P001

An in vivo mimicking growth medium for Streptococcus pneumoniae – the effect of growth conditions on the pneumococcal proteome

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a. Introduction: Standard Streptococcus pneumoniae culture methods use nutrient rich growth media developed to grow any laboratory strain or clinical isolate. However, the environmental niche occupied by pneumococci, the upper respiratory tract, is poor in nutrients, likely affecting pneumococcal phenotype and therefore processes such as colonization. Here, we have grown pneumococci in in vivo-mimicking medium to examine its effect on the proteome.

b. Methods: We have designed an in vivo-mimicking, chemically defined medium (IVM-CDM) containing metal ions and monosaccharide concentrations as measured in the nose using inductively coupled plasma mass spectrometry (ICP-MS) and liquid chromatography mass spectrometry (LC-MS/MS), respectively. In this medium we cultivated carriage isolates BHN100 (serotype 19F) and BHN418 (serotype 6B) and examined the proteome using data independent acquisition mass spectrometry (DIA-MS) and secreted metabolites and compared this with pneumococci grown in the original CDM, unadjusted for metal and sugar concentrations.

c. Results: Nasal monosaccharide concentrations were very low, but the addition of mucins to our IVM-CDM supported pneumococcal growth. The cultures reached lower optical densities in IVM-CDM compared to unadjusted CDM. Proteomic analysis detected around 1000 distinct proteins per sample. Of these, 32 showed different levels during exponential growth in both strains when grown in the different media. In the stationary phase, differences in levels were found for 107 proteins. Based on STRING-db clustering, we identified two clear functional clusters: fatty acid biosynthesis (downregulated in IVM-CDM) and galactose metabolism (upregulated in IVM-CDM). Pneumolysin was found to be downregulated in the IVM-CDM. We also identified the metabolite glycerophosphocholine to be secreted specifically in IVM-CDM conditions.

d. Conclusion: Growth in the in vivo-mimicking medium altered metabolic pathways and the expression of multiple proteins, showing the impact of growth conditions on experimental outcomes. Using appropriate growth conditions mimicking the upper respiratory tract environment may further our understanding of colonization processes.

P002

PCR-directed prediction for Neisseria gonorrhoeae culture using ESwab.

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Introduction: The global emergence of multidrug resistant Neisseria gonorrhoeae (NG) highlights the need for vigilant surveillance of antimicrobial susceptibility of NG. The golden standard for monitoring antimicrobial resistance (AMR) is culture followed by antibiotic susceptibility testing. NG is a notoriously fastidious microorganism and recovery rates are generally rather poor. In the past we have evaluated, and subsequently implemented, the use of quantitative PCR (qPCR) data from routine diagnostics for detection of NG in order to optimize the recovery rates of our NG culture. In this study we have analyzed our data since introducing ESwab (Copan diagnostics) in a further effort to optimize our NG culture.
Methods: Data were obtained from routine PCRs on COBAS 4800 (Roche diagnostics) for detection of NG and from corresponding NG ESwab cultures performed between January 2022 and December 2023 for the Department of Sexual Health of South Limburg. The data were analyzed anonymously retrospectively.
Results: We analyzed 2271 NG PCR positive samples. NG culture was performed in 1058 of these samples, of which 385 (36%) were positive. The recovery rate for samples with a cycletime (Ct) below 25 was 94%. The recovery rate for samples with a Ct between 25 and 30 was 63%. For samples with a Ct above 30 the recovery rate drops to 23%. Recovery rates for these NG cultures while using ESwab were similar to our previous data when samples were obtained using cotton swabs. Using a cut-off value of Ct 30, 40,8% of samples would have been eligible for culture which would have resulted in a recovery rate of 63%.
Conclusion: This study shows that routinely obtained qPCR data can be used to maximize culture effectivity by guiding which samples to submit for NG culture and AMR testing. Introducing ESwab for NG culture has not improved our recovery rates.

P003

Emergence of two multi-drug resistant and zoonotic Streptococcus suis lineages in Thailand following acquisition of a serotype 2 capsule

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Introduction: Streptococcus suis is a zoonotic porcine pathogen that occasionally causes meningitis and sepsis in humans. Driven by consumption of traditional raw pork products, S. suis has become the leading cause of adult bacterial meningitis in Thailand. The majority of global zoonotic infections are caused by strains from lineage CC1 carrying a serotype 2 capsule, however, zoonotic infections in Thailand are caused by an unusually diverse set of lineages with > 40% of the reported cases being caused by two Thai endemic lineages. In this study, we investigated the evolutionary steps that lead to the emergence of the Thai endemic zoonotic lineages CC104 and CC233.

Methods: We whole genome sequenced 140 S. suis zoonotic and porcine isolates and combined them with a curated dataset of 2762 public S. suis genomes to assess the evolution and spread of multi-drug resistance in lineages CC104 and CC233 using phylogenies and evolutionary models.

Results: We identified multiple antimicrobial resistance acquisition events in the Thai endemic zoonotic lineages which carried resistance determinants against five classes of antibiotics. More importantly both clades acquired key point mutations in the penicillin binding proteins resulting in non-susceptibility against β-lactams, the antibiotic class of choice for treating S. suis infections in both pigs and humans. We modelled the evolution of both lineages which shared a common ancestor that was introduced in Thailand at the end of the 19th Century from Western countries. We dated the emergence of lineage CC104 to 1978 and lineage CC233 to 1997. Both lineages emerged following two independent capsule switching events from serotype 7 to serotype 2 capsules.

Conclusion: Both lineages emerged and became zoonotic after acquiring a serotype 2 capsule. The accumulation of point mutations in the penicillin binding proteins is worrisome as they have conferred these novel zoonotic lineages with non-susceptibility against β-lactams.

P004

Evaluating the translation of the national COVID-19 IPC guidance documents to local guidelines in Dutch hospitals in 2020-2021

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Introduction
The COVID-19 pandemic underscored the vital role of infection prevention and control (IPC) policies in hospitals to protect patients and healthcare personnel. It is unknown whether hospitals followed the national guidance documents. This study aims to evaluate the translation of the Dutch national COVID-19 IPC guidance documents into local Dutch hospital guidelines.

Methods
This multi-center retrospective study compared the IPC policies of 7 Dutch hospitals, of which three academic hospitals and four teaching hospitals, to the national guidance documents. IPC practitioners collected data from hospital's (archived) IPC policy guidelines implemented between 01-03-2020 and 31-12-2021. Topics included the definitions of isolation measures and corresponding initiation and cessation criteria. A schematic summary of implemented policies was made to compare guidelines and to identify similarities and differences.

Results
The national guidance documents evolved and varied over time; however, local hospital guidelines displayed more variations compared to the national guidance document. Variation was found in the specified patient groups for which measures were applicable, in the combination of criteria, and the types of rooms and departments described. For the discontinuation of isolation measures, the number of established patient groups ranged from 1 to 12 groups, encompassing broad categories like "all SARS-CoV-2 PCR positive hospitalized patients" to specific subgroups such as "paediatric patients". Furthermore, local policy could change from stricter to more lenient than the national guideline over time. An in-depth analysis of the underlying guideline differences will be performed.

Conclusions
Local hospital guidelines showed more variability than the national guidance documents in the number of patient groups described and demonstrated more policy changes throughout the study period in both stricter and more lenient directions. Variations were observed within and between hospitals, underscoring the importance of examining additional options or subcategories in national IPC guidance documents to facilitate local implementation, which warrants further investigation.

P005

Graphene sensors for single cell antibiotic sensitivity testing

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Introduction

Antimicrobial resistance is a rapidly rising problem. The challenge lies in the development of rapid diagnostic tools. Whereas same-day determination of micro-organisms from cultures has become standard practice, Antibiotic Susceptibility Testing (AST) tools are still relatively slow. AST tools rely on the detection of growth of the pathogen, that typically requires at least single day, or even longer.

At SoundCell, we have developed a breakthrough technology that can potentially reduce the time for AST from culture to only 1 hour. The technology uses laser interferometry to detect nano-scale vibrations (nanomotion) induced by single micro-organisms on ultra-thin graphene biosensors. Using this technology, we show on that the nanomotion of alive and dead bacteria are different [1]. This has allowed us to uniquely assess the effectiveness of antibiotics in bacterial infections in only 1 hour [2,3].

Methods

Clinical isolates of E. coli, K.pneumoniae, S.agalactiae and MRSA were grown on Muller-Hinton Broth for up to 1-2h at 37C to reach an optical density (OD600) of ~0.1. Then 5ml of the refreshed culture was loaded into the graphene biosensor in the presence of various antibotics. Meropenem (1 mg/L), Penicillin (0,125 mg/L) and Amoxicillin (60 mg/L), were tested.

Results

On the SoundCell setup, resistance to amoxicillin and sensitivity to meropenem and penicillin were detected within 3 and in less than 1h respectively. The results were correctly assigned for all mentioned micro-organisms.

Conclusion

Graphene sensors can perform AST within 1 hour.

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P006

Prevalence of toxigenic Clostridium difficile in hospitalized patients in the southwestern province of Saudi Arabia: Confirmation using the GeneXpert analysis

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Clostridium difficile (C. difficile) is a leading cause of nosocomial infections in hospitalized patients worldwide. The current study aims to investigate the prevalence of the hypervirulent C. difficile strain PCR ribotype 027 (RT027) in the southwestern province of Saudi Arabia, where data is limited. Stool samples were collected from 112 inpatients admitted to different hospitals and were screened for C. difficile GDH + toxin A + B by immunoassay; all positive samples were processed for molecular detection of C. difficile using the GeneXpert assay. C. difficile strains were detected in 12 (10.71%) out of 112 stool samples using the GDH + toxin A + B immunoassay method. The toxigenic C. difficile was confirmed in 5 stool samples using the GeneXpert molecular assay. C. difficile strains were detected in 7 (8.97%) out of 78 stool samples from intensive care unit patients, 3 (25%) out of 12 stool samples from the internal medicine ward, 1 (11.11%) out of 9 stool samples from surgery ward, and 1 (10%) out of 10 stool samples from isolation ward using the GDH + toxin A + B immunoassay method and the toxigenic C. difficile strain was confirmed in 1, 2, 1, and 1 stool samples, respectively, using the GeneXpert molecular assay. Toxigenic C. difficile was confirmed in patients at 4 (51.14%) out of 7 hospitals. In the present study, we also analyzed the clinical information of patients with C. difficile-positive stool samples receiving one or more antibiotics during hospitalization. The binary toxin gene (cdt), the tcdC gene, and RT027 were not detected using the GeneXpert molecular assay among 12 C. difficile-positive samples by immunoassay. This study should aid in the prevention of unnecessary empiric therapy and increase the understanding of the toxigenic C. difficile burden on the healthcare system in the southwestern province of Saudi Arabia.

P007

Treatment of CMV infection in pregnancy: a questionnaire among Dutch gynaeologists

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Introduction
Cytomegalovirus (CMV) is the most common cause of congenital infections in the Netherlands. In the current guideline of the Dutch Association for Obstetrics and Gynecology (NVOG) serological screening or treatment of CMV in pregnancy are not recommended. Recent studies have shown that valaciclovir may have a role in prenatal prevention and treatment of congenital CMV infection, although opinion varies on the implications of these studies. We investigated how Dutch gynecologists manage CMV infection during pregnancy, and their reasons to deviate from the current guideline.
Methods
This cross-sectional survey was conducted among registered gynecologists in the Netherlands affiliated with the Fetal Ultrasound Working Group (WFE). The questionnaire, consisting of 22 questions, was distributed via an online platform (SurveyMonkey®), after approval by the chairperson of the WFE. Participation was voluntary and anonymous.
Results
Of the 148 contacted gynecologists, 33 (22%) completed the questionnaire. Serological screening for CMV was performed at patients' request by 45%, and in risk groups by 33% of respondents. In the past three years, seven respondents (21%) initiated secondary prevention with valaciclovir in ≥1 patients with evidence of a primary maternal infection in the first trimester, and four (17%) started valaciclovir in ≥1 patients with a proven fetal infection. Only three respondents found the current guideline sufficient. One-third advocated for introduction of universal CMV screening in pregnant women, and 21% advised screening in risk groups. Approximately one-third of respondents wanted to include secondary prophylaxis in a new guideline.
Conclusion
This survey among Dutch gynecologists shows considerable variation in views on management of CMV infections during pregnancy. Many gynecologists deviate from the current guideline regarding serological screening and secondary prevention or treatment of fetal infection with valaciclovir. This may lead to inequity of treatment. A revision of the current guideline based on recent publications should be considered.

P008

Detection of carbapenemase-producing Enterobacterales and Pseudomonas aeruginosa with BD Phoenix CPO Detect Test panel.

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Introduction:
The Dutch guideline 'Laboratory detection of highly resistant micro-organisms (HRMO)' recommends strategies to detect carbapenemases. Becton Dickinson (BD) PhoenixTM offers a Carbapenemase-Producing Organism (CPO) Detect panel to detect carbapenemase production in gram-negative rods. In this study we evaluate the performance of the BD PhoenixTM CPO Detect panel to detect carbapenemase-producing Enterobacterales and Pseudomonas aeruginosa in routine clinical practice, in comparison to the recommendations in the Dutch guideline.
Methods:
Susceptibility testing of all Enterobacterales and Pseudomonas aeruginosa isolates was performed with BD PhoenixTM CPO Detect panel. Isolates were subject for further testing for a carbapenemase, according to the report of the BD Phoenix CPO Detect panel and/or based on the minimum inhibitory concentration (MIC) of meropenem and/or imipenem, according to the Dutch guideline.
Results:
In Enterobacterales (n=5767), the BD Phoenix CPO Detect panel marked 54 isolates (0.9%) as potentially carbapenemase-producing Enterobacterales. A total of 37 (69%) Enterobacterales were confirmed carbapenemase positive. A total of 106 (1.8%) Enterobacterales had a meropenem MIC > 0,25 mg/L (screening cut-off value according to the Dutch guideline), of which 35 (33%) Enterobacterales were finally confirmed as carbapenemase positive. Two carbapenemase-producing Enterobacterales had a meropenem MIC 0.25 mg/L, and were only detected with BD PhoenixTM CPO Detect panel.
In Pseudomonas aeruginosa (n=1085) the Dutch guideline only recommends detection of Ambler class B carbapenemases, based on the MIC of imipenem and tobramycin. According to the Dutch guideline, 9 (0.8%) of the P. aeruginosa isolates should be further tested for carbapenemase production. Six isolates (67%) were confirmed carbapenemase positive. The BD PhoenixTM CPO Detect panel marked 21 (1.9%) as potentially carbapenemase-producing P. aeruginosa and detected one more VIM-positive isolate, that was tobramycin susceptible.
Conclusion:
In routine clinical practice, the BD Phoenix CPO Detect panel accurately detects carbapenemase-producing Enterobacterales and Pseudomonas aeruginosa, with reasonable false-positive results.

P009

Phenotypical detection of penicillin-resistance in methicillin-susceptible Staphylococcus aureus with the EUCAST disk diffusion method compared with the BD PhoenixTM.

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Introduction

Penicillin-resistance in methicillin-susceptible Staphylococcus aureus (MSSA) is based on a beta-lactamase, encoded by the penicillinase gene BlaZ. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) describes a method based on disk diffusion of penicillin (1 unit) with examination of the zone diameter and the zone edge to detect penicillin-resistance in MSSA. In this study we compared penicillin MIC values in the Becton Dickinson (BD) PhoenixTM and the EUCAST method to detect penicillin resistance in a selection of clinical MSSA isolates.

Methods

Penicillin susceptibility testing with BD PhoenixTM was tested in 201 clinical MSSA bacteremia isolates (2013-2017). All isolates were subject to whole genome sequencing (WGS) to detect presence or absence of penicillinase. A subset of 38 isolates was tested for penicillin susceptibility according the EUCAST disk diffusion method. To get an impression of the interobserver variability, ten isolates were presented to 14 experienced technicians to interpret the results of the EUCAST disk diffusion method.

Results

In 151 (75%) of the MSSA isolates blaZ was detected with WGS. The MIC of penicillin measured with BD PhoenixTM, was in 68% (n=102) interpreted as resistant with an MIC penicillin > 0.125 mg/L. In the isolates without a BlaZ gene (n=50), 74% of the isolates were interpreted as susceptible according to the MIC penicillin measured with the BD Phoenix. The subset of 38 isolates with the EUCAST penicillin disk diffusion method was correctly identified by one observer, and the subset of ten isolates were all correctly identified by 14 laboratory technicians.

Conclusion

The MIC penicillin reported by BD PhoenixTM is not able to distinguish penicillinase-producing MSSA. The EUCAST disk diffusion method to detect penicillin resistance in MSSA is a reliable method.

P010

Time to diagnosis of periprosthetic joint infections: worth the wait?

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a. Microbiological diagnosis of periprosthetic joint infections (PJI) is often challenging, as culture remains the gold standard. It requires multiple intra-operative samples and multiple culture media. Previous studies have demonstrated that time to diagnosis (TTD) of chronic PJI can take up to 14 days, unlike TTD for acute PJI requiring 5-7 days. Our primary aim is to compare TTD of acute and chronic PJI in a large cohort. Furthermore, the added value of implant sonication culture and enrichment media was evaluated.

b.This single-centre retrospective cohort study included 187 confirmed hip and knee PJI (2019-2023), defined by EBJIS (European Bone and Joint Infection Society) criteria. A minimum of 3 intra-operative samples was required. Each sample was incubated for 2 days on direct culture media (blood, chocolate and Schaedler anaerobic agar) and 14 days using two enrichment media (thioglycollate broth, anaerobic/pediatric blood culture bottle).

Comparison of TTD between monomicrobial early acute (n=52), late acute (n=48) and late chronic (n=59) PJI was performed using the Kruskal-Wallis nonparametrical test. A p-value of 0.05 was considered significant.

c.TTD differs across groups, X^2 (2, n=159)= 11.97, p=.003. Late chronic PJI record a significantly higher median score (Md=3) than late acute PJI (Md=2).

All late chronic PJI diagnoses (n=6) with TTD beyond 7 days were based on growth from enrichment media.

23 (12.3%) PJI diagnoses were confirmed based on growth from implant sonication culture only.

One (0.5%) PJI diagnosis was onfirmed based on growth from thioglycollate broth only.

d. This study confirms that TTD of early and acute PJI is less than 7 days, while TTD of chronic PJI can be longer and requires culture with enrichment media. Definitive treatment of acute PJI can therefore be determined within one week after revision surgery. Our future research will focus on novel diagnostic methods to shorten TTD of PJI.

P011

Within patient horizontal gene transfer dynamics of an NDM-7 plasmid among four different bacterial species.

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Introduction
Antimicrobial resistance is a worldwide threat. Bacteria can develop resistance through the acquisition of plasmids via gene transfer. Within-patient transfer of plasmids has been described before, but the evidence is scarce. In this case report, we present the molecular analysis of Enterobacterales strains cultured from one patient, with no apparent travel recent travel history, who was found to have four different carbapenemase-producing species during hospital admission.

Methods
All cultures of the patient obtained during admission were evaluated. Isolates with an MIC > 1 µg/mL for imipenem, or MIC > 0,25 µg/mL for meropenem were tested for carbapenemase activity using the CIM-test. When positive, the isolates were sequenced, using short and long read sequencing on NextSeq (Illumina) and GridION (Oxford Nanopore) respectively. Hybrid assembly was performed using unicycler, and plasmid sequences were compared among isolates.

Results
Over a period of five months, we identified 47 Enterobacterales isolates from 30 (of which 15 positive) clinical and 21 (of which 13 positive) screening cultures of one patient. Within two weeks after admission, a Citrobacter freundii was identified with an NDM-7 carbapenemase gene. Over time, three other bacterial species with carbapenemase production were cultured: Klebsiella oxytoca, Raoultella ornithinolytica and Serratia marscescens. Each of these four isolates contained an NDM-7 carbapenemase gene on a 45kb incX3 plasmid. For two of these species, a carbapenem-sensitive isolate was cultured before the carbapenemase-positive isolate.

Conclusion
Our results suggests horizontal gene transfer of an NDM-7 encoding plasmid among different species within one patient over the course of several months. This case report underlines the importance to also consider mobile genetic elements in outbreaks of nosocomial pathogens, as transmission vehicles of antimicrobial resistance going beyond the spread of bacterial identical strains.

P012

Environmental cleaning and disinfection practices for respiratory viruses among Dutch hospitals and nursing homes

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In the beginning of the COVID-19 pandemic, national and international guidelines recommended to not only clean, but also disinfect surfaces in a room after a COVID-19 patient was discharged. However, these guidelines were often non-specific and underwent frequent modifications as the pandemic progressed. Consequently, healthcare institutions adopted diverse cleaning and disinfection strategies. Meanwhile, in March 2023, the Dutch guidance document was changed to recommend cleaning only. Our aim was to determine cleaning and disinfection practices in Dutch hospitals and nursing homes concerning SARS-CoV-2, compare these to the first COVID-19 wave, and explore whether they differ for other respiratory viruses.

On June 23rd, 2023, a web-based survey in the Dutch language was developed and distributed among infection prevention and control professionals from hospitals and nursing homes across the Netherlands. The survey included questions about cleaning and disinfection practices for SARS-CoV-2, influenza and respiratory syncytial virus (RSV) at that moment, and questions about practices employed during the first COVID-19 wave.

In total, 81 healthcare institutions responded to the survey (non-academic hospitals (n=56), academic hospitals (n=7), nursing homes (n=11) and others (n=7)). During the first COVID-19 wave, 80 institutions applied cleaning and disinfection. For disinfection, 1000 ppm chlorine was used most frequently, followed by 1-2,5% hydrogen peroxide, and 70% alcohol. In 2023, 35 institutions had changed to cleaning only for SARS-CoV-2, for which 28 used a damp microfiber cloth. For influenza and RSV, cleaning only was applied by 39 and 37 institutions, respectively. Those that still practiced disinfection mostly used 1-2,5% hydrogen peroxide, followed by 250 ppm chlorine.

Despite a cleaning-only recommendation, half of the institutions still applied cleaning and disinfection. Additionally, while the cleaning strategy was quite consistent, a variety of disinfectants was used. Therefore, more evidence is needed regarding the use of disinfectants for respiratory viruses.

P013

To culture or not to culture: correlating Neisseria gonorrhoeae culture positivity with PCR cycle threshold values to promote cost-effective gonococcal resistance surveillance

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Introduction
Antimicrobial resistance in Neisseria gonorrhoeae (Ng) necessitates effective surveillance (GRAS), however, culturing is laborious and costly. Focusing Ng culturing efforts on subpopulations likely to yield positive results can enhance resource utilisation without compromising data quality and clinical care. This study aims to pinpoint a Cycle threshold value (CT-value) inflection point for effective Ng surveillance culturing.

Methods
We analysed combined surveillance data and laboratory data from 2962 clients attending the sexual health clinic in Rotterdam, the Netherlands from 2019-2023. Correlations between Ng CT-values, culture timing and culture positivity were investigated. The analysis included 6641 swabs from urogenital (urethra; 1407 / vagina; 486) and extra-genital (oropharynx; 2324 / rectum; 2194) sites, tested using the Cobas® 6800 Platform (Roche Diagnostics). Culturing for Ng resistance surveillance occurred on the initial test day for clients treated presumptively at first consultation (symptoms or notified for Ng) and during treatment consultation for clients with positive PCR screening results.

Results
Mean CT-values differed significantly for positive and negative cultures and by anatomical site (negative culture: CT value 33.0 (interquartile range (IQR) 24.2- 41.9); positive culture: CT 25.4 (IQR 20.0 - 30.3); $p<0.001$). Oropharyngeal samples had the lowest culture positivity rate (25 %). Only 11/1407 (0.78 %) of urethral culture samples were positive above a PCR CT-value of 30. Culture sensitivity declined with longer PCR-culture intervals. Notably, culture positivity sharply dropped from 23 % to 14 % between CT values 34 and 35. A CT-value cutoff at 34 would have resulting in an estimated € 15 000 savings of laboratory costs over five years, missing only 108/2636 (4 %) of positive cultures.

Conclusion
Establishing a Cobas® 6800 CT cutoff value of 34 is expected to be effective for optimising Ng surveillance resource allocation, translating to a 25 % reduction in basic culturing costs without compromising vital surveillance data.

P014

Assessing the Effectiveness of Automated Surveillance Systems for Detecting Pathogen-Related Clusters in Healthcare Settings

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Detection of pathogen-related clusters in hospitals is key to early intervention to prevent onward transmission. In recent years, there has been increasing interest in utilizing (semi-)automated surveillance methods.

In this retrospective cohort study, we extracted microbiological data from the Utrecht University Medical Center laboratory information management system and demographic data from our electronic health records (January 2014 to December 2021). We applied WHONET-SaTScan, CLAR, and our currently used percentile-based system (P75) for cluster detection. We assessed the methods for identifying potential microbiological clusters when applied to various pathogens with distinct occurrence patterns (scenarios): the introduction of a new pathogen with subsequent low-endemicity (for example Candida norvegensis in hematology), an endemic species (Serratia marcescens in NICU), rising levels of an endemic organism (Aspergillus fumigatus in adult ICU), and a sporadically occurring species (VRE hospital-wide).

All cluster detection methods were congruent in detecting spikes of numbers above baseline endemicity. However, there was a paucity of alerts from WHONET-SaTScan (n = 9) compared to CLAR (n = 319) and the P75 system (n = 472). WHONET-SaTScan did not pick up smaller variations in baseline numbers of endemic organisms as well as sporadic organisms as compared to CLAR and the P75 system. CLAR and the P75 system revealed good congruence in alerts across the studied scenarios pertaining to endemic organisms. For example, in the case of the endemic Serratia marcescens, 80% of the alarms were congruent.

Use of statistically based automated cluster alert systems are comparable to rule-based systems only for endemic pathogens. For sporadic pathogens WHONET-SaTScan returned fewer alerts compared to rule-based alert systems. More research is required regarding clinical relevance, timelines of cluster alerts and implementation. Moreover, future work will include more granular data on patient transfers, identified in a data-driven fashion through network analysis, into the cluster detection algorithms.

P015

Monitoring antibiotic resistance of uropathogens in primary care patients with uncomplicated urinary tract infections within the Regional antimicrobial resistance (AMR) care network North-Holland/Flevoland

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Introduction
Surveillance of antibiotic resistance (ABR) in uropathogens is important for determining empiric antibiotic treatment. Currently, this surveillance is based on data derived from routine diagnostics. However, this might result in overestimation of ABR, since Dutch guidelines recommend to take cultures only in patients that failed on therapy or at risk for complicated UTI. Therefore the aim of our study was to determine ABR in primary care patients with uncomplicated cystitis and no indication for culture, and to evaluate and possibly improve empirical antibiotic policy.

Methods
From January 1st-August 31st 2023, urine samples from primary care patients with uncomplicated UTI were sent to the microbial laboratory for urine culture. All seven laboratories in the region of North-Holland/Flevoland participated, involving seventeen general practices. Growth of >10*5 was considered positive. Data were compared with routine laboratory data from ISIS-AR (December 1st 2022-May 31st 2023). 95% confidence intervals around resistance rates were calculated using the Wilson-score.

Results
In total 247 patients were included; 199 (80.6%) urine samples were positive with 215 different isolates. E. coli was the most prevalent: 77,2% (166/215), only 2.8% extended-spectrum beta-lactamase (ESBL)-producers. The overall susceptibility of E. coli was 100% for nitrofurantoin (166/166), 99,4% for fosfomycin (165/166), 84,9% for trimethoprim (141/166). Comparing the resistance rates in E. coli from our study with ISIS-AR showed overall less resistance with only a significant difference for amoxicillin (25,9% (43/166) 95% CI: 20,0-33,3 versus 35,6% (19703/55345) 95% CI: 35,2-36,0).

Conclusion
Based on our results, both nitrofurantoin and fosfomycin are, in line with the Dutch primary care guidelines suitable choices for the treatment of uncomplicated UTI in the primary care setting. E. coli was identified as the most prevalent uropathogen with overall less resistance compared to ISIS-AR, however only significant for amoxicillin; ESBL-prevalence was comparable with previous research, i.e. 2,2% (van Driel, EJCMID, 2019).

P017

Tracing the origin of NDM-1-producing and extensively drug-resistant Pseudomonas aeruginosa ST357 in the Netherlands

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Background:
In the hospital environment, carbapenemase-producing Pseudomonas aeruginosa (CPPA) may lead to fatal patient infections. However, the exact origin of CPPA often remains unknown. Therefore, the aim of this study was to trace the origin of CPPA ST357, which caused a hospital-acquired pneumonia in a repatriated critically ill patient suffering from Guillain-Barré Syndrome.

Methods:
Antimicrobial susceptibility of the CPPA isolate for amikacin, cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, ciprofloxacin, colistin, fosfomycin, imipenem-relebactam, meropenem-vaborbactam, piperacillin-tazobactam and tobramycin was determined by disk-diffusion, Etest or broth microdilution. Whole-genome sequencing was performed for the case isolate and four distinct CPPA ST357 patient isolates received in the Dutch CPPA surveillance program. Furthermore, 103 international P. aeruginosa ST357 assemblies were collected via the NCBI Pathogen Detection Isolate Browser. CPPA were analyzed using whole-genome phylogenetics in combination with antimicrobial resistance gene (ARG) characterization.

Results:
A Dutch patient who carried NDM-1-producing CPPA was transferred from Kenya to the Netherlands. The CPPA case isolate presented an extensively drug-resistant phenotype, with susceptibility only for colistin or cefiderocol-fosfomycin. Phylogenetic analysis showed genetic variation (μ=1.121-E03) among ST357 isolates from Asia (n=42), Europe (n=36), Africa (n=18) and North America (n=12). However, the case isolate and one Dutch isolate clustered with Kenyan isolates (n=17; μ=4.294-E04). This was consistent with previous hospitalization of the patients in Kenya. An additional Dutch CPPA isolate clustered with Indian isolates (n=3; μ=9.464E-04) and was linked to hospitalization in India. The Kenyan cluster was characterized by the blaNDM-1, aph(3')-VI, ARR-3 and cmlA1 ARGs, which were absent in 81/108 other ST357 isolates. Furthermore, the Dutch isolates in the Kenyan cluster contained a novel blaNDM-1 resistance island: "ΔIS5075– ΔISAba14– aph(3')-VI– ISAba125–blaNDM-1– ble".

Conclusion:
This study presents an extensively-drug resistant subclone of CPPA ST357 with a unique resistance island which has been introduced to the Netherlands via repatriation of critically ill patients from Kenya.

P018

Towards a better treatment of bacterial endophthalmitis

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Introduction

In recent years, ocular surgery has increased dramatically, especially Intra vitreal injection with Anti-VEGF for macular degeneration. A serious postoperative complication is endophthalmitis, an infection of the inner eye, which can lead to functional loss of the eye, mandating immediate action (intra-ocular broad-spectrum antibiotics), as this strongly increases the chance for a successfully therapy. At present, we have to delay antibiotic injection until a biopsy of the vitreous fluid is obtained for identification of the infectious agent. To circumvent this delay we have developed a series of 24 PCRs, that can identify the bacterial species regardless of prior antibiotic treatment, and is referred to as multi-mono PCR (mmPCR).

Methods

The mmPCR identifies seven Streptococcus species, five Staphylococcus species, Pseudomonas spp., Candida spp., Haemophilus influenzae, Enterococcus faecalis, Proteus mirabilis, Cutibacterium acnes and a control PCR for generic 16S rRNA. Vitreous biopsies of 47 patients treated with inner eye antibiotics, were obtained at the Eye Hospital Rotterdam, and subjected to culture and mmPCR.

Results

The mmPCR detected 33 positives (S. epidermidis (19x), S. pneumonia (4x), S. mitis/oralis (2x), E. faecalis (2x), H. influenza (2x), S. aureus (2x), S. parasanguinis, P. aeruginosa) and 14 negatives. Concordant results between mmPCR and culture were obtained in 60% of specimens. As expected, the mmPCR detected an additional 14 positives, most likely attributed to inhibition of the culture by administered antibiotics. Surprisingly, culture showed growth in five patients without clinical presentation suggestive of severe endophtalmitis, and without significant levels of 16S DNA.

Conclusions

The mmPCR is applicable as a diagnostic tool in bacterial endophtalmitis after administration of antibiotics, facilitating the immediate injection of antibiotics in endophthalmitis suspected patients, preventing treatment delay and gaining successful treatment. The clinical evaluation of the new strategy is ongoing, with clinical follow-up for one year after the presentation of the patients.

P019

Perpetual observational study (POS) on Disease X: an adaptive observational protocol to study emerging viral threats

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Introduction:
The COVID-19 pandemic underscored the importance of having adaptable clinical trial frameworks ready for rapid deployment during viral outbreaks. Often, clinical studies do not collect comprehensive biological samples, limiting our capacity to conduct thorough studies on pathogen characteristics, pathogenesis, and host (immune)reponse, which are vital for understanding infection dynamics and for interpretation of virological and serological test results. The ECRAID POS Disease X protocol addresses this gap with an adaptable study protocol, aiming to enhance the detection of novel and unusual viruses through metagenomic sequencing and establish a biobank for future research. The study will initially focus on immunocompromised patients seeking hospital care for suspected viral infections, due to their increased susceptibility and potential of developing novel virus variants.

Methods:
Blood, throat swabs, urine, feces, and, if available, residual materials (cerebrospinal fluid or tissue) are collected in the acute, sub-acute, and convalescent phase. Participants are monitored for up to two years to study the viral evolution in patients with persistent infections. Metagenomic sequencing is applied for virus detection and characterization.

Results:
Here we discuss the challenges that were encountered in the initial phase: (1) designing a Case Report Form and a sampling protocol versatile for any syndrome and pathogen, (2) obtaining regulatory approval for a protocol designed to be flexible, (3) selecting and finalizing agreements with study sites, (4) standardizing sample collection, processing and storage across multiple countries, and (5) creating a framework for international data sharing that complies with international privacy laws.

Conclusion:
This work highlights the complexities of establishing a large-scale study network for emerging viruses, necessary groundwork for rapid responses to future outbreaks. Through the collection of clinical data and comprehensive biological samples we support not just the ongoing quest to detect and study new viruses, but also facilitate rapid data-driven responses to new outbreaks.

P020

Nosocomial transmission of NDM-producing Klebsiella pneumoniae ST147 in a Dutch pediatric cancer center associated with patients from Ukraine

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The prevalence of carbapenemase-producing Enterobacterales (CPE) among hospitalized patients in the Netherlands is low. Due to the Russia-Ukraine war and influx of evacuated Ukrainian patients since 2022, the Dutch national CPE surveillance reported emergence of globally spread New Delhi metallo-β-lactamase (NDM)-producing Klebsiella pneumoniae. To date, transmission of CPE of Ukrainian to Dutch patients has not been reported. Here we studied nosocomial transmission of NDM-producing K. pneumoniae in a pediatric oncology center.

NDM-producing K. pneumoniae isolates were detected with carbapenem inactivation methods combined with Xpert Carba-R PCR. CPE isolates were sequenced using Illumina whole-genome sequencing (WGS) and data used to assess whole-genome multilocus sequence typing (wgMLST)-based genetic relatedness in context of K. pneumoniae ST147 isolates from the national surveillance between 2012-2023.

wgMLST analyses of 8 blaNDM-1-producing K. pneumoniae ST147 isolates from 8 patients hospitalized since March 2022 revealed two genetic clusters (≥2 isolates varying ≤20 wgMLST alleles). One cluster comprised isolates of 3 Ukrainian and 2 Dutch patients, the other isolates of 1 Ukrainian and 1 Dutch patient. One Ukrainian patient had no in-hospital epidemiological link. Gantt charts of all hospital admissions showed Ukrainian patients were already colonized at admission, 2 Dutch patients had bacteremia and 1 was colonized during hospitalization. Sinks in 2 rooms, identified as a possible link, were contaminated with K. pneumoniae with high genetic relatedness, and multiple modes of transmission are possible. wgMLST revealed that K. pneumoniae ST147 from 7 patients grouped in a distinct branch when compared to 163 K. pneumoniae ST147 isolates from national surveillance of 2012-2023.

We identified nosocomial transmission of blaNDM-1-producing K. pneumoniae ST147 from Ukrainian to Dutch patients. Transmission occurred despite standard microbiological screening and contact precautions applied to Ukrainian patients at admission. Healthcare professionals should be aware of the risk that influx of evacuated patients from multidrug-resistant organism endemic areas entails.

P021

Diagnostic Performance of two on-demand PCR Test for SARS-CoV-2 in Pediatric Patients Presenting with Acute Respiratory Tract Infection during the COVID-19 Pandemic

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Introduction
In pediatric patients with acute respiratory infection (ARI), the waves of SARS-CoV-2 are now adding to the complex epidemiology with its diverse pathogens, coinfections and seasonality. Timely testing of a large set of pathogens is necessary to provide results that help decision making in the Emergency department (ER). The results potentially affect treatment, prognosis and placement, but only if they are rapidly available. Biofire Filmarray Respiratory Panel 2.1 (RP) and the Cepheid Xpress SARS-CoV-2 (CX) have near-care and on-demand characteristics. Each PCR provides a different type of result and both PCRs have played an important role in the diagnostic setting of patients with ARI. In our study, we report on the performance of both these PCRs for SARS-CoV-2 in pediatric patients.
Methods
Pediatric patients presenting with symptoms of upper respiratory infection were tested with RP and CX PCRs when these tests became available in Sint Maarten. Samples were taken from the nasopharynx, swabs were placed in universal transport medium.
Results
Between December 2020 and June 2023, 58 patients presenting in the ER with ARI were tested with both RP and CX PCRs. Of these patients, 37 (64%) were male, median age was 18 months (interquartile range 7.5-30.3 months). In 17 episodes, both RP and CX were positive for SARS-CoV-2. In 42 episodes, neither PCR detected SARS-CoV-2 but RP detected at least one other pathogen in 23 of the 42 SARS-CoV-2-negative episodes.
Conclusion
This study showed the high concordance of the two types of PCR in a pediatric population of ER patients presenting with ARI. The one discordant sample had a Ct-value over 40. The captured timeframe included COVID-19 waves with several subtypes of SARS-CoV-2. Both PCRs provided precise and timely information regarding the pathogen that underlies the episode of ARI in 52 out of 60 (87%) episodes.

P022

Understanding the burden of respiratory tract infections in children in St. Maarten Medical Center: new epidemiological insights

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Introduction
The aetiology, epidemiology, seasonality and clinical characteristics of respiratory tract infections (RTIs) in children are not well described in the Dutch Caribbean Islands. This impairs the effective timing of vaccination strategies that should be based on knowledge of local transmission epidemiology. This problem affects the St. Maarten Medical Center (SMMC), a hospital that serves Dutch St. Maarten, as well as the surrounding north-eastern Caribbean islands.
Methods
This cohort study collected data of 173 children (0 – 17 years) that presented to SMMC with an RTI between 2018 – 2022 and underwent a Biofire respiratory panel. Medical records were assessed for standardized collection of clinical information.
Results
187 viruses were detected in 142 patients. Single infections were detected in 60.7%, two or more viruses in 21.4%. Rhino/enterovirus infections were most prevalent, affecting 51.4%. Both rhino/enterovirus and influenza were significantly more present during the dry season (December – April) compared with the wet season (May – November): 73,8% vs 26,2%, $p<0.01$ for rhino/enterovirus and 93,3% vs 6,7%, $p<0.01$ for influenza.
Pre-COVID-19, RSV infections peaked in August. Prophylactic efforts to reduce RSV hospitalization in risk groups, such as premature infants, are currently given on a yearly basis between October and February, thus potentially too late to adequately protect these groups in St. Maarten and its surrounding islands.
Rhino/enterovirus patients presented significantly more often with wheezing (66.3% vs. 43.3%, $p<0.01$) or asthma exacerbations (9.8% vs. 1.1%, $p<0.01$) compared with patients infected with other viruses. Influenza patients were less likely to need admission compared to patients infected with other viruses (7.3% vs. 92.7%, $p=0.018$). RSV patients required oxygen more often (31.4% vs. 15.0%, $p=0.031$).
Conclusion
We conclude that SMMC is notably burdened by RTIs; the knowledge gained from this report can be used to define best practices for (prophylactic) measures,

P023

Prebiotic intervention in patients with metastatic or unresectable colorectal cancer treated with 5-FU based chemotherapy: design of a clinical intervention study.

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Introduction
Standard systemic treatment for metastatic and/or unresectable colorectal cancer (mCRC) often involves fluoropyrimidines, such as 5-fluorouracil (5-FU). Previous observational clinical studies, as well as pre-clinical research, demonstrated that chemotherapy affects the gut bacteria and its metabolites, potentially leading to microbial dysbiosis. Microbial dysbiosis might influence anti-cancer efficacy and toxicity of the treatment. Therefore, targeted microbiota modulation using prebiotic fibers could potentially optimize 5-FU-based chemotherapy.
This explorative intervention study aims to investigate the effects of daily administration of a prebiotic fiber mixture on the intestinal microbiota composition in patients with metastatic and/or unresectable colorectal cancer treated with 5-FU-based chemotherapy. Moreover, the effect of the prebiotic fiber intervention on fecal, blood, clinical, and safety/tolerance parameters will be evaluated.

Methods
62 mCRC patients scheduled for 5-FU-based therapy (FOLFOX, CAPOX, or CAP) with or without bevacizumab will be prospectively enrolled in a double-blinded randomized controlled study. After informed consent, patients will be randomized to either the prebiotic fiber intervention or control group, starting at least 3 days before the 5-FU-based treatment. They will take the prebiotic fiber mixture or control twice daily during the treatment (4 cycles of FOLFOX or 3 cycles of CAP or CAPOX). At several timepoints throughout the intervention, patients will collect fecal samples and complete questionnaires on food habits, patient characteristics, experienced chemotherapy side effects, quality of life, current nutritional status, and physical performance. Microbiota composition and short-chain fatty acid levels will be measured in the fecal samples. In addition, blood samples will be collected and analyzed for various parameters. Body composition and tumor response will be evaluated based on CT scans.

Conclusion
The new knowledge from this exploratory clinical intervention study will be pivotal to advance microbiome-targeting interventions to optimize 5-FU-based chemotherapy.

P024

The impact of an educational VR-module on the infection prevention and control knowledge, attitude and practice among medical students at Radboudumc.

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Background
Virtual (VR) and Extended reality (XR) offers a safe learning environment in which students can experience situations that are difficult to access in regular education. The immersive experience facilitates awareness of the consequences of different choices. The infection prevention and control (IPC) team from Radboudumc developed a VR-module, with the Radboudumc Health Academy. The impact of this module on IPC knowledge, attitude and practice (KAP) was assessed among medical students.

Methods
The VR-module was tested among medical students who did the IPC educational program prior to their clinical rotations. After completion of the module they were asked to complete an online questionnaire to assess the KAP regarding IPC. The knowledge questions were theoretical and applied. The proportion of answers were compared between medical students who completed the IPC-program with and without VR-module using non-parametric statistical tests. Here we present preliminary findings from the VR-group (October 2023). Early 2024 we will collect additional data and complete the analysis.

Results
The questionnaire was filled out by 52 students (regular IPC program n=37; VR-group n=15). The overall response rate was 71% (VR group 52%). In the VR-group, students more often gave correct knowledge answers compared to the regular IPC educational program group. The students in the VR-group indicated the added value of this new method of education because of the practical-situational training. In addition, they provided some suggestions for improvement of the VR-module.

Conclusions
Preliminary findings suggest that students from the VR-group were more confident in assessing IPC measures per situation. However, given the small sample size, additional data is needed to establish a more accurate estimate. The positive students' response about the added value motivates us to further improve this module and supports the plans to incorporate this VR-module in the regular IPC education program for medical students at our faculty

P025

Semi-automated surveillance of Hospital Onset Bacteremia and Fungemia.

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Introduction: In an effort to replace the traditional and time-consuming surveillance of central line-associated bloodstream infections, a semi-automated surveillance system for Hospital Onset Bacteremia and Fungemia (HOB) was developed.

Methods: The registration was set up in the newly implemented MICORE IP module, utilizing routine culture data and consultation records. Positive blood cultures, obtained 48 hours post-admission, were automatically recorded. Subsequently, manual assessment and registration of the HOB focus were conducted.

Results: Following validation, the system was put into operation. The registration requires approximately 4 hours of work per month. In the first year, 228 HOB records were identified, resulting in an HOB ratio of approximately 0.12 per 100 admission days. The top three HOB foci were gastrointestinal at 25%, intravenous line at 19%, and urinary tract infection at 13%. In 18% of the records the focus was categorized as unknown. The leading departments for HOB occurrences were the Intensive Care Unit at 33%, Gastrointestinal-Surgery at 18%, and Hematology at 15%. The top five microorganisms were Enterococcus faecium, Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus, and Enterococcus faecalis.

Conclusion: The implementation of the semi-automated surveillance system for HOB has proven successful, significantly reducing the workload to approximately 4 hours per month. The identified HOB records and associated data provide valuable insights into the distribution of HOB foci, departmental occurrences, and prevalent microorganisms. This approach holds promise for streamlining infection surveillance protocols and improving the overall efficiency of monitoring and managing hospital-acquired bacteremia and fungemia.

P026

A milestone in in vitro culture of Treponema pallidum

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Introduction. The spirochete Treponema pallidum is the cause of syphilis. Investigation of this microorganism has so far been hampered by the absence of an in vitro culture model. Using co-cultures with a rabbit epidermal cell line, a specific culture medium and a microaerophilic environment successful in vitro culture of reference strains of rabbit-passaged T.pallidum was recently described. We aimed to set up this method to culture T.pallidum directly from patients.
Methods. Initial experiments were done using the co-culture system mentioned above and described by Edmondson et al . (2018) and a reference T.pallidum Nichols strain. Lesional fluid was obtained from four patients with an penile ulcer and positive dark-field microscopy. Half of the amount was directly inoculated into the culture system containing amikacin and fosfomycin. The other half was used for the analysis of other co-occurring microorganisms in the sample and their antibiotic resistance patterns and a part was stored at -80°C. In subsequent experiments rabbit fibroblast cells were grown on transwell plates and frozen stocks of spirochetes from two patients were added in culture medium with fosfomycin, rifampicin and levofloxacin. This medium was replaced by antibiotic-free medium after 24 hours. Dark-field microscopy was used to assess the presence of viable spirochetes in cultures.
Results. The reference strain proliferated successfully. In three of the four directly incubated cultures motile spirochetes could be observed up to one week after inoculation, but these were overgrown with contaminants. Using the transwell system, viable and motile spirochetes were visible up to six weeks after inoculation, active multiplication was taking place and no contamination was observed.
Conclusion. Direct in-vitro culture of T.pallidum from patients without rabbit passage is possible.

P027

Not Just Black and White: The Gene-Dosage Spectrum of OTULIN

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Introduction: M1-ubiquitination is a posttranslational modification important in the regulation of immune signaling and cell death. Ovarian TUmor domain deubiquitinases with LINear linkage specificity (OTULIN) is a highly conserved M1-deubiquitinase. Autosomal recessive OTULIN deficiency causes a life-threatening, neonatal-onset OTULIN-related autoinflammatory syndrome (ORAS), characterized by fever, panniculitis, diarrhea, and arthritis. Autosomal dominant OTULIN deficiency, by means of haploinsufficiency, underlies late-onset, life-threatening necrosis of the skin and/or lungs, which is typically triggered by infections with Staphylococcus aureus. We hypothesize that the phenotype and severity of disease in human OTULIN deficiency is determined in a gene-dosage manner. Here, we investigate the gene-dosage spectrum of OTULIN deficiency.
Methods: We describe eight patients with life-threatening necrosis of the skin and/or lungs, whom are carriers (five in heterozygous, and three in homozygous state) of extremely rare, predicted deleterious OTULIN variants. Using HEK293T cells overexpressing the cDNA corresponding to the patients' OTULIN alleles, their expression and capacity to inhibit NFκB-dependent signalling was measured. Endogenous expression of OTULIN was measured using patient-derived dermal fibroblasts and PBMCs.
Results: All variants cluster in the catalytic domain of the protein. All five OTULIN variants carried by probands in heterozygosity (one missense, two nonsense, and two frameshift variants) were shown to be either loss-of-function (LOF) or loss-of-expression (LOE), implying that the genetic mechanism that underlies their autosomal dominant OTULIN deficiency is haploinsufficiency. In contrast, the OTULIN variants carried by probands in homozygosity (all three missense variants) were only mildly hypomorphic.
Conclusion: We characterized the gene-dosage dependent spectrum of human OTULIN-deficiency at the functional and phenotypic levels. Mono-allelic LOF/LOE OTULIN variants, and bi-allelic mildly hypomorphic OTULIN variants cause an overlapping phenotype characterized by severe necrotic disease that is provoked by infectious or mechanical triggers.

P028

Surveillance of multi-drug resistant bacteria and bloodstream infections in hematopoietic stem cell transplantation patients: Insights from a Sub-Saharan African tertiary centre

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Introduction: Bloodstream infections (BSI) are serious complications in hematopoietic stem cell transplantation (HSCT) recipients, contributing to significant morbidity and mortality, especially when caused by multidrug-resistant bacteria (1-3). While previous studies reported a 16-55% risk of BSI post-HSCT (4-11), data on causative pathogens in sub-Saharan Africa is scarce. This study aims to characterize multidrug-resistant pathogens identified in surveillance cultures and blood cultures post-HSCT.

Methods: This retrospective study, conducted at Groote Schuur Hospital in Cape Town (South-Africa), investigates BSIs <100 days post-HSCT in patients admitted between January 2018 and May 2023. These results were compared to MDR screening cultures consisting of nasopharyngeal swabs (at admission) for MRSA and rectal swabs (at admission and weekly repeated) for carbapenem-resistant Enterobacterales (CRE). Antibiotic susceptibility testing was performed with VITEK-2 and E-tests were used to confirm carbapenem resistance (CLSI guidelines). Carbapenemases were identified using lateral flow assay (LFA).

Results: Of the 239 patients included, 129 patients underwent autologous HSCT and 93 underwent allogenous HSCT. Underlying hematologic diseases were multiple myeloma (n=69), Hodgkin's lymphoma (n=36), and acute myeloid leukaemia (n=35). MRSA was detected in 2% of patients tested while 24% were screened positive for CRE during their admission, predominantly with Klebsiella pneumoniae (78%). Carbapenemases were identified in 83% of CRE cases, with OXA-48 emerging as the predominant hydrolysing enzyme (97%). Preliminary results revealed that 32% of the patients experienced ≥1 BSI, excluding suspected contaminated cultures. 10% of the BSI were caused by a CRE (predominantly K. pneumonia). 14 of the 23 deceased patients were diagnosed with a BSI during the 7 days pre-mortem, of which 5 were CRE-related (all K. pneumoniae).

Conclusion: This study describes MDR bacterial colonization and BSI in HSCT patients in Cape Town, South Africa. Identifying specific pathogens, particularly those exhibiting multidrug resistance, underscores the ongoing need for infection prevention and tailored antibiotic stewardship.

P029

Pseudomonas aeruginosa carriage and associated risk factors in patients and healthy individuals from Rotterdam, the Netherlands

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Introduction
Data on carriage of Pseudomonas aeruginosa in patients upon hospital admission and in healthy individuals are scarce, and is often limited to screening for intestinal carriage only. The aim was to determine the carriage rates, overall and per body site (throat, navel, rectum), and associated risk factors of P. aeruginosa in patients and healthy individuals.

Methods
This study was performed at the Erasmus MC University Medical Center (Rotterdam) between September 1, 2022 and March 31, 2023. Throat, navel, and perineal samples, and questionnaires, were collected from patients at admission and healthy individuals living in Rotterdam. Swabs were enriched overnight at 35˚C in 5mL Tryptic Soy Broth with 2 mg/L vancomycin. The broth was inoculated onto M-PA-C agar (BD Diagnostics, the Netherlands) to obtain isolates for identification using MALDI-TOF (Bruker Daltonics, Germany) and antibiotic susceptibility testing using VITEK2 (bioMérieux, France). Carriers of P. aeruginosa were compared to non-carriers. $P<0.05$ was considered significant.

Results
In total, 283 patients and 183 healthy individuals were included. Overall carriage of P. aeruginosa was 12.7% (36/283) in patients and 12.0% (22/183) in healthy individuals. P. aeruginosa was predominantly detected in perineal samples (patients: 32/283, 11.3%; healthy individuals: 14/183, 7.7%), yet P. aeruginosa was also found in throat (patients: 5/281, 1.8%; healthy individuals: 2/183, 1.1%) and navel samples (patients: 2/259, 0.8%; healthy individuals: 6/182, 3.3%). Patients with P. aeruginosa were significantly older compared to non-carriers (P=0.017) and more often had an indwelling medical device (P=0.041). Healthy individuals with P. aeruginosa were more likely to be women (P=0.037) and carriers were also significantly older compared to non-carriers (P=0.005).

Conclusion
The overall P. aeruginosa carriage rates among patients upon hospital admission and healthy individuals living in Rotterdam were similar. Furthermore, results show that by relying solely on perineal screening cultures, P. aeruginosa carriers may remain undetected.

P030

Diagnostic evaluation of the serological detection of type-specific hepatitis B surface antigens in the Netherlands, using the WHO international hepatitis B reference panel

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Hepatitis B virus (HBV) poses a substantial global health challenge, with close to 300 million individuals experiencing chronic infections worldwide. Categorized into ten distinct types (A-I), HBV exhibits a high genetic variability. In the Netherlands, genotype A is most prevalent, followed by D. However, since 2019, a notable increase in genotype F is reshaping the epidemiological landscape of HBV infections.
The impact of HBV's high genetic variability on serological assay performance, specifically in detection efficacy of genotype-specific HBV surface antigen (HBsAg) by assays used in routine diagnostics, remains an understudied area. Here, we aimed to investigate the serological detection performance on type-specific HBsAg used in Dutch Medical Microbiology and Clinical Chemical laboratories.

An external quality assessment (EQA) panel containing sixteen well-characterized serological samples with known antigen amounts, representing various HBV genotypes (WHO international HBV reference panel), including a negative serum control, was designed and distributed to twenty-seven participating Dutch laboratories. Each sample contained between 21 and 32 IU/mL type-specific HBsAg. Laboratories were instructed to perform routine diagnostic tests and report their results.

Three quantitative and eight qualitative diagnostic assays were evaluated. The EQA data revealed consistent detection of all genotypes, including genotype F2. Quantitative assays demonstrated variability in antigen detection of the same genotype among laboratories employing the same diagnostic system, e.g., Liaison XL (DiaSorin). Additionally, all quantitative assays exhibited variations in HBsAg detection rates, with values ranging from 10 to 76 IU/mL of type-specific HBsAg, with the lowest measured values (HBsAg < 29 IU/mL) observed for genotypes A2, B2, D1, D2, D3, and F2.

Our study shows consistent serological detection of the HBsAg of the evaluated HBV genotypes, indicating good type-specific assay performance of HBsAg immunoassays. However, variations in detection levels among different genotypes, and type-specific assay performance discrepancies have been observed and support the need for further investigation.

P031

NDM-1/OXA-48-like carbapenemase-producing Enterobacterales from Ukrainian persons impact national surveillance in the Netherlands, 2022-2023

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Introduction
Since February 2022, the Russia-Ukraine war led to migration and medical evacuation of Ukrainian persons to other European countries. Carbapenemase-producing Enterobacterales (CPE) in these refugees were reported by several countries. We describe the occurrence and genetic characteristics of CPE from Ukrainian persons in the Netherlands in 2022-2023.
Methods
We included CPE isolates submitted to the national CPE surveillance between 24-02-2022 and 01-09-2023. Isolate data was linked with clinical/epidemiological patient data (including Ukraine-link) from the mandatory CPE notifications. A carbapenemase gene PCR and, for the first species-carbapenemase gene combination per person, next-generation sequencing (NGS) were performed for carbapenemase allele identification and (whole genome) multi-locus sequence typing ((wg)MLST).
Results
Among 801 submitted CPE isolates, 22% (n=176) were from 101 Ukrainian persons. Two additional Ukrainian persons with CPE, but without submitted isolates were identified in the mandatory notification system. The mean age of Ukrainian persons was 38 (SD 18) years and 82% was male. Thirty-five (35%) persons had >1 CPE (maximum 10). Seventy percent of isolates were from screening swabs and 30% from diagnostic sample materials, mainly wounds/abscesses (50%). The most common species was Klebsiella pneumoniae (70%), mostly MLST ST147 (36%). Furthermore, blaNDM-like genes (73%; mostly blaNDM-1), followed by blaOXA-48-like (34%; mostly blaOXA-48), were most common. Twenty-three percent had >1 carbapenemase gene. Among 148 Ukrainian isolates with NGS results, 95 (64%) were part of 28 genetic clusters (≥2 isolates varying ≤20-25 wgMLST alleles). Twenty clusters included only Ukrainian persons and 5 clusters included Ukrainian and 1 to 3 persons without a known link to foreign countries. Three non-Ukrainian persons had a known epidemiological link to a Ukrainian person from the same cluster.
Conclusion
Since the Russia-Ukraine war, 22% (n=176) of submitted CPE isolates were derived from 101 Ukrainian persons and 88% carried NDM- and/or OXA-48-like-genes. Transmission to non-Ukrainian persons may have occurred.

P032

Exploring the use of viability-PCR for earlier de-isolation of SARS-CoV-2 positive ICU-patients: the CoLaIC study

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Introduction
Patients admitted to the ICU with COVID-19 are placed in isolation until two subsequent negative SARS-CoV-2 PCRs. The question is if the detected viral RNA represents intact virus or compromised viral particles. To distinguish intact infectious virus from incomplete virus or RNA remnants, a viability-PCR method is developed. We hypothesize that because viability-PCR does not detect viral/RNA remnants, the time to a negative PCR would be shorter using the viability-PCR compared to conventional SARS-CoV-2 PCR. This could potentially imply shorter isolation duration for COVID-19 patients.

Methods
Patients admitted to the ICU in two hospitals in Limburg were prospectively included, respiratory samples were collected three times per week. Samples were aliquoted for viability-PCR and regular SARS-CoV-2 PCR. The vial for viability-PCR was split and either treated with propidium monoazide (PMA) for elimination of incomplete virus or analyzed untreated. The PMA-untreated vial corresponds to the conventional SARS-CoV-2 routine PCR used.
Using time to a negative PCR test as outcome, the Paired Prentice-Wilcoxon-test (PPW-test) was used to test for significant difference in time to negative between viability and conventional SARS-CoV-2 PCR. The PPW-test was performed using Ct 35 as cut-off above which a test result was considered negative. Assuming a negative binomial distribution for the differences in time to negative, mean time difference between viability and regular PCR was estimated.

Results
109 patients admitted to the ICU were included for statistical analysis. Time to negative PCR test result differed significantly between PMA-untreated and PMA-treated samples (PPW-test p-value < 0.001). On average, the first negative viability-PCR test result occurred 3.10 days (standard deviation 1.12 days) before the first negative PMA-untreated PCR test result.

Conclusion
Viability-PCR for SARS-CoV-2 could provide a more accurate indication of presence of intact virus and therefore infectivity, potentially enabling earlier de-isolation of ICU patients and lower risks of transmission.

P033

Culture-free detection of bacteria directly on whole blood: Accelerating sepsis diagnosis in the Intensive Care Unit

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Introduction: Traditional diagnosis of bloodstream infections takes 2-5 days, with microbial growth occupying most of this timespan. For Intensive Care Unit (ICU) patients with sepsis, prompt initiation of antimicrobials is crucial. Consequently, broad-spectrum antibiotics are administered for days until a causative pathogen is identified, a process hindered by contamination of blood cultures. We evaluated a rapid DNA-based method for direct detection of pathogens in whole-blood samples. Such technologies may reduce sepsis patients mortality and morbidity by enabling quicker diagnosis and administration of appropriate antimicrobials.

Methods: A two-way DNA-based approach was developed by depleting human DNA and amplifying bacterial 16S-23S interspace (Molecular Culture®, Inbiome) from 5-10ml blood in 6 hours. This method broadly detects bacterial DNA in blood. In an ongoing ICU study at MUMC+, 149 patients with routine blood cultures for fever or suspected sepsis have provided 491 blood samples. Seventy-two samples were processed with this method, and results were compared to standard blood culture outcomes and routine clinical data.

Results: About 85% of the blood cultures were negative, according to routine expectations. A selection of 39 negative and 33 positive blood culture were processed using the new DNA-based method. Of the 39 negative samples, 33 were concordant between the new and traditional method. Of the 33 positive samples, 19 results matched. Interestingly, in 9 unmatched samples, additional potential causative bacteria like Klebsiella pneumoniae and Enterococcus faecium were identified, consistent with other culture results from the same patients. The unmatched negative samples mostly involved Staphylococcus epidermidis in blood cultures interpreted as contaminants.

Conclusion: This DNA-based technology rapidly detected bacteria using the same blood volume as traditional culture. It also identified additional clinically significant bacterial species not found in routine blood cultures. This approach indicates the feasibility of culture-free detection of bloodstream infections and merits further testing in broader clinical settings.

P035

Are we prepared for Candida auris?

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Background
Infections with Candida auris have been rising worldwide, including in Europe. Of important concern is that C. auris colonizes the skin, is able to survive in the environment and is often multi-drug resistant, enabling C. auris to cause large and persistent outbreaks in healthcare settings. Major C. auris outbreaks have been reported in Italy, Spain and the UK. Dutch hospitals have had multiple imported C. auris colonized patients recently.
The National Institute for Public Health and the Environment (RIVM) is continually working on being prepared for the emergence of new (resistant) pathogenic micro-organisms with the aim of quickly developing effective interventions.
Therefore, RIVM aims to set up the required infrastructure and protocols to allow an adequate response to C. auris outbreaks in the Netherlands.
Methods To prepare for a potential C. auris outbreak we have been establishing mycology wet-lab and dry-lab infrastructure. The mycology wet-lab infrastructure includes furnishing a new mycology laboratory and adapting existing workflows to include C. auris.
The dry-lab infrastructure (Apollo pipelines) was developed to run on the internal RIVM High Performance Computing (HPC) cluster to ensure scalability. This basic workflow includes automated quality control, reference-based assembly, resistance gene typing and clade identification through the RIVM data management system (iRODS).
Results A fully-equipped mycology laboratory was set up, in which we routinely culture C. auris, isolate the DNA and perform whole genome sequencing (WGS). The Apollo pipelines have been set up successfully for C. auris. Additionally, sufficient staffing to perform all analyses and investigations has been established. We can respond to an emergency situation in 48 hours starting from cultured isolate or 72 hours for uncultured.
Conclusion Through this infrastructure and platform, we are able to quickly respond to C. auris outbreaks and trace isolates collected from different sources and map the possible transmission route .

P036

Skin swabs compared to skin scrapings for polymerase chain reaction for dermatomycoses and scabies

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Introduction: Polymerase chain reaction (PCR) on skin scrapings has improved diagnostics of dermatological disease including dermatomycoses and scabies. Skin swabs are easier to perform and less invasive but the sensitivity of PCR cotton swab sampling is currently unknown. The aim of this study was to evaluate the sensitivity of sampling with wet and dry cotton swabs as compared to skin scrapings for the diagnosis of dermatomycoses and scabies.

Methods: In this cross-sectional study all patients with a clinical indication for skin scraping for dermatophytes and/or scabies were included after informed consent. Two additional samples per patient were taken using a dry cotton swab and a wet cotton swab humidified with 0.9% NaCl followed by skin scraping. All samples were then tested using real-time PCR for dermatophytes and/or Sarcoptes scabiei.

Results: In total 37 patients were included, 8 tested on scabies, 26 on dermatophytes and 3 on both. S. scabiei DNA was detected in 4 of the 11 (36%) skin scrapings. In none of the dry swabs (0/11) nor wet swabs (0/11) was DNA of S. scabiei detected; sensitivity was 0%.
The dermatophyte species PCR was positive in 7 (24%) skin scrapings, 11 (38%) dry swabs and 10 (34%) wet swabs. Sensitivity was 86% for the dry swabs and 100% for the wet swabs. Median CT-value of the dry and wet swabs (32.6 and 31.9) were significantly higher compared to skin scrapings (26.7; p=0.021 en p=0.043).

Conclusion: Skin swabs have a higher positivity rate for detection of dermatophyte DNA compared to skin scrapings, although CT-values are also higher. Possibly the larger skin surface swabbed can explain this finding. In conclusion skin swabs can be used for diagnosing dermatomycoses but are not a reliable alternative for scabies.

P037

Rickettsiae serological diagnostics; challenging and improvable

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Introduction

Rickettsiae are obligate intracellular Gram-negative bacteria, which can cause zoonotic infections. There are four groups of Rickettsia of which the spotted fever group (SFG) and typhus group (TG) are most important in the Netherlands. Serology results can be difficult to interpret. The aim of this poster is to exchange thoughts and experiences with other attendees to improve our diagnostics.

Methods

All diagnostic requests for Rickettsia serology from 2022 and 2023 were extracted from the RIVM biorepository for evaluation of the results in relation to clinical symptoms and travel history. Sera were tested with immunofluorescence assays for IgM and IgG antibodies against SFG and TG Rickettsia.

Results

In 2022 and 2023, 91 respectively 165 unique patient requests were analyzed. The difference in quantity is probably caused by travel restrictions in 2022 due to the corona pandemic. Approximately 75% lack clinical symptoms and 70% lack travel history information. In 2022, 82.4% of the sera were negative for both IgM and IgG. Interestingly, only 51.5% were IgM/IgG negative in 2023. A follow-up serum was submitted for <2% of the patients, resulting in 36 follow-up samples. Eight follow-up samples were positive for IgM and/or IgG (22.2%), of which four only showed a positive IgG response in the first sample, whereas the other four were only IgM positive.

Conclusion

Follow-up samples and clinical information are necessary for reliable interpretation of serology results. Solitary IgM responses should be interpretated with caution. The IgM response does not significantly increase in 60% of the follow-up sera of which the cause is unclear. Solitary IgG responses could indicate a recent or past infection. Most sera was negative and depending on symptom duration, a follow-up serum can demonstrate seroconversion. In those early cases the antibody levels could be undetectable, performing a Rickettsia PCR could aid early diagnosis and treatment.

P038

Zooming into the unseen interactions of the gut microbiota

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Surprisingly, the microbiota interaction with the gut is still a black box, where essential processes take place such as the interplays of different species with each other, the effects of microbes on host tissue, and how the gut cells respond to these microbes. The major unknown factor is where and how exactly these interactions occur. We aim to uncover these questions by investigating mouse and human intestinal organoids infected by bacteria, and single bacteria, with various cryo-EM techniques. The studied bacteria are both non-pathogenic bacteria found in human stool and pathogenic bacteria that play a role in rising infection numbers like non-tuberculosis mycobacterial strains. Although a first investigation with confocal microscopy gives insights about the gut interplays, a fundamental role is played by cryo-electron microscopy which allows us to look at these interplays at unprecedented resolution. Infected organoids are selected through cryo-correlative light and electron microscopy since the studied bacteria are fluorescently labeled and targets are correlated to the focused ion-beam scanning electron microscope with 3DCT, a 3D correlation toolbox. Once the sample is milled, the obtained lamellae are suitable for cryo-electron tomography. More specifically, this results in the determination of the exact location where microbe-microbe and microbe-host interactions occur. The addressed dilemmas are which structural factors of microbes and host are responsible for modulating disease, for example cell motility and attachment structures such as flagella, pili and secretion systems. Finally, we will identify how the combination of structures and co-location of microbes and host cells determines the effectivity of pathogen-control. This research enables the understanding of the mechanisms behind pathogen interactions with the intestinal epithelium compared to the physiological bacterial strains. Moreover, it contributes to solutions on how to harness the function of the microbiota for disease prevention and determine their potential for treatment of acute infections.

P039

Mapping the metabolome of Mycobacterium tuberculosis; finding biologically relevant features in untargeted metabolomics data.

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Mycobacterium tuberculosis (Mtb) is among the world's top leading causes of death from a single infectious agent and killed an estimated 1.3 million people globally in 2022 alone. Although effective drugs exist, drug-resistant Mtb is emerging as a major threat to global tuberculosis (TB) control. Therefore, new drugs to treat TB are urgently needed. Incomplete knowledge of Mtb metabolism is one of several barriers in the development of new TB drugs. To lower this barrier, we aim to map the metabolome of Mtb using LC-MS untargeted metabolomics. Although untargeted metabolomics data typically yields several thousand features, a peak or signal detected in the LC/MS, we follow up on the emerging consensus that most of these features are redundant, contaminants and artefacts, and that common microbes contain at most a few hundred truly biological metabolites.

Here, we aim to culture Mtb strain H37Rv in minimal Roisin's media containing combinations of 12C- or 13C-glycerol and 14N- or 15N-ammonium chloride as sole carbon and nitrogen source, respectively. Under these conditions, Mtb metabolites will be labelled according to the number of carbon and nitrogen molecules, while contaminants will remain unlabeled.
After analysis of the resulting metabolomes using our high-resolution Q-ToF LC-MS system, we will use the Peak annotation and verification engine (PAVE) computational workflow to eliminate redundant features, contaminants and artefacts.

We expect our approach to discard most of the features obtained using untargeted metabolomics and generate a list of only a few hundred true metabolites with their carbon and nitrogen atom counts. The deep metabolome profiling approach will reveal known and unknown Mtb metabolites that can form the basis for future work on novel metabolic pathways in Mtb by us and others.

P040

Nucleocapsid-directed antibody testing is unsuitable to estimate hybrid immunity, a longitudinal cross-border study in the Meuse-Rhine Euregion

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Introduction
Insight into longevity of past natural SARS-CoV-2 infection within the population is of interest to assess future disease burden and can aid in policy making on timing of booster-vaccination campaigns. Nucleocapsid-directed antibodies (anti-N) are generated after natural infection and in theory can give an indication of the extent of hybrid immunity within a population. The aim of the present study was to provide data on anti-N seroprevalence and anti-N antibody dynamics in inhabitants of the Meuse-Rhine Euregion.

Methods
Questionnaires and self-finger-prick blood samples of inhabitants of the EMR were collected during two periods: week 22-29 (June-July) 2021 for round 1 and week 40 to 45 (October-November) 2021 for round 2. For participants that tested positive for antibodies against the spike-protein of SARS-CoV-2 (anti-S; n=1.291 for round 1 and n=3.075 four round 2), additional anti-N antibody testing was performed.

Results
For round 1, 53.5% (54/101) of participants who reported a positive PCR had anti-N antibodies. The median duration between a positive PCR and anti-N sample collection was 126 days (IQR 62-182, n=39).

For round 2, 49.2% (32/65) of participants who reported a positive PCR had anti-N antibodies. The median duration between the positive PCR and anti-N testing was 153 days (IQR 96-174, n=31). For 94.4% (51/54) participants with anti-N antibodies in round 1, a corresponding result for round 2 was available. In 42.6% (23/51) of participants with anti-N antibodies in round 1, the result was negative for round 2.

Conclusion
A considerable amount of seroreversions of anti-N within 5 months were observed. Moreover, in about half of individuals who reported recent positive PCR, anti-N could be detected. Due to the varying sensitivity of assays to detect anti-N, and faster decay of anti-N, anti-N testing is not suitable to accurately diagnose past SARS-CoV-2 infection and indicate hybrid immunity within a population.

P041

Using the Sysmex UF-4000 urine flow cytometer for rapid diagnosis of urinary tract infection in the clinical microbiological laboratory

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Background: Urinary tract infections are responsible for a significant worldwide disease burden. Performing urine culture is time consuming and labor intensive. Urine flow cytometry might provide a quick and reliable method to screen for urinary tract infection.
Methods: We analyzed routinely collected urine samples received between 2020-2022 from both inpatients and outpatients. The UF-4000 urine flow cytometer was implemented with an optimal threshold for positivity of ≥100 bacteria/µL. We thereafter validated the prognostic value to detect the presence of urinary tract infection (UTI) based on bacterial (BACT), leukocyte (WBC) and yeast-like cell (YLC) counts combined with the bacterial morphology (UF gram-flag).
Results: In the first phase, in 2019, the UF-4000 was implemented using 970 urine samples. In the second phase, between 2020-2022, the validation was performed in 42958 midstream urine samples. The UF-4000 screen resulted in a 37% (n=15895) decrease in performed urine cultures. Uropathogens were identified in 18673 (69%) positively flagged urine samples. BACT>10.000/µL combined with a gram-negative flag had a >90% positive predictive value for the presence of gram-negative uropathogens. Absence of gram-positive flag or YLC had high negative predictive values (99% and >99%, respectively) and are therefore best used to rule out the presence of gram-positive bacteria or yeast. WBC counts did not add to the prediction of uropathogens.
Conclusion: Implementation of the UF-4000 in routine practice decreased the number of cultured urine samples by 37%. Bacterial cell counts were highly predictive for the presence of UTI, especially when combined with the presence of a gram-negative flag.

P042

Validation of a detection method of Escherichia coli virulence genes directly from feces to comply with Dutch notification obligation in times of IVDR

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Background: Shiga-toxin producing Escherichia coli (STEC) is a zoonotic pathotype of E. coli. STEC infections can be asymptomatic, but can also lead to hemorrhagic colitis and even hemolytic uremic syndrome (HUS). Commercial diagnostic gastro-intestinal panels can be used to detect STEC in feces but methods often either lack an IVDR certificate or do not specify which virulence markers have been detected, which is mandatory to comply with the Dutch notification obligation. Therefore, we developed an multiplex qPCR-assay which allows detection of relevant virulence genes directly from human feces.
Methods: DNA is extracted from feces using the MagNA Pure 96 automated system (Roche) and used as sample material for the multiplex qPCR assay containing primers for stx1, stx2, eae and O157 genes, as well as an internal control. After running the qPCR program with the Lightcycler480 (Roche), results are obtained within ~3 hours.
Results: In order to ensure reliable outcomes, the qPCR assay was validated according to ISO15189 standards. The analytical parameters measurement trueness, accuracy, specificity and sensitivity were determined, using both EQA and diagnostic samples. For measurement trueness a performance score of 100% was achieved, for accuracy, this was 98%. The specificity of both, the complete assay and each target, was determined and a performance score of 100% was achieved for all parameters except for the stx2-target, for which a specificity of 97% was achieved. Probe sensitivity was determined to be >95% for all probes.
Conclusion: The developed assay offers a reliable method for detection of STEC virulence genes directly from feces simultaneously allowing the compliance with the Dutch notification obligation of STEC. The use of the MagNA Pure 96 automated system increases user-friendliness and the short assay duration allows for rapid result reporting. This assay will be implemented in addition to culturing to improve the national STEC surveillance scheme.

P044

Quantifying Genomic Variation in Campylobacter Infections

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Introduction. Campylobacter poses a significant public health threat, with multiple reservoirs and transmission routes identified. Despite the high burden of disease, microbiological characterization methods have had limited success in identifying outbreaks of Campylobacteriosis with most cases appearing sporadic. However, with the adoption of genomic surveillance in a One Health context being adopted in many countries it is hoped this highly discriminatory method may reveal common transmission pathways amenable to intervention. In this study, we aimed to: 1) Quantify the Campylobacter variation present within a patients infection 2) Characterize these variations at the pan-genome level.

Methods. Up to 30 strains were isolated from 53 Campylobacteriosis patients and a total of 882 high-quality isolates from 52 patients were retained after quality control. The whole genome was sequenced with a combination of long and short-read technologies. Various methods, including multilocus sequence typing (MLST), core-genome multilocus sequence typing (cgMLST), pan-genome analysis, and core single nucleotide polymorphism (SNP) phylogenetics, were employed to describe the Campylobacter variation within a single patient.

Results. 49 patients were infected with a strain from a single sequence type (ST) of Campylobacter, with 3 patients infected by strains from multiple STs. Significant internal variation was observed within the core genome (cgMLST) and pan-genome in patient isolates, even those isolates belonging to the same STs. The variances identified from the pan-genome primarily occurred in the chromosome and were predominantly patient-specific. However, we also observed a limited number of shared genomic differences among multiple patients.

Conclusion. Our study has identified the diversity and patterns of Campylobacter within multiple patients. It has implications for determining how many isolates should be obtained from a stool sample of clinical specimens to accurately infer genetic variations.

P045

Impact of antimicrobial use on the gut resistome from 6 weeks to two years of age in a large prospective infant cohort in Zambia

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Antimicrobial resistance (AMR) is a global health crisis and increasingly undermines our ability to treat bacterial infections. Infections with AMR disproportionately impact low- and middle-income countries (LMICs), which have a high burden of infectious diseases, limited access to accurate diagnostics, and constrained health resources. To quantify AMR and understand the eco-evolutionary drivers behind its development we conducted a longitudinal cohort study focusing on the gut microbiome, antibiotic use, and enteropathogenic infection in infants followed from 6 weeks to two years of age in Zambia. The study was nested in a phase-III rotavirus vaccine trial comparing an oral live-attenuated vaccine with a parenteral trivalent P2-VP8 subunit vaccine on severe gastroenteritis. Weekly, 900 vaccinated children from Zambia were surveyed for health status, antibiotic consumption, as well as gut microbiome analysis at key developmental stages (1, 2, 3, 6, 12, and 18 months). In a subset of 156 children, representing 1236 samples, we analysed the faecal microbiome using metagenomic sequencing. We characterized the diversity, presence, and abundance of AMR genes over time and assessed their association with underlying microbiome composition, antibiotic usage, demographic variables, and vaccine efficacy. The study findings will inform future intervention strategies for preventing and treating infections with AMR in vulnerable LMIC populations.

P046

Description of the exceedance of Cryptosporidium infections during the summer of 2023 in the Netherlands

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Cryptosporidiosis results in severe diarrhea and is caused by the parasite Cryptosporidium. The diseases can become chronic and even life-threatening in immunocompromised. In the Netherlands, the majority of Cryptosporidium infections are caused by C. hominis and C. parvum. The Dutch Cryptosporidium surveillance relies on the voluntary submission of Cryptosporidium positive feces by medical microbiology laboratories to the RIVM, of which three reporting laboratories have sent in all their positive samples in the last seven years. On average, we receive 20 reported cases per month, with a yearly increase in cases in August and September. In September 2023, a strong increase to 129 cases was observed, compared with an average of 72 cases during September of 2016-2019 (range: 58-97). During 2020, 2021 and 2022, no increase was observed likely due to COVID-19 restrictions. There were no deviating trends in age or gender distribution observed in the reported cases of the 2023 exceedance compared to previous years. Cryptosporidium species typing by qPCR and sequencing showed that, similar to 2016-2019, the increase in cases was predominantly driven by C. hominis (82% of typed Cryptosporidium). The majority of C. hominis typed samples (63%) were not detected in the qPCR but were successfully typed by sequencing of the 18S gene. The qPCR used to identify C. hominis targets multiple C. hominis Glycoprotein 60 (GP60) variants. GP60 sequencing of the qPCR missed C. hominis samples showed that these C. hominis had indeed a GP60 type not detected by the qPCR (IdA13, IdA16, IeA11G3T3 and IfA12G1R5). These data suggest that other GP60 variants are circulating and could be responsible for the Cryptosporidium exceedance in the summer of 2023. Interestingly, similar exceedances have been reported in the United Kingdom, Sweden and Ireland. A potential link between these exceedances of Cryptosporidium infections still needs to be explored.

P047

Performance evaluation of six real-time PCR assays for the detection of Candida auris DNA

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Candida auris is an emerging, often multidrug-resistant fungal pathogen that poses a significant threat to global health. Accurate and timely detection of C. auris is crucial for effective management and control of outbreaks. Traditional diagnostic methods for fungal infections involve time-consuming, insensitive, and labor-intensive culture techniques. Here, we evaluate the performance of six real-time PCR (qPCR) assays (four commercial (AurisID (OLM), FungiXpert (Genobio), Screening Assay (Pathonostics) and Fungiplex (Bruker) and two lab-developed (ErasmusMC and CDC) assays (LDA) for the detection of C. auris DNA.

The analytical sensitivity (limit of detection (LoD)) was determined using probit analysis with dilution series of counted spore suspensions. Analytical specificity was evaluated using clinical isolates (n=22), including C. auris, other Candida species and dermatophytes. The clinical performance was assessed using eSwabs from 11 patients (n=23), environmental eSwabs from the hospital room of a positive patient (n=17) and negative environmental eSwabs (n=40). Currently there is no clearly defined gold standard, therefore as the ErasmusMC LDA had the lowest LoD, this assay was used as reference.

The LoD ranged from 407-29816 spores/ml for ErasmusMC LDA and Fungiplex, respectively. Analytical specificity ranged between 91.7-100%. All assays detected the five C. auris clades, however low-level cross-reactivity was observed for C. pseudohaemulonii or C. haemulonii in some assays. The qPCR results of the clinical and environmental swabs were superior to those of traditional cultures. Compared to the ErasmusMC LDA, the overall clinical sensitivity of the other five assays ranged from 51.9-88.9% (Fungiplex and CDC LDA, respectively), with a trend toward a higher sensitivity among clinical screening samples rather than from environmental swabs.

The comparative analysis presented here contributes to the ongoing efforts to enhance diagnostic strategies for C. auris, ultimately facilitating early detection and control measures to decrease the spread of this pathogen.

P049

Long-Term Persistence of Pneumococcal Antibody Responses Following PPV23 Vaccination in Older Adults

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Background: In 2020, the 23-valent pneumococcal polysaccharide vaccine (PPV23) was implemented in the National Immunization Program for adults aged 73+ years in The Netherlands. In addition, a PPV23 booster is advised five years after initial immunization. In this study, we aimed to predict the long-term persistence of antibody responses following PPV23 vaccination, particularly at the five-year post-vaccination mark, in these older adults.
Methods: Pneumococcal serotype (Ps)-specific antibody responses to PPV23 were assessed until two years post vaccination in participants aged 72-79 (n=188) of a 30 years longitudinal observational study (Doetichem Cohort Study). PPV23-specific IgG responses were measured using a multiplex bead-based immunoassay. Duration of IgG persistence was estimated using a bi-exponential decay model under a Bayesian statistical framework. Additionally, we estimated the proportion of participants with higher-than-baseline IgG levels five years after vaccination.
Results: Increased PPV23-specific IgG levels were observed until 2 years post-vaccination and showed a large variation between participants. Additionally, variation in serotype-specific antibody levels were found, with lowest responses measured against Ps4 and Ps12f. Furthermore, we estimated median IgG levels to remain above serotype-specific pre-vaccination levels for at least 5 years for all 23 serotypes, and 10 years for 8 of the serotypes. Proportions of participants with higher-than-baseline IgG levels at five years post-vaccination were predicted to range from 50% (Ps3) to 86% (Ps9N).
Conclusion: PPV23 elicits IgG responses in older adults, which in our prediction analyses persists for a minimum of five years across all 23 PPV-serotypes in at least half of the vaccinated individuals. These findings provide valuable insights into the kinetics of pneumococcal vaccine responses in older adults and may be considered for further improvement of vaccination strategies.

P050

Toxoplasmosis in the Netherlands: seroprevalence and risk factors

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Introduction: Toxoplasmosis is an important foodborne and environmental infection in the Netherlands. Over the past two decades, T. gondii seroprevalence has shown a decline in several countries, including the Netherlands. T. gondii infection endures throughout life resulting in persisting IgG. Accurate information about recent infections and behavior at the time of infection can give valuable insights into risk factors.
Methods: We compared the Dutch seroprevalence across three national representative cross-sectional serosurveys, conducted in 1995/1996, 2006/2007 and 2016/2017. We also conducted a case–control study including persons with a recent infection (cases) and individuals with a negative test result for IgM and IgG for T. gondii (controls) between July 2016 and April 2021. Food history and environmental exposure were compared using logistic regression.
Results: The earlier observed decrease in T. gondii seroprevalence between 1995/1996 and 2006/2007 (from 40.5% to 26.0%) did not continue into 2016/2017 (29.9%). Across all studies, higher T. gondii seropositivity was associated with increasing age, lower educational level, not living in the Southeast of the Netherlands, and eating raw or undercooked pork.
Consumption of different meat products was found to be associated with recent infection. In the multivariable model, adjusted for age, gender, and pregnancy, consumption of large game meat (adjusted odds ratio (aOR) 8.2, 95% confidence interval (95%CI) 1.6–41.9) and infrequent handwashing before food preparation (aOR 15.9, 95%CI 2.2–115.5 for never, aOR 4.1, 95%CI 1.1–15.3 for sometimes) were significantly associated with seropositivity.
Conclusion: These results emphasize the risk of consuming raw or undercooked meat and advocate for promoting good hand hygiene as preventive measures against T. gondii infection. Given the persistent seroprevalence of T. gondii in the Netherlands over the last decade, there is a pressing need to enhance public health awareness and implement effective prevention strategies to reduce T. gondii infections.

P060

New microbial interactions in anaerobic systems - Build, destroy, and build again

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Within the anaerobic digestion (AD), the well-known obligate syntrophy between acetogenic bacteria and methanogens is essential for the overall thermodynamic viability of the process. Hypothetically, there are other (facultative) relationships in AD yet to be uncovered. However, the discovery and study of new symbioses in AD face a significant challenge – the inherent microbial complexity and intricacy of the ecosystem. In this work, a top-bottom approach was adopted to discover novel microbial dependencies on methanogens. Anaerobic sludge enrichments were set-up under methanogenic conditions, alongside enrichments where methanogenesis was inhibited by the addition of 2-bromoethane sulfonate (BrES). The first and fourth generations of the enrichments, fed with glucose and starch, were compared in terms of both chemical and microbiological aspects. In presence of 10mM BrES, methane production was successfully inhibited, while both substrates were fully converted into short-chain fatty acids. Hydrogen accumulated only transiently in the BrES-amended cultures, suggesting the presence of non-methanogenic hydrogenotrophs. Analysis of the microbial composition of the enrichments was based on the diversity of 16S rRNA gene and activity (16S rRNA cDNA). The differentiation between microbial compositions in methanogenic and BrES conditions was further investigated with multivariate integrative partial least square discriminant analysis (MINT-PLSDA) to identify lineages of microbes absent in BrES enrichments. We anticipate those microbes may harbour metabolic links to methanogens. Follow-up studies, involving the construction new synthetic anaerobic consortia will enable a more in-depth investigation into these potential novel microbial interactions.

P061

Enhancing slow sand filtration for producing safe drinking water: Insights into Schmutzdecke ecology and bacterial removal efficiency in mini-scale filters

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The demand for safe drinking water is constantly challenged by increasing biohazards. One widely used solution is implementing slow sand filtration (SSF) in water production. SSF has gained popularity due to its low energy consumption and efficient removal of biohazards, especially microorganisms, without using chemicals. SSF involves both physical-chemical and biological removal, particularly in the "Schmutzdecke", which is a biofilm-like layer on the sand bed surface. To achieve the optimal performance of SSF, a systematic understanding of the influence of SSF operating parameters on the Schmutzdecke development and filter filtration performance is required. In our study, we focused on three operational parameters- sand types, sizes, and the addition of inoculum (suspension of matured Schmutzdecke), on the mini-scale filters (9cm length). The effects of these parameters on the Schmutzdecke development and SSF removal performance were studied by biochemical analyses and 16S amplicon sequencing, together with spiking experiments with Escherichia coli in the mini-scale filters. Our results indicate that the mini filters successfully developed Schmutzdeckes and generated bacterial breakthrough curves efficiently. The sand size and type were found to have impacts on the Schmutzdecke development. The addition of inoculum to new filters did not induce significant changes in the microbial community composition of Schmutzdeckes, but we observed faster Schmutzdecke development and better removal performance in some inoculated filters. Our study highlights the value of mini-scale filters for SSF studies, which provide insights into Schmutzdecke microbial ecology and bacterial removal with significantly reduced requirements of materials and effort compared to larger scales. We found that operational parameters have a greater impact on the Schmutzdecke biochemical characteristics and removal performances than on the microbial community composition. This suggests that Schmutzdecke characteristics may provide more reliable predictors of SSF removal performance, which could help to improve safe drinking water production.

P062

Methane cycling by freshwater microbial communities along a natural lanthanide gradient in Western Greenland

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Methane is a more potent greenhouse gas than carbon dioxide and its atmospheric concentration has been rising steadily since 2007. Recently, northern lakes have been implicated as the largest natural methane source at latitudes above 50°N. The discovery of a wide-spread lanthanide-dependent methanol dehydrogenase enzyme (XoxF) used by aerobic methanotrophic bacteria has raised new questions on whether methane cycling is controlled by the supply of these metals. The Greenland ice sheet is melting, and silt-laden glacial discharge is generating wind-blown dust which transports nutrients and micro-elements, particularly lanthanides, into lakes.
In order to investigate the influence of the input of this glacial dust on microbial communities, samples were collected from the top layer of the sediment of five lakes along the dust gradient near Kangerlussuaq, Western Greenland. DNA was extracted from the bulk sediment and used for metagenomic sequencing. Taxonomic classification revealed a highly diverse bacterial community, with Alpha- and Gammaproteobacterial methanotrophs as minority members. A total of 27 XoxF-like sequences were recovered, clustering mostly within the XoxF3 and XoxF5 clades. The observed changes in community across the lakes occurred most prominently in non-methanotrophic organisms: for example, Chloroflexota were more abundant at higher dust inputs, whereas the abundance of Archaea decreased in the lakes closest to the ice sheet. Approximately two-thirds of metagenomic reads could not be confidently assigned below the class level. While this adds uncertainty to these observed trends, it also points to a potential treasure trove of yet-to-be-discovered microorganisms in this rapidly changing, but poorly understood environment.

P063

Novel ammonia-oxidizing cultures obtained through targeted cell sorting reveal niche differentiation between ammonia oxidizing bacteria and comammox bacteria

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Nitrification was long regarded as a two-step process, performed by separate guilds of ammonia and nitrite oxidizing microorganisms. The recent discovery of complete ammonia oxidizing (comammox) bacteria resulted in a paradigm shift. The only available comammox pure culture yielded interesting physiological insights, but more isolates or highly enriched cultures are needed to test whether the observed traits are common to all comammox bacteria. However, classical cultivation methods have for a long time overlooked the existence of comammox bacteria, which illustrates the recalcitrance of these fastidious bacteria to cultivation. Here, we present a workflow for the targeted enrichment and isolation of novel ammonia oxidizing microorganisms, including comammox bacteria, from complex environmental samples. Specific in vivo fluorescent labelling of ammonia monooxygenase, the key enzyme required for ammonia oxidation, was combined with fluorescence-activated cell sorting (FACS) into 96-well plates containing mineral medium amended with ammonium and nitrite. All wells were regularly screened for the production of nitrite and nitrate, with nitrate production distinguishing complete nitrifiers from canonical ammonia oxidizers. This method resulted in six highly enriched comammox cultures and one novel axenic Nitrosomonas ammonia oxidizing bacterium. Physiological and genomic characterization of a comammox Nitrospira enrichment culture and the Nitrosomonas isolate that were both obtained from the same biomass source sheds light on their niche differentiation. While the comammox Nitrospira strain had higher affinities for ammonia (0.26 ± 0.10 µM NH3) and oxygen (3.95 ± 0.90 µM O2) than the Nitrosomonas isolate (1.12 ± 0.26 µM NH3; 7.63 ± 0.53 µM O2), Nitrosomonas had higher ammonia oxidation rates compared to comammox Nitrospira. In conclusion, we demonstrated that our approach is well-suited to isolate (complete) ammonia oxidizers from complex environmental samples, and subsequently obtain novel physiological insights. This ultimately advances our understanding of the role of different ammonia oxidizers in natural and engineered ecosystems.

P064

Dry trickling filtration as a sustainable alternative to aeration in producing drinking water from methane-containing groundwater

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Aeration followed by rapid sand filtration is the conventional method for drinking water production from groundwater. During aeration, methane is stripped from the water, whereas microbial and biochemical processes in the sand filter facilitate the removal of iron, ammonium, and manganese. Due to its high greenhouse gas potential, the released methane significantly contributes to the carbon footprint of drinking water production.

Our study explores the possibility of mitigating methane emissions by applying biological methane oxidation as a sustainable alternative to stripping. We compared the traditional approach involving aeration followed by primary and secondary rapid sand filters, and an alternative method with a primary dry trickling filter and a subsequent secondary rapid sand filter. A multi-omics approach, integrating 16S rRNA gene amplicon sequencing, metagenomics, and metaproteomics, was employed to gain a comprehensive understanding of the microbial communities responsible for contaminant removal.

We found that in the alternative approach, the methane entering the trickling filter selected for a dominant population of methane-oxidizing bacteria, which successfully removed methane. As a result, ammonium and manganese removal were shifted to the secondary rapid sand filter. This contrasts the traditional treatment line, where almost no methane-oxidizing bacteria where present, and ammonium and manganese were successfully removed already in the primary rapid sand filter. Interestingly, different nitrogen cycle microorganisms facilitated ammonium removal in the rapid sand filters of the different treatment lines. Complete ammonia oxidizers of the genus Nitrospira dominated nitrification in the traditional treatment, while this was catalyzed by Candidatus Nitrotoga and Nitrosomonas in the trickling filter treatment line.

In conclusion, our research indicates biological methane removal as a viable alternative to aeration-based stripping, offering a more sustainable method for drinking water production from methane-containing groundwater.

P065

Detection of airborne wild water bird-derived DNA demonstrates potential transmission of avian influenza via air-inlets into poultry houses

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Background
In recent years, many outbreaks of highly pathogenic avian influenza (HPAI) were reported at poultry farms worldwide. HPAI is causing unprecedented mortality in wild bird populations, and is increasingly affecting mammalian species. Current control measures for HPAI in poultry involves culling all birds at affected farms and enhanced biosecurity. Presence of wild birds near poultry farms is associated with increased risk of HPAI introduction in poultry, but evidence on transmission routes is lacking.

Aim
To investigate whether HPAI from wild birds can potentially enter poultry barns through air-inlets by detecting eukaryote host DNA in this airflow.

Methods
Particulate matter (PM) samples were collected in the airflow of three poultry farms with previously diagnosed HPAI that were cleared and rigorously decontaminated prior to sampling. As a positive control for water bird DNA, PM samples were collected at a bird rehabilitation shelter.PM samples were repeatedly collected using active air-sampling for 4-5 consecutive days. Outdoor air samples (N=138) were collected around the barn; indoor (N=89), the incoming air was sampled near air-inlets. Metabarcoding was performed on total environmental DNA (eDNA) by deep-sequencing 18S-rRNA gene amplicons.

Results
DNA of water birds was detected in the air inside all three and outside of two poultry farms. Water bird DNA was present in all indoor and outdoor air samples collected at the bird shelter .

Conclusion
This study demonstrates that HPAI can potentially be introduced in poultry houses through air-inlets via airborne matter derived from wild birds. The eDNA metabarcoding provides a tool to monitor and improve biosecurity targeted to HPAI and other pathogens potentially transmitted through air which are difficult to detect at the event of first transmission.

P066

Extended-spectrum cephalosporins-resistant (ESC-resistant) E. Coli: trends and seasonality over nine years in the Netherlands

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Antimicrobial resistance in livestock may contribute to the prevalence of resistant bacteria in humans with potentially detrimental effects on human medicine. Extended-spectrum cephalosporins-resistant (ESC-resistant) E. coli, including ESBL, AmpC-producing E. coli, are considered of particular importance and are monitored selectively in livestock. We have analyzed data from the antimicrobial resistance monitoring program in livestock in the Netherlands for selectively isolated ESC-resistant E. coli, assessing trends and seasonality over nine years (2014-2022). Time-series generalized linear models, employing a log link and a Poisson distribution, were performed on the frequency of ESC-resistant E. coli (AMR) in five different animal production sectors: broilers, pigs, dairy cattle, rosé veal calves, and white veal calves. The models were specified using the time as a trend (i.e., 36 intervals resulting from nine years split into four seasons), and the seasons as a categorical variable in the fixed effects. The trends in ESC-resistant E. coli per quarter for broilers showed a 6.8% reduction in AMR from 2014 to 2020, followed by a small increase (3.5%) between 2021 and 2022. For veal the trends were similar first increasing by 10% and 7.1% by quarter, followed by reductions of 1.3% and 1.4% per quarter, for rosé and white, respectively. Dairy cattle had a slow increase in AMR over nine years, 1.4% per quarter. No changes were seen in slaughtered pigs. AMR prevalence was positively correlated with warmer seasons (summer and autumn) for dairy cattle and veal calves. No associations were found for broilers and slaughtered pigs. Future studies could explore a possible correlation between the observed AMR seasonality and antimicrobial use and herd health indicators (i.e., disease incidence, and antimicrobial usage) in dairy and calf sectors. The trends for other AMR bacteria in livestock could be explored, to elucidate if other resistant organisms display similar trends in prevalence.

P067

Cultivation and characterisation of a novel marine archaeon belonging to the phylum Thermoplasmatota.

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Thermoplasmatota represents a cosmopolitan archaeal phylum, that has been found in diverse environments, including acid streamers, mammalian guts, soils, hot springs, freshwater as well as marine sediments. Most of these environments have cultured Thermoplasmatota representatives. However, none of the widely distributed sediment dwelling Thermoplasmatota lineages have been cultured to date. Here we describe a highly enriched culture (70% relative abundance) of a Deep Hydrothermal Vent Euryarchaeotal Group 1 (DHVEG-1) archaeon, a member of the phylum Thermoplasmatota, found in marine sediments sampled from Aarhus Bay (Denmark). This first cultivated representative of the order DHVEG-1 (Class E2) grows anaerobically between 20-30oC in a consortium with two bacteria of the phyla Desulfobacterota and Proteobacteria and two archaeal taxa belonging to the phyla Halobacterota and Asgardarchaeota. Genomic analyses indicate the metabolic potential to generate hydrogen and acetate and the presence of archaeal type IV pili. Fluorescence microscopy revealed cells with a very small coccoid cell body of approximately 0.5um and the clear presence of pili-like protrusions. These findings hint to a potential syntrophic lifestyle of this first cultivated member of DHVEG-1 archaea. Follow-up studies, including biochemical analyses and high resolution- and live microscopy methods will provide further insights into the ecology and cell biology of this archaeon, including its potential syntrophic interactions with the rest of the consortium members and the role of the pili-like structures.

P068

Need for partnership, cell signaling and adhesion drives microbial aggregation in anoxic biosystems

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Introduction: Natural and engineered anoxic and methane producing environments harbor microorganisms in organized biofilm aggregates, which ensure efficient exchange of biochemical molecules. However, limited knowledge exist on the mechanisms of aggregate formation in these environments. Here we investigated the biochemical and morphological aspects of laboratory evolved microbial aggregates, that represent a model biosystem for the natural and engineered anoxic ecosystems. Structure and gene expression of the laboratory aggregates was compared to the make-up of the mixed-culture/industrial methane-producing biofilms to confirm the validity of the laboratory approach.

Methods: Co-cultures of fatty-acid oxidizing bacteria and methanogenic archaea were grown in a fed-batch mode at 37°C in a bicarbonate-buffered mineral salt medium containing propionate (20 mM) and 1.5 bar N_2/CO_2 (80/20 (v/v)). Morphology and genetic make-up of the resulting co-culture aggregates was compared to that of the mixed culture aggregates from the industrial and laboratory wastewater treating bioreactors (in-house collected and/or publicly-sourced data). Activity and structure of microbial aggregates was analyzed using high-performance liquid/gas chromatography, fluorescent/scanning electron microscopy, and RNA/DNA sequencing.

Results: Laboratory-evolved co-cultures formed aggregates within 5 months, while continuously-operated mixed cultures set-ups generally required at least 6 months for the initial microbial aggregation. Although morphologically different, mixed-cultures aggregates shared the core of hydrogen-exchanging microorganisms with the laboratory co-cultures aggregates. At the different stages of aggregates maturation we observed differential expression of genes for cell signaling, adhesion and polysaccharide production.

Conclusion: We found similar principles governing the spatial organization of defined and mixed laboratory/industrial methanogenic microbial communities. Involvement of cell signaling and adhesion in studied here aggregate formation suggests that bacteria-archaea aggregation at the energy-limited anoxic/methane-producing environments might follow similar principles to the microbial aggregation occurring in the energy-rich oxic environments. However, the distinct need for the hydrogen-exchanging microbial partnerships places anaerobic aggregation apart from the well-studied oxygen-respiring biosystems.

P069

Nitrification potential in the Stockholm Archipelago

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Background. The microbial nitrogen (N) cycle is one of the major biogeochemical cycles on Earth. In many coastal ecosystems, N cycling is perturbed by anthropogenic activities, resulting in eutrophication and deoxygenation of the water column. Furthermore, the increased nutrient and organic matter input cause high concentrations of ammonium in the water column. In the Stockholm Archipelago, nutrient concentrations in the water are increased due to sewage discharge, provoking an eutrophic and partly oxygen-depleted system. In this study, three sites in the Stockholm Archipelago were investigated to assess the microbe-mediated nitrogen dynamics in the water column. N-cycling under anoxic conditions is not well understood, as while nitrite and nitrate can be removed through denitrification or anaerobic ammonia oxidation, both processes depend on the activity of aerobic nitrifiers to oxidize ammonia to provide them with their substrate. However, although nitrifiers are often abundant in oxygen-minimum zones, their metabolic pathways to oxidize ammonium and nitrite under oxygen limiting conditions remains unclear.
Methods. Oxygen and nutrient concentrations in the water column were measured at high vertical resolution. Water samples were taken for DNA extraction and sent for 16S rRNA gene amplicon sequencing.
Results. Based on the water column profiles, ammonium conversions are different at all sites with the lowest turnover at the anoxic, euxinic site. Interestingly, the highest abundance of nitrifiers in the water column was observed around the oxycline at all sites.
Conclusion. This study provides valuable insights into the nitrification potential in an eutrophic system under oxygen-limited conditions and thereby contributes to a better understanding of the impact of human activities on oxygen and nitrogen dynamics in coastal ecosystems.

P070

Sulfide toxicity adaptation in anaerobic methane oxidizing archaea (ANME)

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Coastal anoxic sediments are becoming hotspots of a potent greenhouse gas: methane. Eutrophication-derived deposition of organic matter leads to a rise in methanogenesis and sulfate reduction, increasing the concentrations of methane and sulfide, respectively. Sulfide is a toxic compound that poses a constrain to coastal microbiomes. Methanotrophs act as natural methane-oxidizing biofilters that use a variety of electron acceptors, including eutrophication-augmented nitrogen compounds. However, despite the increasing evidence of methane and sulfide in coastal sediments, the effects of sulfide stress on methanotrophs have yet to be explored. Here, we studied a model enrichment of nitrate-reducing Anaerobic Methane-oxidizing (ANME) archaea, referred to as 'Candidatus (Ca.) Methanoperedens'. Our aim was to utilize a 'Ca. Methanoperedens' enriched culture to (i) establish sulfide stress thresholds and resistance times and (ii) characterize the physiological response to sulfide stress. Methane oxidation potential was tested on a bioreactor at three different sulfide conditions: control (no-sulfide), stress and resistance. For that, whole bioreactor batch activity assays with 13C-CH4 were employed. The physiology of the response was conducted via an omics-centric approach, combining metagenomics and metatranscriptomics. To allow for a detail enrichment community composition and storage polymer (PHA) usage characterization, we included Fluorescent In Situ Hybridization (FISH), qPCR and PHA quantification. 'Ca. Methanoperedens' methane oxidation potential ceased by half both between control to stress and stress to resistance; thereby, indicating a stepwise inhibition with an underlying resistance to long periods of sulfide exposure. Such detoxification could be the combination of sulfide oxidation-stress alleviation via 'Ca. Methanoperedens' direct or symbiotic (with sulfide oxidizing denitrifiers) action. The ecological resilience threshold and resistance time resolved with the proposed methanotrophic study provides a baseline for fundamental microbial ecology and adaptation directly relevant for aquatic ecology.

P071

Microbial Ballet: The story of an alphaproteobacterium hitchhiker on a gliding flavobacterium

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Microbes are social, interacting organisms forming communities of single or mixed species. Many inter-microbial interactions happen through the exchange of nutrient or sensory molecules and some are mediated by motility. Cellulophaga lytica PlyA2 is a marine, gliding flavobacterium which displays vivid structural color due to its highly ordered colonies. Pseudosulfitobacter pseudonitzschiae SW is a marine, non-structurally colored alphaproteobacterium which hitchhikes on expanding, gliding colonies of cellulophaga. Besides the hitchhiking behavior, together they show strong comigration and structural color modification, suggesting cellular reorganization. The aim of this work is to disentangle the relationship of the strains of interest by analyzing them at the metabolic level. Whole genome sequencing and shotgun proteomics were employed on both strains when in mono- and co-culture, followed by comparative functional analysis. Protein expression patterns showed that P. pseudonitzschiae SW is strongly impacted by the presence of C. lytica PlyA2 (279 vs 23 differentially expressed proteins in co-culture). Most of the significantly different proteins of P. pseudonitzschiae SW were distributed into COG categories associated with the general metabolism, such as amino acid transport and metabolism (13%), energy production and conversion (8%) and translation (7%). Similarly, 13 KEGG pathways mostly related to amino acid metabolism, ribosomes and quorum sensing were overrepresented, indicating a potential role in the observed synergistic growth and communication between the strains. This was also supported by the high number of homoserine lactone biosynthetic gene clusters in P. pseudonitzschiae SW induced in the presence of C. lytica PlyA2. From a metabolic perspective, our findings suggest that the two strains have an unusual, highly-asymmetric, non-antagonistic relationship in which P. pseudonitzschiae SW remodels its proteome extensively and increases its population size and C. lytica A2 changes population and proteome minimally but reorganizes its colony sufficiently to change the optical properties.

P072

Guyparkeria halophila as a very efficient microbial platform for the circularization of carbon dioxide and thiosulfate into fine chemicals for the pharmaceutical industry

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The utilization of carbon dioxide (CO_2) for valuable chemicals production has emerged as a significant cornerstone for achieving a circular economy. However, existing CO_2 conversion processes face challenges related to cost-effectiveness, primarily due to the limited use of model microorganisms, production of low-value compounds, and the requirement of affordable energy sources for CO_2 fixation. This study intended to overcome these limitations by advocating for CO_2 fixation using nearly free energy sources, specifically industrial contaminants, like thiosulfate ($S_2O_3^{-2}$). In addition, it will promote the simultaneous elimination and valorization of both waste compounds, CO_2 and $S_2O_3^{-2}$, throughout their conversion into fine chemicals, such as ectoines. These chemicals, naturally produced by prokaryotes under high salinity environments, are crucial ingredients in pharmaceuticals and cosmetics, with a retail value of 1000 € Kg^{-1}. Finally, unexplored halophilic thiosulfate-oxidation bacteria, harboring genomes containing genes for ectoines synthesis, will be identified using novel genomic tools. In total, six microbial genomes were identified as potential candidates to carry out the process. After laboratory validation of ectoine production at 3% NaCl, the fastest growing strain, Guyparkeria halophila, was selected for product optimization at three different salinities (6, 9 and 15% NaCl) using $S_2O_3^{-2}$ and CO_2 as the only substrates. Results showed that G. halophila accumulated significantly higher ectoine yields at 15% of NaCl. Finally, batch bioreactors were implemented combining the optimal conditions previously determined to observe ectoine production and contaminants depletion over time. Under these conditions, G. halophila reached maximum ectoine contents up to 40% (473.9 ± 37.1 mg of ectoine·g $biomass^{-1}$). These results not only constitute the highest ectoine yields so far reported by autotrophs and most of heterotrophs, but also, the first proof of a novel valorization platform for CO_2 and $S_2O_3^{-2}$, establishing a foundation for a new economic niche focused on transforming CO_2 into pharmaceuticals.

P073

Protein structure reveals remote Asgard archaeal homologs of eukaryotic signature proteins

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The eukaryotic cell evolved from the Asgard archaea, a diverse clade whose genomes encode homologs of proteins that play important roles in the complex organization of eukaryotic cells, so-called eukaryotic signature proteins (ESPs). The identification of ESPs is hampered due to extensive sequence divergence during eukaryogenesis. Here, we used de novo protein structure modeling to identify 856 previously unreported structural isomorphs of ESPs (iESPs) within an expanded Asgard archaeal genomic dataset. While most previously identified ESPs were involved in cellular processes and signaling, iESPs are enriched in information storage and processing functions. We combine sensitive sequence similarity detection algorithms with phylogenetic analysis to elucidate the evolutionary history of previously undescribed archaeal homologs of eukaryotic proteins operating in endosomal sorting, ribosome biogenesis, and ubiquitination. By expanding the complement of eukaryotic proteins in Asgard archaea, this work indicates that the archaeal ancestor of eukaryotes was more complex than previously assumed.

P074

Airborne virus exposure mitigation by advancing respiratory protective equipment testing with a fluorescent tracer

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During the COVID-19 pandemic, it became clear that crowded poorly ventilated indoor environments represent a significant risk for aerogenic virus transmissions. Better understanding of the role of aerosols in this transmission may lead to more effective exposure mitigation strategies. In this study we focused on respiratory protective equipment (RPE) by introducing a 'stress test' based on the use of a fluorescent tracer. The rationale behind this setup is based on aerosols as small droplets with a virus load that depends on the type of body fluid and characteristics of the infected host.

A medical nebulizer was used to generate inhalable water droplets with a particle size distribution ranging from 6.5 to 14.8 µm, containing 1% fluorescein. These droplets were sprayed towards a facemask placed on a mannequin head. We assessed the sum of filter penetration and face seal leakage by determining the recovery of the fluorescent tracer on a membrane filter placed in the mouth opening of the mannequin head.

The results reveal a 58% higher leakage in surgical masks compared to FFP2 respirators, translating to overall filtration capture efficiencies of 97% and 98%, respectively. Our study shows that the use of a fluorescent tracer may have value as a new approach to test RPE for virus protection efficacy.

The fluorescent tracer, fluorescein, can be used to quantitatively predict exposure to aerosols by measuring leakage of different RPE's. We will further develop the method for quantitative assessment of RPE performance testing as well as diagnostics of face seal leakage. Moving forwards, we plan to refine and expand this concept through testing on human volunteers.

P075

Synthetic co-cultivation enables the production of odd-chain carboxylates and alcohols from carbon monoxide

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The growing demand for sustainably produced chemicals underscores the need for innovative technologies addressing carbon recycling from recalcitrant wastes and biomass. A promising strategy involves the gasification of these feedstocks into syngas, followed by microbial fermentation. However, the capacity to diversify product range from syngas using single strains remains limited. Here, we introduce a synthetic microbial consortium that produces odd- and even-chain carboxylic acids and higher alcohols solely from CO — the main component of syngas. The consortium is composed of three Clostridial species: the acetogen Acetobacterium wieringae, the propionigenic Anaerotignum neopropionicum, and Clostridium kluyveri, well known for its chain elongation metabolism. Metabolite cross-feeding within this consortium enables the formation of odd-chain products, such as valerate and pentanol, which are unusual in CO-fermenting systems. Specifically, pentanol accumulated to 4.2 mM (0.4 g L-1) in a bioreactor fermentation with solely CO as substrate; a significant achievement despite the low specificity obtained for this product (4% C-mol/C-mol). Our findings also reveal that, while in monoculture the metabolism of A. wieringae is fully acetogenic, in co-culture it shifts partially to solventogenesis, generating ethanol that is used by the partnering strains.

The co-culture established in this work represents a proof-of-concept and a starting point for optimisation that, as we also show, can be guided by genome-scale metabolic modelling. We display the practical utility of the genome-scale metabolic models (GEMs) of A. wieringae and the tri-culture, which facilitate the study and optimization of the process. Computational simulations suggest a balanced species ratio for consortium stability and provide insights into the effect of H2 supplementation. This research contributes to advancing microbial communities as catalytic platforms for syngas fermentation and highlights the value of modelling in bioprocess design.

P076

A new perspective on hydrogen and methane oxidation by type II methanotrophs

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Hydrogen gas (H_2) is present as a trace gas in our atmosphere. It is a powerful electron donor that is ubiquitously used by microorganisms in a wide array of environments. Genomes of several aerobic methane (CH_4) oxidizing bacteria from the class Alphaproteobacteria contain multiple genes encoding [NiFe] hydrogenases, including Methylocystis bryophila H2sT, Methylocapsa aurea KYG, and Methylosinus acidophilus 29. Here we show that these three methanotrophic species instantly oxidize H_2 upon cultivation under CH_4-limited conditions, suggesting a constitutive hydrogenase expression. The maximum H_2 oxidation rate accelerated almost 3-fold within four hours and was highest under low-oxygen (1%) conditions. Interestingly, the maximum H_2 oxidation rate of M. bryophila H2sT was independent of the growth rate, which contrasts our findings of the maximum CH_4 oxidation rate being significantly reduced at low growth rates. Moreover, addition of sulfide (H_2S), a highly reduced molecule produced in anoxic environments, inhibited H_2 oxidation but to a lesser extent than CH_4 or methanol oxidation. The ability to conserve energy from H_2 can increase fitness and enhance growth of methanotrophs in ecosystems with varying CH_4, H_2S, and oxygen fluxes. These findings are a first step towards a revision of the ecological role of mesophilic methanotrophs, and the importance of H_2 in ecosystems where CH_4 concentrations are naturally low.

P077

Portable Genomic Epidemiology: Assessing In-Field Microbial Detection for Cost-Effective Outbreak Surveillance

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Genomic epidemiology is vital in investigating the spread of diseases during an outbreak and for future preparations to tackle emerging outbreaks. Utilizing an in-field microbial detection approach coupled with next-generation sequencing (NGS) is an effective method. This technique functions as a surveillance system, delivering data cost-effectively and rapidly.

We benchmark a portable in-field microbial lab to a traditional molecular lab for DNA isolation, sequencing and microbial identification. Samples from lake water, wastewater treatment plant sludge and retail meat products were chosen due to their potential to harbour infectious bacteria linked to outbreaks. Microbial detection in these areas provides information for environmental monitoring, public health and food safety assisting in outbreak preparedness. DNA was isolated using the Bento-Lab and traditional lab and sequenced using the MinION with Flongle adaptor. Taxonomic classification assessed the ability of microbial detection for both lab types. Results from the portable lab were compared to the traditional lab using four metrics: DNA concentration and purity, read length and taxonomic classification.

All DNA isolated were pure. DNA concentrations were 4.5%-35% higher in the Bento-lab compared to the traditional lab. We produced 20K reads and 1Gb of data for both lab types. For both lab types, we identified bacteria to species level. 49 different bacterial species were identified from lake water using the Bento-lab, 12 species were shared with samples from the traditional lab. Bacteria in the Bento-lab samples had higher diversity and richness. We identified E. coli and Salmonella enterica in the lake water, sludge and retail meat product samples.

We showed that species-level microbial identification can be achieved using an in-field portable lab technology with limited equipment and resources. Portable in-field DNA sequencing has great potential for various research areas, including outbreak detection, identifying emerging pathogens, microbiome surveillance and many more.

P078

Ecological characterization of the rhizosphere microbiome of wheat grown under drought conditions in successive cultivation

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Current efforts to ensure sustainable crop production include characterization of plant rhizosphere microbiomes. In fact, different microbial groups influence plants by inducing or helping to relieve stress. Further, microbes respond to plant-derived signals, which may result in abundance shifts of selected taxonomic groups. Successive cultivation of plants under different treatments can enhance such alterations in the microbiome composition via repeated reinoculation of the rhizosphere microbiome of select plants. In our first successive cultivation trial experiment, wheat was inoculated with an 84-member bacterial isolate library in sterile soil under non-stressed conditions. This approach demonstrated that plants resulting from repeated selection for fast growth developed a significantly different bacterial composition in the rhizosphere compared to the samples with repeated selection for slowed plant growth. In this work, we hypothesize that applying stress conditions highlights key bacterial groups that contribute to plant stress resilience. Our experimental setup included a randomized selection lineage under non-stressed conditions, a selection lineage for robust drought-resilience, and a selection lineage for poor drought-resilience. Moreover, at the end of each cycle, the plants exposed to drought were watered in order to determine their rescue rate. 16S rRNA and ITS amplicon sequencing of rhizosphere and bulk soil allows identification of microbial groups with a different relative abundance between the treatments, particularly differentiating the treatments involving drought compared to well-watered conditions. The characterization of key microbial players in the rhizosphere, represents a solid starting point for further in planta studies under drought conditions, and highlights the value of performing successive cultivation in plant microbiome studies.

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P079

Priority effects, nutrition and HMO-metabolism drive Bifidobacterium longum subspecies dynamics in the infant gut microbiome

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Background
Bifidobacterium longum is crucial for early-life microbiome development. Its subspecies (Bifidobacterium longum subsp. infantis and subsp. longum) exhibit significant genomic diversity, employing distinct ecological and metabolic strategies (e.g. utilization of human milk oligosaccharides, HMOs). An understanding of the factors governing B. longum colonization is required to promote infant health. We analyzed mother-infant gut microbiome pairs to assess the environmental and genomic features influencing B. longum subspecies colonization success and succession.
Methods
The study included 24 mother-infant pairs from the Amsterdam Infant Microbiome Study (AIMS), performing metagenomic sequencing on stool samples at late pregnancy (mother), 1 and 6 months after birth (infant). Metagenome-assembled genomes (MAGs) were assessed to identify characteristics of B. longum subspecies in relation to early-life gut colonization. LASSO regressions identified significant features associated with B. longum subspecies abundance, accounting for priority effects, nutritional intake, microbial interactions and HMO-utilization potential.
Results
B. longum subsp. longum was the most abundant and prevalent gut bifidobacteria at 1 month, and was replaced by B. longum subsp. infantis at 6 months of age. B. longum subspecies have significant differences in their potential to break down HMOs and vertical transmission, higher abundances of B. longum subsp. longum in the maternal gut microbiota, breastmilk and a broader range of HMO-utilizing CAZymes promote its abundance at 1 month of age. At 6 months, B. longum subsp. infantis resplaces B. longum subsp. longum due to nutritional intake, HMO-utilization potential and diminished priority effects.
Conclusion
Our results highlight the importance of priority effects, nutrition and HMO-utilization potential in determining the predictable colonization and succession trajectories of B. longum subspecies in the infant gut. As substrate utilization and metabolite production varies among species, subspecies and even same-species strains, distinct early-life colonization and succession dynamics could potentially influence the benefits they provide to the infant.

P080

Microbial nitrogen cycling potential in marine sediments from seasonally hypoxic Lake Grevelingen

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Coastal ecosystems experience increased eutrophication and deoxygenation due to anthropogenic activities. Such shifts in environmental conditions can change the redox zonation of an ecosystem, which can impact its biogeochemical functioning. Nitrous oxide (N2O), an extremely potent greenhouse gas, is produced in various reactions of the nitrogen cycle. Therefore, a predictive understanding of the impact of eutrophication and deoxygenation on microbial nitrogen cycling and the formation and removal of N2O in coastal waters is crucial.

Sediments were collected from the seasonally hypoxic, marine Lake Grevelingen in March and September 2023 when the bottom waters were oxygenated and depleted in oxygen, respectively. Porewater was analyzed for oxygen, pH, trace elements, and nitrogen compounds. Nitrogen cycling potential of the sediment at different depths and seasons was studied in batch incubations supplemented with various nitrogen compounds in the presence and absence of oxygen.

While the sediment ammonium concentrations increased with depth up to 15 mM in the first 40 cm, nitrate and nitrite were depleted within the first cm. High nitrate and N2O reduction rates were observed for all studied sediment sections. Interestingly, the anoxic sediments showed ammonium oxidation potential when supplied with oxygen and ammonium, but the oxic sediments did not. The microbial diversity and nitrogen cycling potential of the in situ microbial community is being analyzed via 16S rRNA amplicon and metagenome sequencing. Subsequent transfers of active batch incubations have resulted in the enrichment of MBAE14 bacteria, which are being characterized physiologically and genomically.

Despite differences in season and depth, there is a high potential for nitrate and N2O reduction in the sediments of Lake Grevelingen, whereas the ammonium oxidation potential seems to be restricted to anoxic sediments. This is important information to make better predictive models of the future state of our coastal waters and the reduction of their N2O emissions.

P081

Human milk oligosaccharides shape infant gut microbiota

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Human milk oligosaccharides (HMOs) play an important role in forming the gut microbiota in early life. The use of HMOs by individual infant gut or probiotic species has been extensively studied, but how HMOs influence infant gut microflora at the community level and the underlying mechanisms are less known. Here, we constructed an infant microbial synthetic community (SynCom) consisting of 8 representative species belonging to Firmicutes, Bacteroidetes, and Actinobacteria, representing the infant gut. Inoculum were grown in media with seven different HMOs individually, and bacterial cells were collected after 24h of anaerobic culturing. Nanopore sequencing was used to determine the community composition. Supernatants were used for metabolite analysis via HPLC. Results show that B. fragilis is the dominant species in each HMO condition. However, when the community was cultured by tetrasaccharides, the relative abundances of specific Bifidobacteria species were raised from 0.19% to 7-9%, which indicates its supportive effect on Bifidobacterium. In the meantime, the relative abundance of the butyrate producer was significantly increased to 4.5% when compared with other HMO conditions. Correspondingly, butyrate production was increased to 0.66 mM, while no butyrate was observed under other conditions. In summary, we constructed a B. fragilis-dominant infant SynCom and observed that different HMOs may have different abilities to shape infant gut consortia structures and functions. The SynCom could serve as a model infant gut microbiota to test nutrients in the context of precision nutrition.

P082

Effects of intensive forest management of drained peatland on the microbial potential for anaerobic oxidation of methane

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Gas fluxes from boreal peatlands have the potential to influence the concentration of greenhouse gases in the atmosphere. In addition, some of the boreal peatlands are under intense human influence and are used for timber production. The most widespread timber harvesting practice, clear-cutting, has effects on soil and groundwater regimes and microbial functions that are not fully understood. We investigated whether clear-cutting and subsequent water table elevation create a larger niche for anaerobic methanotrophic microbes that mitigate the effect of methane emissions from peatlands. Clear-cutting forest management significantly influenced the composition of microbial community across the soil profile. Soil depth and pH were found to be drivers of community composition. We detected the presence of the genus Candidatus Methanoperedens – a member of the anaerobic methanotrophic archaea, in submerged peat layers. The rate of methane production in the submerged peat layers was slow two years after clear-cutting. However, methane oxidation could be detected in anoxic microcosms with excess of added nitrate as an electron acceptor. This study shows that although methanogenesis is not high in newly submerged soil layers, anaerobic methanotrophs are present there. Their activity therefore potentially reduces the amount of methane produced in deeper layers and buffers the impact of clear-cutting on the habitat's greenhouse gas flux balance.

P083

An easy way for long-term storage of Aphanomyces astaci, the causative agent of crayfish plague.

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The water mould Aphanomyces astaci, also known as causative agent of the crayfish plague, is an oomycete that parasitizes crayfish. For native species, such as the European crayfish (Astacus astacus), this water mould is lethal. To study A. astaci and its spores, this pathogen is cultured in broth or on agar plates in the laboratory which is very time and labor-intensive. In order to facilitate long-term storage of the mould, we investigated whether the hyphen can survive on agar after storage in the refrigerator at 4°C.

To test this, A. astaci (VEN5/14a) was stored at 4 °C for 18 months. First, the oomycete was cultured on peptone glucose agar. With the fresh cultures, sporulation experiments were performed; A. astaci was able to form spores which indicated that long-term storage still results in viable and pathogenic A. astaci. Subsequently, DNA for identification of the species was isolated with the DNeasy plant mini kit. Both Oidtmann and Vralstad PCR assays were performed and confirmed that the strain was indeed A. astaci. Furthermore, DNA amplicons obtained after the Oidtmann PCR were used to confirm A. astaci by Sanger sequencing. Together, we could demonstrate that after 18 months storage the laboratory strain was still viable and identified as A.astaci.

These findings indicate that A. astaci laboratory cultures don’t need to be kept in culture continuously. However, in the outdoor environment these findings might have serious consequences for restocking native crayfish populations.

P084

A variable microbial methane biofilter in the water column of a brackish coastal ecosystem

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Background. Eutrophication and warming enhance microbial methane production in sediments of coastal ecosystems and can thereby increase methane fluxes out of the sediment into the water column and atmosphere. Methanotrophic microorganisms in the water column can form a so-called methane biofilter and mitigate these methane fluxes. The microbial community composition, distribution, biogeochemistry, and efficiency of this methane biofilter remain largely unexplored. Here we investigated the water column methane biofilter at three sites with contrasting water column chemistry and mixing frequencies (ranging from well-mixed to near-permanently stratified) in a eutrophic, brackish coastal ecosystem (Stockholm Archipelago) during summer 2022.
Methods. We measured oxygen, methane, sulfide, and major nutrients in the water column at high vertical resolution (every 1-2 m). To analyze the microbial community composition, we collected DNA samples for 16S rRNA gene amplicon sequencing. To measure in situ methane water-air fluxes we deployed a floating chamber attached to a trace gas analyzer. The potential for aerobic methane removal was determined in incubations onboard the research vessel.
Results. We found high water-air methane fluxes at all three sites, with the highest emissions at the near-permanently stratified site. The methane biofilter mostly consisted of methanotrophic bacteria (MOB) at all sites, but the community composition and vertical distribution differed. Intriguingly, the abundance of the putatively aerobic MOB did not decrease in the anoxic water at the seasonally stratified site but significantly decreased at the near-permanently stratified site. There, the oxycline was shallower and sulfide had accumulated in the bottom water. Here, MOB were likely outcompeted by other microbes such as sulfur-cycling bacteria.
Conclusion. Taken together, this suggests that the methane biofilter can counteract high benthic methane fluxes during summer stratification, but if the stratification and anoxia persist over at least several years, the biofilter can lose its capacity to remove methane.

P085

Co-utilization of glucose and formate in aerobic mixed communities: formate assimilation via tetrahydrofolate system

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Formate, sustainably producible from CO2 through electrochemical reduction using off-peak green energy, has mostly been investigated as a co-substrate in aerobic pure cultures of yeast and fungi. It increases assimilation of primary substrates like glucose by supplying additional energy resulting from formate oxidation, which is suggested to lead to an increased biomass yield. Partial assimilation of the carbon originating from formate is also reported, but it is poorly understood when and why formate is assimilated rather than oxidised under aerobic conditions. Understanding the ecological principles that drive this distinction is crucial to design industrially relevant bioprocesses. Therefore, this study focuses on microbial communities as an alternative approach to elucidate the role of formate as a co-substrate.

Four mixed communities were cultivated on 10 mM glucose or formate and glucose at molar ratio 2:1 in a continuous bioreactor with both dilution rates (D) 0.125/h and 0.3/h. Metabolic specialisation, where different species consume different substrates, was prevented at the higher dilution rate due to the relatively low maximum growth rate of formatotrophs. Although not actively prevented, metabolic specialisation was not observed at the lower growth rate either. Metagenomic and proteomic analyses were conducted for all four communities to investigate the active formate metabolism. Results indicated that formate was assimilated via the tetrahydrofolate system, as formate oxidising enzymes were not detected in significant levels, nor associated to the dominant genera. However, the reductive glycine pathway was completely expressed at D = 0.125/h. Other formate assimilating pathways were not or only partially detected; inability to express these pathway to their full extent was confirmed by KEGG annotations of the associated species.

To conclude, this study found that co-feeding glucose and formate to microbial communities resulted in enrichment cultures where formate is assimilated, suggesting a selective advantage over formate oxidation.

P086

Biological synthesis of the valuable compound hydrazine in anaerobic ammonium oxidizer Kuenenia stuttgartiensis

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Anaerobic ammonium-oxidizing (anammox) bacteria convert the substrates ammonium and nitrite to dinitrogen gas via the intermediates nitric oxide and hydrazine in the absence of oxygen. It is striking that anammox bacteria use hydrazine as a free metabolic intermediate in this reaction, because it is highly reactive and toxic. Hydrazine is a commercially valuable product as well, used in, amongst other things, pesticides, pharmaceuticals and monopropellant rocket fuel, and can only be made via energy-intensive chemical processes [1]. To produce hydrazine, anammox bacteria harbor a biochemically unique enzyme: hydrazine synthase. Little is known about its specific mechanism but based on its crystal structure it is postulated that hydrazine is produced in a two-step mechanism [2]. (1) Nitric oxide is reduced to hydroxylamine at active site heme γI. (2) Hydroxylamine diffuses through an intra-enzymatic tunnel to the second active site heme αI, where it is condensed with ammonium to hydrazine. In this project, we used hydrazine synthase of the anammox model organism Kuenenia stuttgartiensis to investigate this proposed two-step mechanism. First, a method to isolate active hydrazine synthase in anaerobic conditions mimicking that in the cell was set up. Then, a method to measure hydrazine production in low concentrations (0-1 µM) was validated and used to follow hydrazine synthase activity. We show that hydrazine is produced from hydroxylamine and ammonium (step 2 of the reaction) at a rate of 5.5 nmol/min/mg hydrazine synthase. Interestingly, the enzyme has a high affinity for its intermediate hydroxylamine (1.12 µM). Our results show that hydrazine synthase can use externally supplied hydroxylamine as a substrate to produce hydrazine in vitro. Thus the enzyme can perform the second step of the proposed two-step mechanism, which further corroborates that hydrazine is synthesized in this manner by anammox bacteria.

P087

Genome evolution of Asgard archaea: a window into eukaryogenesis?

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Asgard archaea are a group of archaea that represent the closest prokaryotic relatives of eukaryotes. The eukaryotic branch likely sprouted from within the Asgard archaeal class Heimdallarchaeia. Eukaryotes are considered far more complex than prokaryotes and their genomic repertoire vastly expanded due to numerous gene duplications, transfers, inventions and fusion events during the early stages of eukaryogenesis. The expanding repertoire of Asgard archaeal genomes, including several closed genomes and genomes from novel groups, is likely to improve our view on Asgard archaeal genome evolution, including how different genetic innovations have shaped the genome content of Asgard archaea and, potentially, that of proto-eukaryotes. In this study, we perform large-scale phylogenetic analyses and use gene tree-aware reconciliation methods to reconstruct ancestral genomes and illuminate the evolutionary genome dynamics of Asgard archaea. The first results of these analyses specifically hint at an increased rate of gene duplications in Asgard archaea compared to other archaea. This suggests that the mode of genome evolution of Asgard archaea is, to some extent, reminiscent of that of eukaryotes. With further analyses on gene inventions, duplications, fusions and transfers in Asgard archaea we expect to provide additional pieces of the enigmatic puzzle of eukaryotic cellular origins.

P088

Available nitrogen source contributes to shaping methane-consuming microbial communities in lake sediments

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In this study, we addressed the role that available nitrogen plays in determining the composition of methane-consuming communities in a well-studied model system. We applied a multi-phase approach combining microcosm enrichments and synthetic model communities with high throughput sequencing.
We first subjected sediment samples from Lake Washington to a 30-day incubation under conditions mimicking limited oxygen availability observed in the natural environment. Various growth media were utilized, including full and half nitrate mineral salt medium (NMS) as well as nitrogen source-free mineral salt medium (MS). Analysis of the 16S rRNA gene via Illumina sequencing on the 9th and 30th day revealed that Methylomonas strains exhibited increased competitiveness when atmospheric N2 served as the sole available nitrogen source, consistent with our previous findings. However, the addition of half nitrate significantly inhibited the growth of a Methylomonas strain, resulting in a dominance of Methylobacter and Methylotenera species. This effect was even stronger in the presence of higher nitrate concentrations (1g KNO3/L) in the medium.
Subsequently, we conducted a follow-up experiment using synthetic communities of three bacterial strains, incubated with NMS and MS for a 30-day period. Analysis of the 16S rRNA gene on the 7th, 15th, and 30th day showed that Methylomonas remained the dominant bacteria in the absence of nitrate. However, in the presence of nitrate as an available nitrogen source, Methylomonas did not dominate the community. Instead, associations between Methylomonas and Methylotenera were formed under nitrate addition.
Our findings suggest the presence of a cross-feeding mechanism between bacterial communities, whereby the genus Methylobacter serves as a host for the survival of the non-methanotrophic genus Methylotenera under nitrate supplementation. These results provide valuable insights into the complex interactions and dynamics of methane-consuming communities in response to nitrogen availability.

P089

Cultivating the unculturable: bioelectrochemical systems for enriching and characterising anaerobic methanotrophs

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Introduction. In anoxic environments, methane is oxidised to CO2 by methanotrophic microorganisms using various electron acceptors thus preventing the emission of this potent greenhouse gas. Recent studies have revealed the ability of anaerobic methanotrophs, specifically 'Candidatus Methanoperedens' species, to utilise poised electrodes as alternative electron acceptor although it remained uncertain whether they could proliferate in bioelectrochemical systems (BES). Here we show that bioelectrochemical systems are promising tools to enrich for 'Ca. Methanoperedens' and can be used to further characterise their growth and physiology.

Methods. Bioreactor enrichment cultures of the archaeal nitrate-reducing 'Ca. Methanoperedens nitroreducens' (doubling time ~30 days) were cultivated in triplicate BES under an anodic potential (0 V vs. SHE) for two months, while tracking current production. Additionally, a bacteria-suppressing antibiotics cocktail was introduced to further select for archaea.

Results. The onset of current production occurred immediately after inoculation and within one month of cultivation the current started to increase exponentially (doubling time 8.2±0.6 days). After two months a maximum current density of 291 mA m-2 was reached, which is the highest current density recorded so far for anaerobic methanotrophs. Confirmation that methane primarily served as the electron source for current generation was established by substituting methane with argon. Throughout the cultivation, the methane dependent current increased from 35±10% to 96±1%, indicating a strong enrichment of methanotrophs, which was corroborated by metagenome sequencing that revealed that 'Ca. Methanoperedens' was enriched from 12% relative abundance in the bioreactor to 56±10% in the BES.

Conclusion. This study underscores the efficacy of BESs in enriching slow-growing anaerobic methanotrophic archaea and highlights its potential as a tool to study these organisms.

P090

Isolation of a novel thermophilic CO-utilising acetogen

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Thermophilic CO-utilising acetogens hold significant promise for syngas fermentation, ultimatelly allowing the sustainable conversion of waste-to-chemicals. Thermophiles, compared to mesophiles, offer advantages for this process, including more favourable thermodynamics, lower contamination risk and cost-effective product separation via bio-reactive distillation. Nevertheless, their application is currently under-explored due to the very low number of thermophilic isolates capable of CO utilisation. In this study we isolated a novel thermophilic CO-utilising bacterium, strain AZ2, from a terrestrial hot spring in the island of São Miguel, Azores, Portugal. Strain AZ2 is an obligately anaerobic, spore-forming bacterium. According to genome-based analyses, it represents a new species in the uncharacterised UBA 2545 genus in the Moorellaceae family. Strain AZ2 was able to grow fermentatively on CO, producing acetate and trace amounts of propionate. The latter has never been shown before as a direct product of CO fermentation. Furthermore, we confirmed that the closest isolated relatives of strain AZ2, Thermanaeromonas toyohensis, T. burensis, and Thermanaeromonas DSM 2356, can also grow on CO, producing either acetate or hydrogen. With our study, we expanded the list of known thermophilic CO-utilizing acetogens not only by isolating a novel strain that can grow on CO but also by demonstrating this metabolism in previously unexplored thermophilic bacteria.

P091

Electroactive Anammox: the next generation of anaerobic ammonium oxidation wastewater treatment?

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Anaerobic anammox bacteria harbour an enormous potential to be applied in mainstream wastewater treatment as they could remove remaining ammonium from the wastewater with less environmental impact than current praxis. Yet to date a robust technology has not been delivered to market that displaces current aerobic standard technologies.
It has recently been shown that anaerobic ammonium oxidation can be used to generate electricity. This work is beginning the development and use of bioelectrochemical systems (BES) as a technology that could offer such a breakthrough for the field. Focusing on the understanding of the anaerobic ammonium oxidation microbial communities and their potential to operate more robust and stably BES bioreactors and therefore its potential as a robust mainstream technology.
Duplicate two-chamber bioelectrochemical systems (BES) were trialled here in a strict anaerobic environment. The system was inoculated with a laboratory enrichment of Candidatus Brocadia and batch fed a synthetic wastewater with up to 20mM ammonium concentration . The system was operated at a potential of +600 mV versus standard hydrogen electrode (SHE), with continuous current measurement via a potentiostat. A platinum wire connected the stainless steel mesh as a cathode in a phosphate buffer (0.15 mM pH 7.5) and a carbon cloth was also connected via plantium wire in the anode chamber where the inoculum and wastewater medium (7.0 – 7.3 pH) was situated. The BES bioreactors were monitored for its ammonium degradation as well as continuous current measurement as a proxy for extracellular electron transfer (EET). The microbial community was further evaluated through fluorescent in-situ hybridisation (FISH) microscopy. It is clear the system has potential to offer in this field as a manner in which to offer robust anammox treatment.

P092

Human milk oligosaccharides drive resource sharing in an infant gut bacterial synthetic community

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Quickly after birth, the infant gut is inhabited by microorganisms that form the infant gut microbiota. Human milk, the 'golden standard' of infant nutrition contains more than 200 structures of Human Milk Oligosaccharides (HMOs) that feed and shape the early gut microbiota. It is known that members of the Bifidobacterium and Bacteroides species can degrade HMOs and thrive in the infant gut. However, how this leads to the formation of microbial communities is not known. Here we investigate how different HMO structures modulate the development of microbial communities, as well as the function of each bacterial member within them.

We created a synthetic community comprising 13 bacterial strains that are normally found in the gut of vaginally born, breastfed infants. Our synthetic community was subjected to continuous and sequential batch fermentations in a defined medium with two different HMO mixes as sole carbon source. Comparison with profiles of maximum 5-month-old infants' feces, showed that our synthetic community successfully captured the core genera and most abundant metabolites.

In continuous fermentations, Bifidobacterium spp., and Bacteroides spp. became dominant. Upon changing the concentration of the different HMOs, we monitored a shift within the relative abundance of the different Bifidobacterium species. Additionally, Bacteroides fragilis outcompeted the other Bacteroides spp. in our community. Acetate and propionate were the major organic acids produced with 1,2-propanediol, propionate and propanol significantly differing between conditions. The presence of strains that do not possess an HMO degrading capacity provide evidence for successful cross-feeding of simple carbohydrates, organic acids and gases that are produced by the key degrading species in our community. Our study offers a viable model for complex microbial interactions, and insights into how the infant gut microbiota collaboratively degrades the HMOs. This model can now be used to study different nutritional concepts and microbial interactions.

P093

Fighting Citrus Canker: Ten years of research on novel phenolic antibacterial compounds

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Citrus canker - phytopathogen: Xanthomonas citri (Xac) - is one of the most economically damaging diseases affecting citriculture worldwide. As increasing environmental awareness challenges current phytopharmaceutical approaches, new methods are required to combat this pest in a greener way. Our team, in consortium with Brazilian partners, has been studying novel phenolic compounds that mimic natural compounds as potential alternatives to the copper salts that are currently used. We present ten years of research done towards producing a viable option for the market.

Several phenolic compounds with side alkyl chains of variable length have been synthesized, including alkyl gallates (G1-G13), alkyl dihydroxybenzoates (DHBs), 4-alkoxy-1,2-benzene diols (BDOs), and chalcones. The antimicrobial activity against Xac and other model organisms (Bacillus subtilis) was tested to determine the minimal inhibitory and bactericidal concentration. Investigations on the mode of action focused mainly on membrane integrity and disturbances in cell division. The most promising compounds were tested in greenhouses for their capacity to prevent disease. Investigations on resistance development and (phyto)toxicity tests were also performed.

Our results show that compounds with two or three hydroxyl groups on the aromatic ring and an alkyl chain length of 6-8 carbons had the best antimicrobial activity. Membrane permeabilization was found to be the main mode of action. Greenhouse trials show that compounds, such as hexyl gallate (G6), limit the number of canker lesions on plant leaves and protect the trees against infection with Xac. No increased resistance and toxicity towards plants were found with this compound.

These results indicate the suitability of novel phenolic compounds as substitutes for solutions that are currently used. Our next steps are aimed towards product formulation and investigations on the persistence and degradation of the compounds in the environment.

P094

Anaerobic cell extraction of live biomass from marine sediments

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Advancements in imaging and cultivation techniques have led to the successful ecophysiological characterization and isolation of novel prokaryotes from natural environments. Especially anaerobic marine sediments, which were challenging to access became available for cultivation through an increase of sampling expeditions exploring subsurface habitats.

However, methodologies used for high throughput characterization such as fluorescence activated cell sorting and imaging techniques like electron microscopy are susceptible to sediment particles, which can adsorb fluorescent dyes and probes, cover microorganisms and cause misidentifications. Consequently, it is important to separate the microbial cells from the sediment particles during sample preparation. Previously described methods to separate microbial cells from sediment particles have used fixatives or focused on the enumeration of cells and did not regard the viability of these cells.

The aim of this project is to evaluate and optimize
cell extraction techniques for the retrieval of live biomass in an anaerobic environment.

In this study various cell detachment techniques, including sonication, the addition of mild detergents and shaking followed up by a cell separation step using nycodenz or polytungstate gradient centrifugation are used. To evaluate and improve the cell extraction methods, viability and activity assays such as Syto9/propidium Iodide (PI), Redox sensor green/PI and propidium mono azide (PMA) in combination with flow cytometry, microscopy, digital PCR and 16S rRNA gene amplicon sequencing are utilized.

Preliminary results indicate that different cell extraction techniques influence both cell count and community composition of the extracted cell fraction. We observe that density centrifugation employing nycodenz yielded high cell counts sufficiently removing particles for downstream analysis.

The obtained results allow us to generate an adaptable and effective cell extraction protocol that can be used to efficiently characterize and isolate viable microorganisms of interest from marine sediment and other complex matrices, while highlighting the bottlenecks of live cell extractions.

P095

TodSyn: A Synthetic community mimicking the Toddler gut bacterial community to study in vitro Antibiotic-Associated Disturbance recovery strategies.

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The most often prescribed medications for Dutch children are antibiotics. Prolonged use of antibiotics during childhood has been linked to increased health risks in later life. We hypothesized that these risks might reduce by supporting the gut community in its recovery after antibiotic treatment. We aim to study the effectiveness of different strategies using an in vitro setting to increase the recovery speed of the gut community after antibiotic treatment. Therefore a Synthetic community mimicking the Toddler bacterial gut community (TodSyn) was designed. This allows to study the bacterial gut community in a controlled setting. The use of a synthetic community over fecal samples is also more reproducible, and less time expensive.

Members for the TodSyn, were selected based on: (1) abundance in the toddler gut, (2) genome availability, (3) cultivation ability. The abundance of the bacteria was based on the reported gut microbial composition of 12 months old children as presented by Bäckhed (2015). Further selection was conducted based on their trophic roles and functional redundancy between the group of selected bacteria such as trophic interaction, e.g., cross-feeding on another species' metabolites and the ability to degrade carbohydrates and/or SCFA and lactate. The TodSyn redundantly occupies various trophic guilds.

TodSyn contains 9 species. The abundance of Bifidobacterium breve was also found to fulfill the criteria, however the recovery strategy that will be tested with this community will involve an intervention with Bifidobacterium breve, therefore this bacteria was excluded from the community.

TodSyn is a synthetic community that resembles the gut microbiome of toddlers. It contains species from multiple trophic levels to support cross-feeding and functional redundancy. It mimics a simplistic community to study the ecological interactions between species that can be tested under different conditions before application in human subjects.

P096

Nitrogen removal microorganisms in cold wastewater treatment plants

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Nitrification is a core process in waste water treatment plants (WWTPs). Nitrification is performed by the sequential activity ammonia oxidizing bacteria and archaea which convert ammonia into nitrite. The produced nitrite is subsequently oxidized to nitrate by nitrite oxidizing bacteria. Complete ammonia oxidizers can perform both reactions. Even though nitrification is known to occur at lower temperatures, a decrease in nitrification rates in WWTPs is often observed in winter when the temperature of the water drops below 15°C. In this study, we investigated the microbial community of three WWTPs located in Weissenborn (WP), Olching (OP) and Geiselbullach (GP), Germany, with efficient nitrogen removal even at a 5°C. Microbial community analysis performed by 16S rRNA amplicon sequencing revealed that the relative abundance of the canonical ammonia oxidizer Nitrosomonas was not significantly different in the three plants. The relative abundance of Nitrospira was highest in WP whereas the other two WWTPs were mainly dominated by the nitrite oxidizer Candidatus Nitrotoga. PCR analysis confirmed the presence of comammox Nitrospira in WP. This research will provide new insights into efficient nitrification at cold temperatures, and therefore contribute to more sustainable wastewater treatment.

P097

Bacteriophage-associated antibiotic resistance genes are abundant before but not after wastewater treatment

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Antimicrobial resistance (AMR) is a major global challenge impacting public health. Wastewater is the major source of antibiotic resistance genes (ARGs) in urban settings, but little information is known about the abundance of ARGs in the viral and free-floating extracellular DNA fractions. These compartments are relevant to horizontal gene transfer (HGT) events, which are central to AMR evolution and environmental dissemination.
Transduction (i.e., HGT mediated by viruses) has received recent attention due to its ARG mobilization and transmission potential. However, due to their sampling approach, most studies only addressed the transduction potential of already internalized bacteriophages, possibly dormant, and thus not actively spreading ARGs.
This study aimed to elucidate the contribution of the viral and free-floating extracellular DNA fractions in the spread of AMR in wastewater environments. To achieve this, wastewater influent, effluent, and activated sludge were sampled, and qPCR and high-throughput metagenomics were used to assess the abundance and diversity of ARGs carried by bacteria, extracellular DNA, and bacteriophages. These fractions were analyzed separately after a combination of (i) filtration with 0.22 µm membranes (to retrieve bacterial intracellular DNA), (ii) crossflow ultrafiltration followed by PEG precipitation and digestion with DNAse (viral DNA), and (iii) anion-exchange chromatography followed by ethanol precipitation and proteinase K digestion (extracellular DNA).
This study evidences the marginal contribution of the viral fraction of wastewater to the overall dissemination of environmental AMR via wastewater. In contrast, surveillance should better integrate extracellular DNA fractions.

P098

Butyrate and EPS production in Eubacterium-dominated enrichment cultures fed with carbon monoxide and acetate

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Conventional microbial enrichment strategies rely on the adaptation of an initial inoculum to desired substrates and/or environmental conditions. This usually leads to long lag phases, and the success of enrichment process depends on the presence of microbes in the inoculum that thrive under the established conditions. To address this, we investigated a co-inoculation approach by augmenting inoculum sludges with microbes known to grow under the desired conditions. This not only promotes a thriving culture but may also foster beneficial interactions between native and co-inoculated microbes, strengthening the microbial community. Our objective was to enrich CO-utilizing microbes for applications in syngas-driven chain elongation. As starting inoculum we used anaerobic sludge, which was augmented with several combinations of chain elongator Clostridium kluyveri and a mix of acetogens (Eubacterium limosum, Acetobacterium wieringae strain JM, Clostridium autoethanogenum, and Clostridium carboxidivorans). Control experiments with only sludge were also conducted. All cultures were incubated with CO (0.4-1.7 bar, incrementally increased over several transfers) and acetate (200 mM). Cultures were transferred upon full CO consumption.
After four transfers, all tested inocula, both sludge alone and augmented sludges, were able to growth in the presence of 1.7 bar CO. However, cultures augmented with E. limosum were faster to adapt to high CO pressure. Over time, a consistent pattern emerged in the enrichments, wherein acetate and CO were converted to butyrate – a typical metabolism of E. limosum. Interestingly, similar behaviour was observed in both in Eubacterium-augmented cultures and other enrichments without Eubacterium augmentation. Physiological data was corroborated by 16S rRNA gene sequencing, which revealed an abundance of Eubacterium species in all the enrichments. Additionally, the enrichments harboured a significant abundance of non-CO utilizers from the genera Aminobacterium, Oscillibacter, and Proteiniphilum. It is hypothesized that these species thrive on intermediate metabolites and exopolymeric substance (EPS) produced by Eubacterium species.

P099

Electrogenic Enrichment of Anaerobic Methane Oxidizing Archaea from Sediment of an Oligotrophic Brackish Basin

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Methane (CH4) is the most reduced one-carbon compound and the second most powerful greenhouse gas on Earth. In aquatic ecosystems, the equilibrium between its production (methanogenesis) and consumption (methanotrophy) influences atmospheric emissions. In coastal sediments rich in CH4 and metal oxides, anaerobic oxidation of CH4 can occur concomitantly with metal oxide reduction. However, to better understand this intriguing metabolism, highly enriched or axenic cultures of the responsible marine anaerobic methanotrophic (ANME) archaea are desired. Here we employed two-chamber bioelectrochemical systems (BES) to enrich metal oxide-dependent ANME archaea from anoxic coastal sediments by using an anode instead of metal oxides as electron acceptors. Pre-incubations of methane-fed serum bottles, containing anoxic coastal sediments from the Bothnian Sea, utilizing either iron or manganese oxides as electron acceptor, served as inoculants for four BESs (two duplicates). These BESs were continuously sparged with a mixture of 95% CH4 and 5% CO2. We employed an antibacterial combination of streptomycin, vancomycin, ampicillin, and kanamycin to inhibit the electrogenic heterotrophic bacterial community. Electrochemical measurements revealed currents of up to 1.2 mA/m2 and 0.8 mA/m2 in the iron and manganese oxide BESs, respectively, with the anodes poised at 0 V versus the standard hydrogen electrode. Based on the observed currents and the supplementation of our BESs with the antibiotic mixture we suspect that the observed current is generated by an archaeal biofilm that established on the anodes. In upcoming studies, we aim to evaluate the dependency of generated currents on CH4 supplementation and quantify the relative enrichment of ANME archaea on the anodes compared to the original inoculum via metagenome sequencing. With this research, we aim to deepen the understanding of the (electrogenic) metabolism of ANME archaea, their role in the coastal methane filter, and their potential for CH4-driven current generation.

P100

Metabolism of C3 compounds in the thermoacidophilic methanotroph Methylacidiphilum fumariolicum SolV

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Volcanic ecosystems are characterized by low pH values, high temperatures and substantial gas emissions. These gases include CO_2, CH_4 and short chain hydrocarbons such as ethane and propane, providing a suitable niche for chemolithoautotrophic microorganisms, which mitigate their emission to the atmosphere. Contrary to methane oxidation, microbial conversion of short chain hydrocarbons has barely been studied. Recently, it was shown that “Methylacidiphilum” isolates were capable of converting C3 compounds such as propane, 2-propanol, acetone and acetol[1]. Therefore, we examined the potential of type-strain Methylacidiphilum fumariolicum SolV to metabolize C3 compounds. Cultivation experiments showed that SolV is capable of utilizing 2-propanol and acetone as carbon and energy source, with growth rates of 0.055 h-1 and 0.047 h-1, respectively. Through transcriptomics a pathway for the conversion of these C3 compounds was proposed. Initially, 2-propanol is converted to acetone by a 2-propanol dehydrogenase (Mfumv2_1600). Acetone is subsequently converted to acetol by a novel acetone monooxygenase, encoded by the pmoCAB3 gene cluster (Mfumv2_1604-1606)[1]. This acetol is then converted to methylglyoxal by an acetol dehydrogenase (Mfumv2_1609-1610). This acetol dehydrogenase was purified directly from native SolV biomass and its activity confirmed with a Vmax of 4.5 U/mg and Km of 2 µM. It belongs to the glucose-methanol-choline (GMC) oxidoreductase family and contains an FAD and FeS cluster as cofactors. SolV acetol dehydrogenase is heat-stable showing activity up to 80 °C with an optimal temperature of 70 °C. Strikingly, preincubation of the enzyme at high temperatures (55-70 °C) resulted in a 7-fold increased activity, which remained even after storage of the activated enzyme at 4 °C. UV-Vis spectroscopy of the untreated compared to the heat-treated enzyme showed slight spectral differences in the 400 nm region and an increased shoulder at 450 nm, indicating small conformational changes surrounding the cofactors upon heat-treatment.

[1] Awala et al., 2021 10.1038/s41396-021-01037-2

P101

Synergizing laboratory and computational methods in constructing plant growth-promoting Bacillus SynCom

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The genus Bacillus is endowed with remarkable genetic and metabolic diversity and is known to have multiple beneficial traits that contribute to plant growth. Several species of the Bacillus genus have been reported as plant-growth promotion rhizobacteria and are being utilized in commercial agricultural applications. The mechanisms of Bacillus spp. mediated plant growth promotion include induction of systematic resistance, secretion of plant hormones, solubilization of nutrients, and production of antimicrobial compounds ranging from antimicrobial metabolites to organic compounds. Meanwhile, root colonization and biofilm formation have been recently reported as additional biocontrol mechanisms in species such as B. velezensis, B. subtilis, and B. atrophaeus.

In this study, we collected more than 300 isolates of the Bacillales family from diverse sources, including soil, plant rhizosphere, marine sediments, termite, and bird uropygial gland. We determined the complete genomes for 121 Bacillales isolates. Through a combination of co-culture experiments and genome-scale metabolic modeling, we aim to define the pairwise interactions among these Bacillales isolates, the potential for metabolite production, and the prediction of growth in complex synthetic communities. Coupling of laboratory experiments and in silico computational approaches will aid the construction of synthetic Bacillus-dominant communities that exhibit increased reproducibility and robustness in promoting plant growth. Root colonization, biofilm formation and metabolic profiles of Bacillus SynCom will be tested to assess their impact on plant health and growth. Our study innovatively bridge the gap between laboratory experimentation and genome-scale modeling to provide guidance on constructing synthetic communities that effectively promote plant growth.

P110

The Role of Poultry Feed in a Major Salmonella Outbreak Linked to Eggs

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Introduction: Salmonella is one of the most common causes of foodborne outbreaks. This study describes an outbreak investigation that aimed to identify the source of one of the largest S. Enteritidis outbreaks in the Netherlands.

Methods: National Salmonella surveillance registries were used to identify S. Enteritidis patients. Outbreak cases were those with isolates, typed with whole-genome sequencing (WGS), belonging to one of two outbreak clusters (A and B) based on core-genome Multilocus Sequence Typing (cgMLST) with a cluster cut-off of ≤5 alleles since June 2023. A case-control study was performed, with questionnaires focusing on egg consumption. Controls were randomly sampled from the population registry (matched on age, sex and municipality). Trace-back and trace-forward investigations were performed by the Netherlands Food and Consumer Safety Authority (NVWA) based on case questionnaire data and non-human isolates (mainly poultry boot swabs) belonging to the outbreak clusters based on WGS.

Results: By January 2024, 151 outbreak cases have been identified (75 male and 76 females), with a median age of 40 years. Cluster A (n=103) was primarily related to consumption of barn eggs (OR 5.9; 95% CI 2.2-16.0; p<0.001) and B (n=48) to organic eggs (OR 38.0; 95% CI 5.5-261.7; p<0.001). Salmonella isolates from 12 laying hen farms and egg shells used for poultry feed were related to the outbreak based on WGS, indicating that this outbreak had multiple sources.

Conclusion: Current evidence suggests that the outbreak strains have spread to different laying hen farms in the Netherlands through contaminated egg shells that were inadequately treated prior to use in poultry feed. Interventions have been implemented to correct this and identify potentially infected laying hen farms. Despite source identification, the outbreak strains may still be present in laying hen farms not yet identified. Though the rate of human cases has declined, additional cases are anticipated.

P111

Why do babies cry? Exploring the role of gut microbiota and other biopsychosocial factors in colic and other gastrointestinal symptoms in the KOALA Birth Cohort.

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Introduction:
Gastrointestinal symptoms are a common problem during infancy, including infantile colic, which can be loosely defined as prolonged and recurrent crying, without obvious cause. The causes indeed remain unclear despite much research. Prior work has linked maternal mental health to infant crying symptoms, but results on infant nutrition are inconclusive. More recently, several small studies have described associations between gut microbiota and colic, and we aimed to utilise a larger cohort to examine the role of the early microbiota and other biopsychosocial factors in infant gastrointestinal health.

Methods:
Using fecal 16S rRNA gene amplicon sequencing data from 1,012 infants in the KOALA birth cohort, we examined associations between the 1-month gut microbiota and parent-reported infant functional gastrointestinal symptoms, including colic, constipation, and cramps. These analyses were adjusted for other biopsychosocial predictors identified in analyses of 2,665 participants. In 257 infants, we also explored associations between breastmilk Human Milk Oligosaccharides (HMOs) and gastrointestinal symptoms.

Results:
High relative abundance of Staphylococcus was associated with less constipation in the first three months of life. Conversely, Ruminococcus gnavus group was associated with more colicky symptoms, particularly between four and seven months. These microbiota associations were adjusted for maternal distress, breastfeeding, antibiotics, and other potential confounders. Maternal distress at two weeks postpartum was associated with all symptoms throughout infancy. The HMOs LNH and LNnH were associated with less constipation in the first three months.

Conclusions:
Our results support the general conclusion that gut microbiota are relevant in infantile colic and constipation, but more work is needed to elucidate the underlying mechanisms.

P112

Automation of the library preparation for whole-genome sequencing of bacterial genomes using an open-source programming liquid-handler robot

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Introduction

Whole-genome sequencing (WGS) is currently making its transition from research tool into routine clinical microbiology practice. Library preparations (LP) represent the most critical steps in the WGS procedure but are also the most labour-intensive steps that are prone to human error when performed manually. Automating the LP using robotic liquid-handlers will result in increased quality and decreased hands-on time.

Methods

The manual Illumina® DNA Prep protocol was translated into an automated protocol on the flowbot® ONE. The performance of this protocol was evaluated by prepping 16 diverse bacterial strains both manually and automatically, after which the library DNA yields and NGS results obtained were compared. Eight bacterial strains were prepped twice in two independent flowbot® ONE runs to assess reproducibility of results. In addition, the turn-around time (TAT) and hands-on-time (HOT) of both workflows were compared to each other.

Results

The automated workflow resulted in high and reproducible library DNA yields that were comparable to those obtained with the manual workflow. No dropouts were encountered for all LPs. After sequencing, similar assembly quality values were obtained for each of the 40 individual DNA libraries that met our diagnostic quality criteria for WGS. The high-quality sequence data obtained using both workflows was further confirmed with a 100% agreement of the final NGS-result (i.e., complex types). With the automated workflow, the TAT to process eight samples was comparable to the TAT using the manual workflow. However, the HOT for processing eight samples was reduced to 25 minutes for the automated workflow, compared to 125 minutes for the manual workflow.

Conclusion

The newly developed and validated automated LP workflow on the flowbot® ONE generates robust and high-quality DNA libraries. Automating these critical LP steps will further reduce sequencing costs (by reducing HOT) while delivering consistent results in terms of reliability and reproducibility.

P113

Seeing it cristea clear – electron tomography of growing cristae in malaria parasite mitochondria

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During the asexual blood stage, malaria parasites are known to be devoid of mitochondrial cristae. As the parasite prepares for a life in the mosquito, however, mitochondrial morphology drastically changes. Mature gametocytes have been shown to be packed with tubular cristae that are believed to enable the parasite to cope with the decrease in glucose availability in its mosquito host. While these two contrasting states of mitochondrial morphology are described, information about the intermediate steps of gametocyte formation is missing. The use of electron tomography allows me to trace and describe the development of cristae during a range of intermediate steps of gametocyte differentiation. First results indicate the presence of mitochondrial cristae already in the very roots of sexual commitment, even before sexually committed merozoids egress from their last round of asexual reproduction.

P114

Carbapenem-resistant bacteria isolated from imported food products in the period 2017-2023

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Global transmission of antimicrobial resistance (AMR) by international food transport can be a risk for human health. As an import country, the Netherlands monitors imported food products from outside the European Union, such as seafood, seaweed and herbs for the presence of AMR as part of the national AMR monitoring program. Carbapenems are used as last resort treatment against severe infections. Therefore, carbapenemase-producing Enterobacterales form a threat to public health. Between 2017-2023, imported food products were actively monitored for the presence of carbapenem-resistant E.coli, Enterobacter and Klebsiella by selective culturing. The results are presented here.

Carbapenemase-producing bacterial strains were isolated from imported food product samples by a non-selective enrichment and selective isolation on CHROMID CARBA plates. Species identification was performed using MALDI-TOF. Susceptibility testing was performed by broth microdilution according to ISO standards using European standardised antibiotic panels (Sensititre©, Thermo Fisher Scientific). Illumina Short- and ONT long-read sequencing and (hybrid) assembly were used for genomic characterization.

Between 2017 and 2023 fourteen carbapenemase-producing isolates were found in imported food products. In total 1815 seafood products, 179 seaweed products, and 1138 herb samples were analyzed, resulting in twelve carbapenemase-positive isolates from shrimp and tilapia (imported from Asia), one isolate from samphire (imported from the Middle-East), and one isolate from coriander (imported from Africa). Out of the fourteen isolates, twelve were identified as Enterobacter cloacae, one as Enterobacter bugandensis, one as Escherichia coli. WGS analyses revealed that the isolates obtained from coriander and samphire contained IMI-3, whilst the strains from seafood contained FLC-1, IMI-1, IMI-2, IMI-3, NDM-1, NDM-5, OXA-10 and OXA-48.

Between 2017 and 2023, we isolated and characterized 14 carbapenem-resistant bacterial strains from imported seafood, seaweed and herbs. Although a possible link with human infections remains to be investigated, our findings underline the importance of monitoring imported (sea)food products for antibiotic-resistant bacteria.

P115

Functional analysis of spore germination proteins and their interactions in Bacillus spores.

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Bacterial endospores are sturdy structures that resist environmental challenges such as thermal insult, enzymatic degradation and harsh chemicals. They are considered survival capsules of the organisms that generate them i.e. aerobic Bacilli and strictly anaerobic Clostridia common to the gut. Their core, equivalent of the vegetative cells' protoplast, has a water content between 25-45% of wet weight, compared to ~80% in vegetative cells, and consists for 10% dry weight of the spore specific compounds dipicolinic acid. The challenge for the spores is to respond to favorable environmental changes rapidly and efficiently whilst mitigating as much as possible untimely germination events thus preventing too early outgrowth and poor sporulation efficiency. Upon germination by a variety of germinants (i.e. amino acids and purines), spores take up water rapidly, restoring their water content to that of growing cells and releasing the stored dipicolinc acid. Here we show the molecular events at the basis of spore germination in pathogenic Bacillus cereus and the model organism Bacillus subtilis. We visualize germinant receptor proteins in bacterial spores, study their interaction with scaffold proteins in the formation of a germination protein complex (germinosomes) and probe the dynamic changes that occur during germination in both the germinosomes and the calcium dipicolinic acid (CaDPA)specific channel protein SpoVA using fluorescent microscopy, live-imaging and Förster Resonance Energy Transfer (FRET). The data show that L-alanine binding GerRB in B. cereus clusters in foci proofing that germinosomes similar to B. subtilis exist in this pathogen. Co-expression of all three proteins of the tricistronic germinant receptor protein operon shows the highest level of gerRB fluorescence corroborating B. subtilis data on germinant receptor proteins being interdependent for stability. GerRB and gerD show FRET indicating proximity of < 10Å. GerRB and SpoVAEa colocalize, indicative of putative interaction of the germinant receptor and the CaDPA channel protein.

P116

Coccidiosis prevention strategies shape the microbiome, resistome and mobilome composition in the broiler gut.

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Coccidiosis, which is caused by single-cell eukaryote parasites, is a common disease that affects poultry production, with a worldwide financial burden of around 2 billion per year. Prevention strategies, such as anticoccidial drugs (coccidiostats) and vaccination, are widely used to control the effects of this disease.
It has been shown that some of the coccidiostats possess antimicrobial activity and that bacteria can evolve resistance toward these molecules, potentially providing bacterial cross-resistance to other antimicrobials or co-selection of other antimicrobial resistance genes (ARGs).
To investigate the impact of two coccidiosis prevention strategies, i.e. coccidiostat mix of 50% narasin and 50% nicarbazin and the vaccine Paracox 5, on the gut microbiome, ARG distribution and mobile resistome fraction of broiler chickens we perfomed metagenomic sequencing (Novaseq) on 100 caecal content samples and 12 pools of 5 faecal dropping samples from Ross 308 broiler chickens. MetaPhlAn 4.0, kma and MetaMobilePicker were used to perform bioinformatic analyses, and R version 4.2.2 was used for all statistical analyses.
Overall, we observe that 21 and 11 genera are relatively higher abundant in chickens that were vaccinated or that received coccidiostat, respectively. We detect 21 differentially abundant ARGs between treatment groups and found significant correlations between the observed taxonomy and resistome composition, indicating that the bacteriome shaped the resistome. Finally, we identified 14 plasmid fragments carrying ARGs in faecal dropping samples, highlighting potential environmental dissemination of mobile ARGs.
To conclude, our findings demonstrate that different anticoccidial strategies result in differences in the chicken gut microbiome and resistome composition with potential impact on the dissemination of ARGs.

P117

The contribution of intercontinental travel on the resilience and stability of the gut microbiota

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Introduction: As global travel becomes increasingly accessible, individuals frequently visit diverse environments. In this process, they expose themselves to new dietary habits, lifestyles, natural environments, and infections which may affect their gut health. Studies investigating the impact of travel on the gut microbiota are limited. We therefore analysed faecal samples from cohorts of intercontinental travellers before, during, and after their journeys to investigate whether and how intercontinental travel alters the composition of the gut microbiota.

Methods: Our study included 637 travellers who donated faecal samples and filled in questionnaires prior to their travel (T0), immediately post-travel (T1) and 1-month post-travel (T2). In addition, a smaller cohort of 11 travellers were included with daily self-collection of faecal samples prior, during and after travel. All faecal were profiled by 16S rRNA gene amplicon sequencing to examine the microbial diversity, composition, and community structure.

Results: The microbial richness and diversity in post-travel samples were significantly decreased compared to the pre-travel samples but demonstrated resilience, as shown by a restoration at 1 month upon return. In addition, a shift in microbiota community structure was identified through principal components analysis (PCA) between pre-and directly post-travel samples. Antibiotics use and stomach related issues during travel were the largest contributors to gut microbiota perturbations while marked changes in relative abundance of several genera between pre-and directly post-travel were associated with age and sex. Samples from the daily measured cohort displayed stable diversity throughout travel. In contrast, inter-and intraindividual variation was observed in microbial community composition with distinct clustering of pre-travel, during travel and post-travel samples as displayed in the PCAs of each individual set of longitudinal samples.

Conclusions: Distinct alterations in microbiota profiles of travellers were identified, emphasizing the dynamic response of the gut microbiome to intercontinental travel stressors.

P118

Enhanced extracellular matrix production provides protection to cell wall-deficient Escherichia coli

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Recurrent urinary tract infections (rUTIs) pose a substantial medical challenge, with Escherichia coli responsible for 75% of cases. This recurrence within six months of the initial infection is related to elevated frequencies, increased mortality rates, and rising annual costs observed over recent decades. Notably, most first-line antibiotic treatments primarily target cell-wall synthesis, which can lead to the formation of cell wall-deficient (CWD) cells. To study how such cells can sustain, we obtained an E. coli strain capable of efficiently proliferating without its cell wall. One of the mutations lead to enhanced expression of rcsA, encoding an important regulator involved in responding to cell envelope stress. By combining scanning electron microscopy, fluorescent microscopy, and time-lapse imaging we showed that this strain had an increased extracellular matrix production. Individual knockouts of several biofilm pathways, including curli protein, cellulose, and colanic acid, resulted in the inability to grow without a cell wall, emphasizing their essential contribution to wall-deficient proliferation. Interestingly, a long-term evolution experiment showed an increase in this protective extracellular matrix as well as an improved ability to survive in harsh conditions.

These findings suggest a refined and complex protective system, and highlight E. coli's ability to adapt to wall-deficient conditions. Furthermore, they showcase the need for innovative therapeutic strategies to address biofilm-related challenges.

[1] Manuscript in preparation
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P119

Unveiling the mystery behind the relationship between infants' diet, gut microbiome and fiber degradation capacity

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Introduction: The infant's gut microbiome undergoes sequential maturation throughout the first years of life, a process with lasting consequences for health. Birth mode and infant feeding type play crucial roles during microbiota assembly subsequently the introduction of complementary foods, triggers a profound microbial shift towards an adult-like microbiota. Despite these insights, our understanding of diet-microbe interactions in early life remains incomplete.

Methods: Within the LucKi Birth Cohort Study, we explored associations between early-life dietary patterns and infant microbiome development in 105 infants. Analysis involved profiling 389 fecal samples (4, 5, 6, 9, 11, and 14 months) collected during solid food introduction, using whole metagenome shotgun sequencing. Contigs created were mapped to the polysaccharide degrading enzyme database, and statistical tests compared the profiles, resulting in inferred fiber degradation profiles. Dietary clusters were identified using dynamic time warping and clustering dendrogram based on a distance matrix.

Results: Three dietary clusters emerged, reflecting the timing, variety, and type of introduced foods. Each cluster exhibited significant increases in species richness at different time points. Generalized mixed effect multiple regression models revealed significant interactions between dietary classes and age for Akkermansia muciniphila and Bacteroides thetaiotaomicron in all clusters. Hierarchical clustering identified four blocks of dietary fiber (DF)-species correlations. At 6 months, the first dietary class displayed notable differences in DF degradation, with increased capacities for arabinan, pectin, arabinoxylan, and xylan, and reduced capacities for chitin, resistant starch, inulin, and xanthan as compared to the other two dietary clusters.

Conclusion: The current study unveils the complex relationship between diet, gut microbiota, and fiber degradation capacity. Introducing a diverse range of solid foods enhances fiber degradation capacities and microbiome functionality, underscoring the ongoing adaptability of the infant's microbiome to its environment.

P120

Pathway variations and enzyme kinetics in methanol conversion by Desulfofundulus kuznetsovii strains

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Methanol serves as energy and carbon source for microorganisms in various environments. It is produced during degradation of pectin and lignin from dead plant biomass or geochemically from CO_2 and H_2 in deep subsurface environments. Anaerobic methanogens and acetogens convert methanol using a pathway involving a vitamin B12-dependent methyltransferase (MT). The MT-system was thought to be prevalent in anoxic environments for methanol conversion, whereas in oxic environments methanol is generally oxidized by alcohol dehydrogenases (ADH). However, the sulfate reducer Desulfofundulus kuznetsovii strain 17T shows both pathways. In D. kuznetsovii strain 17T, the MT pathway is dependent on vitamin B12.

We recently isolated a strain of D. kuznetsovii – strain TPOSR. Interestingly, the genome of strain TPOSR lacks essential genes in the MT operon. It therefore relies on a vitamin B12-independent ADH for methanol metabolism. Comparisons of strains 17T and TPOSR can help elucidating the advantage of MT and ADH pathways, for example in competition with other microbial groups.

First, we compared the proteome of strains 17T and TPOSR, in presence and absence of vitamin B12 and cobalt (vitamin B12 precursor), to identify genes involved in methanol metabolism. These strains possess 7 annotated ADH genes, but one of them is strongly associated with growth on methanol (NHM28481). NHM28481 were cloned into plasmids and transformed to E. coli BL21. Cellular protein was extracted and heterologous ADH was purified using HIS-tag. Activity of purified ADHs with several substrates, including C1 to C6 alcohols, was positive and NAD+ dependent. We currently look at enzyme kinetics for these reactions.

P121

Cytomegalovirus induced hearing loss: a human stem cell derived inner ear organoid model

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Cytomegalovirus (CMV) infection is the most important non-genetic cause of congenital hearing loss, yet the pathogenic mechanism remains elusive. Given the species specificity of CMV, conventional animal models offer limited insights. Therefore, there is a need for a human disease model. In this study, inner ear organoids (IEOs) derived from human induced pluripotent stem cells (hiPSC) were used to simulate infection of the fetal inner ear.

HiPSC were directed to differentiate into IEOs and cultured for 75 days, resembling the human inner ear in the first trimester. IEOs were cut into 300 µm sections using a vibratome and inoculated with 10^4 pfu of two different CMV strains, TB40e-GFP or AD169. IEOs were cultured for 7 or 21 days, after which they were fixed using 4% buffered formaldehyde. The IEOs were embedded in paraffin and cut into 5 µm sections. Immunofluorescent staining for CMV proteins and relevant inner ear proteins was applied to ascertain the infected cell types and structure of the IEOs.

PCR analysis of the supernatant revealed an increasing viral load after the inoculum was removed, suggesting a productive infection. CMV infected cells were identified in the otic vesicles, predominantly affecting the mesenchymal cells and non-sensory epithelial cells. Several infected hair cells were observed. Potential indirect effects of the infection in the structure of the otic vesicles were also seen.

This is the first description of IEO infection with CMV. The results resemble the limited number of autopsy studies in which infection of mainly non-sensory epithelial cells was described. This organoid model may be used for in-depth pathogenic investigations or potentially serve as a platform for pre-clinical trials assessing therapeutic options.

P123

Glycan-specific IgM is critical for human immunity to Staphylococcus aureus

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Introduction: Staphylococcus aureus is a major human pathogen but the immune factors that confer protection remain elusive. Antibodies play a key role in bacterial killing through antibody opsonization and complement activation, which enhances bacterial uptake and neutrophil recruitment. However, high opsonic IgG titers showing protection in preclinical S. aureus animal immunization studies have consistently failed to translate to protection in human clinical trials. In healthy individuals a large proportion of the anti-S. aureus IgG pool is directed against Wall Teichoic Acid (WTA), an abundant cell wall-anchored glycopolymer and prospective vaccine antigen. Here, we aimed to gain insight into the protective capacity of WTA-specific antibodies by studying the complete WTA-directed antibody repertoire in healthy controls and patients with invasive S. aureus infection, and correlating WTA-specific antibody responses to disease outcome.

Methods: WTA-specific antibody responses (IgG1-3, IgM and IgA) were measured in EDTA-plasma samples from 31 healthy individuals and 36 patients with culture-confirmed S. aureus bacteremia on the intensive-care unit (ICU) using a bead-based flow cytometry assay. Beads were coated with synthetic WTA fragments carrying one of three representative glycan modifications: α1,4-N-acetylglucosamine (GlcNAc), β1,4-GlcNAc, β1,3-GlcNAc.

Results: IgM and IgG antibodies specific to WTA were universally present in plasma from healthy individuals. Functionally, WTA-specific IgM outperformed IgG in opsonophagocytic killing of S. aureus, was not hindered by expression of protein A (SpA), and conferred passive protection against S. aureus infection in vivo. In the clinical setting, WTA-specific IgM responses, but not IgG responses, were significantly lower in S. aureus bacteremia patients compared to healthy individuals, correlated with mortality risk and showed impaired bacterial opsonization.

Conclusion: Our findings support a protective role for WTA-specific IgM antibodies against S. aureus infection, may guide risk stratification of hospitalized patients and inform future design of antibody-based therapies and vaccines against serious S. aureus infection.

P124

Detection of yellow fever virus genome in urine: review of current knowledge.

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Yellow fever (YF) virus is a mosquito borne arbovirus with a positive strand RNA genome, and is the prototype member of the Orthoflavivirus genus. It is endemic in the (sub)tropical regions of Africa and South America and is prone to cause epidemics. Molecular (confirmatory)testing of YFV by reverse transcriptase-polymerase chain reaction (RT-PCR) was recently adopted by WHO using blood. Urine is a non-invasive, easily accessible diagnostic specimen which has been proven to be useful in diagnosing several flavivirus infections. The real advantage of urine is the longer detectability of virus compared to blood. Up to present, systematic data on the usefulness of urine in YF virus diagnostics was lacking.

We have carried out an extensive literature search using the key words “yellow fever AND urine” in PubMed/Medline, Embase and Web of Science. The search resulted initially in 113 after de-duplication. All titles and abstracts were screened and 14 were analyzed in detail.

Following natural infection, the detection rate of YFV in blood was 82% (86/105 samples) versus 70% (110/158) in urine from patients with acute mild/severe infections. Following symptom onset, YFV could be first detected at 3.6 vs 6.7 days in blood vs. urine and last detected till 19.9 vs 35.7 days respectively (73/129 patient followed up). Virus could be isolated from urine, blood and semen. Following vaccination, virus was detected longer in patients with vaccine adverse events (VAE) compared to healthy vaccines (33.1 vs. 24.8 mean days).

Live attenuated YF vaccine can be detected in blood and urine in both healthy vaccines and with VAE. Vaccination history is important both for serological and molecular diagnostic methods but PCR and/or sequencing can distinguish vaccine from circulating strains. Based on the evidence collated in this review, we would recommend to consider urine as a standard, complimentary specimen for YF molecular diagnostics.

P125

Risk factors for colonization with NDM-5 carbapenemase: a matched case-control study

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Introduction
New Delhi Metallo-beta-lactamase-5 (NDM-5) is a carbapenemase posing a significant threat to for the treatment of infectious diseases. From march 2018 to june 2022 an outbreak with Citrobacter freundii carrying NDM-5 took place in a general hospital in the Netherlands. A matched case control study was conducted to identify risk factors.

Methods
For each case two controls were selected, matched on date of admission. Variables were selected based on literature and local expertise. In the preceding period a number of sewage blockings and in-hospital floods had occurred. The environments which were subsequently tested positive for NDM-5 were taken int account as a variable in the analyses. All data were collected from electronic patient files. A p-value threshold of 0.20 was used for inclusion in the multivariable logistic regression model.

Results
A total of 60 NDM-5 cases were detected and all were found to be genetically related. The median age (IQR) was 77.0 years (4-18) and 36 (60%) was female. The univariable analysis showed several associated risk factors (odds ratio [95% confidence interval]): admission on a NDM-5 positive room (toilet, sink or shower drain) (7.27 [2.74-25.19]), incontinency (3.8 [1.88-7.81]), use of the commode chair (4.3 [2.23-8.44]) and use of antibiotics (3.02 [1.59-5.90]). The multivariable analyses demonstrated age (1.05 [1.01-1.09]), number of exposure days to the hospital environment (1.19 [1.08-1.32]) and body mass index (1.11 [1.02-1.23]) as risk factors for obtaining NDM-5 during hospital admission. No effect-modifiers were identified.

Conclusions
This study shows that during an environmental driven outbreak patients with a prolonged hospital stay, use of antibiotics and co-morbidity (i.e; overweight, diabetic and incontinence) have the highest risk of colonization with NDM-5. From an infection prevention perspective, these results are crucial for identifying high-risk patients and implementing appropriate infection prevention and screening measures.

P126

Elucidating the role of the DivIVA protein in shaping the mycelial architecture of filamentous Actinobacteria

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The cell wall is a shape-defining structure enveloping almost all bacteria, protecting them from environmental stresses. Growth of the cell wall is coordinated by large protein complexes. While most bacteria grow by inserting cell wall material in a diffuse manner, some bacteria grow by synthesizing cell wall material exclusively at their cell poles, such as filamentous actinobacteria. These mycelium-forming bacteria establish extensive networks of interconnected filaments. Polar growth in these bacteria is coordinated by the coiled-coil protein DivIVA. This protein is found at the apex of growing tips and at distinct sites along the lateral walls of hyphae where new branches are established. The mechanism by which polar growth influences mycelial morphology remains largely elusive. In this study, we employed a distinctive strain capable of both wall-dependent and wall-independent growth to examine the key functions of DivIVA. Utilizing this adaptable strain, we isolated a DivIVA variant that facilitated filamentous growth, but remarkably largely without formation of branches. Notably, this novel phenotype resulted from only two amino acid substitutions in the inter-coil region of DivIVA. Collectively, these findings underscore the utility of our switchable strain in elucidating essential gene functions and highlight the diverse roles of DivIVA in shaping mycelial architecture. Our study introduces novel insights for modulating the morphology of these prolific bacteria.

P127

Rapid molecular assay for spontaneous bacterial peritonitis diagnosis

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Spontaneous bacterial peritonitis (SBP) presents a significant complication in individuals with cirrhosis and ascites, leading to high mortality rates. Although early diagnosis and antibiotics treatment could reduce these effects, traditional SBP diagnosis relies on culture. Therefore, the use of faster molecular assays holds the potential to enhance prognosis. The Molecular Culture test identifies a broad range of bacterial species in ~4h. It uses a PCR-based assay targeting the 16S-23S interspace rDNA region with phylum-specific fluorescently labelled primers. In this research, we assessed the diagnostic performance of Molecular Culture for rapid SBP diagnosis.

The residual material of 247 peritoneal samples were sent for routine diagnostics to the Medical Microbiology laboratory at Amsterdam UMC (VUMC) and later subjected to the Molecular Culture test.

Molecular Culture's sample positivity and species identification outcomes were compared to those of traditional culture, as performed in standard of care (SOC). Percent positive agreement (PPA) between Molecular Culture and SOC at the sample level was 91,4% (IC 95%, 82.5% to 96%), and negative percent agreement (NPA) was 63.8% (IC 95%, 56.5% to 70.6%). Six samples were reported as culture-positive/Molecular Culture-negative, which consisted of low bacterial loads and one species outside the scope of the assay. The assay successfully identified the most prevalent species found in culture, yielding a PPA of 65.1% (95% CI 52.8% to 75.7%). The majority of the missed bacterial detections came from polymicrobial samples where Molecular Culture correctly identified at least another species. Additionally, Molecular Culture yielded 118 extra bacterial detections. Most of these detections were reported in culture-negative samples, of which 60% reported medium to high leukocyte counts, indicative of bacterial infection.

Molecular Culture performed better than the standard of care. It provided a greater resolution for polymicrobial samples with key insights for species identification, demonstrating its potential to accelerate SBP diagnosis.

P128

Glycan-specific monoclonal antibody-based serotyping of Listeria monocytogenes

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The Gram-positive bacterium Listeria monocytogenes is a foodborne pathogen that can cause listeriosis primarily in newborns, pregnant women and elderly people. So far, twelve serotypes have been characterized. The differentiation of the serotypes is based on the composition and glycosylation of wall teichoic acid (WTA) glycopolymers, referred to as somatic (O) antigens. Based on epidemiological studies, approximately 40% of human infections are caused by serotype 1/2 strains. WTA of serotype 1/2 strains is composed of polymerized ribitol-phosphate subunits that are decorated with N-acetylglucosamine (GlcNAc) and L-rhamnose (Rha) moieties. Loss of rhamnosylation, yielding serotype 3, results in increased susceptibility to antimicrobial peptides and reduced virulence. Correspondingly, serotype 3 strains rarely cause human infections. However, growth conditions can affect WTA compositions through gene regulation, which may underlie observations that serotyping is often unreliable and insufficiently reproducible. Therefore, we aimed to optimize identification of serotype 3 strains. We used three monoclonal antibodies (mAbs) with known WTA-GlcNAc specificity that showed dose-dependent recognition of L. monocytogenes of serotypes 1/2 and 3. There was no binding to other L. monocytogenes serotypes. Two mAbs showed stronger binding to serotype 3 compared to 1/2 at defined concentrations, suggesting that the WTA-rhamnose modification interfered with mAb binding. Serotype discrimination could be converted into an easy-to-use agglutination assay to improve serotyping of clinical isolates. Using fluorescence cell sorting of an L. monocytogenes serotype 1/2 mutant transposon library, we isolated mAb-binding mutants, i.e mutants that lost WTA-rhamnose. This way, we aim to identify genes that might contribute to rhamnose glycosylation and virulence of serotype 1/2. In conclusion, we have shown that glycan specific antibodies can be used to identify specific Listeria monocytogenes serotypes and genes contributing to the glycosylation patterns.

P129

Identification and characterisation of novel monoclonal antibodies against Klebsiella pneumoniae from B cells

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Klebsiella pneumoniae is an opportunistic pathogen and its antibiotic resistance is an increasing threat to human health. Antibody therapy is a promising alternative strategy to treat or prevent antimicrobial resistant bacterial infections. After binding their antigen, antibodies can induce a broad range of effector functions, ranging from neutralising the bacteria to activating immune cells and the complement system. However, it remains elusive what antigen-antibody combinations can potently trigger complement-mediated lysis of Gram-negative bacteria, like K. pneumoniae. We aimed to identify novel monoclonals antibodies (mAbs) against K. pneumoniae to study potent antibody-antigen combinations that activate complement. Therefore, we single cell sorted healthy donor B cells that recognise K. pneumoniae via their B cell receptor and produced mAbs from these. Previously, we have sorted IgG B cells, and produced IgG antibodies from these. We then identified IgGs against the most abundant and accessible surface structures of K. pneumoniae, the O-antigen and capsular polysaccharide (CPS). IgM is a more potent complement activator than IgG, and here we aimed to identify novel mAbs from IgM B cells. We identified thirteen new IgMs against K. pneumoniae and we then further characterised these antibodies through identification of their target antigen and evaluated their ability to activate the complement system on K. pneumoniae. Using K. pneumoniae mutants, we identified that the IgMs, like the IgGs, targeted either the bacterial CPS or the O-antigen. Further characterisation showed that the O-antigen targeting mAbs could potently induce the complement system to kill K. pneumoniae, whereas the CPS-targeting mAbs could not. These novel mAbs can be employed to further investigate what renders some antibodies effective in inducing complement-mediated killing of Gram-negative bacteria, whereas other cannot.

P130

Host-association and genomic diversity of hypervirulent Klebsiella pneumoniae plasmids in humans and pigs

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Klebsiella pneumoniae (Kp), a ubiquitous pathogen found in diverse ecological niches, poses a threat to human and animal health. Hypervirulent Kp (hvKp) has emerged as a major concern due to its ability to acquire virulence and antimicrobial resistance (AMR) genes via plasmids. We investigated genomic diversity and host association of hvKp plasmids in humans and pigs. A total of 41 clinical hvKp pig isolates, originating from Dutch passive surveillance necropsies from 2013 up to 2020, were sequenced using Nanopore technology and compared to a large dataset of related publicly-available Kp genomes from humans (n=230) and pigs (n=76) for phylogenetic and plasmid analysis. Molecular analysis showed that 87% of the Dutch pig isolates belonged to sequence type (ST) 25 (ST25). These pig isolates expressed the K2 hyper-capsule-type and harbored a plasmid encoding the aerobactin lineage (iuc3) that is associated with IncFIB(K) and IncFII plasmid incompatibility groups. The aerobactin lineage iuc3 was significantly more prevalent in ST25 pig isolates (98%, 40/41) than in human isolates (10%, 24/230). Despite sharing the same ST25, the majority of human isolates displayed lower virulence scores (0-1), while all pig isolates exhibited higher virulence scores (3-4) due to the presence of aerobactin iuc3. Our findings suggest that the presence of the aerobactin lineage iuc3 on a plasmid is host-associated with pigs and is correlated with hypervirulence. This finding is important for understanding and investigating the role of such plasmids in hvKp virulence and transmission.

P131

Evaluation of Molecular Culture for pleural infection diagnosis

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Diagnosis of pleural infections relies on routine culture, however, the yield is often low due to receipt of antimicrobials or to nutritionally fastidious microorganisms. Molecular assays have the potential to drastically improve diagnosis. We developed the Molecular Culture test, a 4-hour PCR-based assay that combines length polymorphisms of the 16S-23S interspace rDNA region with phylum-specific fluorescently labelled primers to identify bacteria to the species level. In this study, we evaluate the diagnostic accuracy of this novel test on pleural effusions.

501 pleural effusion samples were subjected to Molecular Culture. Positive and negative outcomes of Molecular Culture were compared to routine culture results. A subset of samples was sequenced for database improvement and species-matching algorithm training. A subset of positive samples was used for comparison of species identification to routine culture diagnostics.

55 out of 440 samples were positive in routine culture (12.8%) whereas 134 samples (30.5%) were positive with Molecular Culture. 46 samples were found positive by both methods (10.5%). PPA between Molecular Culture and culture was 83.6% (95% CI 71.7-91.1%) and NPA was 77.1% (95% CI 72.7-81.3%) at the sample level. The 9 culture-positive/Molecular Culture-negative samples were primarily low-load skin bacteria.

101 unique patient samples were utilized for species identification comparison. Culture results were only considered when deemed clinically relevant, such that low-load skin contaminants were removed, yielding a PPA of 55.2% (95% CI 39.5-70.9%). Molecular Culture yielded 56 additional bacterial detections compared to culture. Discrepancies were more prevalent in the polymicrobial samples: PPA for monomicrobial samples was 74.1% (95% CI 60.8-87.4%), whereas PPA was 41.9% (95% CI 30.7-53.1%) for polymicrobial samples.

Molecular Culture showed a sensitivity exceeding that of culture, identifying many culture-negative samples as positive. Combined with its fast turnaround time, Molecular Culture may provide a much-needed option for faster diagnosis of patients suffering from pleural infections.

P132

Improved meningitis diagnostics of culture-negative CSF samples with Molecular Culture

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Introduction: Diagnosis of bacterial meningitis is traditionally performed by culturing cerebrospinal fluid (CSF) samples. However, culture often has a slow turn-around time and can give false negative results when fastidious micro-organisms are present or when patients have been treated by antibiotics before sampling. Molecular assays, based on DNA detection, have the potential to drastically improve diagnosis. We developed the Molecular Culture test, a PCR-based assay that combines length polymorphisms of the 16S-23S interspace rDNA region with phylum-specific fluorescently labelled primers to identify bacteria to the species level. This assay takes approximately four hours, thus offering a much faster turn-around time than culture. In this study, we evaluated the diagnostic performance of Molecular Culture on a set of culture-negative CSF samples.

Methods: Residual material of 354 culture-negative CSF samples from patients suspected of meningitis were subjected to DNA isolation followed by Molecular Culture. Species identification was performed by automated software based on an extensive bacterial database.

Results: 31 (9%) culture-negative samples were found positive by Molecular Culture. Within these samples, 9 unique species were identified. Additionally, 5 samples contained unknown bacteria of the FAFV (Firmicutes, Actinobacteria, Fusobacteria and Verrucomicrobia), Bacteroidetes, or Proteobacteria phyla.

Conclusion: Molecular Culture showed improved detection of bacterial species, with 9% of culture-negative CSF samples being positive with Molecular Culture. Combined with its fast turnaround time, Molecular Culture may offer a crucial alternative for promptly diagnosing and treating patients afflicted with bacterial meningitis.

P133

Successful host adaptation of Escherichia coli IncK2 plasmids in chicken.

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Antimicrobial resistance is a global health threat and because it is often encoded on plasmids, it is crucial to understand the dynamics of plasmid spread and host adaptation. It was shown that IncK plasmids can be divided into two separate lineages named IncK1 and IncK2. IncK2 plasmids are found predominantly in poultry. The relatively high body temperature of chicken may influence IncK2 plasmids fitness cost, copy number, and stress response in the Escherichia coli host. These data explore IncK2 plasmid's success and persistence in E. coli of chicken origin.

This study included 58 IncK2 carrying isolates of human (n=16), poultry (n=34), cattle (n=3), pig (n=4) and environmental (n=1) origin from 11 European countries and Lebanon, as well as 14 publicly available (plasmid database PLSDB) IncK2 plasmid sequences. 58 IncK2 carrying isolates analyzed in this study were sequenced using both Illumina and Nanopore technology. A phylogenetic analysis of all plasmids was performed in order to determine the genetic relatedness of IncK2 plasmids isolated in different countries and from different sources. Additionally, a genome wide association study (GWAS) was performed on annotated sequences to assess if an association exists between specific genetic features of E. coli or the plasmid and chicken, that could explain the suspected IncK2 plasmid adaptation to the chicken reservoir. Multiple genes are significantly associated with IncK2 plasmid or its host E. coli and the chicken reservoir. Moreover, based on predicted function of genes significant in GWAS, components of chicken diet like raffinose, L-arabinose and phytic acid seem to select for E. coli strains carrying IncK2 plasmids.

This study shows that potential adaptation of plasmids to a chicken host is a complex process that involves both physiological and genetic determinants of the plasmid and the bacterial host. Obtained results suggest chicken host specificity of IncK2 plasmids.

P134

Longitudinal genomic analysis of carriage and invasive S. pyogenes isolates reveals emergence of distinct emm1.0 variants and subtypes coinciding with national upsurge in invasive disease

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Introduction
Since 2022, several countries reported striking increases in invasive group A streptococcal (iGAS) infections. We hypothesized that the emergence of more virulent strains, such as new S. pyogenes variants or changes in recently-emerged emm1 type variants (M1UK, M1DK), could be underlying the recent outbreak in the Netherlands.

Methods
We analyzed shifts in emm(sub)type prevalence using 2,692 invasive and 349 S. pyogenes carriage isolates collected between January 2009 and March 2023. Genomes of 505 emm1 isolates were additionally analyzed by whole-genome sequencing (WGS) to determine the emergence and genetic diversification of emm1 in relation to the 2022-2023 outbreak.

Results
The nationwide iGAS upsurge in 2022-2023 correlated with a marked expansion of subtype emm1.0 among national invasive strains, from 17% (18/103 isolates) in January-March 2022 to 59% (361/616 isolates) in January-March 2023 ($p<0.0001$). In contrast, overall S. pyogenes carriage rates and the proportion of emm1 in carriage isolates remained stable between 2009-2023. Among invasive emm1 isolates, the M1UK variant became dominant in 2016 and outcompeted the contemporary M1global lineage in winter 2022-2023. This shift was not detected in asymptomatic carriers. Phylogenetic comparison showed M1UK iGAS isolates from 2022-2023 separate into two distinct groups: a branch of 2022-2023 isolates that are highly clonal, and a group consisting of smaller distinct branches, including a novel emm1 subtype, emm1.134, characterized by four lineage-defining SNPs. Accessory genome analysis showed that DNase spd1 and superantigen speC were acquired in 9% (46/505 of all emm1 isolates, and 100% (15/15) of suspected M1DK isolates.

Conclusion
The nationwide iGAS outbreak between 2022-2023 is linked to clonal replacement and suspected increased virulence of two new successful M1UK clones. The rise of these clones may have implications for secondary attack rates and the rationale for prophylaxis for close contacts.

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P135

Nanopore sequencing of Influenza A virus for swine influenza surveillance

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Introduction: Influenza A viruses (IAV) that circulate in pigs harbor the potential to cause pandemics thus it is crucial to monitor their circulation. Currently, RT-qPCR targeting RNA segment 7 is used for detection of IAV. For virus subtyping, a second PCR with subtype-specific primers is performed. However, no information on possible genome reassortment and mutation can be obtained using these assays. Whole genome Nanopore sequencing does offer detailed information on the virus make-up and could provide a time efficient alternative to the currently employed procedure.
Methods: Samples from virus stocks and swine samples of differing Ct values were amplified with the SuperScript III One-Step RT-PCR-System with Platinum Taq DNA Polymerase. Five primer sets for amplification were evaluated. Sequencing was performed with rapid (RBK) and native barcoding kits (NBD), flow cells or flongles and the Mk1c MinION. Genome Detective and the Nextflow Influenza Typing Workflow wf-flu were used and compared for data analysis.
Results: Overall, the MBTuni-12.5 primer set achieved the most favorable results for depth and coverage of gene segments throughout the IAV genome. Library preparation with the NBD resulted in more data thus higher depth and coverage of the RNA segments compared to the RBK. Data analysis with Genome Detective offered a clear overview of depth and coverage for each segment and produced typing results for HA and NA segments of samples up to a Ct value of 38. Wf-flu however, failed to provide typing results for the HA segment even for virus stock samples and depth of coverage for this segment was often low or non-existent.
Conclusion: Swine samples with low viral loads were successfully sequenced with the established protocols. The MBTuni-12.5 primer set for amplification in combination with NBD for library preparation and Genome Detective for data analysis were found to achieve the best results.

P136

The role of macrophage expressed gene 1 (MPEG1) in severe Staphylococcus aureus infections in humans

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Introduction: The molecular basis of interindividual clinical variability upon infection with Staphylococcus aureus is unclear. Most individuals carry S. aureus asymptomatically, but only a minority develop life-threatening disease. Severe staphylococcal disease can result from single-gene inborn errors of immunity (IEIs). We aim to elucidate the human genetic etiologies of life-threatening staphylococcal disease.

Methods: A 1.5-year-old boy from a consanguineous family was hospitalized for sepsis due to a necrotizing pneumonia and concurrent bacteremia with S. aureus. His disease was clinically accompanied by a macrophage-activating-like syndrome. Because of a suspicion of an IEI, whole-exome sequencing (WES) was performed.

Results: A panel-based analysis for known IEIs was negative, but an open analysis of the patient's WES-data revealed two homozygous, ultra-rare and predicted deleterious missense variants in macrophage expressed gene 1 (MPEG1). MPEG1 encodes for Perforin-2 and contributes to intracellular killing of bacteria. MPEG1 knockout mice are highly susceptible to S. aureus infections. In humans, heterozygous MPEG1 variants are associated with relatively mild skin and pulmonary infections, but MPEG1-population genetics metrices are within the range of genes underlying IEIs with autosomal recessive inheritance. We hypothesize that autosomal recessive MPEG1 deficiency may underlie life-threatening S. aureus infections in humans. We aim to characterize the patient's alleles using a gene-editing strategy in THP-1 cells and by recombinantly expressing the patient's Perforin-2 variants. Next, we will validate the defect using the patient's cells. Preliminary findings indicate that, when compared with cells from healthy controls, the patient's dermal fibroblasts are hypersensitive to cell death following infection with S. aureus.

Conclusion: The study of IEIs can serve as a compass for the clarification of human-specific host-pathogen interactions, such as is the case for S. aureus. This study may reveal MPEG1 as a new gene underlying a previously unknown IEI.

P137

The role of microbiologists on a planet in crisis: scientific activism to educate the public, reach out to society and advocate sustainable developments.

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The current state of the climate- and environmental crisis calls for immediate societal change to be implemented, based on scientific evidence (IPCC Assessment Report 6). Currently, too few of these changes have been implemented to limit global heating to 1.5C (UNEP Emissions GAP report 2023) and the societal change that is required to achieve this is not proceeding at the speed required. Historically, rapid societal changes were often driven by small groups of citizens engaging in civil disobedience. Examples from the 20the century are Mahatma Gandhi's non-violent resistance which led to India's independence and Martin Luther King's campaign of non-violent disobedience which advanced civil rights for people of colour in the United States.

Microbiologists worldwide are contributing to climate change research in the fields of nature adaptation, mitigation, green energy production and infectious disease. We postulate that microbiologists can add an extra contribution to educating the general public about the urgency of the climate crisis as well as inciting government and institutional action, by engaging in climate activism. There are many examples of renowned scientists participating in different forms of activism. A famous example being the Russell-Einstein manifesto from 1955, where the scientific community came together to warn about the dangers of nuclear war. In the past 2 years, science activism worldwide, including in The Netherlands, has increased in numbers and effectiveness. Scientists from many different fields, including microbiology, are sharing expertise to educate the public, to incite and lead sustainable developments and to advocate for climate action. The effect of science activism on the public debate was recently confirmed by a second place for Scientist Rebellion NL in the Trouw Duurzame Top100 list 2023.

P138

The importance of biofilms in prosthetic joint infection: an in vitro titanium disk test system

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Introduction: Prosthetic joint infection affects 1-2% of total prosthetic joint replacement patients, often linked to challenging biofilm formations on implant surfaces. Biofilms are generally difficult to eradicate using antibiotics alone, necessitating additional surgical procedures. To investigate alternative optimal antibiotic treatment and simulate infection, a static model is used with different titanium disks to optimize conditions for biofilm formation.

Methods: American Type Culture Collection (ATCC) bacterial strains, including S. aureus ATCC25923, S. aureus ATCC29213, K. pneumoniae ATCC13883, K. pneumoniae ATCC700603, were cultivated at 37^{o}C. This was done on titanium disks with different surfaces (polished titanium surface, plasmapore-coated, and corundum-blasted), in 12-wells plates with two-days or seven-days incubations in tryptic soy broth. Colony forming unit counts assessed bacterial growth, while scanning electron microscopy (Jeol JSM-IT200) visualized biofilm surface coverage in µm².

Results: S. aureus ATCC25923 exhibited a 1,7-fold increase in biofilm surface coverage (135,6 µm²), compared to S. aureus ATCC29213 (80,6 µm²). K. pneumoniae ATCC700603 showed a 1,4-fold reduction in biofilm surface coverage (275,5 µm²), compared to K. pneumoniae ATCC 13883 (398,3 µm²). However, K. pneumoniae ATCC700603 showed more extracellular polymeric matrix (EPS) on both corundum-blasted and plasmapore-coated surfaces, compared to K. pneumoniae ATCC13883 on corundum-blasted surface, particularly evident during plate shaking while incubating. Prolonged incubation (seven days) of S. aureus ATCC25923 resulted in a 1,4-fold increase in biofilm surface (393,5 µm²) compared to a two-day incubation (290,4 µm²). K. pneumoniae ATCC700603 displayed a 3,8-fold difference in biofilm surface between seven-day (1047,5 µm²) and two-day (275,5 µm²) incubations.

Conclusion: The optimal growth conditions for forming biofilm on titanium disks are dependent on different bacterial strains, impacting biofilm formation greatly. Optimal growth conditions involve shaking plates during incubation. Seven-day incubation leads to bigger biofilms. Future experiments assess antibiotic susceptibility differences between biofilms and planktonic bacteria, using S. aureus ATCC25923 and K. pneumoniae ATCC700603.

P139

Identifying the intracellular microbiome in inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic disease with recurrent inflammation along the gastrointestinal tract. Its two main entities are Crohn's disease and ulcerative colitis. Both diseases are characterised by dysregulated immune responses and altered microbiomes, but the exact mechanism behind flare frequency and intensity has not been elucidated yet.

In healthy people, the GI tract is lined with a protective mucus layer, consisting of highly glycosylated mucin proteins, antimicrobial peptides, and immunoglobulins, that separates the intestinal microbiota from the intestinal epithelial cells. In IBD patients, the intestinal mucus layer is less efficient in keeping bacteria at bay. By 16S fluorescent in situ hybridisation (FISH), we observed bacteria inside intestinal epithelial cells of IBD patients. We hypothesise that, due to the defective mucosal barrier during IBD, some intestinal bacteria invade intestinal epithelial cells, and that these bacteria are more likely to contribute to disease flares than bacteria found in the intestinal lumen.

Here we present a method to isolate the intra-epithelial bacteria from patient biopsies. In this proof of principle bacteria were cultured from fresh biopsies of two Crohn's disease patients. Pinch biopsies were taken from inflamed distal ileum, adjacent non-inflamed ileum, and non-inflamed colon. First, biopsies were enzymatically treated to obtain single cells. These were incubated with a mix of antibiotics that cannot cross human cell membranes, to kill extracellular bacteria. The inflamed sample was then separated into immune (CD45+) and non-immune (CD45-) cells. Subsequently, all samples were plated onto different growth media and incubated anaerobically. In the end, bacteria could be cultured from all biopsies and 16S sequencing identified 6 different species in patient 1. These results indicate that Crohn's disease patients likely harbour intracellular bacteria in their intestinal cells. Further research is needed to verify these results and explore possible links to clinical presentation.

P140

Mapping the vaginome using nucleic acid amplification testing and Nanopore sequencing

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The vaginome plays an essential role in the health of women. An unhealthy vaginome (imbalance in micro-organisms) can increase the chance of sexual transmitted diseases, including Human Papillomavirus (HPV). Around 90% of the population gets infected with HPV at least once in their life. In most cases, HPV infections are cleared within 1 to 2 years, otherwise cervical dysplasia or cancer may develop within 10-15 years. The vaginome might be involved in development of cervical dysplasia, and can currently be sorted into 5 community state types (CSTs) based on microbial community composition and abundance.

This study aimed to find a method to reliably characterize the vaginome of women attending the Dutch Cervical Cancer Screening Program. Secondly, possible correlations between the vaginal microbiome, HPV infections, and cervical dysplasia were investigated.

A sample selection of 992 cervical swabs from the National Dutch Screening Program were used, including 492 HPV-positive and 500 HPV-negative swabs with different gradations of cervical dysplasia. The swabs were tested on the presence of Lactobacillus, Gardnerella vaginalis, and Atopobium vaginae, using the Aptima® Bacterial Vaginosis (BV) assay with the automated Panther system.

DNA was isolated by using different isolation methods: MolYsis, Ceres, and eMAG. DNA samples were analyzed by Nanopore sequencing (Mk1B using Native Barcoding kit) with Epi2Me Labs for data analysis.

Within the nanopore sequencing runs, over 95% of all produced reads were classified as human DNA with all extraction methods used. In one of the HPV-positive samples, 47 reads were classified as HPV16, the type mostly associated with cervical cancer. The Nanopore sequencing data was compared with the BV-assay. BV-positive samples in the Aptima assay match the found bacteria with Nanopore Sequencing.

Currently, we are evaluating a targeted approach with amplicons of 16S, 18S/ITS1 and HPV. The best method will be used on the full sample set.

P141

Graphene quantum dots have microbicidal activity against Candida species

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Candida species are opportunistic fungal pathogens which, upon invasion, can cause severe infections associated with high morbidity and mortality. Standard antifungal treatments, such as echinocandins and azoles, are increasingly ineffective, particularly against the “new” pathogen Candida auris, which makes treatment of candidiasis difficult. Especially immunocompromised patients are vulnerable to Candida infections. Graphene quantum dots (GQD) may provide an alternative or addition for antifungals. GQD consist of a single layer of carbon atoms in a honeycomb-like structure with photo-activation properties. Upon photo-activation, GQD can generate reactive oxygen species (ROS) with broad microbicidal activity, including fungi.

We aimed to test a newly developed carboxylated form of the GQD, colloidal GQD-COOH, as well as a novel GQD-COOH coating for their fungicidal activity against Candida albicans and C. auris. To test the microbicidal activity of colloidal GQD-COOH, we used the minimal microbicidal concentration assay. After 30 minutes of photo-activation with a 435nm blue LED light, the lowest concentration of colloidal GQD-COOH which killed 99.9% of fungi was 6.25 µg/ml for C. auris and 12.5 µg/ml for C. albicans. Furthermore, we developed a novel coating consisting of alternating layers of GQD-COOH and polymer, and tested this coating applied on glass slides for its fungicidal activity using the Japanese Industrial Standard assay. The GQD-COOH coating showed promising fungicidal activity against both C. albicans and C. auris. Therefore, the GQD-coating has potential for future application in e.g. wound dressings or catheters.

P142

In-host adaptation of Aspergillus fumigatus in chronic fungal airway infections in CF patients

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Cystic fibrosis (CF) patients can encounter chronic respiratory infections, with Aspergillus fumigatus being a common opportunistic pathogen. This study aimed to identify chronic A. fumigatus isolates with the same genotype but isolated at different timepoints in a CF patient. This was done for multiple CF patients. We hypothesized that chronic isolates would exhibit in-host adaptations after prolonged exposure to the CF lung environment.

Using Short Tandem Repeat analysis, we screened a total of 83 CF patients with at least 5 A. fumigatus isolates gathered between 2010 till 2013 in the Wilhelmina Kinderziekenhuis and in the Canisius Wilhelmina Ziekenhuis resulting in 85 chronic isolates. To investigate the in-host adaptation for these chronic isolates, we conducted a comprehensive set of phenotypic tests including the assessment of susceptibility to hydrogen peroxide and menadione oxidative stress, sensitivity to Congo red indicative of cell wall integrity, response to diverse pH levels, and tolerance to copper exposure.

Our findings reveal distinct phenotypic variations among A. fumigatus isolates, suggesting potential adaptation mechanisms over time within the CF host environment. The most recent isolates exhibited altered responses to oxidative stressors, suggesting enhanced antioxidant defenses or in some cases the opposite. Furthermore, differences in cell wall integrity, pH adaptation, and copper tolerance were observed, indicating potential host-driven selection pressures influencing A. fumigatus phenotypes.

This study contributes valuable insights into the phenotypic dynamics of chronic A. fumigatus infections in CF patients, shedding light on the adaptive strategies employed by the fungus within the host environment. Understanding these phenotypic adaptations may have implications for targeted therapeutic interventions and the development of more effective treatment strategies for persistent A. fumigatus infections in the context of cystic fibrosis.

P143

Molecular Dynamics of Commensal Neisseria Species in the Human Oropharynx in Response to Antibiotic Treatment.

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INTRODUCTION Ceftriaxone-resistant Neisseria gonorrhoeae (NG) poses a threat to first-line empirical treatment of gonorrhoeae. Ceftriaxone resistance is partially conferred through mosaic penA alleles, which are mainly obtained through transformation of DNA from commensal Neisseria species colonizing the oropharynx. Neisseria subflava in particular has been shown to both exhibit high-level ceftriaxone resistance and contribute to mosaic penA formation, thus forming a potential reservoir of particular interest. Therefore, this study aimed to determine the relative abundance of commensal Neisseria species including Neisseria subflava and abundance dynamics in response to antibiotic treatment.

METHODS 96 oropharyngeal swabs were selected from follow-up consultations of 10 patients who visited our STI clinic between 2019 and 2021. Selection was based on frequency of positive PCR tests for NG. Commensal Neisseria species identification and quantification was performed through targeted metagenomic sequencing of penA and flanking regions. Patients were treated with ceftriaxone 500mg IM when testing positive for NG.

RESULTS The mean relative abundance across all samples was highest for Neisseria subflava (97,8%); other commensal Neisseria species varied in mean relative abundance from 0,02 to 1,13%. Relative abundances before and after treatment were compared using the Wilcoxon signed rank exact test. The relative abundance of Neisseria subflava differed significantly between consultations before and after treatment with antibiotics (P-value < 0.05), with a mean relative abundance of 98,0% before treatment, compared to 85,4% after treatment. Relative abundances for other commensal Neisseria species did not significantly differ after treatment.

CONCLUSION Commensal Neisseria form a reservoir of AMR for NG. Our results show that Neisseria subflava is highly abundant in the oropharynx compared to other commensal Neisseria species and may thus form a more readily accessible reservoir for AMR. Furthermore, Neisseria subflava decreased in relative abundance after treatment, suggesting that many strains remain relatively susceptible to ceftriaxone despite reported intrinsic resistance.

P144

Putative players in metabolic health: Gut-bacteria derived membrane vesicles have distinct immunogenicity dictated by bacterial producer strain

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Introduction
The microbiota play a pivotal role in human health. In metabolic diseases the composition of the gut microbiota is often altered. Gut-bacteria produce bacterial membrane vesicles (bMVs) containing bacterial cargo. In animal models, gut-bMVs can exit the intestine to reach host organs. Here they can affect energy metabolism leading to diabetic phenotypes. In humans, effects of bMVs on the host in the context of metabolic disease are uncharacterized. In this work, we relate the gut-bMV repertoire to the composition of bacteria in 12 participants with prediabetes (BMI > 25 kg/m2) and 12 controls. In addition we characterize immunogenicity of these bMVs in vitro.

Methods
Fecal samples were collected and bMVs were purified through (ultra)centrifucation and size exclusion chromatography. DNA was obtained from bacteria and bMVs and subjected to 16S rRNA variable region amplification and Illumina sequencing. Feces-derived bMVs were incubated with human THP-1 macrophages and differentiated adipocytes. Cytokine expression and secretion (IL6, TNFα, IL1β) was related to vesicle composition.

Results
Per gram of feces, ~10^11 bMVs were isolated. Bacterial and bMV composition are dissimilar. Gram-negative anaerobes (Bacteroides, Alistepes, Barnesiella, Coprobacter, Odoribacter) are overrepresented in vesicle compositions. No differences in bacterial or vesicle composition can be observed between participant groups. Immunogenicity of bMVs on macrophages and adipocytes is largely explained by proportional abundance of Lachnospiraceae vesicles. Intriguingly, abundance of Akkermansia vesicles appears negatively correlated to IL1β production by macrophages, but not for IL6 and TNFα.

Conclusions
Gut-derived bMVs add complexity to microbiome-host interactions as their composition is different from the composition of the bacteria that produced them. Although bMV compositions themselves are not different between investigated participant groups, their immunogenic potential is dictated by their bacterial origin and might affect host (immuno)metabolism. Research into translocation of gut-bMVs to organs in humans is warranted, to investigate effects on host metabolism.

P145

Resistance reservoirs: transmissible antimicrobial resistance mechanisms in intensive care sinks

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Introduction: Antimicrobial resistance (AMR) threatens public health and healthcare around the globe. AMR can spread between bacteria via mobile genetic resistance elements (MGRE). Previously, a MGRE with fourteen genes against seven classes of antibiotics has been identified conferring resistance to many first-line antibiotics. To maintain effective treatments and mitigate the spread of resistance, it is important to investigate possible reservoirs and potential resistance mechanisms in the patient and hospital environment.

Methods: Sinks, taps, and surfaces used by patient and personnel of intensive care units of the MUMC+ were sampled every eight weeks. Per round, 143 samples were enriched and cultured on chromID ESBL-agar plates (bioMérieux). Colonies were identified using MALDI-TOF (bioMérieux). Resistance phenotypes, MGRE presence, and genotype of the bacteria were determined via disc diffusion, colony PCR, and Illumina whole genome sequencing, respectively.

Results: Multidrug resistant bacteria resided primarily in sink drains, where over 90% (42/45) of the drains contained resistant isolates with on average 2.5 isolates per drain. CTXM-15 was present in 3% (3/106) and 11% (13/122) of the identified isolates in the two executed sampling rounds. Presence of the previously identified MGRE was similar, with only 3% (3/106) and 10% (12/122). The number of unique bacterial species in which the complete MGRE was present increased between the sampling rounds from 11% (2/18) to 27% (4/15). Furthermore, the most abundant bacterial species that accounted for over 25% of the isolates, Citrobacter freundii complex, had notable resistance numbers while they did not harbor the MGRE.

Conclusion: These results demonstrate that hospital sink drains are a reservoir of resistant bacteria, including extended spectrum β-lactamase containing bacteria (ESBLs), and potentially carry two distinct transmissible resistance mechanisms. Therefore, it is important to further investigate how this reservoir in the patient environment might influence effective and safe healthcare.

P147

Lethal ROS production upon membrane depolarization of dormant Bacillus subtilis cells

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The bactericidal activity of several commonly used antibiotics have been shown to partially rely on the production of reactive oxygen species (ROS). Bacterial persister cells, an important cause of recurring infections, are tolerant to these antibiotics because they are in a dormant state. However, even dormant cells must maintain a membrane potential. Here we used Bacillus subtilis as model system to study the effect of membrane depolarization on dormant cells. Surprisingly, we found that membrane depolarization also leads to ROS production. In contrast to previous studies, this does not require the Fenton reaction and results primarily in superoxide radicals. Genetic analysis revealed that Rieske factor QcrA, the iron-sulfur subunit of complex III, is a primary source of superoxide radicals. Interestingly, the membrane distribution of QcrA changed upon membrane depolarization, suggesting a dissociation of complex III. Our data reveal an alternative mechanism by which antibiotics can cause lethal ROS levels, and may partially explain why membrane-targeting antibiotics are effective in eliminating persisters. The current status of the project will be presented.

P148

Mechanism-oriented antimicrobial screening by transcriptome profiling, machine learning and cytological profiling

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There is a need for novel antimicrobial compounds. Although antimicrobial natural products are found routinely, identifying the active molecules in complex mixtures is an arduous task and known compounds are often found again.

To address this problem, we've developed a novel workflow combining transcriptome profiling and machine learning to identify promising antimicrobial compounds from nature. This approach categorizes antimicrobials into broad activity classes with high accuracy, based on transcriptome data, and is effective even with unknown compounds. Its robustness across various concentrations and different transcriptomic techniques is ensured by careful feature selection and generalizable model parameters.

Combining this with bacterial cytological profiling allowed us to successfully pinpoint an array of interesting natural products from the Westerdijk Institute's fungal extract databank and elucidated mechanisms of action for some known antimicrobials.

In conclusion, this work shows the effectiveness of multi-disciplinary strategies in antimicrobial drug discovery and mechanism of action studies.

P149

The Salmonella adhesin Rck mediates entry through the epidermal growth factor receptor in a MUC13-dependent manner

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In the intestine, epithelial cells are separated from commensal and pathogenic bacteria by a protective layer of secreted and transmembrane mucins. The transmembrane mucins harbor a highly O-glycosylated extracellular domain, transmembrane domain, and cytoplasmic tail that can contribute to intracellular signaling. Some transmembrane mucins are used by bacteria as receptors for invasion. For example, the enteropathogen Salmonella enterica requires MUC1 to trigger its invasion into enterocytes. Recently, Salmonella was reported to also invade cells via a zipper mechanism, in which the outer membrane protein Rck binds to the epidermal growth factor receptor (EGFR), and initiates receptor-mediated endocytosis. Multiple transmembrane mucins, including MUC1 and MUC13, have EGF-like domains and/or have been shown to interact with signaling receptors of the EGFR family. Therefore, we want to investigate the role of MUC1 and MUC13 in Rck/EGFR-mediated invasion.

To study Rck-mediated invasion, we expressed full-length Rck, or truncated Rck that cannot mediate invasion, in Escherichia coli and Salmonella enteritidis. Gentamicin protection assays were performed to quantify the number of intracellular bacteria in the human colon cell lines HT29-MTX and HRT18 and their MUC1 knockout (KO) and MUC13 KO derivates. These showed that Rck-mediated invasion was similar in MUC1 KO cells compared to WT cells. However, the invasion was dramatically decreased in MUC13 KO cells. In addition, we inhibited EGFR kinase activity with gefitinib before infection to confirm the role of EGFR in Rck-mediated invasion. Rck-mediated invasion was decreased dramatically in gefitinib-treated WT cells. Furthermore, the gentamicin protection assay on MUC13 KO HRT18 cells complemented with MUC13 showed that bacterial invasion levels were restored to the WT level. These data demonstrate that MUC13, and not MUC1, promotes Rck-mediated invasion of Salmonella.

P150

Interactions with Porphyromonas gingivalis outer membrane vesicles influence the clumping and virulence of Fusobacterium nucleatum

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Periodontitis is an inflammatory disorder caused by polymicrobial biofilms that are formed in the subgingival crevices. To date, more than 400 bacterial species have been identified in subgingival biofilms. The respective polymicrobial communities develop through interspecies interactions that are modulated pathogens. Porphyromonas gingivalis is a keystone Gram-negative bacterial pathogen that has been implicated in the onset and progression of periodontitis, and periodontitis-associated diseases including rheumatoid arthritis, cancer and even Alzheimer's disease. Its key virulence factors are proteases, the so-called gingipains, which are secreted into the growth medium mostly in association with outer membrane vesicles (OMVs). On the other hand, the Gram-negative bacterium Fusobacterium nucleatum is an opportunistic pathogen that is also involved in periodontitis and that serves as a bridge species between early and late colonizers of the oral cavity. To date, little is known about how these bacteria interact with each other. To investigate these interactions and the possible involvement of OMVs from P. gingivalis, we employed the F. nucleatum type strain ATCC22856 and the P. gingivalis strain W83. In addition, we employed a spontaneous F. nucleatum mutant that lost the ability to auto-aggregate. Our results show that OMVs from P. gingivalis bind to the surface of F. nucleatum, thereby affecting the ability of wild-type F. nucleatum to auto-aggregate. Importantly, we also show that, upon co-infection of human salivary gland epithelial cells, the presence of P. gingivalis or its OMVs will inhibit the internalization of F. nucleatum, whereas the presence of F. nucleatum increases the ability of P. gingivalis to invade these cells. Altogether, our observations show that P. gingivalis can enhance its virulence with support of F. nucleatum.

P151

Measuring translation efficiency and ribosome pausing during long fed-batch cultivation of B. subtilis using ribosome profiling

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Long fed-batch fermentations are used to produce enzymes by Bacillus subtilis and other industrially relevant microorganisms. Translational regulation can play a significant role in the synthesis efficiency of proteins. We were interested to know whether ribosome pausing varies in cultures grown for long periods by employing ribosome profiling. First, we benchmarked our method by using the antibiotic mupirocin which inhibits the synthesis of isoleucine tRNA, causing the ribosome to stop at Ile codons. This worked well, and subsequently, we measured pause sites on the amylase mRNA during a 160-hour fed-batch fermentation. It appeared that pause sites do not correlate with the presence of rare codons, indicating that other factors are responsible for pause sites. Pause sites show some enrichment in guanosine residues but the conservation is insufficient to accurately predict ribosome pause sites. These results show that modern deep-sequencing techniques such as ribosome profiling can provide important insights on protein production in bacteria.

P152

The gut microbiome and health status in the Dutch population: determining key associations with health and disease

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Introduction: the gut microbiome is suggested to play a crucial role in human health, influencing various physiological processes and disease susceptibility. However, defining what constitutes a healthy microbiome throughout the lifespan remains an ongoing challenge. This study (n = 3,746) explores the intricate interplay between the gut microbiome and a set of parameters defining the health status in the Dutch population, across a broad age range (0 – 87 years). We aimed to elucidate associations between gut microbiome and health parameters to contribute to a definition of a "baseline" faecal microbiome across all age groups, and the extent to which different environmental factors influence it. Methods: we analyzed the gut microbiome of participants >14 years old, using both 16S rRNA sequencing (n = 3,746) and metagenomic approaches (n = 200). Univariate analyses were used to identify key variables significantly influencing health status. Nineteen distinct disease categories, stratified into acute and chronic groups, were considered together with self-rated health score and obesity. These four disease scores individually served as proxies for health, accounting for related medication use. Results: we found significant associations between the gut microbiome most of the disease categories of which diabetes was one of the most explanatory variables. Self-rated health score was the strongest variable in the dataset both for Shannon diversity and composition. Many potentially beneficial groups, including butyrate producing bacteria were negatively associated with insulin use. Conclusion: our findings present key variables that play a role in shaping the gut microbiome, providing valuable insights into the interplay between microbial composition and diversity and various health conditions. This study provides a foundation for further investigations into personalized microbiome-based interventions for improving health outcomes.

P153

Waning of respiratory syncytial virus antibody levels during the COVID-19 pandemic in the Dutch population

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Introduction: The measures undertaken to curb SARS-CoV-2 transmission during the COVID-19 pandemic had great impact on the spread of the respiratory syncytial virus (RSV). Strongly reduced notifications of RSV infections were followed by an out-of-season peak once measures were lifted. Non-pharmaceutical interventions of this scale provided a unique opportunity to improve our understanding of susceptibility to RSV infection on an individual and population level. Here, we investigated the impact of non-pharmaceutical interventions on RSV-specific humoral immune responses to identify groups at increased risk of infection.
Methods: Between June 2020 and June 2022, 927 participants of a nationwide longitudinal study (PIENTER-Corona) in the Netherlands (age: 1-90 years) donated blood 6 or 7 times, every 3-6 months. Levels of IgG antibodies to the pre-fusion (F) protein of RSV were measured with a fluorescent bead-based multiplex assay (MIA). Changes in antibody levels within individuals over time were analyzed to study waning immunity and infections.
Results: Anti-RSV-pre-F-specific antibody levels were present in all participants and detected at a constant level with increasing age. During the pandemic, a significant decrease in antibody levels was detected in children up to 15 years of age ($P<0.05$), while this was marginal in adults. The decrease in antibody levels was steepest in the youngest children and progressively diminished with increasing age. The increased notification rates of RSV infections among children in the summer of 2021 was paralleled by specific antibody increases mostly in children. In the winter of 2021/2022, infections were also detected in adults, whereas in the spring of 2022 infections were mainly observed in children.
Conclusion: These data show that the interventions during the COVID-19 pandemic resulted in waning immunity to RSV, especially in children. Longitudinal analyses of antibodies contribute to the understanding of the kinetics of RSV infections over time and between age groups.

P154

Clinical applications of Nanopore sequencing for the detection of antibiotic resistance genes in Mycobacterium tuberculosis

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Mycobacterium tuberculosis (Mtb) is one of the leading causes of death worldwide by an infectious disease. Emerging rifampicin-resistant tuberculosis (RR-TB) and multi-drug resistant tuberculosis (MDR-TB) poses a great threat for the future because of fewer treatment options. Early diagnosis of RR-TB and MDR-TB infections are essential to guide empirical treatment in order to reduce further transmission of drug-resistant strains. Drug-resistance testing is frequently done via whole genome sequencing of cultured strains using the Illumina sequencing platform. Due to the dependency of culturing this is time-consuming and can take up to several weeks. In addition, the high costs associated with Illumina sequencing prohibit its use as part of a more standard diagnostic workflow in ISO-certified diagnostic microbiological laboratories. Even though diagnostic laboratories already use rapid molecular tests, such as the GenXpert MTB Ultra (Cepheid), these can only detect mutations in the rifampicin determining region (RRDR) in the rpoB gene. At The Municipal Health Service of Amsterdam (GGD Amsterdam) we developed a novel multiplex PCR combined with amplicon-based nanopore sequencing, covering 12 resistance genes and a total of 10 drugs, namely: rifampicin (RIF), isoniazid (INH), ethambutol (EMB), pyrazinamide (PZA), streptomycin (SM), bedaquiline (BDQ), linezolid (LZD), moxifloxacin (MFX), levofloxacin (LFX) and clofazimine (CFZ). We estimated the sensitivity by comparing 13 freshly cultured, patient-derived, drug-resistant Mtb strains to previously obtained Illumina sequencing data of the same strains as part of national TB surveillance. We analyzed data real-time during sequencing, resulting in a median turnaround time of approximately 1-2 days. With this method we were able to obtain an elaborate resistance profile with Oxford Nanopore Technology (ONT). Further research will involve the direct application of our multiplex PCR on clinical materials and compare clinical sensitivity and specificity to the auramine staining, culture and GenXpert MTB Ultra.

P155

Screening basidiomycetes and testing their potential to produce mycelium materials.

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The utilization of mycelium materials fosters a shift towards a sustainable circular economy. The current species used in mycelium material production is relatively limited. To discover more species with the potential to produce mycelial materials, our study isolated and identified 12 basidiomycetes, of which 7 species previously unexplored as either pure mycelium materials, mycelium composites or both.

The solid-state fermentation is used to test the colonization rate of these 12 species on hemp shive. Trametes species especially Trametes betulina exhibited showed superior colonization. The property assessment of mycelium composites included water absorption, thermal conductivity and compression resistance. The composite materials from these 12 fungal species demonstrated elevated water absorption, reaching 659.7% to 963.4% of the original weight after 15 days. Composites from Trametes versicolor, Bjerkandera adusta, and Ganoderma resinaceum exhibited thermal conductivity values ranging from 0.0278 ± 0.0014 to 0.0280 ± 0.0013 W/m K. Their thermal insulation performance surpassed that of certain natural insulating materials such as hemp and cellulose.

Additionally, these 12 isolated species were cultivated in shaking MEB (malt extra broth) cultures. Species Trametes betulina, G. resinaceum, Ganoderma adspersum, Chondrostereum purpureum, Pleurotus ostreatus and Phlebia tremellosa were selected to produce pure mycelium material based on their promising biomass yields 5.84 ± 0.97 up to 7.34 ± 0.64 g/L for dry weight. The pure mycelium material from P. tremellosa is the most rigid one due to its biggest Young's modulus of 334.1 ± 97.0 MPa, whereas the material from G. resinaceum with the lowest Young's modulus of 28.7 ± 5.1 MPa is relatively pliable among the tested materials.

Moreover, my research highlights that the co-cultivation of two distinct fungal species has the potential to improve mycelium biomass yield since the biomass dry weight of three certain co-cultures is higher than that of their monocultures.

P156

Bartonella henselae IgM after a tick-bite in the Netherlands

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Introduction
Bartonella henselae can cause cat-scratch disease (CSD) in humans and is primarily transmitted by cats. For CSD diagnoses in individuals without cat contact, ticks may serve as an alternate transmission route. Previous studies indicated the presence of Bartonella species in ticks, with less than 0.2% of Ixodes ricinus ticks in the Netherlands harbouring B. henselae. The current study aims to assess if individuals have serological evidence of Bartonella infection after a tick bite.

Methods
In total n=277 serum samples were selected from a nationwide prospective observational study among patients who had consulted a general practitioner for a tick bite (TB; n=143) or erythema migrans (EM; n=134) in 2008 in the Netherlands and from whom blood was been collected at baseline and 3 months later. As a control group, 277 age- and gender-matched sera from a cross-sectional population-based seroprevalence survey conducted in the Netherlands in 2006-2007 were used. All sera were analyzed for the presence of B. henselae-specific IgM antibodies by an in-house ELISA.

Results
At baseline, B. henselae-specific IgM was present in 20.6% of TB-patients and in 12.9% of EM-patients, respectively.Three months later, B. henselae-specific IgM seropositivity had increased to 27.7% among TB-patients and had decreased to 5.4% among EM-patients. For population controls, B. henselae-specific IgM seropositivity was 6.5%.

Conclusion
The dynamics in Bartonella-specific IgM seropositivity over time among TB- and EM-patients indicates a potential role of tick bite by the possible transmission of B. henselae. Further research is necessary to assess the significance of these findings in relation to the low incidence of 0.2% of B. henselae in ticks. Additionally, it is imperative to interpret the results of this study in conjunction with clinical symptoms for a more comprehensive understanding.

P157

Molecular detection of Ixodes ricinus-borne pathogens

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Introduction

The extent to which infections with Ixodes ricinus-borne pathogens (TBPs), other than Borrelia burgdorferi s.l. and tick-borne encephalitis virus, cause disease in humans remains unclear. One of the reasons is that adequate diagnostic modalities are lacking in routine diagnostic or research settings. To meet this diagnostic requirement, three multiplex (MPX) molecular qPCRs were set up for the simultaneous detection of Anaplasma phagocytophilum, Borrelia burgdorferi s.l., Borrelia miyamotoi (MPX1), Babesia species, Spotted Fever Group Rickettsiae (MPX2), Neoehrlichia mikurensis, Spiroplasma ixodetis, and Bartonella species (MPX3) in human samples.

Methods

Due to the scarcity to non-existence of confirmed patient materials, the analytical validation was performed using synthetic controls. Furthermore, negative human clinical samples (blood and cerebrospinal fluid) were spiked with the synthetic controls to determine the matrix effect.

Results

The acceptable limit of detection when multiplexing the different pathogens, the good inter-assay variability and the absence of false positive results makes the qPCRs potentially suitable for human diagnostics. Successful detection of spiked materials showed that the detection of the TBPs is not inhibited by the presence of human clinical samples. Clinical validation has started, but due to the shortage of confirmed positive samples, we offer the qPCR free of charge for diagnostics of clinically suspicious samples until September 2024.

Conclusion

The multiplex qPCRs evaluated in this study are technically suitable for laboratory diagnostic assessment of clinical samples suspect for TBP infection. However, further clinical validation and independent confirmation of our results is planned, pending the availability of human samples.

P158

Cryo-ET imaging of mycobacterial infections and visualisation of the type VII secretion system

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Within the genus Mycobacterium are highly pathogenic species, which are the causative agents of diseases such as tuberculosis, leprosy, and so-called 'non-tuberculous diseases'. The World Health Organisation reports that tuberculosis alone is lethal to approximately 1.5 million people annually. As with many deadly bacteria, the occurrence of antibiotic-resistant mycobacteria is increasing, placing additional pressure on the development of new treatments.

Mycobacteria cause disease by colonising macrophages predominantly in the lungs. In order to survive and infect their host, mycobacteria rely on large, membrane-bound protein complexes known as Type VII Secretion Systems (T7SSs). T7SSs are required to block merging of the macrophage lysosome and phagosome, for translocation to the host cytosol, and for infection of other macrophages, as well as for the uptake of metabolites and nutrients. Because of their vital role in both mycobacterial homeostasis and host infection, T7SSs present a potential target for the development of novel treatments against tuberculosis, leprosy, and other life-threatening mycobacterial diseases.

In my project I will investigate the potential of T7SSs as targets for anti-mycobacterial drugs. To this end, I will use cryogenic electron microscopy (cryo-EM) and -tomography (cryo-ET) in combination with correlative light-electron microscopy (CLEM), scanning electron microscope focused ion beam milling (SEM-FIB milling) and sub-tomogram averaging (STA) to image mycobacteria and their Type VII Secretion Systems in situ for the first time. To do this, mycobacterial cells will be vitrified on their own, inside of infected zebrafish, and inside of human macrophages. With these data, I will investigate the morphological changes of mycobacteria and macrophages, and the structural changes of the T7SSs, upon treatment with newly developed anti-mycobacterial drugs.

P159

High prevalence of toxigenic Corynebacterium diphtheriae in wounds of refugees arriving in the Netherlands in June and July 2023

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Background: An increase of diphtheria by Corynebacterium diphtheriae in refugees was reported from several European countries in 2022-2023. We studied the prevalence of toxigenic C. diphtheriae among refugees arriving in the Netherlands and validated a molecular assay for rapid detection of diphtheria in clinical specimen.

Methods: Refugees aged ≥ 16 years, arriving at national first-registration centers and consulting a GP for wound(s) between June 5 and July 30, 2023, were asked to participate. A swab was collected from the most suspicious wound and the throat. Primary diagnostics was performed using culturing combined with PCR on first-day culture and on C. diphtheriae isolates. Positive isolates were sequenced. In parallel a quadruplex qPCR assay, targeting all relevant diphtheria species and the toxin-gene, was applied directly on clinical materials.

Results: Of 61 participants, nearly all were male (95%) and mainly from Syria (68%). Ages ranged between 16-54 years (median 23 years). From 45 participants both a wound and throat swab, from 15 participants only a wound swab and from 1 participant only a throat swab was examined. In 6/60 (10%, 95% CI: 3.8%-20.5%) wounds, toxin gene-bearing C. diphtheriae was detected. Throat carriage was present in 3 of these cases. Sequence type (ST)377, a frequently found ST among refugees in Europe, was found in cases from Syria (n=4/40). Cases from Eritrea (n=2/4) were genetically identical to each other and from a non-defined ST. We also showed that molecular diagnostics directly on clinical material has equal sensitivity and specificity as compared to the gold-standard; molecular diagnostics on cultured swabs.

Conclusion: The prevalence of toxigenic C. diphtheriae in wounds and throats of refugees arriving in the Netherlands is of concern. We expect that easy accessible testing of refugees with wounds for diphtheria, allows early detection and minimizes the risk of further spread.

P160

qPCR-based detection of Streptococcus pneumoniae in respiratory samples collected from adults with and without Community Acquired Pneumonia (CAP)

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Introduction
Despite the availability of pneumococcal vaccines, Streptococcus pneumoniae remains the primary bacterial
cause of Community Acquired Pneumonia (CAP). We applied molecular methods to define rates of pneumococcal carriage in community-dwelling adults with CAP versus a control group.

Methods
Pneumococcal DNA was detected with quantitative polymerase chain reaction (qPCR) assays targeting the piaB and lytA genes in minimally processed (raw) nasopharyngeal, oropharyngeal and saliva samples collected at the onset of respiratory symptoms from n=219 adults with radiographically confirmed CAP, and from n=240 age- and sex-matched individuals without respiratory symptoms (controls). Sputum samples of n=130 CAP patients that produced sputum were also tested. The enrolment took place between April 2018 and March 2020.

Results
Seventy-eight (35.6%) of 219 CAP patients and 22 (9.2%) of 240 controls have at least one sample positive for
pneumococcal DNA. Among CAP patients, 23.7% had sputum, 20.1% oropharyngeal, 19.6% nasopharyngeal
and 15.0% saliva sample positive. No sample type outperformed all other respiratory samples and agreement
among sample types was low (Fleiss' Kappa 0,47). However, among sputum-producing patients testing sputum exhibited significantly increased sensitivity (89.7% versus ≤48.3%) and exhibited near-perfect agreement to a composite reference (κ 0.91). Among controls, 5.0% have oropharyngeal, 3.8% nasopharyngeal and 3.3% saliva samples positive for pneumococcus, without statistical differences. For each sample-type, fraction of samples positive for pneumococcus was significantly higher among CAP patients compared to individuals without respiratory symptoms (Fisher Exact; $p<0.001$).

Conclusions
Among sputum-producing CAP patients testing sputum exhibits superior sensitivity, whereas among overall
CAP patients testing other respiratory samples can be informative.

P161

Accuracy of Streptococcus pyogenes carriage detection with culture and with qPCR in oropharyngeal and nasopharyngeal samples collected from children

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Streptococcus pyogenes (Group A streptococcus, GAS) causes life-threatening infections but is also a colonizer of humans. Asymptomatic S. pyogenes colonization (carriage) remains poorly understood. We evaluate the accuracy of GAS detection using diagnostic culture and a qPCR-based approach applied to samples collected from 24-month-old children.

Oropharyngeal and nasopharyngeal swabs were collected from n=331 Dutch children in a cross-sectional study. First, samples were cultured on blood agar (BA) without and with gentamicin (SB7-BA). Overnight cultures were screened for beta-haemolytic colonies and isolates susceptible to bacitracin were classified as GAS. Next, DNA was extracted from all-colony-growth harvests of SB7-BA plate and tested in qPCRs targeting speB and spy1258 genes unique for GAS. Harvests positive by qPCR from which GAS was not yet isolated were cultured on CHROMagar-StrepA medium in a second attempt to isolate live GAS. Accuracy of different approaches was assessed using as isolation of live GAS from a person as reference.

Thirty-two children (9.6% of 331) were identified as carriers based on isolation of viable GAS at the primary diagnostic step from either oropharynx (n=24) or nasopharynx (n=20). qPCR-guided culturing resulted in an additional n=21 oropharyngeal and n=5 nasopharyngeal GAS isolates, increasing the percentage of culture positive children to 14.8% (49/331). Primary cultures of oropharyngeal and nasopharyngeal samples exhibited substantial and moderate agreement with a reference (Cohen's □=0.62 and □=0.54, respectively). qPCR-guided culturing increased accuracy of GAS detection by culture in both, oropharyngeal (□=0.95, almost perfect agreement) and nasopharyngeal samples (□=0.63). When compared to primary diagnostic cultures, the application of qPCR has substantially improved accuracy of GAS detection in oropharyngeal samples (□=0.91) but not in nasopharyngeal samples. Overall, n=54 children (16.3% of 331) have been identified as carriers by any method applied.

The accuracy of GAS carriage detection can be greatly improved by complementing culture of oropharyngeal samples with qPCR.

P162

Akkermansia in Parkinson's disease: friend or foe?

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Introduction: Parkinson's disease (PD) is a debilitating condition characterized by the progressive loss of dopaminergic neurons in the brain, leading to its typical symptoms like motor-related impairments such as tremors, but also non-motor symptoms such as constipation. It is well established that PD is associated with alterations in gut microbial composition. Strikingly, also Akkermansia muciniphila, a mucin degrading bacterium, is widely reported to be increased in abundance, opposing to most other diseases. It is known that A. muciniphila displays vast genomic and phenotypic diversity. We were thus asking whether PD patients might possess specifically adapted A. muciniphila strains.
Methods: We used a previously recruited cohort of 19 PD patients and their matching healthy household relatives (HC), exhibiting a broad range of clinical PD related features. Stool samples were incubated in mucin containing medium and a variety of colonies was picked. Isolates were observed microscopically and growth-curves were recorded using different carbon sources. In parallel DNA was isolated and whole genome sequencing was performed, followed by comparative genomic analysis.
Results: To this end we performed isolation of 17 A. muciniphila strains from 5 individuals (2 HC / 3 PD). The isolated strains were characterized phenotypically, which showed differences in their preferred carbon source as well as their cell morphology. Whole genome sequencing revealed that PD patients harbored strains from phylogroup AmII and AmI, while from HC we only isolated strains from AmIV. We were able to confirm changes in vitamin B12 metabolism as well as specific sialidases and fructosidases, which are important for glycan usage.
Conclusion: Here we show that PD patients might possess specifically adapted strains to their gut environment. Ultimately, our findings may help to better understand the role of the microbiota in PD and in developing targeted therapies to improve patient's symptoms.

P163

Universal targeting of Staphylococcus aureus through antibodies binding wall teichoic acid

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Staphylococcus aureus (S. aureus) is an opportunistic pathogen causing a diverse range of conditions like skin infections and sepsis. Recently, WHO prioritized S. aureus as a bacterial pathogen for which new treatments are urgently needed to curb the reduced treatment options due to antibiotic resistance. A possible alternative treatment may consist of antibody-based immunotherapy where patients receive S. aureus-targeting monoclonal antibodies (mAbs). So far, clinical trials have failed to show consistent results for the use of mAbs to treat S. aureus infections.
An abundantly expressed surface antigen is wall teichoic acid (WTA), a glycopolymer with limited structural variation through glycosylation. The available anti-WTA mAbs strongly promote complement activation and phagocytic killing of S. aureus. However, all these WTA-specific mAbs are glycoform-specific and thus do not cover all S. aureus strains. Therefore, our goal is to generate mAbs targeting S. aureus WTA irrespective of glycoform.
Two llamas were immunized with simplified and stable synthetic WTA mimics (sWTA) of all known S. aureus WTA glycoforms. After four immunizations, anti-WTA responses were confirmed in serum. Phage libraries were constructed displaying the full repertoire of variable domain of heavy-chain-only antibodies (VHH). WTA-specific phages were selected from the library using sWTA-coated beads and specificity was confirmed by sWTA ELISA. After sequencing and recombinant production, anti-WTA VHHs were tested for binding to sWTA-coated beads and S. aureus strains. One of the selected VHHs recognized sWTA irrespective of glycostatus, and also naturally-expressed WTA displayed on the S. aureus surface but only after increasing VHH's avidity.
In conclusion, we successfully generated an antibody universally recognizing S. aureus WTA in a glycoform-independent manner. Follow-up experiments will assess and optimize therapeutic potential, to eventually allow mAb-based S. aureus therapies.

P164

Implementation of a national Campylobacter laboratory surveillance based on whole genome sequencing

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Background: In the Netherlands, Campylobacter infections impose the highest disease burden among food-borne bacteria, and individual cases are not notifiable. In 2021, a sentinel laboratory surveillance for Campylobacter was initiated, employing whole genome sequencing (WGS). Here we outline the implementation of the first three years, aiming to assess the level of clustering and investigate outbreaks.

Methods: From May 2021 to December 2023, seven Dutch medical microbiology laboratories submitted over 4,000 Campylobacter isolates to the RIVM. The genus was confirmed with Bruker Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF) and a random selection of 400-500 isolates underwent WGS annually. Illumina technology and an in-house Juno pipeline were employed, following ISO 15189 standards. Ridom SeqSphere was used to establish the 7-locus MLST and cgMLST (637 loci) for cluster identification (AD=13).

Results: In total, 699 of 1,254 (54%) isolates were part of one of 135 clusters. Of these, 90% were C. jejuni, and 10% C. coli. The median age was 42 years (IQR: 25-62), with 52% males. Of all clusters, 103 comprised of ≤5 isolates, 27 of 6-20 isolates, and 5 of >20 isolates, with a median of three (range: 2-33). An outbreak investigation was conducted for a cluster (n=15) near the German border, comprising 11 females with a median age of 43. Despite questionnaire data not indicating a source, an isolate from imported chicken was identified as a potential source. Two-third of clusters (66%) persisted for more than one year, and 27% for all three years.

Conclusion: These data highlight the existence of predominantly small clusters and a few larger clusters, suggesting that Campylobacter epidemiology is primarily driven by a diffuse spread of a diverse range of strains from numerous sources, rather than by larger point-source outbreaks. A One Health approach may improve source identification by matching clinical isolates with animal or environmental sources.

P165

Presence of Brucella abortus small-RNA in extracellular vesicles from infected macrophages show promise as potential biomarker for brucellosis

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Brucella abortus is a zoonotic, facultative intracellular bacterium that causes brucellosis in cattle. Although the Netherlands is officially free of brucellosis, positive serological tests are occasionally reported. Because the specificity of these serological tests is low, their results have to be validated by PCR or culture of the bacterium from infected tissue of a sacrificed animal. To prevent unnecessary euthanasia of healthy animals, we aim to improve B. abortus diagnostics by directly detecting the presence of the bacterium in living animals using novel bacterial-derived biomarkers. These biomarkers include RNAs that are secreted in extracellular vesicles (EVs). EVs are cell-derived nanoparticles that are secreted by living cells and serve a role in intra- and interspecies extracellular communication. EVs have also been shown to play a major role in the pathogenesis of bacterial infections, and pathogen-derived EVs, or their components, circulate in the body fluid of the host. In this study, we used an in vitro infection model of a bovine macrophage cell line infected with B. abortus to identify RNAs that could serve as novel biomarker. After infection, we isolated and characterized EVs from the culture medium, and determined the small-RNA profile of these EVs. Preliminary results show that B. abortus small-RNAs are secreted via EVs during macrophage infection, with a relatively high abundance of bacterial tRNA-derived fragments. Furthermore, host cells respond to infection by inducing the secretion of small nucleolar RNA (snoRNA) via EVs. These results give new insight in the small- RNA composition of EVs secreted by bovine macrophages during bacterial infection and provide a first direction for the identification of potential diagnostic biomarkers for B. abortus.

P166

Discovery of 6-GABA-trehalose, a novel M. tuberculosis stress response metabolite

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Tuberculosis (TB) is a severe global health burden that kills over 1 million people each year. TB is caused by Mycobacterium tuberculosis (Mtb), a bacterium that is becoming resistant to the once effective antibiotics that were developed over 50 years ago. With drug resistance rising, there is an urgent need for new drugs to treat TB. A large and untapped reservoir of novel drug targets may be hidden within the large number of Mtb genes that have no known function. We aimed to discover such potential novel drug targets by screening for novel Mtb metabolites and linking them to their biosynthetic enzymes. We focused on novel metabolites involved in the pathogenic lifestyle of Mtb by exposing it to stresses that mimic the macrophage phagolysosome and screening for unknown stress-responsive metabolites using untargeted metabolomics. Our approach revealed an abundant unknown metabolite that is produced rapidly upon exposure to nitric oxide and hypoxia. Importantly, Mtb also produced the unknown metabolite in a physiologically relevant human monocyte-derived macrophage infection model. Using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), we next identified the unknown metabolite as 6-GABA-trehalose (6-GT), a metabolite that has never been reported before. Biochemical tests with Mtb protein lysate revealed that 6-GT is formed from GABA, trehalose and ATP by an enzyme that we named 6-GABA-trehalose synthetase (6-GTS). Protein fractionation combined with proteomics identified the 6-GTS to be the gene product of Rv1722, a gene without known function. To conclude, we have discovered a previously unknown stress-responsive Mtb metabolite, 6-GABA-trehalose, and its biosynthetic gene, Rv1722, that appears to play an important role in Mtb stress adaptation. Future research will focus on resolving the function and structure of Rv1722 to determine if Rv1722 may indeed serve as a drug target and to provide a foundation for designing an Rv1722 inhibitor.

P167

Localization of antibodies and complement molecules on Gram-negative bacteria

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Increasing numbers of infections with antibiotic resistant Gram-negative bacteria require the development of alternative treatment strategies. One such alternative could be the use of therapeutic antibodies that can activate the complement system. Complement molecules can potently kill Gram-negative bacteria via the formation of lytic pores, called Membrane Attack Complexes (MACs). To guide the development of therapeutic antibodies, it is key to understand how antibodies induce complement-mediated killing on a molecular level. Here, we used widefield fluorescence microscopy to study complement activation and MAC formation on Escherichia coli (E. coli). In order to identify potential preferential binding spots of antibodies and complement molecules on the bacterial surface, bacteria were incubated with antibodies and complement factors that were either directly labeled or stained with a secondary antibody. By using a fully purified complement assay, we were able to control each component of the cascade individually and study preferential binding at low concentrations that are just sufficient to damage the bacterial inner membrane. In addition, we used a naturally membrane impermeable DNA dye to correlate MAC deposition with bacterial inner membrane damage. Altogether, our results suggest that MAC deposition starts at the bacterial (division) poles, caused by preferential binding of the activation product C3b at those spots. In conclusion, we show how imaging techniques can give crucial molecular insights into complement mediated killing of Gram-negative bacteria.

P168

Effect of introduction of MenACWY vaccination on circulating Neisseria meningitidis carriage strains in the Netherlands

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The Netherlands replaced the MenC vaccine with a MenACWY vaccine in the national immunization programme (NIP) in 2018. Carriage studies amongst young adults identified a similar prevalence of meningococcal carriage rate of around 25% before and after MenACWY introduction in the Dutch NIP. Here, we assessed the strain characteristics of the obtained isolates.

The obtained meningococcal carriage isolates, 68 strains pre-MenACWY introduction and 146 strains post-MenACWY introduction, were whole genome sequenced (Illumina technology) to determine sequence type, clonal complex, finetype of PorA/FetA, in silico genogrouping. We also predicted coverage by both MenB protein vaccines.

Carriage prevalence declined for the combined vaccine types from 13/299 (4%) pre-MenACWY introduction to 1/601 (0.2%), mainly driven by a decline in MenY (10/299 (3%) to 1/601(0.2%)) and MenW (from 3/299 (1%) to undetected). MenA and MenC were absent in both studies. MenB strains predominantly belonged to clonal complexes ST-32, ST-41/44, and ST-213 and the distribution over the genetic lineages was not affected by the introduction of MenACWY. Coverage by either 4CMenB or menB-FHbp increased from 10/13 (77%) pre-MenACWY introduction, to 25/28 (89%) post-MenACWY introduction. Carriage of genogroupable MenE increased from 0.7% to 5%. Twenty-two out of 31 MenE strains isolated post-MenACWY introduction belonged to clonal complex ST-60, where P1.5,2;F1-7 was the dominant finetype (16/22, 73%). Eighty-six percent of the strains (19/22) were serologically classified as MenE. Twenty-nine MenE strains (94%) were predicted to be covered by menB-FHbp, but not by 4CMenB.

Following implementation of the MenACWY vaccine in the NIP, carriage of vaccine-type meningococci was nearly eliminated. No significant genetic shifts in circulating MenB strains were observed but we did detect increased circulation of MenE strains attributable to clonal complex ST-60. Consistently, three cases of invasive disease by MenE belonging to clonal complex ST-60 were reported in 2023, all in immunocompromised patients.

P169

Sequencing-based surveillance of circulating Bordetella pertussis strains in the Netherlands from 2015-2023

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Introduction

Whooping cough is a notifiable disease in the Netherlands. Since the introduction of the acellular pertussis vaccine there is active surveillance on the circulating strains, with an emphasis on presence of vaccine antigens in these strains and early detection of possible vaccine-escape mutants. During the COVID-19 pandemic pertussis circulation was reduced in the Netherlands. Here, we report on the re-emerging strains and compare them to the pre-pandemic pertussis population.

Methods

We analyzed circulating B. pertussis isolates using both genotypical and phenotypical methods. A population structure was determined using a read mapping approach with the B1917 global strain as a reference genome. We additionally provide a detailed analysis of the trends in vaccine antigen expression, both phenotypically using Luminex and WGS-based.

Results

Approximately 300 pertussis isolates were sequenced between 2015 and 2023. Importantly, none of the included isolates were epidemiologically linked to another. First, we generated a population structure of the Dutch pertussis isolates to place recent isolates in a broader context. Pre-pandemic, we found two dominant lineages with a known marker SNP in fim3 gene dividing both lineages. Noteworthy, the fimbriae are components of pentavalent whooping cough vaccines. To investigate pertactin deficiency, the most common vaccine escape mechanism, we determined phenotypic pertactin expression for all isolates, while simultaneously predicting pertactin expression based on WGS-data. In the earlier years (2015-2017) 10% of strains were deficient in pertactin, this increased to 24% in later years (2018-2020). Post-pandemic strains, thus far, showed 12% pertactin deficiency.

Conclusions

The pre-COVID-19 pandemic circulating B. pertussis strains are genetically homogenous. We see a trend towards more pertactin deficient strains in later years. Since the pandemic, all epidemiologic evidence suggested little to no circulation of pertussis. We will report on the re-emerging pertussis strains in the Netherlands and the genetic lineages that are resurfacing.

P170

A strategy to develop a safe probiotic targeting LA-MRSA starting from nasal microbiome data

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Introduction
LA-MRSA inhabiting the porcine respiratory tract forms a zoonotic risk. Amplicon sequencing and qPCR guided our isolation and identification of bacteria suitable for probiotic interventions against MRSA. Probiotic candidates were subsequently assessed for safety and efficacy.

Methods
Nasal swabs were taken longitudinally from 252 piglets, from 36 sows, from 9 farms in Germany, Ireland, and the Netherlands (n=4032). Duplicate samples were taken for bacterial isolation and DNA extraction. V3-V4 16S rRNA and tuf gene Illumina sequencing, and S. aureus specific qPCR data was generated. Species antagonistic to S. aureus (rmcorr) were isolated, and their antimicrobial resistance and hemolysis was determined. WGS identified strain taxonomy, antimicrobial resistance and virulence genes, following EFSA guidance. Three candidate probiotic strains were used as a nasal cocktail treatment in a small-scale in vivo safety test, followed by a field-trial treating 360 piglets (50% placebo) in 6 farms in Ireland and the Netherlands. Treated and untreated animals were followed by longitudinal swabbing.

Results
Amplicon sequencing supplemented with S. aureus specific qPCR data, identified 54 species in the nasal microbiome that were negatively associated with S. aureus. An isolation effort combined with suitability screens, resulted in a probiotic cocktail of three lactic acid bacteria (LAB). A safety test found the cocktail to be safe for nasal application in newborn piglets (Rattigan et al.,2023). The cocktail, as 1 mL nasal drops, was used to treat 180 piglets in 6 European farms. Using qPCR, we quantified the reduction of of S. aureus/LA-MRSA in the pig farms.

Conclusion
Investigation of the developing porcine nasal microbiome resulted in probiotic candidates aimed to reduce S. aureus. These candidates are safe for nasal application in neonatal piglets. The level of reduction of S. aureus/LA-MRSA obtained after application of the probiotic cocktail will be presented.

P171

Performance of molecular culture on interlaboratory comparison programs

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Introduction: Diagnosis for bacterial infections traditionally relies on culturing of patient samples. However, culture often has a slow turn-around time and gives false negative results when unculturable micro-organisms are present or when patients have been treated with antibiotics before sample collection. DNA-based molecular assays, such as the Molecular Culture assay, offer a promising solution. This PCR-based assay utilizes 16S-23S rDNA polymorphisms and phylum-specific primers, creating unique bacterial fingerprints.
The absence of reference profiles for some bacterial species underscores the need for a well-curated database. Our study showcases Molecular Culture's precision through participation in SKML and UK NEQAS interlaboratory programs, aimed at routine culture, and provides evidence for accurate identification of a broad range of bacteria.

Methods: 63 freeze-dried samples were analyzed which were sent by program partner laboratories. The results obtained from Molecular Culture were compared to those given by SKML and UK NEQAS. Additionally, in most samples, Molecular Culture amplicons were sequenced with the MinION (Oxford Nanopore) and compared to a proprietary database to confirm the identification generated through Molecular Culture.

Results: In all 63 samples, Molecular Culture successfully detected bacterial signals in the correct phylum category, yielding 100% sensitivity and accuracy at the phylum level.
Using the current database for Molecular Culture, 48 of 63 samples (76%) were directly identified correctly to the species level. Combined with nanopore sequencing, all species were identified to the species level.

Conclusion: Molecular Culture can accurately detect a broad range of bacteria and identify them to the species level. When Molecular Culture yielded an incomplete or incorrect result, nanopore sequencing of amplicons yielded a correct identification. By routinely combining Molecular Culture with nanopore sequencing, the reference database of Molecular Culture can be continuously improved, making it increasingly viable to perform only Molecular Culture for species identification, leading to fast turnaround times.

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Exploring thermotolerance in Listeria monocytogenes: In silico and functional analysis of plasmid-encoded ClpB

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Introduction: Infection with foodborne Listeria monocytogenes is rare but can result in severe disease with high mortality rates. While the heating of food products is generally effective in eliminating L. monocytogenes, certain strains appear to be more heat resistant than others. The conserved chromosome-encoded ATP-dependent chaperone ClpB is linked to induced thermotolerance in L. monocytogenes. In this study, we explored the potential contribution of a novel plasmid-encoded ClpB in enhanced thermotolerance.

Methods: L. monocytogenes genomes were sequenced with the Nanopore platform, assembled with Flye, and annotated with RAST. In silico analysis included alignments in Geneious, InterProScan domain- and AlphaFold2 protein structure prediction, and Foldseek structure comparison. Heat-challenging experiments assessed the heat resistance of strains with plasmid-encoded ClpB and controls.

Results: Genomes of strains isolated from a food product post-heating revealed the presence of both chromosomal clpB (2,602 bp, clpB-c) and a smaller plasmid-derived clpB gene (1,120 bp, clpB-p). Alignments showed that clpB-p represented a truncated form of clpB-c with ~41% DNA sequence similarity in the latter half of clpB-c. Domain and structure predictions highlighted a shared ATPase, three common ClpA/B family regions along with structural resemblance between a domain of ClpB-c and ClpB-p. Both proteins were predicted to be subunits of a hexamer, forming a pore structure with disaggregase and chaperone functionality. Interestingly, ClpB-c was reported to be regulated (natively repressed) through a coiled-coil structure, an element that was notably absent in ClpB-p. Heat-challenging experiments demonstrated a two-log reduction in survival for clpB-p negative compared to clpB-p positive strains.

Conclusion: In L. monocytogenes isolates that survived food product heating, a plasmid encoding a truncated form of ClpB was identified with predicted disaggregase and chaperone functions. The absence of a coiled-coil structure suggests that this truncated protein is a natively unrepressed chaperone that contributes to thermotolerance.