats with pockets, and pocketless polo shirts. Sampling focused on four locations: A - Abdomen, B - Shoulder (sleeve), C - Pocket side, and D - Wrist (sleeve). Semi-quantitative cultures and resistance testing were conducted by the Institute for Infectious Diseases at Bern University, with identification of possible skin contaminants based on Centers for Disease Control and Prevention guidelines. A descriptive analysis of participants and of the distribution of bacterial contamination was followed by logistic regression to identify predictors for clothes change frequency and contamination.

Results: A total of 100 samples were collected from localizations A and B, 75 from localization C, and 17 from localization D. The vast majority of samples (97%) exhibited microbiological contamination, primarily attributed to common skin commensals. The proportion of samples with pathogen growth varied from 12% at site D to 29% at site B and C. Staphylococcus aureus was the most frequently identified pathogen at each localization, varying from 6% at site D to 17% at C. Clinically relevant multidrug resistant organisms were not identified. The logistic regression analysis revealed a possible association between shorter path times to the automated laundry system (less than 5 minutes vs. more than 5 minutes) and frequency of clothing changes. No predictors for contamination due to relevant pathogens were identified.

Conclusions: HCWs clothes become rapidly contaminated, but mostly by skin commensals. A facilitated access to automated systems could boost the frequency of clothing changes.

P150

An initial too small empirical antibiotic agent does not increase the risk for therapy failures after surgery for orthopedic infections

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Aim

In Infectious Diseases, the choice of postoperative antibiotic agents bases on (local) epidemiology and the expected pathogen group. In contrast, surgeons with few experiences in orthopedic infections prefer a broad-spectrum empirical antibiotic agent presuming that an unintended wrong coverage might hamper their therapeutic success.

Methods

We included all consenting patients with operated orthopedic infections in the last five years; with a minimal follow-up time of six months. We excluded episodes with incomplete documentation or infections without debridement in the operating theatre, and stratified the initial empirical treatment into two major groups: correct empirical choice (or targeted therapy from the start) versus an insufficient empirical choice (with ulterior correction upon arrival of the intraoperative results). We computed the delay until a correct antibiotic therapy as a continuous (in days) and as categorized variables (groups ≤ 1 day, 2-5 days, and more than 5 days). The outcomes were therapeutic failures, lengths of hospital stay, number of surgical revisions, and adverse events in relation with the initial antibiotic regimen.

Results

Among 482 independent infection episodes, 199 (41%) were implant-related. The median duration of postoperative antibiotic regimen was 42 days. In 206 cases (43%), the initial empirical choice was insufficient, with a mean and median time until switching to a correct (targeted) treatment of 8 and 4 days, respectively. Overall, we noted 33 therapeutic failures (7%) during or after therapy. In group comparisons, there was no difference between a correct or wrong initial treatment in terms of ultimate failures (18/276 vs. 15/206; χ2-test, p=0.74), adverse events (15% vs. 7%, p=0.11), length of hospital stay (median 9 vs. 9 days, p=0.96), or the need for supplementary surgical debridement (median 0 vs. 0 intervention, p=0.51). In multivariate logistic regression analysis, the duration of insufficient antibiotic treatment failed to alter the incidence of “failure” (odds ratio 0.9, 95% confidence interval 0.9-1.1).

Conclusion

After surgery, an unfortunate initial empirical antibiotic choice does not increase the risk of therapeutic failures, adverse events, length of hospital stay or the number of additional debridement.

P151
Outbreaks at a Swiss tertiary care hospital

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Aims
Outbreaks of healthcare-associated infections represent significant risks to patients, hospital operations and public health. The surveillance and characterization of outbreaks at the hospital and ward level help identify areas and specific pathogens associated with frequent outbreaks. This, in turn, enables the initiation of targeted infection prevention interventions. This study aims to describe the epidemiology of outbreaks in our tertiary care hospital over a 7-year period.

Methods
The prospective surveillance data of outbreaks of multidrug-resistant organisms (MDRO), respiratory viruses, and Norovirus since 2017 were analyzed. We defined an outbreak of MDROs as the appearance of the same sequence type and no or less than 2 single nucleotide polymorphism (SNP) differences in at least two patients with an epidemiological context. Norovirus, Influenza or SARS-CoV-2 were classified as outbreaks in case of two or more cases of healthcare-associated infections within one department within 5 days.

Results
From 2017 to February 2024, we recorded 85 healthcare-associated outbreaks (median 8 per year, range 2-26) with 270 involved patients (median 2, range 2-19). MDROs were responsible for 9 outbreaks (median 2 per year, range 1-3) with 47 patients involved (median 3, range 2-19). Respiratory viruses accounted for 69 outbreaks (median, range) with 190 patients (median 2, range 2-7). Influenza was responsible for most outbreaks between 2017 and 2020 (n=10, 40%), while afterwards, SARS-CoV-2 was dominant (n=48). Furthermore, 7 (8.2%) Norovirus outbreaks were recorded.
Most outbreaks (n= 26, 30.6%) were noted in 2022, with 92% being SARS-CoV-2-associated.
In total, eight (9.4%) outbreaks were recorded in intensive care units (ICU), 43 (50.6%) in medical departments, 15 (17.6%) in mixed departments, and 7 (8.2%) in surgical departments.
The largest outbreak with 19 patients was a transmission of a Vancomycin-resistant Enterococcus faecium (VRE) VanA, ST 612 in one of the ICUs in 2020.

Conclusion
Most detected outbreaks involved relatively few people, both outside and within the COVID-19 pandemic. Whether an early detection of transmissions is responsible has to be investigated.
Targeted interventions should be planned to prevent and minimize further outbreaks in the most at-risk departments. A national platform for outbreak reporting and visualization could enable early detection and intervention in larger outbreaks.

P152

Correlation of antimicrobial consumption with Clostridioides difficile incidence across the departments of an academic medical centre

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Background
The aim of the study was to correlate Clostridioides difficile infection (CDI) incidence with total antibiotic consumption and use of specific antibiotics or antibiotic groups across 17 clinical departments of an academic hospital.
Methods
Retrospective correlation study with data on CDI and antibiotic prescriptions from 1.1.2008 to 31.12.2021. CDI episodes were defined using CDC criteria. Antibiotic consumption was reported per WHO in defined daily doses (DDD). A mixed effects logistic regression model was fitted with each department as random effect to determine CDI incidence as a function of year and adjusted for antibiotic consumption.
Results
Amoxicillin-clavulanate showed the highest annual consumption across the 17 clinical departments (median 13.5 DDD/100 patient-days), followed by ceftriaxone and fluoroquinolones. The highest antibiotic consumption was encountered in plastic & hand surgery, the lowest in neurology.
The average CDI incidence was highest in nephrology (22.3/10’000 patient-days) and lowest in otorhinolaryngology (0.1/10’000 patient-days).
We observed a correlation between overall antimicrobial consumption and CDI incidence ($p < 0.001$). When plotting each department’s CDI incidence against the departmental average annual consumption, no significant trend was found; however, there was a trend for the correlation between CDI and selected antibiotic usage, such as carbapenems ($p=0.01$), ceftriaxone ($p=0.08$), cefepime ($p=0.01$), macrolides ($p=0.01$), and piperacillin-tazobactam ($p=0.07$).
Conclusions
We detected a correlation between antibiotic consumption and CDI incidence across the departments of an academic hospital; however, we could only correlate departmental CDI incidence with the usage of select antibiotics. Further research is needed on how specific antibiotic usage predisposes which patient populations to CDI.

P153

Giving doctors feedback on the handling of urinary catheters – A intervention study to reduce urinary catheter days and catheter-associated urinary tract infections

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Introduction

To prevent catheter-associated urinary tract infections (CAUTIs), urinary catheters should be restricted to patients that require them and remain as short as possible. CAUTIs contribute to substantial morbidity/mortality. In this quality improvement project, we provide feedback to resident physicians about the indication for urinary catheter, the number of catheters in patients hospitalized in their wards, and the number of CAUTIs. The aim of this study was to assess the knowledge of residents on indications for urinary catheters and other aspects of CAUTIs before and after providing monthly CAUTI feedback.

Methods

We conducted a survey in all residents before the intervention. We initiated a monthly CAUTI feedback on a dedicated floor in 11/2023. We repeated the survey in residents that received feedback during their time working on the intervention floor in 01/2024.
We assessed their responses to (1) indications for urinary tract infections, (2) the estimated percentage of patients receiving urinary catheters during the hospitalization, (3) the percentage of CAUTIs and the risk for bacterial growth per urinary catheter day. Groups were compared using Fisher’s exact test.

Results

The baseline assessment was completed by 38 residents (35% response rate). In total 13 residents completed the survey in 01/2024 (41% of residents working on the floor). The only indication that was reported by almost all residents was acute urinary retention (95%). Almost all residents identified urinary incontinence and comfort as false indications. In total 55% reported ICU care to be an indication for urinary catheter. Although we observed for all indications an increase in the proportion of correct answers in residents working in the intervention ward, the increase was only significant for accurate measurement for urinary output (50 to 84%, $p =< 0.05$). Residents overestimated the proportion of patients with urinary catheter , the proportion of nosocomial infections due to CAUTIs , and the risk of bacterial colonization per catheter day.

Conclusions

Residents overestimated the prevalence of urinary catheter, the risk of CAUTIs, and the risk for bacterial colonization. Interventions with monthly feedback increased the awareness correct indications for urinary catheter with a significant increase in the awareness of urinary catheters for accurate measurement of urinary output.

P154

Successful introduction of an electronic monitoring tool in a prevention bundle for catheter-associated urinary tract infections to support continuous surveillance

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Aim
Catheter-associated urinary tract infections (CAUTI) are one of the most common nosocomial infections. We aim to present our data and experience with an automated CAUTI-surveillance system.
Methods
In our 500-bed tertiary care hospital, we implemented a CAUTI prevention bundle including a stringent indication list, an electronic alert, and giving permission to nurses to remove catheters without physician oversight in 2013 and its efficacy has been shown before. In 2023, we introduced an automated CAUTI-surveillance tool extracting clinical data, catheter-alerts (warrants information on indication for continued catheters), as well as microbiological data of which we retrospectively analyzed data between 2021 and 2023. ICU patients are not included due to a different electronic patient record system. Suspected CAUTI (sCAUTI) were defined as urinary catheter in situ for at least 2d and a positive urine culture (2d after insertion and 24h after removal). Probable CAUTI (pCAUTI) additionally warrants fever >38° within 96h of a positive urine culture. All sCAUTI cases were manually validated using the CDC definition. resulting in verified CAUTI cases (vCAUTI)
Results
In total, 17921 urinary catheters with a total of 72459 catheter days were included. No relevant changes in frequency were observed over the study period. 353 cases of sCAUTI resulted in 131 pCAUTI, of which 117 were vCAUTI resulting in a pCAUTI-rate of 1.8/1000 catheter days and a vCAUTI-rate of 1.6/1000 catheter days. Median catheter dwell-time was 3d (IQR 2-5) overall and 7.5d (IQR 5-13) in vCAUTI. vCAUTI-prevalence was highest in the neurological wards with 8.1/1000 catheter days. Response to the automated catheter alert was generally high, although not quantified and therefore we will additionally analyze catheter-indication in vCAUTI-cases, thereby allowing an estimate of potentially preventable infections.
Discussion
With considerable effort implementing a prevention bundle, CAUTI-rate was 1.6/1000 catheter days in our hospital. Comparability with literature is limited as only febrile episodes are included due automated data extraction. The benefits of manual validation need to be discussed as pCAUTI-rate is comparable. Electronic CAUTI-surveillance enables infection prevention to rapidly identify wards with higher incidence and prevalence, thus allowing specific interventions to reduce CAUTI incidence.

P155
Healthcare workers' attitudes towards antimicrobial-coated hospital textiles

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Aims
Contaminated surfaces and hospital textiles are increasingly considered contributors to transmission of pathogens in hospitals. Fabrics with antimicrobial properties could reduce the risk for pathogen transmission. We aimed to assess the attitudes of healthcare workers (HCWs) towards antimicrobial coating of hospital textiles.

Method
We conducted a mixed-methods study at the University Hospital Zurich with a qualitative phase to develop a questionnaire and a quantitative phase to assess outcome measures there.
First, a convenience sample of HCWs answered hypothetical questions about germicidal work clothes. Based on inductive content analysis a questionnaire was prototyped, user-tested, refined, and distributed to HCWs. Answers were assessed by 5-point Likert scales. Fisher's exact test was used to evaluate differences.

Results
The questionnaire was completed by 65 HCWs between June and November 2022. Hands were considered most important for pathogen transmission but 80% of participants also attributed some importance to clothes and curtains.
Answering the general question about hypothetical, highly effective coated work clothes or curtains, 75% of HCWs showed a positive or very positive overall attitude towards such textiles.

High agreement (> 50% agree/strongly agree) was found for the statement regarding positive effects on transmission interruption. Statements with very low agreement involved cost (coated textiles would be cheaper overall) and duration of use (coated textiles would need to be changed/cleaned less). About 20% of HCWs were concerned about adverse dermatological effects and negative impact on their skin microbiome.
Most questions were answered similarly for clothes and curtains, except HCWs were more skeptical that coated clothes could be manufactured to be sufficiently effective (29.7% vs. 14.3%, $p = 0.036$).

Concerning the use of for antimicrobial-coated work clothes, 21 participants (35%) were in favor of using these across all professions in the hospital. 17 HCWs (28.3%) found coated work clothes useful in close patient contact, 16 (26.7%) in close contact to patients at increased risk for infections and 6 HCWs (10%) saw no need at all.

Conclusion
Although overall attitude of HCWs towards antimicrobial-coated hospital textiles was positive, concerns about impact on skin and microbiome as well as doubts about effectiveness should be carefully addressed by manufacturers to ensure acceptability of end-users.