O01

Carbapenem-resistant Pseudomonas aeruginosa in humans, the hospital environment and the water chain in Rotterdam, the Netherlands

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There is a lack of insight into sources and transmission routes of carbapenem-resistant Pseudomonas aeruginosa (CR-PA). The aim was to determine CR-PA carriage rates in patients and healthy persons, and to gain insight into the presence and sources of CR-PA in the wet hospital environment and the water chain.

This study was performed between March 1, 2022 and March 31, 2023 at the Erasmus MC, Rotterdam. Throat, navel and perineal samples, and questionnaires, were collected from patients upon hospital admission and healthy persons living in Rotterdam. Clinical CR-PA isolates identified through routine diagnostics were also collected (first isolate only). Samples were taken twice within one year from sink and shower drains in all patient rooms and bathrooms from general wards used for patient inclusion. Water samples were taken monthly from the hospital drinkwater inlet, hospital and municipal wastewater treatment plants (WWTPs), and the receiving river. $P<0.05$ was considered significant.

In total, 469 patients and 194 healthy persons were included. Carriage rates were 0.4% (2/469) in patients upon admission and 0.5% (1/194) in healthy persons. Thirty-seven clinical isolates were collected within one year. CR-PA was identified in 1.2% (9/747) and 0.5% (4/741) of drains during sampling rounds one and two, respectively. CR-PA was predominantly found in bathroom sink drains (round 1: 44.4%; round 2: 50.0%), yet overall no significant difference in contamination between sink and shower drains was observed ($P=0.725$). The largest concentration of CR-PA was identified in the influent of hospital wastewater. WWTPs removed CR-PA (onsite hospital WWTP) or reduced (municipal WWTP) CR-PA concentrations. However, CR-PA was still detected in treated effluent from the municipal WWTP and in the receiving river.

Overall, CR-PA carriage rates were low. The presence of CR-PA in the hospital and aquatic environments may pose a risk for humans when interacting with these environments.

O02

In-house pipeline provides robust and rapid genomic drug resistance predictions of clinical Mycobacterium tuberculosis complex isolates

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Introduction: Rapid and accurate antimicrobial susceptibility testing (AST) of Mycobacterium tuberculosis complex (MTBC) is essential for tuberculosis (TB) control. Whole-genome sequencing (WGS) in a near-patient setting can be a rapid alternative to slow phenotypic methods, by detecting mutations in resistance-associated genes. In our country with low prevalence of resistance, negative predictive value is the critical asset for AST by WGS. We compared phenotypic and genotypic AST results for eight anti-tuberculous drugs: isoniazid, rifampicin, ethambutol, moxifloxacin, amikacin, kanamycin, ethionamide and streptomycin.

Methods: Clinical MTBC isolates obtained in routine diagnostics between 1 January 2020 and 31 October 2023 were included. Only isolates with a known phenotypic (microdilution, using Sensititre MYCOTBI plate MYCOTB) and genotype result (using Illumina NextSeq 500) AST were enrolled. The genomic resistance profile was determined by comparing detected mutations in resistance-associated genes with established list of resistance-causing mutations of the World Health Organization (WHO) (version 2021.7; category 1 and 2 was considered as resistant) using the in-house developed pipeline 'MyCodentifier'. Turnaround times (receiving time of sample until drug susceptibility result) for phenotypic as well as genotypic results were evaluated. WGS was performed once weekly.

Results: In total, 194 MTBC isolates were included. The negative predictive value (NPV) of WGS was ≥97.7% for all anti-tuberculous drugs, except for ethionamide (NPV 86,8). Turnaround times for genotypic AST was a median of 25 days (range 4-93 days, average 27.9 days), while phenotypic AST needs 34 days (range 6-109 days, average 37.7).

Conclusion: In a near-patient setting in low endemic and low resistance TB area, when genotypic AST by WGS detects no mutations in their resistance-associated genes according to the list of WHO, susceptibility to amikacin, ethambutol, isoniazid, kanamycin, moxifloxacin or rifampicin can be assumed and does not require phenotypic confirmation. Using genotypic AST, turnaround time can be shortened by weeks.

O03

Interspecies interactions of cystic fibrosis-associated pathogens alter antibiotic pharmacodynamics

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Polymicrobial infections (PMIs) are common in patients with cystic fibrosis (CF). Although PMIs are associated with poor antibiotic treatment response, effects of the ecological interactions between co-infecting pathogens on antibiotic sensitivity are poorly characterized. To this end, we systematically quantified these effects bidirectionally for a panel of CF-associated pathogens and antibiotics using a custom designed co-culture platform and evaluated the clinical relevance of these findings using pharmacokinetic-pharmacodynamic (PK-PD) modeling.

A high-throughput co-culture platform was developed to characterize pairwise interactions of CF-associated pathogens in synthetic CF sputum medium, while optically monitoring their growth dynamics. Using this platform, all 21 pairwise interactions of seven CF-associated pathogens were studied in absence and presence of one of four antibiotics (ciprofloxacin, meropenem, tobramycin, ceftazidime). Using the obtained growth profiles we fitted pharmacodynamic models for each antibiotic-species combination studied. Changes in antibiotic sensitivity were derived by computing the fold change (fc) of the half-maximal effective antibiotic concentration (EC50) for the focal species in co-culture relative to mono-culture.

Across all studied pairwise pathogen interactions and antibiotics (n=186), 29% of all interactions decreased antibiotic sensitivity with fcEC50>1.5, whereas 20% increased antibiotic sensitivity with fcEC50<0.75. We found that 20% of all interactions were bidirectional, leading to either a shared increase (5%), decrease (8%) or mixed (7%) response in antibiotic sensitivity. Effects of interspecies interactions on antibiotic sensitivity were most pronounced for tobramycin with 43% of interactions causing an increased EC50, and for meropenem with 33% of the interactions causing decreased EC50. Pronounced species-specific effects included an increased EC50 for Burkholderia cepacia (ceftazidime, tobramycin), Stenotrophomonas maltophilia (tobramycin), and Staphylococcus aureus (tobramycin) in the presence of all studied pathogens.

In conclusion, changes in pharmacodynamic response mediated by interspecies interactions of CF-associated pathogens are common and diverse. Our findings provide a starting point for designing tailored antibiotic treatment strategies for CF-PMIs.

O04

Looking beyond the parent drug: A study of the contribution of TBAJ-876 and its TBAJ-876-M3-metabolite to the total bactericidal activity in a tuberculosis mouse model.

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Background
TBAJ-876, a new diarylquinoline anti-tuberculosis drug, was developed as a possible safer alternative to bedaquiline. In vivo, diarylquinoline metabolites are produced and these have anti-tuberculosis activity themselves as well. Differences in parent drug-to-metabolite ratios between mice and humans might impact model-based predictions on drug activity in humans. Hence, this study explores the contribution of TBAJ-876 and its major metabolite, TBAJ-876-M3 (M3), to the total TBAJ-876 bactericidal activity in a murine tuberculosis model.

Methods
BALB/c mice, intratracheally infected with Mycobacterium tuberculosis, received treatment with TBAJ-876 (1.56, 6.25, or 25 mg/kg) or M3 (3.125, 12.5, or 50 mg/kg) via oral gavage. After 1, 2 or 4 weeks of treatment, pulmonary mycobacterial loads were determined by lung cultures. Bactericidal activity was defined as the load reduction compared to start of treatment. Additionally, plasma drug concentrations were measured at several time points after the last dose administration. The overall bactericidal activity following TBAJ-876-treatment is driven by both TBAJ-876 and M3. Therefore, the contribution of M3 to the overall activity was estimated based on M3-exposure following TBAJ-876 treatment, and corresponding M3-activity observed in M3-treated animals.

Results
TBAJ-876 and M3 showed strong dose- and time-dependent bactericidal activity. Lungs of mice treated with 50 mg/kg M3 for 4 weeks were culture negative. Following TBAJ-876 treatment, exposures to M3 were 2.2-3.6x higher than to TBAJ-876. Based on drug exposure and bactericidal activity, TBAJ-876 activity appeared to be largely driven by M3.

Conclusions
TBAJ-876 and M3 are highly active against M. tuberculosis within this murine tuberculosis model. Activity observed after TBAJ-876 treatment seemed predominantly driven by M3, given its high exposure and potent activity, emphasizing the need to consider metabolites and their potentially distinct exposure and activity profiles compared to parent drugs to enhance the predictive value of mouse-model driven predictions on drug activity in humans.

O05

Mycobacterium bovis infection of Dutch domestic cats resulting in human exposure

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Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB). The Netherlands is officially bovine tuberculosis (bTB) free since 1999. M. bovis has a broad range of hosts and therefore does not only infect cattle, but also other mammals. Wildlife may act as a reservoir that fuels bTB positive herds. Besides livestock and wildlife, companion animals are also permissive for bTB and therefore a potential risk for human bTB.
In December 2022, an euthanized 3-month-old kitten was submitted to the Pathology department (Utrecht University). Upon necropsy, multiple granulomas were detected in lungs and lymph nodes. Microscopic analysis revealed acid fast bacilli, which was confirmed by specific IS6110 PCR. Differential PCR classified the pathogen as M. bovis. After this index case three more cats from the same household were euthanized and tested M. bovis positive. The remaining cat and dog received antibiotics. Four out of six human contacts tested positive for tuberculin skin test (TST) and three for Interferon Gamma Release Assay (IGRA). One contact had lung abnormalities and biopsy tested positive for M. bovis. All contacts received antibiotics. In parallel, another household presented a cat with similar symptoms. Necropsy indeed revealed the presence of granulomas in lungs and lymph nodes which tested positive for M. bovis. No further cases were identified in other cats and human contacts from this household. Interestingly, isolates from household I and household II dissociated with 500 SNPs suggesting two independent M. bovis strains. The M. bovis sources were not identified.
With this case report we would like to stress that bTB can still occur in a bTB free country in non-bovine mammals. Furthermore we want to raise awareness that transmission may occur from domestic animals to humans.

O06

Embracing the Mycobacterial Wall-Deficient Lifestyle: Unveiling Secrets Through Transcriptomics

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Mycobacteria, including notorious pathogens such as Mycobacterium tuberculosis, Mycobacterium avium and Mycobacteroides abscessus, are responsible for devastating diseases such as Tuberculosis. The intricate mycobacterial cell wall assumes a critical role in providing structural support, influencing pathogenesis, and safeguarding against environmental challenges during infection. Recognized as indispensable, the enzymes orchestrating cell wall synthesis stand as one of the prime targets for effective antibiotics. Surprisingly, our recent findings reveal that a multitude of mycobacterial species, including those previously mentioned, can generate viable cell wall-deficient (CWD) cells in response to cell wall targeting agents and mycobacteriophages. Employing cryogenic electron tomography coupled with deep learning segmentation, we have corroborated the absence of conventional cell envelope structures in CWD cells. Instead, a discernible thin, unidentified layer above the plasma membrane is detected, coinciding with peptidoglycan fluorescence. Transcriptomic analysis of CWD cells in the model organism Mycolicibacterium smegmatis indicates substantial metabolic rerouting and a shift to anaerobic respiration. Intriguingly, parallels are observed with the in vivo transcriptome of M. tuberculosis, including elevated expression of inorganic nitrogen assimilation and antioxidant ergothioneine biosynthesis. Furthermore, the mycobacterial CWD transcriptomic signature highlights upregulation of the type VII secretion system ESX-4, implicated in DNA uptake during Distributive Conjugal Transfer, accompanied by heightened activation of transposases and integrases. These outcomes imply that transition to a CWD lifestyle stimulates DNA recombination, potentially fostering mutations that confer resistance to antibiotics. Collectively, our findings underscore the widespread adoption of the mycobacterial lifestyle within the Mycobacteriaceae, prompting contemplation of its integration into foundational mycobacterial research. This paradigm shift may yield valuable insights into the dynamics of mycobacterial adaption and resistance mechanisms.

O07

Unraveling the chromosome of a predator: a dramatic shift in chromosome organization goes hand in hand with transcriptional changes in Bdellovibrio bacteriovorus

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The obligate predatory bacterium Bdellovibrio bacteriovorus goes through dramatically different life cycle stages. It goes from a non-proliferative attack phase to the growth phase: here it attaches to a prey bacterium (e.g. E. coli), invades its periplasm, grows into a long filament and finally divides into an even or odd number of daughter cells that burst out of the prey's remains. The predator's wide host range includes pathogens as Salmonella and Vibrio cholerae, making it an interesting target in the search for new antimicrobials. However, how B. bacteriovorus invades and kills its prey is largely unknown. One of the unanswered questions is how the switch from the attack phase to the growth phase is regulated.

Recently, parts of the replication and chromosome cycle of B. bacteriovorus were elucidated. One of the striking observations was that the attack phase nucleoid is so compact that it excludes free proteins. We aimed to understand the role of chromosome organization during the B. bacteriovorus life cycle. For this, we used RNA-sequencing and chromosome conformation capture (Hi-C) of seven key points of the predator's life cycle: starvation, attachment, onset of replication, onset of chromosome segregation, growth, division & exit, and newborn progeny.

Using quantitative microscopy, we show that the B. bacteriovorus nucleoid opens up upon replication. Hi-C shows that during growth a dramatic reorganization of the nucleoid takes place, while during division and exit, gene expression shuts down in preparation for the attack phase. During the attack phase, non-specific DNA-binding proteins bind only transcribed regions, suggesting limited chromosome accessibility. Finally, the temporal expression patterns allowed us to predict genes specifically involved in prey entry or exit. Concluding, this study gives the first full-cycle overview of the transcriptome and chromosome conformation in B. bacteriovorus and shows that they are intimately linked.

O08

Structure and function of a putative water channel in Bacillus subtilis germinant receptor protein GerAB

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With no known water channels in the model spore-forming organism Bacillus subtilis, the molecular mechanism of spore water uptake during germination is unknown. Recent work showed that a subunit of the prototypical B. subtilis spore germinant receptor GerA, the integral inner membrane protein GerAB, initiates spore germination in response to L-alanine. Our previous work found that GerAB contains what appears to be a water channel. Using Molecular Dynamic (MD) simulation, we found water passing through the GerAB protein in silico. At the same time, utilizing Steered MD simulation, we now also pull single water molecules through the GerAB channel and calculate the free energy of water permeation. These computational methods, as well as the predicted GerAB structure, provided indications that GerAB residues Y97, L199 and F342 may be crucial in the water channel's function. Mutagnesis of the three residues to alanine corroborated the assumptions with in vivo data. The so called triA mutant where Y97, L199 and F342 were all mutated to alanine showed virtually no germination anymore measured as water uptake in a live-imaging experiment setting. In contrast response to the germinant mixture Asparagine, Glucose, Fructose and Potassium (AGFK) was maintained though remarkable only at 82% efficiency of the wild-type. Y97A showed 93% germination efficiency with AGFK but only 1.5% with L-alanine. Preliminary western-blot analyses indicate that the germination complex is likely present in Y97A supporting the functional role of this residue in water passage through the B. subtilis spore protein. L199A and F342A showed similar germination patterns but the presence of a full germinsome was not sure in these mutants. Future experiments include kinetic analysis of spore germination events including confirmation of B. cereous FRET and co-localization data of germinosome and spoVA channel components (see abstract Brul et al.) in B. subtilis germinosome and the SpoVA channel protein.

O09

The two-component system ArlRS is essential for wall teichoic acid glycoswitching in Staphylococcus aureus

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Staphylococcus aureus is among the leading causes of hospital-acquired infections. Wall teichoic acids (WTA) are covalently-anchored ribitolphosphate polymers of critical importance to the S. aureus cell wall and host interactions. Approximately one-third of S. aureus isolates can decorate WTA with a mixture of α1,4- and β1,4-N-acetylglucosamine (GlcNAc) through the activities of the specific glycosyltransferases TarM and TarS, respectively. Importantly, α1,4- and β1,4-GlcNAc WTA modifications impact S. aureus such as antibody binding and phage infectivity and are influence by environmental conditions, e.g. high salt, switching WTA glycosylation from α1,4-GlcNAc to β1,4-GlcNAc dominance. The aim of this study was to identify regulatory mechanisms underlying WTA glycoswitching.

The Nebraska Transposon Mutant Library (1,920 arrayed mutants of TarMS+ strain JE2) was screened by colony blotting for differential expression of WTA-linked α1,4- or β1,4-GlcNAc using monoclonal fragment antigen-binding (fab) clones 4461 (α-GlcNAc) and 4497 (β-GlcNAc). Potential hits were validated by flow cytometry and confirmed using defined gene-deletion mutants. Infectivity by phage Stab20, which is hindered by WTA α-GlcNAcylation, was assessed for select mutants. S. aureus USA300 single mutants (ΔtarM, ΔtarS), and a double mutant (ΔtarMS) were used as positive and negative controls, respectively.

The library screen identified 150 and 128 potential hits for regulation of tarM and tarS, respectively, among which mutants of the two-component system (TCS) ArlRS. Deletion of arlRS or all 15 non-essential TCSs in S. aureus MW2 abrogated WTA β1,4-GlcNAc decoration and Stab20 phage infectivity. Complementation with an arlRS-expressing plasmid restored β1,4-GlcNAc WTA and phage infectivity in both knockouts indicating non-redundant regulation of WTA decoration by ArlRS. Finally, we observed that ArlRS expression is a prerequisite for the Mg2+-induced glycoswitch from α1,4-GlcNAc to β1,4-GlcNAc WTA glycosylation through transcription factor MgrA. These data suggest that the two-component system ArlRS is essential for β1,4-GlcNAc glycosylation and glycoswitching of S. aureus WTAs.

O10

Functional profiling of CHAP domain-containing peptidoglycan hydrolases of Staphylococcus aureus USA300 uncovers potential targets for anti-staphylococcal therapies

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Functional profiling of CHAP domain-containing peptidoglycan hydrolases of Staphylococcus aureus USA300 uncovers potential targets for anti-staphylococcal therapies

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Introduction: The bacterial pathogen Staphylococcus aureus (S. aureus) employs a thick cell wall for protection against physical and chemical insults. This wall requires continuous maintenance to ensure strength and barrier integrity, but also to permit bacterial growth and division. The main cell wall component is peptidoglycan. Accordingly, the bacteria produce so-called peptidoglycan hydrolases (PGHs) that cleave glycan strands to facilitate growth, cell wall remodeling, separation of divided cells and release of exported proteins into the extracellular milieu. A special class of PGHs contains so-called 'cysteine, histidine-dependent amidohydrolase/peptidase' (CHAP) domains. In the present study, we profiled the roles of 11 CHAP PGHs encoded by the core genome of S. aureus USA300 LAC. Methods: Mutant strains lacking individual CHAP PGHs were analyzed for growth, cell morphology, autolysis, and invasion and replication inside human lung epithelial cells. Results: The results show that several investigated CHAP PGHs contribute to different extents to extracellular and intracellular growth and replication of S. aureus, separation of dividing cells, daughter cell separation once the division process is completed, and autolysis. In particular, the CHAP PGHs Sle1 and SAUSA_2253 control intracellular staphylococcal replication and the resistance to β-lactam antibiotics including oxacillin. Conclusions: This makes the S. aureus PGHs in general, and the Sle1 and SAUSA300_2253 proteins in particular, attractive targets for future prophylactic or therapeutic anti-staphylococcal interventions. Alternatively, these cell surface-exposed enzymes, or particular domains of these enzymes, could be applied in innovative anti-staphylococcal therapies

O11

Uncovering the Role of IgA-coated Bacteria: Establishing an Improved High-Throughput IgA-SEQ Protocol

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Introduction

The composition of the gut microbiota is essential for systemic homeostasis. Dysbiosis is believed to contribute to intestinal and systemic diseases such as inflammatory bowel disease (IBD) and cancer. Studies show that certain bacterial species within the intestinal tract can chronically activate the immune system, resulting in low-grade intestinal inflammation. However, it remains unclear how this influences diseases such as IBD and cancer.

Methods

IgA-SEQ is a technique to 'immunoprofile' the microbiota by identifying species that display immunostimulatory behaviour. (In)direct stimulation of B cells results in the production of high-affinity antigen-specific IgA that is secreted into the gut, targeting the bacteria that initiated the response. IgA-SEQ technology combines antibody-based bacterial cell sorting and 16S rDNA gene sequencing to specifically identify IgA-coated bacteria from fecal material. Current IgA-SEQ protocols involve lengthy, low-throughput FACS-based cell sorting, and downstream applications are limited due to the low number of IgA-coated bacteria that can be sorted.

Results

We established two optimized magnetic-based cell sorting protocols to address the limitations described above. We compare the original FACS-based protocol with the magnetic-based protocols. We show high purity and reproducibility of magnetically sorting IgA-coated bacteria in simplified artificial settings, in addition to complex human fecal samples. High numbers of IgA-coated bacteria can be isolated using these protocols, allowing for a range of previously unachievable downstream applications. As proof-of-principle, we performed metagenomic analysis on the IgA-coated fraction of a set of human fecal samples. Moreover, we show that it is possible to perform the isolation in anaerobic conditions, allowing anaerobic bacteria to survive the sample preparation.

Conclusion

Our data validates the high-throughput magnetic-based cell sorting methods for the isolation of IgA-coated bacteria, addressing the limitations of the original FACS-based IgA-SEQ protocol. Currently, we are using these techniques to elucidate the role of IgA-coated bacteria in IBD and cancer.

O12

De novo acquisition of antibiotic resistance in six species of bacteria

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De novo acquisition of antibiotic resistance in six species of bacteria
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Bacteria can become resistant to antibiotics in two ways, acquiring resistance genes by horizontal gene transfer and de novo development of resistance upon exposure to non-lethal concentrations. The importance of the second process, de novo build-up, has not been investigated systematically over a range of species and may be underestimated as a result. To investigate the DNA mutation patterns companying de novo acquisition process, six bacterial species encountered in the food chain were exposed to step-wise increasing sublethal concentrations of six antibiotics to develop high level resistance. Phenotypic and mutational landscapes were constructed based on WGS sequencing at two time points at the evolutionary trajectory. In this study, we found: 1) all of the six strains can develop high levels of resistance against most antibiotics. 2) increased resistance is accompanied by different mutations for each bacterium-antibiotic combination. 3) The number of mutations varies widely, with Enterobacterales species having by far the most. 4) In the case of fluoroquinolone resistance a mutational pattern of GyrA combined with ParC is conserved in five of six species. 5) mutations in genes coding for efflux pumps are widely encountered in gram negative species. The overall conclusion is that very similar phenotypic outcomes are instigated by very different genetic changes.

Key words: de novo resistance, antibiotic resistance evolution, mutational pattern, species-specific mutations, food chain.

O13

Bacteriophage therapy reduces bacterial load in Staphylococcus aureus burn wounds infections in an ex vivo human skin model

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Yearly 11 million people require medical treatment for burn wounds globally. Mortality rates are high and mostly due to bacterial infections. Staphylococcus aureus is a major causative agent of burn wound infections. Antibiotic resistance and biofilm formation complicate treatment of these infections with antibiotics. An alternative for antibiotics is the use of bacteriophages that infect and kill bacteria.
To gain more insight into the efficacy of bacteriophage therapy for burn wound infections, an ex vivo model was set-up using surplus human skin obtained after elective surgery. A burn wound was applied to the skin, which was inoculated with a methicillin-resistant S. aureus (MRSA) strain. After one hour, the skin was treated with either bacteriophages (phage ISP or RPCSa2) at different multiplicity of infection (MOI), antibiotics (Fusidic acid), or phosphate buffered saline. Colony forming units (CFU) were determined after 24 hours.
A single treatment with bacteriophages or antibiotics significantly decreased CFU after 2, 4 and 24 hours. When the skin was treated every three hours (three times in total), an increased phage efficacy was observed for phage RPCSa2, but not for phage ISP. Finally, when the skin was pre-treated with bacteriophages one hour prior to S. aureus exposure, a significant reduction in bacterial load was observed after twenty four hours for both bacteriophages at two out of three MOI tested. These findings suggest that bacteriophage therapy can be an effective treatment for burn wound infections caused by S. aureus, especially when used in a prophylactic manner.

O14

Akkermansia muciniphila drives mucin glycan degradation in a cooperative synthetic in vitro mucosal microbial community of the human gut

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A distinct microbial community resides in the human gut outer mucosal layer. Some of these microbes degrade the mucin glycans of this layer. This is part of the normal turnover process of mucus and results i.a. in the production of beneficial short-chain fatty acids (SCFA). Due to the complexity and diversity of mucin glycans, mucin degradation requires a broad range of bacterial extracellular glycan-degrading enzymes. Consequently, we hypothesised that mucin degradation occurs in a network of mucosal microbes with concerted action of enzymes. We assembled and studied an anaerobic in vitro synthetic mucin-degrading community in bioreactors.

We created a synthetic community of microbes of interest to model ecological interactions between microbes and mucus. This 15-member mucin-degrading synthetic community (MDSC) consisted of seven mucin degraders and eight cross-feeders. The community was grown in triplicate anaerobic bioreactors with continuous mucin supply for 120 hours. We tracked species-level relative abundance through 16S rRNA gene amplicon sequencing and qPCR, we followed metabolite production with HPLC and we evaluated community function with metaproteomics. Mucin degradation was assessed by LC-MS/MS and MALDI-TOF MS.

The community reached stable state at t=72h. During stable state (t72-t120), the community was dominated by specialist mucin degraders Akkermansia muciniphila and Ruminococcus spp, and generalist glycan degraders Bacteroides spp. Butyrate producers and hydrogen consumers could cross-feed on the products of mucin degradation. The community consistently produced SCFAs. Furthermore, we observed near complete degradation of mucin glycans. The community expressed a plethora of mucin-targeting enzymes, including sialidases, fucosidase, galactosidases, hexosaminidases, sulfatases and peptidases.

We established a synthetic mucin-degrading microbial community as a model for ecological interactions in the mucus layer. Specialist A. muciniphila plays a key role, but other mucin degraders and cross-feeding microbes occupy their own niche. Taken together, we provide evidence for a network of collaborating microbes in the mucus layer.

O15

Effect of sulfide on the microbiome of the green alga Caulerpa prolifera

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Caulerpa is a genus of green macroalgae that live in tropical and subtropical coastal waters. It is an intriguing organism because, although it has plant-like structures, it is one giant cell containing nuclei, chloroplasts, mitochondria, and symbiotic bacteria – the ‘holobiont’. The role of the symbiotic bacteria is a mystery. However, they might play a role in the growth and development of the host, in the adaptation to environmental change and hence, in the ecological success of these algae. Caulerpa prolifera has inhabited the Portuguese coast since its discovery in the 19th century. The alga is known to colonise new areas, especially those where endemic species of seagrasses are no longer present and change the sediment’s physical and biochemical properties, increasing the hydrogen sulfide levels in the sediment. Here, we tested the hypothesis that bacterial symbionts play a role in the tolerance to high sulfide levels. We conducted a mesocosm experiment incubating C. prolifera at different sulfide concentrations and measured the effect on photosynthesis and growth. Subsequently, we extracted DNA and RNA from the leaves and roots and performed 16S amplicon sequencing to determine the microbial community composition and the active members therein. Our results showed that C. prolifera maintains photosynthesis and growth even at high sulfide concentrations. Notably, its roots host a diverse array of sulfide-oxidizing and sulfate-reducing bacteria which taxonomic profile changes dramatically with the change of sulfide concentration in the sediment. This suggests an adaptive mechanism for modifying sediment properties and symbionts’ potential ability to perform a sulfide oxidation defence for the alga. These results underscore the significance of microbial partners in Caulerpa's survival and adaptation to sulfide-rich environments, offering new insights into the complex interactions within marine holobionts.

O16

Synthetic community mitigates ammonia emissions produced by livestock fecal microbiota

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Introduction: Ammonia emissions are a cause of particulate matter, eutrophication, and a hazard to animal and human health. In the livestock sector, ammonia is mainly generated by the nitrogen metabolism of manure microbiota. Synthetic communities (SynComs) have been applied to reduce ammonia emissions from livestock manure. However, little is known about interactions between SynComs and manure native microbiota, the interactions between SynComs and the manure environment, or the ammonia mitigation mechanism.
Methods: A synthetic community of three lactic acid bacteria (LAB) species was designed to mitigate ammonia emissions from livestock manure. Multi-omics including 16S rDNA amplicon, metagenomic, and metatranscriptomic sequencing were employed to explore the influences of the LAB SynCom on taxonomic and metabolic profile of manure microbiota.
Results: The LAB SynCom treatment led to a reduction in ammonia emissions by up to 95%. The taxonomic analysis revealed that the LAB SynCom reshaped the manure microbial community structure and might inhibit dominant ureolytic bacteria. Furthermore, we find that the LAB SynCom treatment significantly suppressed the abundance and expression of genes involved in pathways of forming ammonia and its precursors. The antibacterial peptide Plantaricin A and lactate were identified as the key inhibitory compounds and were found to act synergistically in inhibiting the growth of ammonia-producing bacteria. Moreover, we find that ammonia produced by manure bacterial community was converted into ammonium via hydrogen ions of lactate generated by the LAB SynCom and then served as a nitrogen source for LAB growth and metabolism.
Conclusion: Colonization of manure by the LAB SynCom is enhanced by uptake of ammonia produced by manure bacteria. The LAB SynCom enhanced the mitigation of ammonia emissions by suppressing the nitrogen metabolic network via Plantaricin A and lactate. This study revealed multi-interactions between the extrinsic synthetic community and environment microbiota and the influence of their interactions on environment.

O17

Membrane changes during syntrophic growth of an archaeal/bacterial consortium: a model for eukaryogenesis

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The first eukaryotic cell is assumed to have arisen from a symbiosis of an archaeal cell, likely an Asgard archaeon, and a bacterial partner. As Asgard archaea are proposed to contain eukaryotic-like intracellular structures, it has been hypothesized that many eukaryotic proteins involved in regulated membrane contacts have their origins in archaea. Yet, little is known about the role of cell-to-cell contacts for the emergence of eukaryotes. Due to the lack of cultivated and genetically tractable Asgard archaea or closely relevant lineages, model systems of syntrophic interacting microorganisms can help to shed light on how cell-to-cell interactions between different species arose. Here, we specifically focus on determining which membrane lipids and proteins are involved in cell-to-cell interactions. To this end, we use syntrophic co-cultures of the sulfate-reducing bacterium Desulfovibrio vulgaris and the methanogenic archaeon Methanococcus maripaludis. Evolved co-cultures after several generations under syntrophic conditions, are being analyzed by transcriptomics and proteomics to identify differentially expressed proteins connected to cell-to-cell interactions with a focus on membrane proteins. These analyses are further complemented with lipid analyses to determine changes in the cell membrane of the syntrophic co-culture partners as a response to changes in the membrane proteins. To visualize cell interactions fluorescence microscopy is used. Membrane changes could be shown between mono to coculture and during syntrophic adaptation. This in-depth analysis of a model syntrophic co-culture will provide clues on how interdomain cell-to-cell interactions lead to the emergence of the first eukaryotic cell and which role early archaea had in it.

O18

Engineered acetogens for use in synthetic co-cultures

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The production of biocommodities from C1 substrates using acetogens is outstanding, although limited by their narrow product spectrum. This narrow spectrum was broadened by applying metabolic engineering approaches; however, titers are still low. Alternatively, acetogens can be combined with other bacteria in synthetic co-cultures. This approach brings the challenge of controlling the co-culture dynamic to achieve stable production. Hence, applying engineered acetogens in synthetic co-cultures might unlock their potential to be used at an industrial scale by recombinantly producing bioproducts from greenhouse gases.
In the presented work, we applied molecular tools for the industrial-relevant acetogen Clostridium autoethanogenum, the natural butyrate producer Eubacterium limosum, and the narrowly studied CO-tolerant Acetobacterium wieringae JM strain. That includes the establishment of a fluorescent reporter protein (FAST) as well as performing genomic integrations applying CRISPR-Cas- and gene deletions using homologous recombination-based methods.
While all strains were successfully engineered on a plasmid-based manner and showed FAST mediate fluorescence, flow cytometry analysis revealed heterogeneous populations. Therefore, the CRISPR-Cas-based genome engineering tool ‘SIBR-Cas’ was used to integrate FAST into the genome of C. autoethanogenum. The engineered C. autoethanogenum:FAST strain showed a homogenous fluorescent population, though fluorescent sign needs still to be augmented. In addition, genes of the butyrate production operon of E. limosum were targeted using a homologous recombination approach, resulting in the creation of two knockout mutants. While strain E. limosumΔcrt, where the gene encoding a crotonase was disrupted, is still capable of producing butyrate, deletion of the gene encoding the butyryl-CoA dehydrogenase in E. limosumΔbcd resulted in the loss of production. This strain serves as the foundation to investigate butyrate metabolism and redirect its metabolism to more favorable, non-native products.
Our results substantially contribute to the development of novel and robust, highly engineered acetogen, which can be employed in co-cultures to produce valuable industrial platform chemicals.

O19

Anti-Staphylococcal antibodies effectively potentiate complement activation and phagocytosis of Coagulase Negative Staphylococci in term and preterm neonatal cordblood

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Introduction

Coagulase Negative Staphylococci (CNS) are the most common pathogens found in neonatal intensive care units. Since hypogammaglobulinemia is an important risk factor in preterm neonates, clinical studies have thus far focused on antibody supplementation with pooled intravenous immunoglobulins from healthy donors, but with little success. Monoclonal antibodies (mAbs) successfully prevent infectious diseases such as Respiratory Syncytial Virus. A trial with anti-Staphylococcal mAb Pagibaximab failed to prevent neonatal sepsis. We hypothesize that the effectivity of mAb therapy depends in part on complement-enhancing potential.

Methods

Classical (CP) and alternative pathway (AP) activity in very preterm (<32 weeks gestational age (wGA)), near-term (32-37 wGA) and term (37+ wGA) neonatal plasma was determined by CH50 and AP50 respectively.

Two different IgG1 mAbs (CR5133 and CR6453) that recognize Staphylococcal surface components and Pagibaximab were selected for this study. The effect of Fc-mutations that improve IgG hexamerization in CR5133 and CR6453 on complement activation was also tested. To do so, bacteria were opsonized with mAbs in the presence of neonatal cord blood plasma, and their effect on C3b deposition was studied by flow cytometry. Finally, we studied the phagocytic capacity of neonatal neutrophils in whole blood, using fresh preterm and term neonatal cord blood samples.

Results

We show that compared to healthy adults, term and near-term neonates show decreased CP and AP activity. The effect was even more pronounced in very -preterm neonates. We observed that the hexabody variants of CR5133 and CR6453 show more complement activation than wildtype antibodies, IVIG and Pagibaximab. We also show that complement activation is essential to induce efficient phagocytosis. Furthermore, we showed that phagocytosis of S. epidermidis could be enhanced by mAbs in preterm and term neonatal whole blood.

Conclusion

Our findings provide insights that are crucial for optimizing anti-staphylococcal mAbs as prophylactic agents for neonatal bloodstream infections.

O20

Frontline immunity against Streptococcus pyogenes by human Langerhans cells

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Introduction

Streptococcus pyogenes or Group A Streptococcus (GAS) is a major cause of non-invasive and invasive infections resulting in an estimated 500,000 deaths worldwide annually. The highly variable streptococcal M protein, encoded by emm, is a major virulence factor of GAS. The skin and pharynx are prime entry sites for GAS. Subsequent local infections are on the obligatory causal pathway to more severe disease manifestations. Langerhans cells (LCs), characterized by the expression of the C-type lectin receptor langerin, are the only innate sentinel cells in the skin epidermis and are present in oral mucosa. LCs can take up, process and present antigens to local memory T cells as well as naïve T cells in the draining lymph nodes. We investigate how LCs recognize GAS strains to locally detect and eliminate invading bacteria.

Methods

We used soluble human recombinant langerin to investigate the interaction with a diverse range of wild-type as well as genetically-modified GAS strains. Furthermore, we captured the complete transcriptional response of primary human LCs stimulated with UV-killed GAS using bulk RNA sequencing.

Results

Recombinant human langerin recognized a wide range of GAS isolates and M-types, but binding was not universal. Ligand binding occurred through the canonical carbohydrate-recognition domain since binding was abrogates by mannan. On the GAS surface, emm3 deletion abrogated langerin binding. Transcriptome analysis revealed that primary LCs produced pro-inflammatory molecules such as CCL3 and CCL4, as well as IL10 upon stimulation with UV-killed GAS.

Conclusion

Human LCs and their characteristic receptor langerin, interact with and respond to GAS of diverse, but not all, M-types. We are currently pinpointing the GAS ligand of langerin and hypothesize that this LC-GAS specific interaction supports host defense to prevent bacterial dissemination.

O21

The capsule serotype of Streptococcus pneumoniae affects respiratory epithelial cytokine responses and downstream immune activation

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The capsule of Streptococcus pneumoniae, an essential virulence factor for serum resistance, encompasses over 100 serotypes, each exhibiting distinct pathogenic traits: some serotypes are known to often cause invasive disease, while others associate with asymptomatic colonisation of the upper respiratory tract. The epithelium of the respiratory tract serves as a barrier against invasive disease, but also orchestrates the primary immune reaction against potential pathogens, leading to the migration of immune cells to the site of infection. Therefore, we investigated the epithelial immune responses induced by the pneumococcal polysaccharide capsule and its downstream effects on immune activation.
Isogenic capsule switch mutants were made in a TIGR4 background and included serotypes associated with invasive disease (1, 4, and 8) and with colonisation (unencapsulated, 6B, and 35B). This enabled us to specifically study the effect of the polysaccharide capsule on epithelial immune responses in the absence of other differences in virulence factors. Primary nasal epithelial cells differentiated on an air-liquid interface (ALI) from 7 donors were infected with these capsule switch mutants and basolateral medium was collected at 48 hours post-infection. Analysis of 16 cytokines in these media showed capsule-dependent differences in the concentrations of 10 cytokines. Particularly, the mutant without capsule expression exhibited the highest induction of CCL2, CXCL1, and CXCL8 compared to the other mutants, highlighting the capsule's importance in pneumococcal-epithelial cell interactions. Additionally, in an immune cell migration pilot experiment we observed that these distinct epithelial cytokines differentially attracted CD8 T cells, NK cells, monocytes or neutrophils. Overall, our findings show that the pneumococcal capsule is an important virulence factor in epithelial immunity, influencing the attraction and activation of downstream immune cells. Our future research aims to further examine the migration kinetics and understand how epithelial cytokines induced by the capsule mutants impact the activation of downstream immune cells.

O22

Fusobacterium nucleatum-released ADP-heptose upregulates PD-L1 in intestinal epithelial cells via the activation of ALPK1

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Fusobacterium nucleatum is a Gram-negative oncobacterium that is associated with colorectal cancer. The molecular mechanisms utilized by F. nucleatum to promote colorectal tumor development have largely focused on adhesin-mediated binding to the tumor tissue and on the pro-inflammatory capacity of F. nucleatum. However, the exact manner in which F. nucleatum promotes inflammation in the tumor microenvironment and subsequent tumor promotion remains underexplored. Here, we show that both living F. nucleatum and sterile conditioned F. nucleatum culture medium promotes CXCL8 release from the intestinal adenocarcinoma HT29 cell line. We determined that the identified responsible and secreted F. nucleatum molecule had the same characteristics as the pro-inflammatory metabolite ADP-heptose, since the observed pro-inflammatory effect was Alpha-kinase 1 (ALPK1)-dependent in both HEK293 and HT29 cells. In addition, we determined that not only F. nucleatum promoted an ALPK1-dependent pro-inflammatory environment, but that also other Fusobacterium species such as F. varium, F. necrophorum and F. gonidiaformans generated similar effects, indicating that ADP-heptose secretion is a conserved feature of the Fusobacterium genus. By performing transcriptional analysis of ADP-heptose stimulated HT29 cells, we found several inflammatory and cancer-related pathways to be differentially regulated, including DNA mismatch repair genes and the immune inhibitory receptor PD-L1. Finally, we show that stimulation of HT-29 cells with F. nucleatum resulted in an ALPK1-dependent upregulation of PD-L1. These results aid in our understanding of the mechanisms in which F. nucleatum can affect tumor development and therapy, and pave way for future therapeutic approaches.

O23

Salmonella enterocyte invasion through MUC1: contributions of the extracellular domain and cytoplasmic tail to invasion and signaling

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Salmonella Enteritidis (Salmonella) is a common food-borne enteropathogenic bacterium that can bypass the mucus layer and invade intestinal epithelial cells. Highly glycosylated mucin proteins are expressed on the apical surface of enterocytes, together called the glycocalyx. Previously, we discovered that the Salmonella giant adhesin SiiE interacts with the glycosylated transmembrane mucin MUC1 which induces apical invasion into enterocytes. Here, we investigate if the MUC1 glycosylated extracellular domain (ED) and/or the cytoplasmic tail (CT) with signaling capacity contribute to Salmonella invasion and/or induction of downstream signaling. As our model, we use HT29-MTX (MUC1-WT) cells, CRISPR/Cas9-MUC1 knockout cells (ΔMUC1), CRISPR/Cas9-MUC1-CT (MUC1-ΔCT) cells that lacked the CT and mucinase-cleaved MUC1-ED (cleaved-ED MUC1) cells leaving the transmembrane domain and CT intact. A significant reduction in bacterial infection was observed in ΔMUC1 and cleaved-ED MUC1 cells, suggesting that MUC1-ED has an essential receptor function. No difference in bacterial infection was observed between MUC1-WT and MUC1-ΔCT cells, showing that the CT is not essential for SiiE-MUC1 invasion. The MUC1-CT was also not required for secretion of IL-8 as measured by ELISA. To determine the contribution of the MUC1-CT, we performed a large RNAseq experiment with uninfected and infected cells. The most striking observation was that the regulation of certain genes by the NFκB family was dependent on the presence of the MUC1-CT. Immunoblot analysis demonstrated that the NFκB transcriptional subunits p50, p52, p65, RELB and c-REL are equally expressed and translocated in all cell types. However, the NFκB cytoplasmic inhibitory subunits p100, p105 and IκBα are significantly upregulated in MUC1-ΔCT and ΔMUC1 cells compared to MUC1-WT cells in the absence of Salmonella but are equally upregulated after Salmonella invasion. Based on these results, we conclude that the MUC1 extracellular domain is essential for Salmonella invasion and MUC1-CT has an anti-inflammatory function by suppressing the NFκB pathway.

O30

Archaeal Parisitism - Insights into the Interactions Between Ca. Nha. antarcticus and Hrr. lacusprofundi

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The DPANN archaea comprise several archaeal phylum-level lineages many members of which have reduced genomes and small cells. All cultivated DPANN are symbionts of other archaea that require direct cell-cell interactions with their host for proliferation. Thus far, the dynamics of DPANN host interactions remain enigmatic. We applied a combination of live fluorescence, cryo-correlated light and electron microscopy, and bioinformatics approaches to investigate the interactions between Ca. Nha. antarcticus and Hrr. lacusprofundi. Our results reveal that Ca. Nha. antarcticus acts in a parasitic manner which causes death of the host cell within hours of attachment at rates similar to those seen with lytic viruses. Furthermore, the results indicate that during this process nanohaloarchaeal cells enter the cytoplasm of the host. This behaviour implicates Ca. Nha. antarcticus as a top-down regulator of natural host populations and suggests DPANN archaea could significantly impact nutrient cycling and community structure in globally distributed ecosystems.

O31

Community outbreak with Panton-Valentine Leukocidin-encoding livestock-associated methicillin-resistant Staphylococcus aureus in the Netherlands

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Introduction
Infection with livestock-associated (LA)-MRSA is usually less severe and human-to-human transmission of LA-MRSA is usually limited, compared to non-LA-MRSA. However, Panton-Valentine Leukocidin (PVL)-positive LA-MRSA strains cause more severe infection and lack livestock association. Here we describe a community outbreak of PVL-positive LA-MRSA severe infections without livestock contact.

Methods
MRSA isolates are routinely sent to the RIVM for multiple locus variable-number tandem repeat analysis (MLVA) typing as part of TypeNed surveillance. In the course of the outbreak, PVL PCR and LA-MRSA/clonal-complex 0398 PCR was performed on MRSA isolates with an antibiogram similar to the outbreak strain by the regional laboratory. Whole genome sequencing (WGS) was additionally performed on all isolates by the RIVM. The municipal health service (MHS) conducted extended source tracing, concentrated around direct skin and/or animal contact of cases.

Results
In November 2023, the first 5 patients with skin lesions with PVL-positive, MLVA type MC0398/MT2306 MRSA were notified by the laboratory to the MHS. Up to January 17th, a total of 18 cases were identified, of which 17 had skin lesions. Six patients required surgical intervention, one patient also had bacteraemia. Disease onset was between September-December 2023. WGS confirmed a genetic cluster. 14 cases cooperated in source investigation; 10 cases had visited a specific massage parlour prior to disease onset. The parlour was visited by the MHS. One of three employees examined for carriership and 3/16 environmental samples tested positive for the same MLVA type MRSA. The parlour was disinfected thoroughly. The infected employee and all cases were advised eradication therapy.

Conclusion
This PVL-positive LA-MRSA-strain caused unusually severe skin infections for LA-MRSA. The outbreak was linked to a massage parlour and confirmed human-to-human transmission in the community. These findings suggest changing pathogenicity and transmissibility of LA-MRSA, which emphasizes the need for early detection and rapid source tracing.

O32

The added value of a regional, prospective whole genome sequencing based surveillance for methicillin-resistant Staphylococcus aureus (MRSA) outbreak detection in five hospitals in the Netherlands

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Prospective WGS surveillance has the potential to enhance the identification of hospital outbreaks. This study aimed to assess the added value of a regional, prospective WGS based surveillance for MRSA outbreak detection.

A prospective genomic MRSA surveillance study was performed between 1 June 2022 and 31 May 2023, across five hospitals in the Netherlands. Only one isolate per year per case was included. WGS was performed using Nextera XT on an Illumina MiSeq. Genomic clusters were established using whole genome multilocus sequence typing with a cluster cut-off of 15 alleles. Genomic and epidemiologic data was combined to identify transmissions. The detection of a cluster was classified to have added value for infection control policy when: 1) a cluster of possible nosocomial transmission was identified 2) a cluster of possible transmission in the community was identified.

A total of 323 MRSA isolates were eligible for WGS, with 169 isolates (52%) undergoing sequencing. Sixty-nine isolates (41%) clustered with at least one other isolate. This resulted in the identification of 24 clusters (each comprising 2 to 8 cases), of which six clusters (25%) spanned multiple hospitals (Table 1). In most cases, a cluster was already suspected based on epidemiological data (e.g.household members). For nine clusters (38%) WGS proved added value for infection control. WGS confirmed nosocomial transmission in three hospitals and two nursing homes. In five clusters, three of which spanned multiple hospitals, it was evident that MRSA infections originated outside the hospital settings (community). These clusters of infections were subsequently reported to the Public Health Service for further investigation.

The prospective WGS based surveillance indicates minimal MRSA transmission in general within the five participating hospitals. The primary added value of the surveillance lies in identifying community clusters that extend across multiple hospitals, cases that might otherwise go unnoticed.

O33

Is LA-MRSA still livestock-associated?

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Introduction: In 2018 a One Health surveillance of livestock (LA)-MRSA in humans and animals in the Netherlands was started with a consortium of the RIVM, WBVR, WFSR and NVWA to monitor the nature of MRSA transmission between animals and humans.

Methods: Samples from animals, meat, dust from livestock farms (poultry, pigs, cattle), slaughterhouses (dairy cows, broilers) and persons working on the farms were cultured using (pre-) enrichment and selective plates. LA-MRSA isolates were compared with isolates collected in the Dutch national MRSA surveillance. Next-generation sequencing (NGS) data of a subset of MRSA belonging to genogroup 0398 (GG0398) were generated. Whole-genome multi-locus sequence typing (wgMLST) was performed on NGS data from LA-MRSA from the national human MRSA surveillance (n=1770), animals (n=528), persons working/living on the farms (n=28), meat (n=97) or dust collected at slaughterhouses and livestock farms (n=158) to assess genetic relatedness.

Results: A high MRSA farm prevalence was observed on finishing pig farms (75.8%), whereas the MRSA prevalence was lower on veal calf farms (25.4%), dairy farms (6.1%) and MRSA was not found on broiler farms. From 2018-2022, 2,764 meat samples were analyzed and 284 (10.3%) were MRSA-positive. wgMLST showed that isolates originating from animals, meat and humans grouped together in LA-MRSA GG0398. Remarkably, one branch comprised only LA-MRSA from humans, and these isolates were often Panton Valentine Leucocidin (PVL)-positive, without a link to animals/farms. No PVL-positive LA-MRSA originating from animal sources were found. As there were no clusters containing animal isolates only and some animal-related isolates were closely related to human isolates, it is likely that transmission between animals and humans occurred. In addition, (indirect) transmission between different animal species occurred as some animal isolates differed by less than 16 wgMLST alleles.

Conclusion: We showed that PVL-positive LA-MRSA were human-associated and not livestock-associated anymore and human-to-human transmission seems probable.

O34

Needles in a haystack: Identifying new human genetic etiologies underlying severe Staphylococcus aureus infections.

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Introduction:

Staphylococcus aureus although an asymptomatic commensal in many, affects a tiny proportion of the population with life-threatening infection. The causes of this interindividual variability are poorly understood. Rare, single-gene defects (also known as inborn errors of immunity (IEIs)) predispose otherwise healthy people to S. aureus infections but collectively account for only a minority of cases. Patients with uncharacterized etiologies present as experiments of nature from which previously unknown IEIs can be uncovered.

Methods:

Whole exome sequencing (WES) data of the 118 patients with severe necrotizing S. aureus infections in the lungs and/or skin was systematically scanned in a genome-wide level to filter for very rare variants and predicted to be deleterious. The same refinement criteria were carried out with cohorts of control exomes with Mycobacterium tuberculosis infections and Hidradenitis Suppprativa, an inflammatory skin disease. The resulting lists of genes from the different cohorts were compared against each other to assess and rank the burden of mutations in genes.

Results:

We have identified rare, predicted deleterious and rare variants in genes: Interleukin 1 Receptor-Like 1, Spectrinβ N1, Ankyrin 2, C-X-C Motif Chemokine Ligand 1 which are carried by the patients in heterozygosity; variants in F-Box and Leucine-Rich Repeat Protein 19, Phosphatidylinositol-4-Phosphate 3-Kinase Catalytic Subunit Type 2, and Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3 which are carried by patients in homozygosity; variants in Rho Guanine Nucleotide Exchange Factor 6 which are carried in hemizygosity.

Conclusion:

We identified predicted deleterious variants in host genes, whose pathological/immunological consequences may underlie severe S. aureus infections. Ongoing functional characterization of the alleles will inform our understanding of the mechanism of disease in the patients affected. It will eventually inform about the essential role of human genes in immunity against S. aureus infections.

O35

A hospital outbreak with methicillin-resistant Staphylococcus argenteus in the Netherlands, June 2023

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Introduction Staphylococcus argenteus is part of Staphylococcus aureus-complex, and causes similar disease. Methicillin-resistant S. argenteus (MRSArg) is rare in the Netherlands. Of 52,467 isolates submitted to the national MRSA surveillance between 2008 and 2021, 54 (0.10%) were identified as MRSArg. Here we describe the first hospital outbreak with MRSArg in the Netherlands.
Methods Species determination was performed using MALDI-TOF MS (Bruker Daltonics). Cefoxitin disk diffusion and molecular screening was performed by Xpert® MRSA NxG. MRSArg were sequenced by whole genome sequencing (WGS). WGS data was used for multilocus sequence typing (MLST), whole-genome MLST (wgMLST), PVL encoding genes, SCCmecFinder, ResFinder and CARD. Antibiotic susceptibility testing was performed using Vitek® 2XL (BioMérieux).
Results Recently, a health care worker (HCW) was tested positive for MRSArg carriership. Since there was an epidemiological link between the HCW and a patient with a positive MRSArg blood culture, cohort screening and an outbreak management team was initiated. The cohort screening revealed an additional 8 HCW of 293 screened persons and 3 patients of 201 screened patients as carrier of MRSArg. All MRSArg positive persons (n=13; 8 female/5 male; age range 22-86, median age 55) received carriership eradication treatment. wgMLST identified a genetic cluster in which the 13 MRSArg isolates varied 1 to 2 wgMLST alleles, all with MLST ST2250. The isolates carried the dfrG, blaZ genes and the mecA gene in an IVc(2B) SCCmec type-cassette, but not PVL. Six isolates carried a V588F mutated ileS gene (97,3% identity/100% query coverage) with MICs for mupirocin between 2 - 32 mg/L, the remaining 7 isolates were sensitive.
Conclusion Here, we report the first hospital outbreak by MRSArg in the Netherlands. The mutated (V588F) ileS gene is possibly implicated in the intermediate mupirocin susceptibility. No additional MRSArg were detected in the hospital, indicating that the infection prevention measures were appropriate.

O36

Antagonism or Synergy: Unraveling the intertwined relationship between Bacillus, and Trichoderma against plant pathogens

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Agricultural practices using the combination of the bacterium Bacillus and the fungus Trichoderma demonstrate their growth promotion and biocontrol effects. Co-cultivation of these two microorganisms leads to a more effective promotion than the individual cultures, despite the inherent antagonistic relationship between the bacterium and the fungus. To explore the molecular and chemical details of this bacterial-fungal interaction (BFI), we employed transcriptomics, HPLC-MS, Scanning Electron Microscopy, and specific gene knockout approaches. Here, we report that surfactin, a lipopeptide produced by Bacillus velezensis, promotes Trichoderma harzianum to produce azaphilone, which actives reactive oxygen species (ROS) immunity in the fungus. During this synergistic BFI, the growth of the pathogen Fusarium oxysporum f. sp. cubense (FOC) is inhibited in addition to the degradation of the FOC virulence factor, fusaric acid. The Bacillus-Trichoderma interaction creates a dynamic equilibrium, ultimately benefiting plant health and suppressing a plant-pathogenic fungus. Our study offers a new perspective and research direction for understanding BFI in complex soil environments.

O37

Antimicrobial susceptibility to last-resort antibiotics in carbapenemase-producing bacteria from Ukrainian patients

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Introduction

Since March 2022, there is an emergence of multidrug-resistant micro-organisms (MDRO) in Europe because of an increasing number of Ukrainian patients with MDRO. The goal was to collect phenotypic susceptibility data to assess implications for clinical practice.

Methods

For 122 MDRO, carbapenemase-producing Enterobacterales (CPE, n=96), Pseudomonas aeruginosa (CPPA, n=20), carbapenem-resistant Acinetobacter baumannii-calcoaceticus (CRAB, n=6), collected from March to December 2022, broth microdilution (BMD), fosfomycin agar dilution, gradient strip ampicillin-sulbactam, sulbactam-durlobactam, ceftazidime-avibactam-aztreonam and disk-diffusion (DD) for cefiderocol was performed. Epidemiological data was obtained as part of mandatory reporting by Municipal Health Services (CPE), and surveillance questionnaires (CPPA/CRAB).

Results

Of all isolates, 65% (79/122) were blaNDM-positive. For meropenem, aminoglycosides, ceftazidime-avibactam, ceftolozane-tazobactam and imipenem-relebactam susceptibility rates were low (0-30%). For cefiderocol, results depended on reading with or without microcolonies, applying EUCAST or CLSI breakpoints, and whether DD or BMD was used, e.g. for Klebsiella pneumoniae, 30-97% were susceptible, depending on these variables. For colistin, 103/111 (93%) non-intrinsically resistant CPE/CPPA/CRAB isolates were susceptible. For the majority of CPE, a low minimal inhibitory concentration (MIC) of <0.5 mg/L was measured for tigecycline and ceftazidime-avibactam-aztreonam. For CPPA, cefiderocol tested susceptible in 65-100% of isolates. For CRAB, ampicillin-sulbactam MICs were ≥128 mg/L, for sulbactam-durlobactam 1-2 mg/L. Admission at a Ukrainian hospital in the last year was a risk factor for being MDRO positive.

Conclusion

There is extensive phenotypic resistance to last-resort antibiotics in MDRO from Ukrainian patients. For cefiderocol, interpretation of susceptibility results depends on several variables. In case of infection, treatment options are colistin, cefiderocol (CPE/CPPA/CRAB), tigecycline (CPE/CRAB), ceftazidime-avibactam-aztreonam (CPE), sulbactam-durlobactam (CRAB).

O38

Large-scale computational analyses of gut microbial CAZyme repertoires enabled by Cayman

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Introduction: Carbohydrate-active enzymes (CAZymes) are crucial for digesting glycans, but bioinformatics tools for CAZyme profiling and interpretation of substrate preferences in microbial community sequencing data are lacking. To address these limitations we developed a CAZyme profiler (Cayman) and a hierarchical substrate annotation scheme. Cayman is freely available from https://github.com/zellerlab/cayman.

Methods: Leveraging our newly developed tools, we genomically surveyed CAZymes in human gut microbial genomes (n=107,683) and performed 2 meta-analyses based on gut metagenomes. The first one was performed on CAZyme repertoires of Western versus non-Western gut metagenomes (n=4,281) and the second one compared colorectal cancer patients (CRC) to controls (n=1,998).

Results: First, investigating CAZyme repertoires of bacterial genomes combined with our substrate annotation scheme provides the most comprehensive view of CAZyme repertoires of human gut microbes so far. We furthermore discovered several bacterial genera (Hungatella, Eisenbergiella) with high numbers of mucin-targeting CAZymes, making them previously undescribed, putative mucin-foragers. Second, our Western/non-Western meta-analysis surprisingly revealed higher CAZyme richness in Western populations. We follow this surprising finding up by an inference pinpointing which gut microbial taxa are the major contributors to community-wide CAZyme shifts in order to explain the observed differences between Western and non-Western microbiomes. Lastly, we find a consistent CRC-CAZyme signature characterised by enrichment of enzymes for the degradation of animal-derived and host-derived glycans and depletion for enzymes facilitating digestion of dietary fibre, in line with dietary risk factors for CRC as established by epidemiological studies.

Discussion: We present the first easy-to-use command-line tool for profiling CAZymes from metagenomes. We demonstrate how it facilitated the discovery of several novel aspects of CAZyme biology of the human gut microbiome leveraging large-scale metagenomic data. We anticipate that Cayman will be broadly useful for studying diverse microbial communities and their glycan metabolism.

Uncovering the origin and evolution of oxygen-impermeable membranes in multicellular cyanobacteria

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Introduction

Some cyanobacteria produce specialized non-photosynthetic cells for nitrogen fixation, called heterocytes. Heterocytes are surrounded by heterocyte glycolipids (HGs), which contribute to the protection of the nitrogenase enzyme from oxygen. Due to their preservation in the environment, HGs have been used as indicators of nitrogen fixation, to differentiate specific families and genera of heterocytous cyanobacteria based on their structural diversity, and to reconstruct past surface water temperatures. However, despite the relevance of HGs for nitrogen fixation and the emergence of multicellularity within cyanobacteria, and their potential as biomarkers, knowledge on their origin and subsequent evolution, and related structural diversity, is lacking.

Methods

We reconstructed the acquisition and evolution of the biosynthetic capability to produce HGs by screening ~3,600 cyanobacterial genomes and plasmids for the co-localization of key genes involved in HG formation and their deposition in the cell envelope of the heterocyte. We further analysed the lipid composition of 24 heterocytous and of 2 non-heterocytous cyanobacterial cultures using high-resolution accurate mass/mass spectrometry to elucidate the connection between biosynthetic capability encoded on the genome and biosynthetic product.

Results

We found 465 genomic clusters of HG biosynthesis genes (‘hgl islands’) within heterocytous cyanobacteria, and also unexpectedly 35 in non-heterocytous cyanobacteria. A duplication of the hgl island predating heterocyte formation resulted in two islands in many heterocytous cyanobacteria today. Phylogenetic analyses combined with comprehensive HG identification revealed that HG structure evolved quickly and convergently within heterocytous cyanobacteria, challenging the common use of HGs as taxonomic biomarkers. In addition, genomic data suggest that HGs might have originated from ancient molecules that predate multicellularity and were not involved in nitrogen fixation, remnants of which may still be produced by select non-heterocytous cyanobacteria today. Together, our results open a new chapter in the understanding of the evolution of cyanobacteria.

O40

Mapping the Glycan Landscape: A Genomic Approach to Unravel Extracellular Polymeric Substance Biosynthesis in Environmental Micro-organisms

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Introduction
The chemical composition and properties of Extracellular Polymeric Substances (EPS) and their dependence on growth conditions remain poorly understood in environmental biotechnology. This knowledge gap hinders the growing interest in resource recovery and using EPS as sustainable biopolymers in industrial applications.
Here, we present a method for screening bacterial genomes with respect to their potential to produce polysaccharides or other glycans, a major component of EPS. Rather than starting at the immense number of potential glycan structures, we set out to understand the glycan composition from their nucleotide-sugar precursors.

Method
We compiled an HMM database consisting of genes associated with nucleotide-sugar biosynthesis, carbohydrate active enzymes (CAZymes) and additional TIGRFAMs entries associated with EPS biosynthesis. As subject, we used a set of MAGs from “Ca. Accumulibacter”, an environmentally dominant polyphosphate accumulating organism, found in estuarine environments and wastewater treatment plants. Using HMMer, MAGs were scanned for occurrences of these query genes, which were grouped into putative gene clusters based on their genomic proximities.

Results
Analyzing 82 MAGs from members of the “Ca. Accumulibacter” genus, we identified the spectrum of nucleotide-sugars that these bacteria can produce. The presence/absence pattern was generally consistent with previously observed prevalence of nucleotide-sugars within the bacterial and archaeal domains.
Moreover, it was found that the genes for rare monomers tend to occur in clusters with genes for CAZymes and other glycan biosynthesis-related proteins, while genes for common monomers tend to be isolated.
Finally, a set of recurring gene cluster architectures was identified, such as clusters containing genes associated with the PEP-CTERM system, which has been described to be strongly associated with EPS biosynthesis.

Conclusion
With this approach, we can determine the set of available glycome building blocks, identify their genomic context, and identify candidates for novel EPS gene clusters, aiding the understanding of EPS biosynthesis pathways.

O41

Unveiling the hidden world of bacterial membrane-spanning lipids: Adaptive responses, and novel insights for branched GDGT production in the environment

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Membrane-spanning lipids (MSLs) are commonly present in most archaea, but they are rarely seen in bacteria. In bacteria, the MSLs are formed by tail-to-tail condensation of fatty acids (FA) to form diabolic and iso-diabolic acids, which are the precursors of branched glycerol dialkyl tetraether (brGDGTs) lipid molecules. Normally, brGDGTs biomarkers are used for reconstructing past temperatures due to their sensitivity to environmental conditions. Nevertheless, the identification of bacteria capable of synthesizing these compounds and their precursors has been limited. Furthermore, the physiological processes regulating their production are still unknown. By investigating the biosynthetic genes, namely mss and ger, the genes encoding for the tail-to-tail condensation, and the ether lipid synthesis, we unveil novel bacterial strains capable of synthesizing brGDGT precursors. Among these strains, two of them (Sporanaerobacter acetigenes and Keratinibaculum paraultunense) were seen to produce iso-DA and alkyl ether lipids as part of their membrane lipid composition. Further testing of these strains under different abiotic stresses (i.e., temperature, pH, oxygen) revealed that the production of MSLs varies according to the condition, but it also depends on the strain producing these lipids. Our results indicate a relationship between abiotic stressors and MSL production dynamics, highlighting the adaptive nature of these organisms in response to environmental challenges. In conclusion, the variability in MSL production under different conditions provides new insights into microbial lipid dynamics that could enhance the accuracy and reliability of brGDGT-based temperature reconstructions.

O42

Alterations of glycan composition in aerobic granular sludge during the adaptation to seawater conditions

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Bacteria can synthesize a variety of glycans, being found attached to proteins and lipids or as loosely associated polysaccharides to the cells. The major challenge in glycan analysis in environmental samples lies in developing high-throughput and comprehensive characterization methodologies to elucidate the structure and monitor the change of the glycan profile, especially in protein glycosylation. In the current research, the dynamic change of the glycan profile of a few extracellular polymeric substance (EPS) samples from aerobic granular sludge (AGS) was investigated by high-throughput lectin microarray and mass spectrometry. AGS was grown in a lab-scale bubble column reactor fed with acetate and under increasing seawater conditions (10-35 g/L). The AGS was collected at 3 stages, before, during and after adaption to seawater. The EPS was extracted under alkaline and heat condition. It was found that there were glycoproteins in all of the EPS samples. In response to the exposure to seawater, the amount of glycoproteins and their glycan diversity displayed an increase during the adaptation phase indicated by a strong signal for 55 lectins. The yield of the EPS before and after exposure to seawater was increased by 2-fold. After stable conditions, the EPS showed less glycan diversity and a significantly different glycan profile than before seawater exposure. These results show an approach to identify and monitor the diversity and dynamic alteration of the glycan profile of the EPS in response to environmental stimuli.

O43

E. coli meet your match: Production of monoclonal antibodies from E. coli-specific B cells to combat bacterial infections.

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E. coli's genetic adaptability drives its prevalence and the emergence of highly virulent and drug-resistant strains, posing a significant health threat. Monoclonal antibodies (mAbs) show promise as an alternative therapy due to their high affinity for bacterial antigens, with a reduced risk of triggering bacterial resistance. mAbs can neutralize bacteria, activate the complement system, or induce phagocytosis by neutrophils.
To develop mAbs against E. coli, we selectively identified B cells that specifically recognise clinically relevant E. coli strains and utilised their B cell receptor (BCR) information to recombinantly express mAbs. Initially, we stained peripheral blood mononuclear cells (PBMCs) from healthy volunteers or a patient who experienced E. coli bacteremia, using an E. coli lab strain or an E. coli clinical isolate, respectively. Both E. coli strains were labelled in two fluorophore colours each, as B cells binding to both fluorescently labelled E. coli colours were considered specific. This specificity was validated by the enrichment of memory B cells in the double-fluorescent B cell populations, implying that B cell-bacteria interactions occur via affinity-matured BCRs. We successfully identified double-fluorescent B cells against the E. coli lab strain and the E. coli clinical isolate. We continued by single-cell sorting these double-fluorescent B cells, amplifying their BCR genes, and cloning for expression in HEK-293 cells to produce mAbs. Currently, we have produced a mAb exclusively targeting the E. coli lab strain, without cross-reactivity to the clinical isolates.
In conclusion, our results demonstrate the successful generation of a mAb against E. coli lab strain, marking a promising initial stride in the development of therapeutic antibodies. Future steps involve producing mAbs against E. coli clinical isolates and investigating their role in complement activation and phagocytosis.

O44

Flumequine, a fluoroquinolone in disguise

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Introduction

Antimicrobial compounds are effective drugs, unfortunately, their success also seems to be their downfall as antimicrobial resistance (AMR) results in a rising risk for treatment failure. Fluoroquinolone resistance (indicator: ciprofloxacin) in commensal E. coli isolated from broilers (meat-producing poultry) in the Netherlands remains relatively high. Remarkable, since in the Netherlands fluoroquinolones are classified as a 3rd choice antimicrobial in livestock, with legal restriction for use. However, flumequine, a quinolone which harbours a fluoride atom in its chemical structure, is classified as 2nd choice antimicrobials enabling the usage of flumequine in poultry. Therefore we investigated whether flumequine selects for E. coli with resistance mechanisms similar to fluoroquinolone resistance.

Methods

Initially we conducted in vitro experiments in which we exposed E. coli to concentrations of flumequine and enrofloxacin below MIC levels. Subsequently we studied the selection and induction of fluoroquinolone resistant E. coli in a more complex setting by performing caecal fermentations treated with flumequine and enrofloxacin. Lastly we examined genomes of E. coli isolates from broilers for de novo single nucleotide polymorphisms (SNP) induction in the gyrA gene combined with phenotypic resistance. The isolates were isolated from broilers treated with clinical concentrations of enrofloxacin or flumequine.

Results

Use of flumequine as an antimicrobial compound leads to approximately 40 percent increase of resistant E. coli in the caecal fermentation, comparable with the enrofloxacin treatment. After exposure to flumequine and enrofloxacin we detected the same SNPs (S83L, D87G) in gyrA (responsible for fluoroquinolone resistance) in our in vitro experiments. Lastly we identified phenotypic resistant E. coli isolates from broilers treated with enrofloxacin and flumequine and the same resistance-causing SNPs were observed.

Conclusions

Flumequine has the same selective properties as enrofloxacin. In line with Dutch policy for use of fluoroquinolones in livestock, the classification of flumequine as a 3rd choice antimicrobial is justified.

O45

Novel cell platforms to valorize carbon dioxide into fine chemicals for the pharmaceutical industry

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The transformation of CO_2 off-gas emissions into valuable compounds represents a key target for the development of a sustainable and circular bioeconomy. In this study, we explored for the first time a novel market opportunity based on the conversion of CO_2 into ectoine (1000 €/kg) and hydroxyectoine (1200 €/kg), osmolytes produced by prokaryotes in high salinity environments. To this aim, halophilic microbes able to use CO_2 as a carbon source and H_2 as a green-energy source were identified by databases and their genomes mined for the genes of ectoines synthesis pathways (ectABCD). A total of 11 species had the genes to synthesize ectoines fixing CO_2 aerobically. Further laboratory analyses in 1 L batch bioreactors fed with 10% of CO_2 showed that the most promising bacteria for the implementation of this novel bioconversion process were Hydrogenovibrio marinus, Hydrogenibacillus schlegelii and Rhodococcus opacus. After salinity and H_2/CO_2/air ratio optimization, H. marinus accumulated the highest amount of ectoine among the three tested strains at 6% NaCl (79.6 ± 10.5 mg ectoine g biomass-1) and had the fastest growth with the shortest lag phase. Notably, ectoine content was similar for R. opacus and H. schlegelii at all the salinities tested, but hydroxyectoine levels increased at higher salinities. R. opacus accumulated maximum hydroxyectoine values at 7% NaCl (52.1 ± 4.3 mg hydroxyectoine g biomass-1) whereas H. schlegelii achieved maximum content at 5% (62.0 ± 7.9 mg of hydroxyectoine g biomass-1). These hydroxyectoine values fall within the range of yields obtained from rich carbon sources and were obtained as isolated osmolytes. Overall, this research set, by means of the use of an innovative genomic technique and bioengineering, the possibility to find novel microorganisms able to transform CO_2 into fine chemicals with important value for the economy and society.

O46

Prediction of clinical outcome of Escherichia coli O157:H7 infection using Machine Learning

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Introduction
Shiga toxin-producing Escherichia coli O157:H7 (STEC) is a globally dispersed zoonotic pathogen, causing a range of symptoms from mild to bloody diarrhoea and haemolytic uremic syndrome (HUS). STEC's primary virulence factor, Shiga-toxin (Stx), inactivates host ribosome, leading to cell apoptosis. Although the Stx subtype is a key predictor of disease severity, differences in virulence with the same Stx profile are often observed.
Here, we employed a Random Forest (RF) algorithm to predict disease severity based on whole genome sequencing (WGS) data.
Methods
WGS data from 1030 STEC isolates in the UK, reported as bloody diarrhoea (BD) (n= 597), diarrhoea (D) (n=387) and HUS (n=44), were divided into training (n=817) and test (n=213) sets, stratifying by clinical outcome and population structure.
DNA kmers of varying lengths were binary encoded and used as predictive features. Feature selection involved Chi-square tests and MUVR.
A RF model was trained and optimised using 10-fold stratified cross-validation. Training sets in each fold were randomly upsampled. Model's feature importance was assessed using Gini impurity values. Evaluation on the test set revealed the final model's performance.
Results
Overall accuracy of the RF classifier was 0.76, with a weighted average F1-score of 0.75. BD had the highest F1-Score (0.81), followed by D (0.67) and HUS (0.41). Combining BD and HUS as 'high risk' and D as 'low risk' resulted in an accuracy of 0.78, outperforming traditional risk assessment approaches like Stx subtyping and sublineage determination.
The most important feature was a 100-bp kmer, aligning downstream of the stx2a gene. Strains with stx2a lacking this kmer showed a distinct clinical profile (2.6% HUS, 56.7% BD, 40.8% D) than those with it (14.3% HUS, 71.0% BD, 14.7% D).
Conclusions
Our RF algorithm based on WGS data outperformed traditional STEC risk assessment methods and revealed a potential predictor of disease severity.

O47

Automated surveillance of hospital onset bacteraemia: first results

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Background: Hospital-onset bacteraemia (HOB) is currently under study as a hospital-wide target to include in (fully automated) surveillance of healthcare-associated infections. In general terms, HOB is defined as a positive blood culture, more than two days after admission. In case of a common commensal, a second positive culture within two days is required to be considered as infection. We aimed to develop an automated HOB surveillance and describe the first epidemiological results. We aligned our definitions as much as possible with the draft definitions formulated by the PRAISE (Providing a Roadmap for Automated Infection Surveillance in Europe) Network.
Methods: Data on hospital admissions and blood cultures in the period 2017-2021 were collected in four hospitals (1 academic medical centre (AMC) and three teaching hospitals (TH)). HOBs were identified by a fully automated surveillance system. HOB rates (number of HOBs/1000 patient-days) were calculated by ward type and (group of) micro-organism.
Results: The hospital-wide incidence of HOB was between 0.9 and 2.0 per 1000 patient days, with a higher HOB rate for the AMC compared to TH. The Intensive Care Unit (ICU) ward had the highest HOB rate in all hospitals (range 5.6 – 18.4), with a higher ICU HOB rate in 2020 and 2021 (COVID-19 years). The micro-organisms with the highest hospital wide HOB rate were coagulase-negative Staphylococci (range 0.11-0.38) , Enterococci (range 0.12-0.37), Enterobacterales (range 0.20-0.40), polymicrobial HOBs (range 0.13-0.30) and Staphylococcus aureus (range 0.11-0.21).
Conclusion: We developed an automated surveillance system for HOB, that was able to detect possible increases and discriminate between ward types. Further research should focus on the acceptance and interpretability, as well as risk adjustment of surveillance results.

O48

Waning of monkeypox virus-specific antibodies one year after MVA-BN vaccination

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Smallpox vaccination efforts were stopped in the 1970s following the eradication of variola virus. Consequently, poxvirus-specific immunity is virtually absent in generations born after the cessation of vaccination. While zoonotic transmission of monkeypox virus (MPXV) has been observed in several regions in West and Central Africa, sustained transmission outside these endemic areas was rare until the 2022-2023 global outbreak of mpox, which was declared a public health emergency of international concern by the World Health Organization. In order to provide protection and contain the outbreak, individuals at-risk of contracting mpox, primarily men who have sex with men (MSM), were in many countries invited for vaccination with modified vaccinia virus Ankara-Bavarian Nordic (MVA-BN, also known as Imvanex), a third-generation smallpox vaccine authorized and in use as a vaccine against mpox. We previously demonstrated that MVA-BN vaccination resulted in only low levels of MPXV-neutralizing antibodies at 28 days post second vaccination, but strong virus-specific CD4+ T-cell responses. The durability of these responses is currently not known. Here, we describe the one-year follow-up of a cohort of healthcare workers and an at-risk cohort of MSM, who were vaccinated with MVA-BN during the 2022-2023 mpox outbreak. We demonstrate a sharp decline in binding and neutralizing antibody levels in both groups, with about two-thirds of the individuals having binding antibody titers below the lower limit of detection. Assays to detect virus-specific T-cells are ongoing. The roles of MPXV-neutralizing antibodies and MPXV-specific T-cells as correlates of protection against disease and/or transmission in humans are currently unclear. This, combined with occasional clusters of new infections, including among previously vaccinated or mpox-convalescent individuals, highlights the necessity to further investigate the implications of waning humoral immunity against mpox regarding reduced vaccine effectiveness, the potential need for booster vaccinations in high-risk groups, and the risk of an mpox re-emergence.

O48

Genomic surveillance of multidrug-resistant organisms based on long-read sequencing

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Introduction

Multidrug-resistant organisms (MDRO) pose a significant threat to public health world-wide. Public health laboratories perform surveillance to monitor trends in antimicrobial resistance and transmission of MDRO including resistance plasmids. The aim was to develop genomic MDRO surveillance based on long-read sequencing only.

Methods

Genomic DNA of 221 MDRO was automatically extracted and purified using the Maxwell. The MDRO included 70 Klebsiella pneumoniae (Kpn), 69 Escherichia coli (Eco), 11 Enterobacter cloacae complex (Ecl), 9 Citrobacter freundii (Cfr), 11 Pseudomonas aeruginosa (Pae), 7 Acinetobacter baumannii (Aba) and 44 methicillin-resistant Staphylococcus aureus (MRSA). MDRO were sequenced using both short-read (Illumina NextSeq 550, Nextera DNA Flex Library Prep kit) and long-read (Oxford Nanopore Technologies, Rapid Barcoding Kit 24 V14, SQK-RBK114.24, MinION flow cell R10.4.1) whole-genome sequencing (WGS). Basecalling was performed using Dorado 0.3.2 duplex mode and Rerio model for a subset (n=25) of Kpn. Long-read data was assembled using Flye, Canu, Miniasm and Unicycler. WGS data with >30x coverage was used for multilocus sequence typing (MLST), whole-genome MLST (wgMLST), and identification of resistance genes (Abricate).

Results

Comparison of wgMLST profiles based on long-read and short-read WGS data revealed that n=43/70 (61%) Kpn, 65/69 (94%) Eco, 11/11 (100%) Ecl, 8/9 (89%) Cfr, 1/11 (9%) Pae, 7/7 (100%) Aba and 43/44 (98%) MRSA yielded nearly identical genomes. Kpn basecalled with Rerio significantly improved 23/25 (92%) performance. Long-read-based wgMLST varied 24 (Kpn-duplex), 10 (Kpn-Rerio), 4, 3, 3, 52, 2, and 2 wgMLST alleles compared to short-read-based wgMLST, respectively. MLST sequence types were concordant between long-read and short-read WGS data. Antimicrobial resistance genes were detected in long-read sequencing data with high sensitivity/specificity.

Conclusion

We demonstrate that automated DNA extraction followed by long-read sequencing is an accurate method compared to short-read sequencing for genomic surveillance of MDRO. Kpn and Pae require improvement of basecalling algorithms, as Rerio illustrated.