P001

Salinization of a freshwater peatland leads to increased methane production in quaking bog peat

Naomi Boerma[1], A. Legierse[1], T. Gremmen[2], Dr. G. van Dijk[2], Dr. M.A. Glodowska[1]
[1]Radboud Institute for Biological and Environmental Sciences, Radboud University, [2]Research Centre B-WARE B.V.

Salinization of freshwater wetland ecosystems through rising sea levels is a growing concern. Carbon stored in the sediment of these ecosystems is vulnerable to decomposition, releasing greenhouse gases (GHG) like carbon dioxide (CO2) and methane (CH4). The formation of newly formed peat in the form of quaking bogs is key in carbon sequestration in these wetlands. However, a knowledge gap remains on how salinization affects microbial processes that drive CH4 cycling in quaking bogs. In Westzaan, North of Amsterdam, a long-term salinization experiment revealed increased presence of CH4 in brackish quaking bog root zones, contrasting with other results from this study that found decreased emissions of CH4 from brackish ditches. We aimed to identify key conditions driving CH4 cycling in quaking bog root zones through a combination of field measurements and laboratory experiments. This involved analysis of the geochemistry of the sediment and porewater, qPCR analysis of pmoA (aerobic methanotrophy) and mcrA (anaerobic methanotrophy and methanogenesis) as well as 16S amplicon sequencing. Additionally, activity of methanotrophs using 13CH4 and methanogens was studied via incubation experiments. Increased methanogenesis was found in brackish quaking bog incubations compared to freshwater incubations, supported by relatively higher porewater CH4 concentrations in the brackish quaking bog. In incubations amended with 13CH4, no formation of 13CO2 was observed in either freshwater or brackish quaking bogs, suggesting that anaerobic methanotrophy is not occurring. Finally, depletion of CH4 was observed in aerobic incubations with CH4, indicating aerobic methanotrophy, but no rate differences were observed between brackish and freshwater incubations. Together these results suggest that the origin of the increased CH4 in the root zones is an increase in methanogenesis rather than a decrease in methanotrophy in brackish quaking bogs. This research contributes to filling current knowledge gaps on the potential effects of salinization of freshwater peatlands on carbon biogeochemistry.

P002

Establishing key microbiota and biodegradation kinetics for organic micropollutant removal during riverbank filtration

Veronika Frančeková[1], Prof. Dr. H. Smidt[1], Principal scientist P. van der Wielen[2,3]
[1]Wageningen University & Research, [2]KWR Water research institute, [3]Radboud University

Riverbank filtration (RBF) is a nature-based water treatment mechanism used for green drinking water supply. It serves as a pre-treatment for surface water, requiring no additional chemical- or energy-intensive steps, before reaching drinking water treatment plants for final treatment. In RBF, water is drawn from the river/lake bed through a colmation layer into the aquifer, where surface water mixes with groundwater. During this infiltration, a combination of chemical, physical, and biological processes gradually improves water quality. However, the efficiency of RBF depends on factors such as the properties of the colmation layer, temperature, redox conditions, and nutrient concentrations. RBF has been shown to effectively remove organic micropollutants (OMPs), including pharmaceuticals, pesticides, and industrial chemicals, which have become contaminants of emerging concern. Although OMPs occur at low concentrations (µg/L to ng/L range), their long-term exposure, increasing concentrations due to widespread use, and unknown toxicity are raising concerns for both human health and the environment.
Despite the potential of RBF for OMP removal, the specific microorganisms responsible for biodegradation and the growth kinetics of these processes remain unclear. This project aims to resolve these knowledge gaps by identifying potential microbial players involved in OMP removal using metagenomic and ribosomal RNA gene sequencing, followed by targeted gene analysis. Secondly, culture-based approaches will be used to assess microbial growth kinetics and the impact of environmental conditions on OMP biodegradation. The final goal is to apply the findings from this study to optimize conditions for enhanced OMP removal at existing RBF sites and inform the location and design of new sites, ultimately providing safer pre-treated water with minimal subsequent treatment steps.

P003

Electroactive Anammox: low-concentration ammonium treatment

Dr. B. Conall Holohan[1], Mr. P. Smid[1], Prof. Dr. C. U. Welte[1]
[1]Microbiology Department, Radboud University

Nitrogen in surface water is causing the nitrogen crisis in The Netherlands, and indeed worldwide. Anaerobic ammonium oxidising bacteria (anammox) harbour untapped biotechnological potential for their application in mainstream wastewater treatment as they could remove remaining ammonium from the wastewater with a lower environmental impact than current methods. However, to date there is no robust technology to do so.

Here, we demonstrate a proof-of-principle of bioelectrochemical ammonium removal. Two-chamber bioelectrochemical system were inoculated with a laboratory enrichment of Candidatus Kuenenia and fed with synthetic wastewater with 10□M up to 2mM ammonium, similar to sewage levels. The system was operated at a poised anode potential of +600 mV versus standard hydrogen electrode (SHE), with continuous current measurement via a potentiostat.

The BES bioreactors successfully treated the ammonium (98% removal) and converted it to current (up to 7 mA/m-2), a proxy measurement for extracellular electron transfer (EET). The activity of the anammox community was confirmed through the addition of nitrite (an alternative electron acceptor) resulting in a lack of current production. Furthermore, the microbial community was investigated through fluorescent in-situ hybridisation (FISH) microscopy, showing anammox on the anodes. In our study we demonstrate that anammox can treat low-concentration ammonium in bioelectrochemical environments without nitrite, offering a novel solution for treating low ammonium concentrations in wastewater, combatting the nitrogen crisis.

P004

Are newly discovered fish gill endosymbionts the key to sustainable aquaculture?

Pascal Huizing[1], Wouter Mes[2], Sebastian Lücker[1], Maartje van Kessel[1]
[1]Radboud University, [2]Radboud University

Aquaculture is one of the largest and fastest-growing food-producing sectors worldwide. When fish are cultured at high densities and fed with high protein diets, the main nitrogenous waste product of fish, ammonia, can reach high concentrations in aquaculture tanks. Ammonia is toxic to fish, and reducing its concentration in the water is vital for the aquaculture industry. The gills of fish harbor nitrogen-cycling microbial symbionts. Unlike typical vertebrate-associated microbes found extracellularly, these bacteria thrive inside gill cells, forming a unique mutualistic system. The bacteria are shown to be ammonia-oxidizing Nitrosomonas spp. working in concert with denitrifying bacteria to detoxify the excreted ammonia into harmless dinitrogen gas. Little is known about their physiology, phylogeny, and colonization processes. To study the colonization of fish, we grew zebrafish larvae until 5 days post-fertilization (dpf) in gnotobiotic conditions and studied the presence of Nitrosomonas-like bacteria in control, conventionalized, and gnotobiotic groups using PCR targeting ammonia monooxygenase subunit A (amoA), a marker gene for ammonia-oxidizing bacteria. We determined the likely transmission pathway as being vertical through the deposition of Nitrosomonas on fertilized eggs, most likely via the water. To study the transmission of the symbiotic Nitrosomonas in more detail, we aim to isolate these symbionts by combining classical cultivation techniques and activity-based fluorescent labeling. Here, bacteria harboring the ammonia monooxygenase (AMO) enzyme are specifically tagged with a fluorophore. Subsequently, fluorescent-activated cell sorting (FACS) is used to sort cells for downstream cultivation. Together, this study helps to shed light on a previously unknown symbiosis from which the insights could prove useful to sustainable aquaculture practices and to help mitigate the environmental impacts of aquaculture.

P005

Enrichment of nitrogen removal microorganisms adapted to low temperature

Shiyi Liu[1], Dr Maartje van Kessel[1], Dr Sebastian Lücker[1], Dr Peer Timmers[2]
[1]Radbound University, [2]IE University

Nitrification is a core process for nitrogen removal in wastewater treatment. It is mediated by ammonia-oxidizing bacteria and archaea, which convert ammonia into nitrite, and nitrite-oxidizing bacteria that oxidize the resulting nitrite to nitrate. Additionally, complete ammonia-oxidizing bacteria can catalyze both reactions. Despite the reported capacity of nitrifying microorganisms to perform nitrification at low temperatures, reduced nitrification rates in wastewater treatment plants are often observed in winter when the water temperature drops below 15 °C. In this study, we investigated the microbial community of a wastewater treatment plant in Weissenborn, Germany, which efficiently removes nitrogen even at 5 °C. The nitrifying community was dominated by Nitrosomonas and Nitrospira, as revealed by 16S rRNA gene amplicon sequencing. We inoculated a bioreactor with activated sludge from this plant to enrich these nitrifying bacteria. The obtained culture currently removes 1.11 mM NH4+ per day at 12 °C and will be used for physiological characterization. This research provides new insights into the microbial community involved in nitrification at cold temperatures and will thereby contribute to optimizing nitrogen removal from wastewater.

P006

From prediction to production: Discovering new RiPPs from plant-associated Paenibacillus

Dr Nataliia Machushynets[1], Dr. M. Zdouc[2], Dr. A. Kloosterman[1], Prof. Dr. M. Liles[3], Prof. Dr. M. Medema[2], Prof. Dr. G.P. van Wezel[1]
[1]Leiden University, [2]Wageningen University, [3]Auburn University

Plants live in close association with millions of microorganisms that provide resilience against pests and diseases through the wide variety of natural products they synthesize. One class of such natural products, known as ribosomally synthesised and posttranslationally modified peptides (RiPPs), provides a unique opportunity to develop safer biologicals, as they frequently display narrow-spectrum antimicrobial activities. Predicting the chemical structures of RiPPs from bacterial genomes is notoriously difficult due to the diverse enzymology involved in producing these metabolites. Therefore we have previously developed decRiPPter (Data-driven Exploratory Class-independent RiPP TrackER), a RiPP genome mining algorithm aimed at discovery of novel RiPP BGCs based on patterns of gene cluster, precursor peptide conservation across species and presence of new enzymology. In this study, we employ Global Natural Products Social molecular networking (GNPS MN) combined with decRiPPter output to probe the diversity of RiPPs from plant-associated Paenibacillus and to discover new RiPPs with potential applications in crop protection. GNPS molecular networking is used to evaluate the production of RiPPs within over 200 Paenibacillus strains and decRiPPter is applied to mine their genomes to identify new candidate RiPP families. We will present our latest results, which have led to the identification of new RiPP candidates.

P007

Microbial nitrogen cycling in eutrophic coastal sediments

Isabel Rigutto[1], Dr. Marit R. van Erk[1], Dr. Tom van Berben[1], Prof. Dr. Ir. Caroline P. Slomp[1], Prof. Dr. Ir Mike S. M. Jetten[1]
[1]Radboud University

Coastal ecosystems experience increased nitrogen-eutrophication and deoxygenation due to anthropogenic activities. Such shifts in environmental conditions can change the ecosystem's redox zonation, impacting its biogeochemical functioning. The extremely potent greenhouse gas nitrous oxide (N2O) is produced in various reactions of the nitrogen cycle. Therefore, a predictive understanding of microbial nitrogen cycling and the formation and removal of N2O in eutrophic and deoxygenated coastal ecosystems is crucial.

Sediments were collected from the eutrophic, 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. High-resolution depth profiles were recorded with microsensors. The 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. The microbial diversity was assessed via 16S rRNA gene and metagenomic sequencing. Nitrogen cycling microorganisms were enriched and isolated in selected incubations.

The sediment ammonium concentration was 15 mM at 40 cm depth and decreased towards the surface, whereas nitrate and nitrite were depleted within the first centimeters. Nitrifiers were detected at all sediment sections, and potential for ammonium oxidation was observed in the presence of oxygen. No potential for anammox was detected. High nitrate and N2O reduction rates were observed for all sediment sections. Bacteria dominated the microbial communities, and denitrification genes were detected in high abundances. Subsequent transfers of active N2O-reducing batch incubations resulted in the enrichment and isolation of novel marine Proteobacteria, which are currently being characterized physiologically and genomically.

There is a high nitrogen cycling potential Lake Grevelingen sediments across seasons and sediment depths. This is important information to make better predictive models of the future state of our coastal waters and the reduction of their N2O emissions.

P008

Growing mud on electrodes: enrichment of methane-oxidizing bacteria from anoxic brackish marine sediments

Msc. Peter ter Horst[1], Msc. R Klomp[1], Msc. A. J. Wallenius[1], Dr. T. Berben[1], Msc. M.A.W. Schutgens[1], Prof. Dr. Ir. M.S.M. Jetten[1], Prof. Dr. Ir. C.P. Slomp[1], Prof. Dr. C.U. Welte[1]
[1]Radboud University

Methane is the second most important greenhouse gas, playing a critical role in global climate dynamics. In aquatic ecosystems, atmospheric emissions are regulated by the balance between biological methane production (methanogenesis) and consumption (methanotrophy). In aquatic environments, methanotrophic bacteria and anaerobic methanotrophic archaea catalyze methane oxidation coupled with the reduction of electron acceptors such as oxygen, metal oxides, natural organic matter, nitrate, or sulfate.

Here, we cultivated a microbial community from anaerobic Bothnian Sea sediments in a two-chamber bioelectrochemical system (BES), generating methane-dependent currents. The sediment was pre-enriched in bottle incubations supplemented with graphene oxide (electron acceptor), methane (electron donor), and carbon dioxide (carbon source). The BES was operated with methane-fed graphite felt anodes poised at 0.4 V vs. SHE, achieving methane-dependent current densities of up to 0.8 $mA \cdot m^{-2}$. Metagenomic analysis revealed an enrichment of multiple genera within the Methylomonadaceae family on the anode of the BES compared to the original inoculation cultures.

These findings suggest that marine methane-oxidizing bacteria could generate electricity under controlled reactor conditions, while in natural environments, their activity is likely driven by the reduction of oxygen, or, in its absence, metal oxides or natural organic matter. This study enhances our understanding of methane-oxidizing bacteria's metabolic versatility and underscores their potential in bioenergy applications and methane mitigation strategies.

P013

Reference matters: how genome selection impacts metagenomic profiling accuracy

Jasper van Bemmelen[1], Ioanna Nika[1], Dr Jasmijn Baaijens[1]
[1]TU Delft

Introduction:
Reference genomes play a central role in metagenomic profiling, where the goal is to identify and quantify the organisms present in a metagenomic sample. Due to this reliance on reference genomes, it is crucial to have access to a representative set of reference genomes. As genome databases are expanding rapidly, their sizes are becoming a computational bottleneck. Profiling tools such as Kraken2 and CLARK address this by representing genomes as sketches instead of full sequences, at the expense of accuracy. In contrast, careful selection of representative reference genomes can also reduce runtime and memory usage and potentially even improve profiling accuracy. While some approaches for reference selection have been proposed, the impact on metagenomic profiling accuracy remains unclear.

Methods:
In this work we review approaches for reference selection in metagenomics and perform simulation-based benchmarking experiments to assess the impact of reference selection on metagenomic profiling results. We simulate SARS-CoV-2 viromes, as well as three bacterial metagenomes of increasing diversity. On these datasets, we evaluate abundance estimation errors for existing reference selection approaches in combination with state-of-the-art metagenomic profilers.

Results:
We observe significant differences in abundance estimation accuracy between reference selection approaches. In general, including more genomes improves profiling accuracy up to a certain extent -- including all available genomes (i.e. no reference selection) often gives sub-optimal results. For the SARS-CoV-2 viromes this effect is most pronounced: reference selection leads to substantial improvements in terms of profiling accuracy.

Conclusion:
The impact of reference set selection on abundance estimation results in metagenomic samples has largely been ignored. Our results show that even relatively simple reference set selection methods can improve abundance estimation accuracy, while also reducing runtime and memory usage. This highlights the need for further studies into reference selection for metagenomics, and integration into state-of-the-art metagenomic profiling tools.

P014

Synchronous emergence of Streptococcus pyogenes emm type 3.93 among invasive infections in the Netherlands and England

Dr Matthew Davies[1], B de Gier[2], K Schipper[1], R L. Guy[3], J Coelho[3], A P. van Dam[1], R van Houdt[1], S Matamoros[1], M van den Berg[1], P E Habermehl[1], K Moganeradj[3], Y Ryan[3], S Platt[3], H Hearn[3], E Blakey[3], D Chooneea[3], B J.M. Vlaminckx[4], T Lamagni[3], Prof N M. van Sorge[1]
[1]Amsterdam UMC, [2]National Institute for Public Health and the Environment (RIVM), [3]UK Health Security Agency, [4]University Medical Center Utrecht

A global increase in the incidence of invasive group A streptococcal (iGAS) infections was observed after lifting COVID-19-related restrictions in 2022 with dominance of M1UK in many countries. After seasonal declines in iGAS incidence during the summer of 2023, simultaneous, rapid expansion of a previously rare emm type 3.93 was seen in both England and the Netherlands from November 2023, causing 20% and 60% of all iGAS cases, respectively, within 4 months. emm3.93 was associated with iGAS in children 6-17 years of age and with increased risk of pneumonia/pleural empyema and meningitis in both countries. No significant excess risk of death was identified for emm3.93 compared to other types. Genomic analysis of historic and contemporary emm3.93 isolates revealed the emergence of three new clades with a potentially advantageous genomic configuration. We performed specialised streptococcal proteomics analysis on genetically closely-related emm3.93 strains with and without this genomic configuration. Preliminary data revealed increased expression of surface-expressed and secreted virulence factors including SpyCep, C5A peptidase, streptokinase, streptolysin S and superantigen SpeA, suggesting that this genomic configuration may be underlying the change in epidemiology. Our findings underscore the value of molecular surveillance, including long-read sequencing, in identifying clinical and public health threats.

P015

Nanopore Sequencing for Rapid VRE Outbreak Detection: Evaluating Data Quality Thresholds

Dr Rebekka Koeck[1], Dr S Matamoros[2], Mr E Beuken[1], Dr C Jamin[3], Dr A Bart[4], Prof. Dr P Savelkoul[1], Dr L van Alphen[1]
[1]Maastricht University Medical Centre, [2]Amsterdam University Medical Center, [3]National Institute for Public Health and the Environment (RIVM), [4]Tergooi MC

Introduction: Hospital-associated outbreaks of vancomycin resistant enterococci (VRE) could pose a serious threat to patients, and rapid identification of transmission is critical to ensure timely implementation of containment strategies. Illumina-based whole genome sequencing (WGS) for clonality assessment of all VRE isolates presents challenges relating to batching requirements and lengthy sequencing times, which could potentially be overcome with Nanopore technologies offering more flexible sample pooling and faster processing.

Methods: DNA was extracted from 14 VRE isolates from three Dutch hospitals (MUMC+, AUMC, TergooiMC), using the MasterPure DNA and RNA Purification Kit (Biosearch Technologies) with lysozyme pre-treatment. Sequencing libraries were prepared using the SQK-RBK114.96 kit (ONT) and sequenced on an R10.4.1 flow cell on a GridION sequencer for 72 hours. The data were pre-processed with thresholds: i) minimum read length of 1000 or 2000 base pairs (bp), ii) minimum average quality score of 10, 12 or 15, iii) top 80, 90 or 100% highest quality bps (chopper, filtlong). The pre-processed fastq files were used for species identification (Mash). Assembled genomes (flye, unicycler) were characterised with MLST and AMR gene (NCBI-amrfinderplus) and plasmid (abricate) detection and used to calculate inter-sample SNP distances (SKA).

Results: All generated assemblies had a genome coverage of >77X and genome lengths appropriate for Enterococcus faecium (3.0 to 3.5Mb). The number of contigs per assembly varied greatly depending on the applied pre-processing thresholds and assembler, but in general, removing shorter and lower quality reads yielded less fragmented assemblies. While SNP differences between non-clonal sample pairs differed by up to 440 SNPs depending on the applied pre-processing, this variation was considerably less for clonal sample pairs (maximum 35SNPs).

Conclusion: Once minimum required sequencing time, optimal data quality, and cluster thresholds have been established for this methodology, Nanopore WGS is a promising method for outbreak detection of many bacterial species.

P017

Nation-wide spread of an outbreak-associated IncFIB-multidrug-resistance plasmid, the Netherlands, 2018-2022

Gijs Teunis[1], Dr. WA van der Reijden[2], Dr. D Notermans[1], Dr. CW Ang[2], F Landman[1], S Witteveen[1], A de Haan[1], J Bos[1], S Lansu[1], Dr. L Mughini-Gras[3], Dr. AC Schürch[4], Dr. APA Hendrickx[1]
[1]National Institute for Public Health and the Environment, [2]Comicro, [3]Utrecht University, [4]University Medical Centre Utrecht

Introduction

A clonal outbreak of multidrug-resistant (MDR) Citrobacter freundii sequence type (ST) 22 was reported between 2018-2022 in the Netherlands and detected in the multidrug-resistant microorganisms (MDRO) surveillance. Here we investigated its plasmidome and compared it with other plasmids from the national MDRO surveillance.

Methods

The national dataset comprised of n=1,315 isolates collected in the MDRO surveillance between 2016-2022 by the RIVM. MDRO were sequenced by Illumina and Nanopore sequencing. Complete plasmids (n=4,596) were clustered using mge-cluster. Antimicrobial resistance genes (ARG) and plasmid replicons were identified using ResFinder and PlasmidFinder.

Results

Six clusters of n=191 plasmids were associated with the outbreak of C. freundii ST22 (n=35 isolates) between 2018-2022. Two clusters represent non-mobilisable plasmids (n=65), without ARGs or replicons. Additionally, two plasmid clusters were mobilisable (n=67), one with Col440I and one without replicons. Both were rarely found outside of C. freundii ST22 isolates. Furthermore, one cluster with conjugative IncX3 plasmids carrying blaNDM-5 (n=30) was found associated with the outbreak, a nationally disseminated carbapenemase-carrying plasmid. Lastly, there was one IncFIB plasmid cluster (n=30) conferring predicted MDR to 7-8 antibiotic classes, which was not found prior, nor outside the outbreak for the first 2 years. Within the outbreak, these IncFIB-MDR plasmids show a stable backbone with module gain/loss events and inversions as well as a variable ARG repertoire. Since 2020, the IncFIB-MDR plasmids (n=8) have been found in C. freundii ST11, ST91, ST303, ST523, and ST579 as well as in different hospitals (n=6) in six of 12 provinces. Importantly, in 2022, the IncFIB-MDR plasmid was found within the outbreak hybridised with the blaNDM-5-IncX3 plasmid.

Conclusion

The outbreak enabled an IncFIB-MDR plasmid to establish in the national Citrobacter population. The MDR-plasmid spread is worrisome because of its ability to integrate new ARGs and conjugation systems.

P018

CefiderocolFinder: a pipeline to detect genetic adaptations implicated in cefiderocol resistance in multidrug-resistant microorganisms of Ukrainian patients in the Netherlands

Bryan van den Brand[1], Dr. D. W. Notermans[1], Dr. N. J. Verkaik[2], Dr. ing. A. P. A. Hendrickx[1]
[1]Rijksinstituut voor Volksgezondheid en Milieu (RIVM), [2]Erasmus Medical Centre

Introduction

Since February 2022 the war in Ukraine led to patient relocations to European countries. The national Dutch surveillance for multidrug-resistant microorganisms (MDRO) has received MDRO from Ukrainian patients since the start of the war, including carbapenemase-producing Escherichia coli (CPEC), Klebsiella pneumoniae (CPKP), Pseudomonas aeruginosa (CPPA) and carbapenem-resistant Acinetobacter baumannii (CRAB).

Methods

MDRO (n=98) collected from Ukrainian patients from March to December 2022 were used, including CPEC (n=16), CPKP (n=60), CPPA (n=17) and CRAB (n=5) of which the majority (61/98) encoded a New Delhi metallo-lactamase (NDM-1, n=50 or NDM-5, n=11). All were tested for resistance for cefiderocol with broth microdilution and interpreted according to EUCAST breakpoints. MDRO were sequenced with Illumina and the data was filtered, aligned, variant called with GATK and annotated with snpEff. Annotations were cross-referenced with described genetic adaptations against cefiderocol. CefiderocolFinder was automated in a Snakemake pipeline with high performance computing support.

Results

Cefiderocol resistance occurred in a minority of MDRO (16/98; 16%). Four of 11 distinct adaptations were present in the MDRO, mainly in CPEC. Surprisingly, no adaptations were found in CRAB (1 resistant) and CPPA (3 resistant). Three of 16 CPEC harboured a frameshifted cirA and ten of the 16 CPEC harboured the insertion of YRIN in the ftsI gene, encoding PBP3. In 15/16 (93%) of the CPEC and 2/60 (3%) of the CPKP, the fepA gene harboured a mutated stop codon or frameshift. In three of 16 CPEC the fhuA gene harboured a frameshift. Most (13/18; 72%) of the CPEC/CPKP isolates that had cirA/ftsI/fepA/fhuA adaptations were susceptible. A substitution of serine to asparagine in baeR was found in ten of 60 CPKP isolates in a different location as described in A. baumannii.

Conclusion

CefiderocolFinder has found genetic adaptations in MDRO from Ukrainian patients with phenotypic cefiderocol resistance but also in susceptible isolates.

P023

Rapid detection of antibiotic resistance in direct urine samples

MSc Marit Bot[1,2], J Flipse[1], SLM Bongers[1,2], L Geraets[3], C Giardino[3], JJ Hoogerwerf[4], J ten Oever[4], HFL Wertheim[2], MPA van Meer[1]
[1]Rijnstate, [2]Radboud University Medical Center, [3]PathoFinder BV, [4]Radboud University Medical Center

Background
The rise of antibiotic resistance in Gram-negative bacteria threatens successful treatment of infections, increasing the need for carbapenems. However, an increased need for carbapenems could lead to overtreatment. Improving correct use of carbapenems for patients with a urinary tract infection can be done by rapid identification of extended spectrum beta-lactamase (ESBL) producing bacteria in direct urine samples. In this retrospective study, we determined the diagnostic performance of the Rapid ESBL NP test and a polymerase chain reaction (PCR)-assay.

Methods
A total of 218 urine samples were collected from two Dutch hospitals, of which 67 contained ESBL-producing bacteria, 101 contained non-ESBL-producing bacteria and 50 were culture negative urines. Detection of ESBL-activity was done with the enzymatic Rapid ESBL NP test (Liofilchem, Roseto degli Abruzzi, Italy), which demonstrates hydrolysis of cefotaxime, and the PCR-assay (Pathofinder BV, Maastricht, the Netherlands), which detects CTX-M genes. Both tests were compared to standard culture-based antimicrobial susceptibility testing with subsequent disk-diffusion based confirmation tests when ESBL was suspected. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Results
After excluding the non-interpretable results (7.3%), the Rapid ESBL NP test had a sensitivity, specificity, PPV and NPV of 96.8% (95%CI: 89.0-99.6), 100% (97.4-100.0), 100.0% (94.1-100.0) and 98.6% (94.7-99.6), respectively. All non-interpretable results were cloudy. Additional experiments showed that filtering these urines caused non-interpretable samples to become interpretable. The CTX-M PCR had a sensitivity, specificity, PPV and NPV of 94.1% (71.3-99.9), 91.8% (84.6-96.4), 66.7% (50.5-79.7) and 98.9% (93.1-99.8), respectively.

Conclusions
The Rapid ESBL NP test had a high sensitivity and specificity and may be a suitable rapid screening tool for ESBL-producing bacteria in urine samples. This should be confirmed in a clinical trial. The PCR-assay has a lower specificity. In clinical practice, this could lead to an increased overtreatment.

P024

Evaluation of two rapid immunodiagnostic tests for the diagnosis of human leptospirosis

Dr. Emma de Koff[1], M.C. Koel[2], E.P.M. van Elzakker[1,2]
[1]Amsterdam UMC, [2]Amsterdam UMC

Introduction: Leptospirosis is serologically confirmed by the microscopic agglutination test (MAT) and/or enzyme-linked immunosorbent assay (ELISA). These tests are labor intensive and require specialized laboratories and technicians, which increases the turnaround time. Timely diagnosis of the infection is important for adequate antibiotic treatment as well as source and contact tracing. We tested the clinical performance of two rapid immunodiagnostic tests that detect the presence of anti-leptospiral IgM.

Methods: Leptospira VirCLIA (Vircell, Granada, Spain) and Leptocheck-WB (Zephyr Biomedicals, Goa, India) were performed on 50 sera from confirmed leptospirosis patients (by MAT and/or ELISA; 25 with disease duration ≤10 days and 25 with disease duration >10 days) and 50 sera negative for leptospirosis (by ELISA). Next, we also performed a prospective evaluation of both tests on 300 sera sent to the Expertise Center of Leptospirosis on clinical suspicion of leptospirosis (August-December 2024).

Results: For Leptospira VirCLIA, we found an overall sensitivity of 88% and specificity of 94% on selected sera. The sensitivity was high regardless of disease duration, with a sensitivity of 84% for patients who had been ill ≤10 days and 92% for patients who had been ill for >10 days. For Leptocheck-WB, we found an overall sensitivity of 76% and specificity of 100%. Leptocheck-WB had higher sensitivity for patients with a disease duration ≤10 days than for patients with a disease duration >10 days (92% vs. 60%; chi-squared test, p=0.020), which could be attributed to a prozone effect. Combining both test results, the overall sensitivity increased to 96% with a specificity of 94%. Preliminary findings of the prospective evaluation confirm these results.

Conclusion: The diagnostic accuracy of Leptospira VirCLIA and Leptocheck-WB was high, especially when performed in combination, even at an early stage of the disease. This yields new opportunities to improve the diagnostic workflow of leptospirosis in The Netherlands.

P025

Challenges in blood culture outcome predictions: applying an emergency department approach to the inpatient population

Msc Youssef El Ghouch[1,2], prof.dr. M.C. Schut[3], MSc A.G. den Hollander[2], dr. M. Schinkel[2], prof.dr. P.W.B. Nanayakkara[2], dr. K.C.E. Sigaloff[2], prof.dr. J.M. Prins[2], dr. R.P. Schade[1]
[1]Amsterdam UMC, [2]Amsterdam UMC, [3]Amsterdam UMC

Machine learning models have demonstrated the ability to accurately predict blood culture outcomes, though their focus has primarily been on patients in the emergency department (ED). Most bloodstream infections occur in admitted patients. However, they are commonly found in hospitalized patients. Studies that developed adequate models to predict blood culture outcomes of inpatients are either restricted to specific patient groups, or include community-acquired. In this study, we explored the feasibility of developing a predictive model for a broader population of hospitalized patients, using a similar approach to the one applied in our center's ED, which achieved an AUROC of 0.81.

Blood cultures collected from admitted patients between 2021-2023 at Amsterdam UMC were used. Samples collected in the first 48 hours of the admission were excluded. Blood cultures with the same sampling time were aggregated to one collection moment. We only included the first collection moment of each admission. To predict the outcome, we utilized demographic, laboratory, and vital signs data measured within 24 hours prior to sample collection. We trained an XGBoost model with a stratified 10-fold and performed iterative imputation. Most contributing features were identified with SHAP-values.

We included 2922 data points of which 16% had a positive outcome. The model reached an AUROC of 0.62, specificity of 0.83 and a sensitivity of 0.33. The most contributing variables were the pulse rate (std), systolic blood pressure (std), admission length, number of measurements of leukocytes, and body temperature (max).

With the low AUROC, we can conclude that it is hard for the model to discriminate between positive and negative blood cultures. The main reason is the lack of clinical measurements in admitted patients before a blood sample is collected. As the present approach does not seem feasible in this population, further investigation is required to find a more fitting approach.

P026

Impact of antibody waning on Bordetella pertussis relapse in The Netherlands between 2022 and 2024

Channah Gaasbeek[1,2], Eric Vos[1], Annika van Roon[1], Jesse Waterweg[1], Hester de Melker[1], Rob van Binnendijk[1], Gerco den Hartog[1,3]
[1]RIVM, [2]Erasmus MC, [3]RadboudUMC

After a period of relative absence of Bordetella pertussis from 2020 onwards, notifications increased significantly in 2023 in the Netherlands. We determined whether waning immunity could have contributed to increased susceptibility to infection with B. pertussis.
Between November 2022 and November 2024, 450 age-stratified randomly selected participants of a nationwide longitudinal study (PIENTER-Corona) (age: 2-87 years) donated blood three to five times every six months. Changes in serum IgG antibody concentrations to Pertussis Toxin (Ptx), Filamentous Hemagglutinin (FHA) and Pertactin (Prn) were used as criteria to hallmark waning immunity, while a >2-fold increase of Ptx IgG antibodies between 2 timepoints with resulting concentrations >20 IU/mL indicate a recent infection. All participants filled in questionnaires to collect sociodemographic and symptoms data.
In November 2022, Ptx IgG concentrations were similar across ages, except for children between 7–14 years who showed significant lower concentrations. Conversely, these children displayed significant higher concentrations compared to other ages in November 2024. During the study period, among 194 individuals <18 years, 54 infections (24.7%) were detected and among 256 individuals between 18–87 years six infections (2.3%) were detected. Infected children showed lower concentrations of anti-FHA and anti-Prn IgG antibody concentrations pre-infection compared to children not infected during the study period. Only 30% of the individuals with an infection, reported cough symptoms.
Reduced B. pertussis circulation between 2020 and 2023 likely resulted in decreased boosting of immunity in children following programmatic booster vaccination at 4 years of age, increasing the proportion of susceptible individuals. However, infected individuals experienced mild disease, indicating effective protection against severe disease among participants in the serosurvey despite delayed exposure following waning immunity. The lower number of detected infections in adults in our cohort suggest sufficient protection against severe B. pertussis infection from past vaccination schedules together with a boost from infections.

P027

Guanidino lipoglycopeptide antibiotic EVG7 prevents recurrent Clostridioides difficile infection by sparing members of the Lachnospiraceae Family

Dr. E. Mons[1], Dr. Jannie G.E. Henderickx[2], I.M.J.G. Sanders[3], C.E. Perkins[4], Dr. W.K. Smits[2,3], Dr. C.M. Theriot[4], Prof. Dr. N.I. Martin[1]
[1]Leiden University, [2]Leiden University Medical Center, [3]Leiden University Medical Center, [4]CVM NC State University

Oral vancomycin effectively treats active Clostridioides difficile infection (CDI), yet is associated to recurrence by disrupting indigenous gut microbiota. Antibiotics are needed that target C. difficile while sparing other gut microbiota members. Here we show that a new semisynthetic vancomycin derivative, EVG7, prevents recurrent CDI in a murine model by sparing members of the Lachnospiraceae family.

C57BL/6J mice received oral gavage with C. difficile spores after cefoperazone pre-treatment. From days 4–9, mice were treated with vancomycin (0.4 mg/mL) or EVG7 (0.4 mg/mL) in drinking water. Weight loss and disease severity were monitored during the experiment. Bacterial enumeration and 16S rRNA Illumina sequencing were performed on fecal pellets (days -7, 0, 4, 9, 14, 18) and cecal content (necropsy day; vancomycin on day 14/15, EVG7 on day 18). Cecal content samples were additionally used in toxin activity testing.

The gut microbiota had significant dissimilarity between the two treatment groups (Permanova, P = 0.001). Compared to vancomycin-treated mice, the cecal content of EVG7 mice had significantly higher Shannon diversity (P = 0.0001) and Chao1 richness (P = 0.0009). Furthermore, lower relative abundance of the C. difficile amplicon sequence variant (P = 0.0001) supported reduced C. difficile CFUs and toxin activity in cecal content of EVG7 mice. Lachnospiraceae family members were more abundant in cecal content of EVG7 mice compared to vancomycin-treated mice. Longitudinal analysis revealed a similar increase of Lachnospiraceae in feces during and after EVG7 treatment, while there was a strong decrease during vancomycin treatment.

Altogether, these data indicate that Lachnospiraceae were spared during and after EVG7 treatment in a recurrent CDI murine model. Since Lachnospiraceae are associated with resistance against C. difficile colonization and are protective against CDI, EVG7 may warrant further investigation as an alternative to oral vancomycin therapy for the treatment of CDI and prevention of its recurrence.

P028

Prevalence of oxacillin-susceptible MRSA in clinical specimens

Phd Roel Nijhuis[1], Thijme Rispens[1], MD, PhD Damian Melles[1]
[1]Meander Medical Centre

Introduction

MRSA can be responsible for difficult-to-treat infections as well as for outbreaks within healthcare settings. Less known, but as important, is the so-called 'stealth MRSA': oxacillin-susceptible mecA-positive S. aureus (OS-MRSA). Oxacillin-susceptibility in OS-MRSA is often the result of mutations in the mecA gene, leading to loss of function of this gene. Remarkably, the mutation can be reversible with antibiotic pressure, turning OS-MRSA into MRSA. In routine diagnostics, OS-MRSA will not be detected as mecA PCR is not tested on all oxacillin-susceptible S. aureus isolates. The prevalence of OS-MRSA varies per study, but prevalence's of ~1% to >10% are reported. The aim of this study was to determine the prevalence of OS-MRSA in clinical specimens in our setting in the Netherlands.

Methods

All S. aureus isolates identified from clinical specimens were tested for methicillin resistance by cefoxitin disc diffusion and evaluating the cefoxitin screen and flucloxacilline MIC as determined by Vitek2 (Biomerieux). Five hundred methicillin susceptible isolates were included in the study and tested for the presence of the mecA-gene by real-time PCR, using pools of 10 isolates to determine the possible presence of OS-MRSA. If the mecA-gene would be detected in a pool of isolates, each isolate was tested separately in the mecA PCR.

Results

From November 2023 to June 2024, 500 isolates were included from 498 patients (51.4% male). Most specimens were obtained within the hospital (54.0%), followed by the general practitioner (40.8%). None of the pooled specimens tested positive in the mecA-gene real-time PCR used at our setting.

Conclusion

OS-MRSA can be of risk for both the individual patient and healthcare settings, as this kind of MRSA cannot be easily detected using routine diagnostic procedures. At our setting in the Netherlands, we evaluated the prevalence of OS-MRSA in clinical specimens and showed that no OS-MRSA was found.

P029

Laboratory diagnosis of Lyme borreliosis: a comparison of three anti-Borrelia antibody assays

Drs R Janssen[1], drs F Bosma[2], ing I Polman[2], dr F Geeraedts[2], Dr. Annelies Riezebos-Brilman[2]
[1]Maastricht University, [2]Labmicta

Introduction:
Routine serodiagnosis of Lyme borreliosis (LB) relies on two-tier testing (TTT) using a sensitive screening assay and confirmation with specific IgG/M immunoblots. Our currently used ELISA was compared with two alternative open access screening assays, to improve accessibility. The pre-set requirements included comparable sensitivity and specificity.

Methods:
A selection was made from diagnostic sera stored at -20°C, including cases with early and late LB (n=75) and controls clinically not suspected to have LB (n=40). Early localized LB cases were diagnosed with erythema migrans (EM; n=40) or lymfocytoma (n=2) and disseminated/late LB cases included acrodermatitis chronica atrophicans (n=6), arthritis (n=9), cardiologic manifestations (n=9), neuroborreliosis (n=7), uveitis (n=2). All sera were tested for IgG and IgM with LIAISON Borrelia IgG/M II (LXL) and Borrelia VirClia Monotest IgG/M (Virclia) and results were compared with routine diagnostic test results, using Serion ELISA classic Borrelia burgdorferi IgG/M (Serion) for LB screening and Microgen recomLine Borrelia IgG/M for confirmation. Positive outcome of LB screening was defined as an indeterminate/positive IgG and/or IgM result in the screening assay.

Results:
Sensitivity of LB screening using LXL and Virclia was respectively 92.9% and 97.6% in early localized LB, and respectively 100% and 97% in late LB. In the control group with negative LB screening using Serion, 20/20 sera were negative for both IgG and IgM in LXL and 17/20 showed negative LB screening using Virclia, including 1 repeatedly aborted test-result. In the control group with positive LB screening in Serion and negative immunoblotting results, LB screening with LXL and Virclia was negative in respectively 14/20 sera (70%) and 12/20 sera (60%).

Conclusion:
For late LB manifestations LXL and Virclia showed similar performance compared to the current assay, however in early localized LB both assays were slightly less sensitive, with an improved specificity compared to the Serion assay.

P030

Validation of a β-glucuronidase activity assay and its association with gut microbiome profiles in colorectal cancer patients undergoing irinotecan-based systemic therapy

E. Russ[1,2], L.E. Hillege[1,2], Dr. J Ziemons[1,2], M.A.M. Stevens[1,2], E. de Jong[2,3], A. Lijnen[4], L Hutchinson[5], D.E. Kok[6], Dr. L. Valkenburg-van Iersel[1,7], Prof. dr. M.R. Redinbo[5], Prof. dr. J. Penders[4,8,9], Prof. dr. M.L. Smidt[1,2]

[1]GROW - Research Institute for Oncology and Reproduction, Maastricht University, [2]Maastricht University Medical Center+, [3]Catharina Hospital, [4]Maastricht University Medical Center+, [5]University of North Carolina, [6]Wageningen University & Research, [7]Maastricht University Medical Center+, [8]NUTRIM - Institute of Nutrition and Translational Research in Metabolism, Maastricht University, [9]Euregional Microbiome Center

Irinotecan-based systemic therapy is commonly used in metastatic colorectal cancer (CRC) patients. However, late-onset gastrointestinal (GI) toxicity is a common and considerable drawback, which may be linked to gut microbial β-glucuronidase (β-GUS) activity. Specific β-GUS enzymes reactivate the irinotecan metabolite SN-38G into the cytotoxic SN-38, leading to mucosal damage and diarrhea. This study aims to 1) validate a β-GUS enzyme activity assay for quantifying SN-38-specific β-GUS activity in a clinical setting and 2) investigate the relationship between β-GUS activity and the gut microbiome's diversity, composition, and functional capacity in CRC patients.

Fecal samples were collected from CRC patients before treatment initiation in the context of on-going cohort studies at Maastricht University and Wageningen University. A total of 180 samples were pre-processed into fecal lysates and normalized to a protein concentration of 1 mg/mL using a BCA protein assay. Subsequently, β-GUS activity was measured using an SN-38-specific enzyme activity assay. In addition, metagenomic shotgun sequencing (Illumina NovaSeq) was performed to quantify gut microbiota diversity, composition, functional capacity and the presence of SN-38-specific β-GUS variants.

Using the enzyme activity assay, SN-38G-specific β-GUS activity was quantified based on fluorescence and using SN-38G as substrate. Based on this, patients with high bacterial β-GUS activity could be identified. Patients with elevated β-GUS activity are hypothesized to exhibit distinct gut microbiome profiles, characterized by specific β-GUS enzyme variants.

The β-GUS activity assay would be a valuable tool for clinical studies investigating the relationship between fecal β-GUS activity and late-onset GI toxicity in irinotecan-treated patients. Integrating β-GUS activity data with metagenomic shotgun sequencing may reveal distinct gut microbiome profiles and SN-38-specific β-GUS variants in patients with elevated activity. This combined approach could enhance understanding of microbiome-related toxicity mechanisms, supporting the development of predictive biomarkers and personalized interventions to improve irinotecan therapy.

P031

Development of an optimized in vitro Staphylococcus aureus biofilm model with relevance to prosthetic joint infections

Lydia Speijker[1,2], Dr. J. Dingemans[1,3], Dr. B. Cillero-Pastor[4], Prof. Dr. J.J. Arts[2,5], Prof. Dr. P.H.M. Savelkoul[1,2], Dr. I.H.M. Van Loo[1,2]
[1]Maastricht University Medical Centre +, [2]Maastricht University Medical Centre +, [3]Jessa Ziekenhuis Vzw, [4]Maastricht University, [5]Maastricht University Medical Centre+

Introduction: Prosthetic joint infections (PJI) occur in 1-2% of total prosthetic joint replacement patients and are often linked to biofilm formation on implant surfaces. Biofilms are difficult to eradicate due to enhanced antibiotic tolerance, necessitating additional surgeries. More insights into biofilm characteristics regarding antibiotic tolerance are therefore needed. However, currently there are no adequate biofilm models for antimicrobial susceptibility testing in a PJI context. Our aim was to develop an optimized in vitro Staphylococcus aureus biofilm model using titanium discs, emphasizing maximal surface attachment and biofilm biomass.

Methods: American Type Culture Collection (ATCC) bacterial strain S. aureus ATCC25923 was cultivated at 37oC on plasmapore-coated titanium discs in 12-wells plates for 24 hours (24h), 48 hours (48h), 72 hours (72h), and 7 days (7d) in tryptic soy broth, shaking at 25 revolutions per minute. Viable bacterial growth was assessed by colony forming unit (CFU) counts. Biomass was quantified using safranin staining, measuring optical density at 540 nanometers. Scanning electron microscopy (SEM) visualized biofilm surface coverage in µm².

Results: Maximum biomass accumulation occurred at 24h (OD540: 0.079, CFU: 1.2×108/ml, SEM: 1046.3 µm2). Subsequently, biomass decreased by 27.9% at 48h, slightly recovered at 72h (2.5%), then declined by 59.5% at 7d. CFU counts showed reductions of 66.7%, 74.2%, and 90.0% at 48h, 72h, and 7d, respectively compared to 24h. SEM revealed surface area reductions of 68.3% (48h), 88.3% (72h), and 69.0% (7d) compared to 24h. These results suggest dynamic biofilm development, with maximal attachment of biofilm observed at 24h followed by gradual degradation, despite biomass fluctuations.

Conclusion: Optimal surface attachment and biofilm biomass was observed at 24h, followed by decline. Our model provides foundation to study antibiotic susceptibility differences between biofilms and planktonic bacteria in a PJI context. Future experiments will assess other surfaces and incorporate molecular biomarkers to determine biofilm growth stages.

P032

Prebiotic fiber mixture modulates ex vivo gut microbiota in fecal samples derived from colorectal cancer patients undergoing capecitabine therapy

M.A.M. Stevens[1,2], L.E. Hillege[1,2], Dr. J. de Vos - Geelen[1,3], Dr. L. Valkenburg - van Iersel[1,3], Prof. dr. J. Penders[4,5,6], Dr. D.J.M. Barnett[4,6], Dr. K. Venema[5,7], L.H.J. Dopheide[8], Dr. G. Roeselers[8], Dr. ir. A. van Helvoort[4,8], Prof. dr. M.L. Smidt[1,2], Dr. J. Ziemons[1,2]
[1]GROW - Research Institute for Oncology and Reproduction, Maastricht University, [2]Department of Surgery, Maastricht University Medical Center+, [3]Department of Internal Medicine, Division of Medical Oncology, Maastricht University Medical Center+, [4]NUTRIM - Research Institute of Nutrition and Translational Research in Metabolism, Maastricht University, [5]Euregional Microbiome Center, [6]Department of Medical Microbiology, Infectious Diseases and Infection Prevention, Maastricht University Medical Center+, [7]Centre for Healthy Eating & Food Innovation, Maastricht University - Campus Venlo, [8]Danone Research & Innovation

Introduction: Advanced colorectal cancer (CRC) is commonly treated with 5-fluorouracil (5-FU) or its prodrug capecitabine, which might affect the gut microbiota and its metabolites. These changes may induce or exacerbate microbial dysbiosis, potentially impacting treatment efficacy and chemotherapy toxicity. Our previous in vitro research has shown that prebiotic fiber mixtures can counteract 5-FU-induced microbiota changes in samples derived from healthy participants. As a next step, we aimed to investigate whether a prebiotic fiber mixture can also counteract 5-FU-induced dysbiosis in fecal samples from CRC patients during capecitabine chemotherapy.
Methods: Fecal samples from four colorectal cancer patients undergoing their third capecitabine cycle were collected and pre-processed anaerobically. Samples were inoculated into the TIM-2 model (validated and computer-controlled TNO in vitro model of the colon), maintained on standard nutrition for 16 hours, and subjected to a 72-hour intervention period. The gut microbiota was fed either standard nutrition alone or supplemented with a prebiotic fiber mixture containing galacto-oligosaccharides, fructo-oligosaccharides, and low-viscosity pectin. Longitudinal lumen (reflecting the colon) and dialysate (reflecting absorbable metabolites) samples were analyzed. Changes in luminal gut microbiota composition were assessed by 16S rRNA gene amplicon sequencing. Cumulative levels of short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs) in the lumen and dialysate were quantified using gas chromatography-mass spectrometry. In addition, functional microbiota profiling will be performed through metagenomics sequencing, metabolite analysis and enzyme activity assays.
Results: The prebiotic fiber mixture modulated capecitabine-treated gut microbiota, promoting the growth of potentially beneficial bacteria, such as Lactobacillus and Bifidobacterium. The concentrations of SCFAs, acetate and butyrate, were higher in prebiotic fiber mixture treated fecal samples compared to untreated samples, whereas the levels of BCFAs were reduced.
Conclusion: Prebiotic fiber mixtures might serve as a promising strategy to promote the growth of beneficial bacteria and mitigate microbial dysbiosis induced by 5-FU-based chemotherapy.

P033

Failure to detect Streptococcus pneumoniae due to false negative PCR results caused by a large insertion in the Spn9802 fragment

Dr. Myrthe Toorop, Margriet E.M. Kraakman[1], Dr. Eric C.J. Claas[1], Dr. Els Wessels[1], Dr. Stefan A. Boers[1]
[1]Leiden University Medical Center

Introduction:

Misidentification of Streptococcus pneumoniae and Streptococcus pseudopneumoniae has been largely addressed with introducing a duplex real-time PCR including a S. pneumoniae specific lytA PCR and a PCR targeting the Spn9802 region. Our laboratory at the Leiden University Medical Center (the Netherlands) identified 107 positive S. pneumoniae duplex PCR tests between 2017-2024 in clinical strains. Interestingly, two S. pneumoniae strains tested positive for lytA, but negative for Spn9802 (sequence type 19331). Additionally, two S. pneumoniae strains from a neighboring hospital exhibited the same PCR characteristics (sequence type 15063). In this study, whole genome sequencing (WGS) was performed to investigate the cause of the Spn9802 PCR negative results.

Methods:
DNA was extracted from the four cultured S. pneumoniae strains using the Easy-DNA gDNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced on a PromethION Flow Cell (R10.4.1) in accordance with SQK-NBD114 sequencing procedures (Oxford Nanopore Technologies (ONT), Oxford, UK). FastQ-formatted sequences were extracted , processed, and analyzed using Flye (version 2.9.3) and Geneious (version 10.2.6).

Results:
The WGS results revealed an 876 bp insertion within the Spn9802 fragment, explaining the false-negative Spn802 PCR results. The insertion sequences showed high sequence similarity among the strains with a maximum difference of only a single nucleotide. In response to this finding, a new PCR primer was developed and implemented in the diagnostic duplex PCR. The updated PCR successfully detected all four S. pneumoniae strains that previously tested negative for the Spn9802 target.

Discussion:
These findings are relevant for S. pneumoniae diagnostics worldwide, since the insertion can lead to false-negative PCR results when single-target PCR tests (only Spn9802 target) are used. Awareness among (molecular) microbiologists about the existence of this variant containing the Spn9802 insertion, and the use of PCR assays that target at least two different genes, may overcome this risk.

P034

Waiting for follow-up blood cultures is not always needed for diagnosis of line-associated bloodstream infections

Dr. Lieke van Balen[1], Dr. R.M. Koeck[1]
[1]Maastricht UMC+

Introduction
When blood cultures grow coagulase-negative staphylococci (CNS) in patients with a central venous catheter (CVC), strategies for distinguishing central line-associated bloodstream infection (CLABSI) from contamination include taking follow-up blood cultures or using the number of bottles as an indicator. In this study, we retrospectively compared these two strategies in haematology patients.

Methods
In our hospital, standard care for febrile neutropenia consists of taking 4-8 blood culture bottles (from the line and by venipuncture), followed by starting betalactam antibiotic therapy. If a blood culture grows CNS, follow-up cultures are taken and possible CLABSI is diagnosed when at least 1 follow-up bottle grows the same species. We collected data from patients with a CVC on the haematology ward from 2019 to 2024. Patients were included if they had at least one positive blood culture from a series of at least 4 bottles (2 sets) taken within an interval of 4 hours, and had follow-up cultures taken within 72 hours after the first series. Presence or absence of CLABSI was categorized using the criteria of the Dutch CLABSI registry (PREvalentie van ZIEkenhuisinfectieS; PREZIES) and the clinical diagnosis from the patient record.

Results
We included 76 patients. The number of positive bottles was significantly related to both PREZIES, the clinical diagnosis and the standard of care method (Chi-square p-values for all three: $p<0.001$). Using a cutoff of 4 or more positive bottles, sensitivity for PREZIES CLABSI was 89% compared to 86% with follow-up cultures, with specificities of 88% (4 positive bottles) and 78% (follow-up cultures). Sensitivity for clinical CLABSI diagnosis was 82% with at least 4 positive bottles and 84% for follow-up cultures, with specificities of 100% (4 positive bottles) and 94% (follow-up cultures).

Conclusion
If 4 or more blood culture bottles are positive, then follow-up cultures are not needed for diagnosing CLABSI.

P035

Invasive pulmonary Arthrographis kalrae infection with cerebral abscesses in an immunocompromised child.

Dr. Beatrice Winkel[1], Dr J. Jans[1,2], Dr M Luesink[3], Prof. Dr. R.J. Nievelstein[6], Dr J. Buil[4], Prof. Dr. F. Hagen[5], Dr. T.F.W Wolfs[1,2], Dr. J.T. van der Bruggen[1]
[1]Umc Utrecht, [2]Wilhelmina Children's hospital/UMC Utrecht, [3]Princes Máxima Center for pediatric oncology, [4]Radboud University Medical Center, [5]Westerdijk fungal biodiversity institute, [6]Wilhelmina Children's hospital/UMC Utrecht

Arthgrographis kalrae is an arthroconidial hyaline fungus found in soil and is considered to have low virulence. It has rarely been implemented in opportunistic infections in immunocompromised individuals. To date, only 17 cases of A. kalrae infection have been reported.

We report a case of invasive A. kalrae infection in a 3-month-old boy with basophilic acute myeloid leukemia undergoing chemotherapy. The patient suffered persisting neutropenic fever despite broad antibiotic treatment. High-resolution computed-tomography scan of the lung showed a large nodular consolidation with irregular borders but no typical halo sign. However, both serum and broncho-alveolar lavage (BAL) samples were positive for galactomannan and BAL samples grew A. kalrae. No Aspergillus species were cultured. Although neurological symptoms were absent, routine magnetic resonance imaging of the brain showed two ring enhancing lesions in the brain parenchyma suspicious of small abscesses. The patient was treated with antifungal double therapy consisting of liposomal amphotericine-B 5mg/kg and isavuconazole. Despite this treatment cerebral lesions increased in volume and serum galactomannan remained high. Resection of the lesions was necessary to attempt cure. Fungal culture of resected brain tissue also grew A. kalrae.
To test wether A. kalrae could be the cause of the positive galactomannan results, we tested ten strains and found nine culture supernatants tested positive in the galactomannan assay. Susceptibility testing of the A. kalrae strain of this patient and 11 additional (non-invasive) clinical A. kalrae strains by broth microdilution according to EUCAST method showed low MIC's for azoles, liposomal amphotericine-B, olorofim, fosmanogepix and terbinafine. High MICs were found for flucytosine and MICs for echinocandins were variable.

We report the first case of invasive pulmonary A. kalrae infection with cerebral abscesses. This case underscores the importance of considering rare fungal pathogens such as A. kalrae in differential diagnoses, particularly for immunocompromised patients presenting with atypical infections.

P036

Fulminant Rothia mucilaginosa meningitis and ventriculitis in immunocompromised patients- a case series

Dr. Beatrice Winkel[1], Dr. B.F. Goemans[3], Dr. I.M. van der Sluis[3], Dr. J. Stutterheim[3], Dr. L.G.M. Daenen[4], Dr. M.A. de Witte[4], Dr M. van der Flier[2], Dr. T.F.W. Wolfs[2], Dr. J.T. van der Bruggen[1]
[1]UMC Utrecht, [2]Wilhelmina's Children's hospital/UMC Utrecht, [3]Princess Maxima Center for paediatric oncology, [4]UMC Utrecht

Rothia mucilaginosa is a Gram-positive coccus found in human upper airways. It rarely causes invasive disease but poses risk in immunocompromised patients where it can cause opportunistic infections. Rothia mucilaginosa associated meningitis is rare, only a few cases have been reported in literature. To date, evidence-based treatment guidelines are lacking.

We report a case series of six immunocompromised patients -four children and two adults- diagnosed with Rothia mucilaginosa meningitis. Underlying hematological illnesses varied. Cerebral imaging showed cerebral bleeding and ventriculitis in all patients. Five strains were found susceptible in vitro to amoxicillin (Minimal inhibitory concentrations (MIC) 0,016-0.5 mg/L). One strain had an MIC for amoxicillin of 2 mg/L and was considered resistant. Treatment regimens used varied. Among regimens used were high dose amoxicillin in combination with rifampicin and intrathecal vancomycin. Three patients received an external ventricular drain and on one patient craniotomy was performed to evacuate empyema. Perioperatively or upon autopsy extensive mucinous depositions were seen in the brain. Mortality was high; four of the six patients died, one child survived without serious sequelae and one child is currently still being treated.

Rothia mucilaginosa has the potential to cause fulminant meningitis and ventriculitis in immunocompromised patients. We present six cases of Rothia mucilaginosa meningitis over the past five years. Notably, in four instances Rothia mucilaginosa bacteremia preceded the onset of meningitis by 3 days to 5 weeks. During this period of five years, we recorded 58 cases of Rothia mucilaginosa bacteremia in total. Currently, we do not know which bacteremic immunocompromised patients are most at risk and may develop central nervous system infection. Therefore, treating physicians should be aware of the possibility of Rothia mucilaginosa meningitis/ventriculitis in immunocompromised patients. A high index of clinical suspicion is warranted especially when a blood culture has become positive in this patient population.

P043

Mycoplasma genitalium molecular typing in men discriminates between phylogenetic clusters based on sexual preference and antibiotic resistance

Nikki Adriaens[1,2], Fenna Bouwman[1], Sylvia Bruisten[1,2], Clarissa Vergunst[1,4], Alje van Dam[1,2,3], Tessa Doelman[2,3], Brenda Westerhuis[1,2]
[1]Public Health Service Of Amsterdam, [2]Amsterdam institute for Immunology and Infectious diseases, [3]Amsterdam UMC location University of Amsterdam, [4]NWZ

Introduction. The rising antibiotic resistance in Mycoplasma genitalium has become a public health concern, requiring close surveillance. mgpB/MG309 typing is commonly used to study genotype distribution and resistance patterns of M. genitalium in men who have sex with men (MSM), however data for men who have sex with women (MSW) is limited. This study aimed to compare mgpB/MG309 typing of M. genitalium isolates between MSM and MSW, examining links between genotypes, antibiotic resistance, and epidemiological factors.
Methods. Previously collected M. genitalium isolates from men diagnosed with urethritis between May 2018 and November 2019 wereanalyzed. Molecular typing was performed by sequencing relevant regions of the mgpB and MG309 loci. Macrolide resistance was assessed by detecting mutations in the 23S rRNA gene via qPCR, while fluoroquinolone resistance was determined through sequencing the parC and gyrA genes.
Results. A total of 62 M. genitalium samples were analyzed from 33 MSM and 29 MSW using mgpB/MG309 typing. At the mgpB locus, 24 sequence types (STs) were identified, with ST4 being most prevalent in MSM and ST2 in MSW. The MG309 locus revealed 12 distinct numbers short tandem repeat numbers, with repeat number 10 most common in both groups. Phylogenetic analysis based on mgpB sequences revealed two clusters. Cluster A included more MSW, whereas cluster B was predominantly MSM ($P < 0.001$). Resistance to macrolides and fluoroquinolones was significantly higher in cluster B ($P < 0.01$ and $P < 0.05$, respectively), including all 13 dual-resistant isolates ($P < 0.01$).
Conclusion. Molecular typing of M. genitalium isolates revealed two clusters differing by sexual preference and antibiotic resistance, highlighting the importance of surveillance of resistance across genotypes. The findings support the hypothesis of multiclonal spread of resistance through independent mutations. Next-generation sequencing is needed to further explore the links between genetic diversity and antibiotic resistance in M. genitalium.

P044

Successful implementation of PCR directed Neisseria gonorrhoeae culture: results from a large 5-year prospective study

Drs Merel Boderie[1], Dr. H.A. van Dessel[1,2], Dr. N.H.T.M. Dukers-Muijrers[2,3,4], Dr. C.J.P.A. Hoebe[1,2,4,5], Dr. L.T.J. Levels[6], Dr. I.H.M. Van Loo[1,2], Dr. P. Wolffs[1,2]
[1]Maastricht UMC, [2]Maastricht University, [3]Public Health Service South Limburg, [4]Maastricht University, [5]Maastricht University, [6]Limburg North Public Health Service

Introduction: Due to the growing emergence of multidrug resistant Neisseria gonorrhoeae (NG) worldwide, it is important to monitor antimicrobial resistance (AMR) data of this difficult to culture microorganism. NG culture recovery rates depend on the bacterial load, by proxy of the cycle threshold (Ct) value obtained by quantitative polymerase chain reaction (qPCR), irrespective of the anatomic site of infection. In this 5 year prospective study we have implemented the use of qPCR data from routine NG diagnostics with the aim to optimize the NG culture recovery rate.
Methods: Data were obtained from routine qPCRs on COBAS 4800 (Roche diagnostics) for detection of NG and from corresponding NG cultures performed between January 2017 and December 2021 for two sexually transmitted infections (STI) clinics in Limburg, The Netherlands. STI clinic 1 was advised to only culture an anatomic site of infection when the qPCR Ct value was ≤ 32. STI clinic 2 continued culture practice as usual.
Results: 2498 NG qPCR positive samples were analyzed. NG culture was performed in 686 (27%) of these samples, of which 227 were positive (mean culture recovery rate 33%). The median Ct value of samples sent by STI clinic 1 and 2 were 28.9 (IQR 2.8) and 32.5 (IQR 8.1) respectively. STI clinic 1 sent in 344 of their 1814 (19%) NG qPCR positive samples for NG culture of which 180 were positive (culture recovery rate 52%). STI clinic 2 sent in 342 of their 684 (50%) NG qPCR positive samples for NG culture of which 47 were positive (culture recovery rate 14%). Results do not include the potential impact of transport times.
Conclusion: This 5-year study demonstrated that routinely obtained qPCR data can be used to maximize culture effectivity by guiding which samples to submit for NG culture and subsequent AMR testing.

P045

PERFORMANCE OF A NOVEL MOLECULAR TEST IN CULTURE-NEGATIVE PERIPROSTHETIC HIP AND KNEE JOINT INFECTIONS

Drs Tessel Sligting[1], Dr. Martine Bos[1], Dr Susana Gardete-Hartmann[2], Dr Sujeesh Sebastian[2], Dr Sofia Berdalli[2], dr Sebastian Simon[2], Prof dr Jochen Hofstaetter[2]
[1]InBiome, [2]Michael Ogon Laboratory for Orthopaedic Research

Introduction: Unexpected negative cultures (UNCs) present significant diagnostic challenges in periprosthetic joint infections (PJI). The Molecular Culture®-ID (MC-ID) test is an innovative diagnostic tool that identifies a wide range of bacterial species in a single test. This retrospective study aimed to assess MC-ID performance in detecting the microbial content of synovial fluid (SF) and matching tissues collected from hip and knee revision arthroplasty with UNCs.
Method: A total of 81 UNCs cases from 63 patients who underwent revision hip and knee arthroplasty were included. Based on the European and Bone Joint Infection Society (EBJIS) criteria, 78 cases were classified as infected and three as likely infected, but infected by clinical observation. MC-ID was performed in SF from 81 cases and matching tissues of 42/81 of these cases.
Results: Overall, 33.3% (27/81) of UNCs cases yielded positive detections, of which 2/27 (7.4%) had two microorganisms identified simultaneously. Microorganisms were found in either 13/27 SF only or 4/27 tissue(s) only, and in 10/27 cases both tissue and SF had matching microorganisms. In 51.8% (14/27) cases, the microorganism(s) detected would have been clinically relevant after re-evaluation of the patient's microbiological history. The prevalence of the 29 species identified in the positive cases was 20.7% (6/29) Cutibacterium acnes, 17.2% (5/29) Streptococcus pneumoniae/mitis, 10.3% (3/29) each of Streptococcus agalactiae and Staphylococcus epidermidis, 5.1% (2/29) each of Haemophilus parainfluenzae, Staphylococcus aureus, and Streptococcus bovis, and 3.4% (1/29) each of Streptococcus dysgalactiae, Enterococcus faecalis, Staphylococcus lugdunensis, Corynebacterium striatum, Bacteroides fragillis, and Corynebacterium coyleae. In total, 23/27 (85%) cases were associated with patients receiving antibiotic therapy at the time of SF collection. Overall, 23/63 (23.3%) of UNCs patients could have benefited from alternative management based on the MC results.
Conclusions: The Molecular Culture®-ID test offers clinical value in diagnosing and managing PJI in cases with unexpected negative cultures.

P046

Evaluation of pertussis PCR assays: differentiating Bordetella species and managing equivocal results

Milou Gerits[1], PhD RHT Nijhuis[2], MD, PhD SFH Raven[1], MD, PhD A Russcher[2]
[1]Public Health Service Region Utrecht, [2]Meander Medical Centre

Introduction: Pertussis, primarily caused by Bordetella pertussis (Bp) and less often by B. parapertussis (Bpp), can cause severe disease in risk groups for which chemoprophylaxis is indicated. Diagnostics include PCR, which often target IS481 for detection of Bp, and may be used in combination with other targets such as IS1001 (present in Bpp) and IS1002 (absent in B. holmesii, present in low numbers in Bp) to differentiate from other Bordetella species. This study evaluates (1) available PCR assays for differentiating Bordetella species, (2) the prevalence of non-pertussis Bordetella species, and (3) optimal handling of equivocal PCR results.

Methods: PubMed was searched to identify studies evaluating pertussis PCR assays. Additionally, results of pertussis diagnostics including PCR targets IS481, IS1001, and IS1002, performed at Meander Medical Center (July 2021 until November 2024), were evaluated to assess the frequency and characteristics of IS481-positive results. A positive and weak-positive Bp result were defined as a positive result for both IS481 and IS1002, and a positive IS481 result with a Ct-value ≥ 30 in absence of IS1002, respectively.

Results: Literature review showed that 14 of 19 included studies used the pertussis toxin gene alongside IS481, while most others used IS481 in combination with IS1002 (5/19) to confirm presence of Bp. Besides Bp, a low number of B. holmesii (0.8%, IQR 0.0-6.1, 7/13) and B. bronchiseptica (0.3%, IQR 0.0-1.1, 4/6) was identified across the studies.
At Meander, 193 of 1096 tests were positive for Bp and 66 tested weak-positive. No Bpp was detected.

Conclusion: B. pertussis remains the dominant cause of pertussis as shown in literature and local data. Evaluating the Meander results showed that infections by other Bordetella species were extremely rare. PCR assays targeting IS481 and IS1001 currently provide sufficient diagnostic accuracy, with minimal risk of B. holmesii cross-reactivity due to its low prevalence.

P048

Genome Wide Next-Generation Sequencing for determining Helicobacter pylori Antimicrobial Resistance

Drs. Huyveneers[1,2], Dr. R.R. Jansen[2], Dr. W.A. van der Reijden[2,3]
[1]St Antonius Hospital, [2]OLVG Lab, [3]Comicro

Helicobacter pylori is a spiral-shaped gram-negative bacteria that infects the gastric and duodenal mucosa and its chronic inflammation can lead to gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Susceptibility-based treatment is limited by the need of invasive gastric biopsies for culturing, and the fastidious nature of the bacteria. We investigated the potential of next-generation sequencing (NGS) to determine the antibiotic resistance in H. pylori. Strains isolated from gastric biopsies with known antimicrobial susceptibility, determined with E-test, were whole-genome sequenced using Illumina technology. Ridom Seqsphere (8.5.1) was used for trimming (Trimmomatic 0.36), quality control (FastQC 0.11.7) and de novo assembly (Velvet 1.1.04). The assembled genomes were aligned to genes (reference strain AE000511.1) associated with resistance for clarithromycin (23S rRNA) levofloxacin (gyrA, gyrB), metronidazole (rdxA, frxA), amoxicillin (pbp1A) and tetracycline (16S rRNA). A total of 20 isolates were examined for mutations. Resistance-associated mutations were detected in 92.3% (12/13) of clarithromycin-resistant strains (A2142G, A2143G) and 80.0% (4/5) of levofloxacin-resistant strains (N87K/I/Y, D91G/N/Y). In 50.0% of the metronidazole-resistant strains (5/10) a severe mutation in de rdxA gene was present (R16H/C, nonsense mutations, insertions/deletions). Among two amoxicillin-resistant strains, one (50.0%) harbored a resistance-associated mutation (N562Y). No mutations in the A926G-A928C region were identified in the only tetracycline-resistant strain tested. Other single nucleotide polymorphisms (SNPs) or combinations of SNPs might be involved in phenotypical resistance, although these cannot be determined by this number of genomes and concomitantly more phenotypically characterized strains has to be included in further studies. Nevertheless, these findings demonstrate that NGS is a promising technique for screening antibiotic resistance in H. pylori, especially when this technique can be optimized as a target captured sequencing technique for non-invasive samples, like stool, vomitus or saliva.

P049

Evaluation of five real-time PCR assays for detection of Candida auris.

Thijme Rispens[1], P.C.R. Godschalk[1], R.H.T. Nijhuis[1]
[1]Meander Medical Center

Introduction

Candida auris is a yeast that can cause invasive infections and hospital outbreaks. In the Netherlands, the prevalence of Candida auris is low, but outbreaks have been reported worldwide. Recently, the Dutch guideline on highly resistant microorganisms (HRMO) has been adapted, now including that patients with hospitalization abroad must be screened for Candida auris. The aim of this study was to evaluate five real-time PCR assays to select the best assay for Candida auris screening.

Methods

Five real-time PCR assays were evaluated, comprising AURISID (CE-IVD; OLM), Altostar C. auris kit (RUO, Altona), ErasmusMC Lab-developed test (EMC-LDT), CandidaGenius kit (RUO, PathoNostics) and C. auris kit (RUO, Revvity). Analytical validation was performed by evaluating the limit of detection, analytical specificity and reproducibility. Subsequently, a clinical validation was performed using 45 negative and 45 spiked eSwab materials. Finally, 14 specimens from a single patient colonized with C. auris were tested with the CandidaGenius, Altostar and EMC-LDT.

Results

The analytical validation showed that the Altostar kit and EMC-LDT had the lowest limit of detection, specificity testing showed no false-positive results in AURISID, Altostar, EMC-LDT and CandidaGenius. Finally, CandidaGenius and Altostar assays had the most reproducible results.
The clinical validation revealed one false-positive result when testing with the Altostar kit, three false-negative specimens were obtained when testing the CandidaGenius assay.
Of the 14 specimens tested from a single patient colonized with C. auris, six, five and three tested positive with the Altostar assay, EMC-LDT and CandidaGenius assay, respectively.

Conclusion

This evaluation showed that the Altostar C. auris assay and the EMC-LDT had the best performance in detecting C. auris and both can be used for screening of C. auris in clinical specimens. In our setting, the Altostar C. auris assay has been introduced to ensure compliance with the IVD regulation in the near future.

P050

Global dissemination and structural variation of an outbreak-associated multidrug resistance genomic region in Enterobacteriaceae.

Drs. Connor Rossel[1], Dr. LB Van Alphen[2], Dr. C Linssen[1], Dr. ER Heddema[1], Prof. Dr. PHM Savelkoul[2]
[1]Zuyderland Medisch Centrum, [2]Maastricht University Medical Center , Care and Public Health Research Institute (CAPHRI)

Background:
A similar multidrug resistance genomic region (MRR) was detected in multiple isolates of three separate outbreaks involving extended spectrum beta-lactam, aminoglycoside and fluoroquinolone resistant Enterobacteriaceae. This study aimed to map the characteristics, occurrence and genomic plasticity of the MRR globally.

Methods:
An antimicrobial resistance gene (ARG) based approach was used to query Google Scholar and PubMed Central (January 2014 to April 2024) for research articles which contained genomic backbones with the MRR. Long-read plasmid and chromosome assemblies were extracted from NCBI and annotated with Bakta, IntegronFinder, ISFinder and TnCentral. The MRR in these backbones were split into 9 distinct ARG blocks based on adjacent transposase annotations and manually assessed for structural variation.

Results:
The plasmid (n=120) and chromosomal (n=8) backbones were predominant in K. pneumoniae complex (75.0%) and originated from North America (n=39), Europe (n=35), Africa (n=16), Asia (n=14), Oceania (n=13) and South America (n=7).
Altogether, the ARG blocks presented a total of 118 structural variants (SV): qnrB1 (SV=27; n=102), blaCTX-M-15 (SV=24; n=138), tet(A) (SV=18; n=94), sul2—aph(3'')-Ib—aph(6)-Id (SV=14; n=121), dfrA14 (SV=14; n=116 occurrences), blaTEM-1b (SV=9; n=120), aac(6')-Ib-cr—blaOXA-1—catB3 (SV=8; n=138), aac(3)-IIe (SV=2; n=92), and aadA1—catA1 (2 variants; n=12).
The MRR presented a large degree of modularity, since there was no single apparent ARG block order. However, three typical co-occurrences were found. Firstly, blaTEM-1b was typically flanked by sul2—aph(3'')-Ib—aph(6)-Id downstream (95.0%) and blaCTX-M-15 upstream (84.1%). Secondly, qnrB1 was flanked by tet(A) upstream (61.7%) and dfrA14 (63.5%) downstream. Thirdly, aac(3)-IIe was often flanked by aac(6')-Ib-cr—blaOXA-1—catB3 upstream (80.4%).

Conclusion:
This study demonstrates that the observed MRR is not outbreak specific, but has disseminated globally. Furthermore, the MRR's structural variability makes detection and interpretation complex. However, the preservation of clinically relevant ARGs in this MRR suggests an effective transfer of the ARG blocks between distinct Enterobacteriaceae isolates.

P051

Evaluation of the EasySeq Full-Length 16S sequencing kit for bacterial identification in clinical samples: a multicentre study

Tim Severs[1,2], J.H.B. van de Bovenkamp[3], L.B. van Alphen[4], L. Gard[2], J.W.A. Rossen[1,2,5], R.Y. Yahiaoui[6], S.P.F. Matamoros[7], J. Dingemans[8], S.D. Pas[9], S.A. Boers[10]
[1]Isala, [2]University Medical Center Groningen, University of Groningen, [3]Labmicta, [4]Maastricht University Medical Center+ (MUMC+), [5]University of Utah School of Medicine, [6]Maasstad Hospital, [7]Amsterdam UMC location University of Amsterdam, [8]Jessa Ziekenhuis, [9]Radboud University Medical Center, [10]Leiden University Medical Center

Introduction

Bacterial culture remains an essential method for pathogen identification but is often inadequate for fastidious bacteria or complex infections. Its sensitivity is further diminished by prior antibiotic exposure. Advanced molecular alternatives, such as targeted next-generation sequencing (NGS) and shotgun metagenomics, offer significant potential but face challenges, particularly the lack of standardisation required to ensure consistent quality. To address this gap, NimaGen recently introduced its full-length 16S kit for Oxford Nanopore Technologies (ONT). This multicentre study evaluates the kit's performance in comparison to nine different commercial and in-house developed 16S workflows, aiming to assess its suitability for clinical diagnostics.

Methods

Nine major medical centres across the Netherlands and Belgium participated in this study, with each centre processing 20 clinical samples collected from sterile sites such as synovial fluid, cerebrospinal fluid, and tissue (n=180), ranging from negative to polymicrobial infections. Each centre employed distinct DNA extraction methods, aliquoted the DNA and then used it as input for their validated in-house molecular diagnostics workflows - including 16S Sanger, 16S Illumina/ONT, 16-23S Illumina sequencing – and the EasySeq™ Full-length 16S Library Prep Kit for ONT for which sequence data were processed with GenomeDetective. In addition, all participating labs processed a Mock standard in triplicate to evaluate interlaboratory performance and reproducibility.

Results

In two out of the three centres analysed so far, the NimaGen kit demonstrated enhanced micro-organism detection. Data from the remaining centres are still under analysis to finalise the comparison.

Conclusion

Despite variability in in-house method across centres, these approaches remain valuable for diagnosing bacterial infections. Preliminary findings suggest the NimaGen EasySeq™ kit improves micro-organism detection and enables more precise species-level identification. To our knowledge, this study represents one of the largest multi-centre studies evaluating direct bacterial identification across a broad range of clinical samples. Comprehensive results are anticipated by January 2025.

P052

The architect of cristae: the atypical Mitochondrial Contact Site and Cristae Organization System of malaria parasites

Silvia Tassan-Lugrezin[1], Irina Bregy[2], Laura van Niftrik[2], Taco Kooij[1]
[1]Radboudumc, [2]Radboud University

Plasmodium falciparum is the most virulent malaria parasite species and causes more than 600,000 deaths annually. P. falciparum harbors a single mitochondrion, which is one of the most important drug targets. When asexual blood-stage parasites differentiate to gametocytes (the parasite stage responsible for transmission via mosquitoes) the Plasmodium mitochondrion undergoes dramatic changes both in morphology and protein composition. In fact, the malaria parasite mitochondrion represents the only naturally occurring model in which cristae, the characteristic invaginations of the inner mitochondrial membrane, are known to be formed de novo. In model organisms, the main complex involved in cristae organization is the mitochondrial contact site and cristae organizing system (MICOS). We identified orthologues of MIC60 and MIC19, two of the main components of the MICOS, in P. falciparum through homology detection and complexome profiling.
To study the putative MIC60 and MIC19 proteins possibly involved in de novo cristae formation, we generated loss-of-function mutants and parasite lines expressing epitope-tagged MIC60 and MIC19 using CRISPR-Cas9-mediated gene modification. Using immunofluorescence microscopy and electron tomography we analyzed mitochondrial morphology and protein localization, in particular during the cristae containing gametocyte stages.
Our findings advanced the understanding of mitochondria biology in P. falciparum and its divergency from model organisms.

P053

Prevalence of Vancomycin Variable Enterococci in the Netherlands

Dr Christian von Wintersdorff[1], M Roelofsen[2], L Versteegh[2,3], C Jamin[1], Y Benyahya[1], M Mulder[1], G Bastiaens[2], M Van Meer[2], J Flipse[1,2,3]
[1]Maastricht University Medical Center+, [2]Rijnstate Hospital, [3]Dicoon

Introduction:
Vancomycin-resistant enterococci (VRE) are known to cause infections that are difficult to treat in immunocompromised individuals. However, some enterococci carry vancomycin resistance genes while appearing phenotypically susceptible, rseferred to as vancomycin-variable enterococci (VVE). Detecting these VVE is challenging for laboratories, as they can go unnoticed with conventional culture-based diagnostic methods.

The prevalence of VVE in the Netherlands remains largely unexplored, as national protocols rely on phenotypic resistance testing. To address this gap, two laboratories in the South-Eastern Netherlands investigated the presence of VVE.

Methods:
At MUMC+ and Rijnstate Hospital, 479 enterococcal isolates, identified as vancomycin-susceptible and recovered from presumed sterile sites, were collected. Resistance testing was performed according to EUCAST guidelines. Presence of van-resistance genes was assessed using PCR.
To examine VVE, resistance induction was performed through successive culturing under vancomycin pressure. Whole-genome sequencing (WGS) was conducted on both the original vancomycin-susceptible isolates and their resistant variants. Additionally, resistance expression was evaluated using a fluorescent vancomycin-binding assay and through quantification of van-gene RNA expression levels.

Results:
Six isolates that were phenotypically vancomycin-susceptible tested positive for vancomycin resistance genes: four E. faecium with vanB, one E. gallinarum with vanC1, and two E. casseliflavus with vanC2/3. Following exposure to vancomycin, three E. faecium isolates consistently developed resistance. WGS analysis revealed clonal relationships among the three isolates, each of which acquired unique mutations in vanS/R regulatory genes during resistance development. Neither relative DNA copy number of vanB nor its RNA expression correlated with observed resistance phenotypes.

Conclusion:
This study highlights the low prevalence of vanB-positive, vancomycin-susceptible E. faecium isolates capable of acquiring resistance under antibiotic pressure in vitro. Considering the rare yet potential clinical significance of these findings, we support the recommendation for molecular screening of enterococcal isolates repeatedly cultured from sterile or deep wound sites, particularly in patients undergoing glycopeptide therapy.

P054

Rapid culture-free detection of bloodstream infections in ICU patients: A comparative study

Msc Anna Wijen[1], Dr. Christian von Wintersdorff[1], Dr. Rob Driessen[2], Dr. Suzan van Mens[1], Dr. Dennis Bergmans[3], Prof. Dr. Paul Savelkoul[1]
[1]Department of Medical Microbiology, Infectious Diseases & Infection Prevention, Research Institute of Nutrition and Translational Research in Metabolism (NUTRIM), [2]Department of Intensive Care and Cardiology, Cardiovascular Research Institute Maastricht (CARIM), [3]Department of Intensive Care, Research Institute of Nutrition and Translational Research in Metabolism (NUTRIM)

Introduction: Traditional bloodstream infection diagnosis takes 2–5 days, mainly due to microbial growth delays. For ICU patients with sepsis, timely antimicrobial therapy is critical. Current culture-free methods have limited detection ranges, complicating infection exclusion. This study evaluates a DNA-based method for broad-range bacterial detection in whole blood, providing same-day results.

Methods: In an ongoing ICU study, 92 blood samples from 50 patients were collected alongside routine blood cultures. After human DNA depletion (Polaris, Philips), a PCR-based method targeting the 16S-23S bacterial DNA interspace (Molecular Culture, Inbiome) was used, with a 6-hour turnaround. The molecular results were compared with blood cultures and further evaluated using patient cultures and clinical diagnoses.

Results: Out of 92 samples, 9 had positive blood cultures, including 7 true pathogens and 2 contaminants. The molecular technique correctly identified 4 of the 7 true pathogens and did not find back the 2 contaminants. The remaining 3 were detected but not identified due to low intensity (considered mismatches). Of the 83 negative blood cultures, 70 were negative by both methods, while 5 molecular results showed possible matches due to initial misidentification or negligible intensity, which could be rectified. Notably, the molecular technique identified 6 additional bacteria missed by blood cultures but confirmed by other patient cultures (considered matches), and another 2 that aligned with clinical diagnoses despite negative blood cultures (considered possible matches). Overall, there was an 89.1% match, 7.6% possible match, and 3.3% mismatch. At the patient level, molecular results would likely have provided the correct infection diagnosis for 48 out of 50 patients.

Conclusion: This DNA-based method provides rapid bacterial detection with the same blood volume as traditional cultures. The results show strong concordance with clinical data, emphasizing its potential to expedite bloodstream infection diagnosis. Follow-up samples and software optimization will be incorporated for further improvement.

P059

How probiotic A. muciniphila can engraft and influence mucosal microbiota in a synthetic community

Msc Maryse Berkhout[1], MSc C de Ram[2], S Boeren[3], Dr CM Plugge[1], Dr C Belzer[1]
[1]Wageningen University & Research, [2]Wageningen University & Research, [3]Wageningen University & Research

Introduction

Akkermansia muciniphila is a human gut bacterium that is associated to host health and therefore proposed as a next-generation probiotic. As a specialist mucin glycan degrader, its preferred niche is the colonic mucus layer. However, it is unknown if probiotic A. muciniphila can engraft and how it influences the resident mucosal microbiota. Therefore, we assessed its compositional and functional influence on a preexisting in vitro synthetic microbial mucin-degrading community.

Methods

We grew a 14-species synthetic mucin-degrading community that completely degrades mucin glycans without A. muciniphila on mucin in continuously operated anoxic bioreactors. After stabilisation, we introduced A. muciniphila (t = 96h). Every 24 hours, we analysed community composition (combination of 16S rRNA gene amplicon sequencing and qPCR) and metabolite production (HPLC), and before (t = 72) and after (t = 216h) addition of A. muciniphila we analysed the metaproteome (nLC-MS/MS) and mucin glycan degradation (PGC-LC-MS/MS).

Results

A. muciniphila engrafted in the synthetic community, as it remained present for five 24h cycles (five volume changes), but it did not dominate. Addition of A. muciniphila did not induce major compositional changes, except for an increase of generalist glycan degrader Bacteroides thetaiotaomicron. On a functional level, the community was steady as well. Most mucin-degrading enzyme groups remained stable: peptidase, fucosidase, galactosidase and sulfatase expression levels were not significantly altered, whereas sialidase and hexosaminidase expression were significantly increased after A. muciniphila addition.

Conclusions

Even though the preexisting synthetic mucin-degrading community could completely degrade all mucin glycans, this community still harboured a niche for A. muciniphila. However, A. muciniphila did not substantially modulate community composition and function. This suggests that A. muciniphila as a probiotic in vivo might engraft without impacting the resident human gut microbiota, but with benefits for the host. Further research is needed to verify this hypothesis in vivo.

P060

Understanding antibiotic-induced blooms driving horizontal gene transfer of antibiotic resistance.

Dr Laura de Nies[1], Dr Mathew Stracy[1]
[1]University Of Oxford

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are critical priority antibiotic-resistant pathogens posing a major threat to public health. Plasmid-encoded ESBL genes spread via conjugation between Enterobacteriaceae species within the intestine of colonised patients, yet the fundamental mechanisms driving this dissemination remain poorly understood. Under normal conditions, Enterobacteriaceae are vastly outnumbered by commensal anaerobes within the gastrointestinal tract, limiting the physical contact between donor and recipient bacteria required for conjugative transfer. Antibiotic treatment can lead to 'blooms' of otherwise low-abundant Enterobacteriaceae, increasing donor-recipient encounters.

We established stool-derived microbial communities from three healthy individuals in vitro. To each community, we introduced an E. coli strain with a conjugative ESBL-plasmid conferring resistance to most beta-lactam antibiotics, including third-generation cephalosporins, and assessed the effect of different antibiotics on community structure, focusing on the role of overgrowth on plasmid transfer.

We detected antibiotic-specific perturbations of the microbiota resulting in different magnitudes of 'blooming' of resistant E. coli. Direct selection for plasmid-encoded resistance has been associated with increased plasmid carriage. However, we found that whilst treatment with third-generation cephalosporins caused overgrowth of the donor strain, it eliminated potential recipients, therefore inhibiting plasmid transfer. Instead, other "indirect" antibiotic selection pressures, which select for Enterobacteriaceae in general rather than for the resistance plasmid, led to co-blooming of donor and recipient strains, increasing conjugation rates. Additionally, we found that microbiota composition determines the magnitude of overgrowth, and consequently, conjugation. In two communities Oxacillin treatment resulted in Enterobacterial blooming and increased conjugation. However, in the third community, characterised by increased Lachnospiraceae and Bacteroidaceae abundance, antibiotic-induced overgrowth was limited.

In conclusion, whilst direct antibiotic selection increased plasmid-carriage, this is due to clonal expansion of the donor strain. In contrast, antibiotic-induced co-blooming of donors and recipients favours plasmid transfer, with both the antibiotic and baseline microbiota composition shaping the magnitude of co-blooming.

P061

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

Ir. Gerben de Zwaan[1], Dr.rer.nat P Geesink[1], MSc Y.M. Kaspareit[1], Dr. G Tahon[1], Prof. dr. ir. T.J.G. Ettema[1]

[1]Wageningen University And Research

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 are providing 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.

P062

Glycan-driven dynamics of the infant gut microbiota are portrayed through synthetic communities

Athanasia Ioannou[1], Ms. M. D. Berkhout[1], Dr. W. T. Scott Jr.[2,3], Mr. Y. Kavanal Jayaprakash[1], Ms. B. Blijenberg[4], Mr. S. Boeren[5], Dr. M. Mank[4], Dr. C. M. Plugge[1], Dr. J. Knol[1,4], Dr. C. Belzer[1]
[1]Wageningen University & Research, [2]Wageningen University & Research, [3]Wageningen University & Research and Delft University of Technology, [4]Danone Global Research & Innovation Center, [5]Wageningen University & Research

Upon birth, the infant gut is quickly inhabited by microorganisms termed the gut microbiota. The gut microbiota relies heavily on glycans for carbon and energy. Human milk, the 'golden standard' for infant nutrition, contains more than 200 structures of Human Milk Oligosaccharides (HMOs). At the same time, the mucosal layer develops and provides an additional substrate for glycan-degrading bacteria. It is known that Bifidobacterium spp., Bacteroides spp., and Akkermansia muciniphila can utilize both mucin and HMOs. However, how this leads to microbial community formation is unknown. Here, we investigate how glycans present in the infant gut in the first year of life create trophic interactions and shape infant gut-related bacterial communities.

We created two bacterial synthetic communities, BIG-Syc and BabyBac, to represent the breastfeeding and weaning period of infancy, respectively. The 13-strain BIG-Syc and 7-strain BabyBac contain primary degraders and cross-feeders, based on the trophic interactions expected in the presence of glycans in the infant gut. BIG-Syc was continuously grown in HMO mixes and BabyBac was subjected to sequential batch fermentations with combinations of HMOs, galactooligosaccharides, fructooligosaccharides, and mucin. Comparison with profiles of maximum 5-month-old infants' feces, showed that BIG-Syc successfully captured the compositional, metabolic, and proteomic aspects of fecal microbiomes.

Bifidobacterium spp., and Bacteroides spp. became dominant in BIG-Syc. Upon changing the concentration of the different HMOs, we monitored a shift within the relative abundance of the different Bifidobacterium species. For BabyBac, the presence and ratio of mucin and HMO proved to be the main driving force for community composition. Growth of cross-feeders on the aforementioned glycans provides evidence for successful cross-feeding of simple carbohydrates, organic acids, and gases. Our study offers a viable model for complex microbial interactions and insights into how dietary- and host-derived glycans differentially affect the composition and functionality of infant gut microbiota-related synthetic communities.

P063

A random forest classifier for fast classification of the microbial composition of complex communities

Sabine Michielsen[1], dr. H Bachmann[1]
[1]Vrije Universiteit Amsterdam

Introduction

Microbial communities found in nature and food fermentations are often complex. An example is the water kefir community, which consists of approximately 30 different species of lactic acid bacteria (LAB), acetic acid bacteria(AAB) and yeasts contained in a self-maintained granule.
In order to understand the community dynamics within the water kefir community, changes in community functions such as granular growth or metabolite production need to be related to shifts in community composition. Currently, there is a lack of fast and inexpensive methods to follow this composition.
Here, we investigate if a combination of flowcytometry and machine learning can be utilized to distinguish organisms at the genus or even species level.

Methods

A random forest classifier was trained using a flowcytometric fingerprint of LAB, AAB and yeast monocultures isolated from water kefir. Subsequently, the accuracy of the classifier was tested using the flowcytometric data obtained from mixed cultures with predetermined ratios of LAB, AAB and yeast, which roughly emulated the composition of the water kefir community.

Results

On the mixed cultures, the initial classifier overestimated AAB by 40%, and yeasts by 20%, indicating low accuracy. The classifier was unable to distinguish between species. The implementation of fluorescent cell staining and a background filtering step improved the prediction significantly. Following implementation, LAB were overestimated by <3%, while the amount of AAB and yeasts was underestimated by <1% and <4%, respectively. At a species level, the classifier was still unable to accurately differentiate species.

Conclusion

After background correction and cell staining, flowcytometry combined with an optimized random forest-based algorithm can be utilized to accurately differentiate taxa within our test set, however, it is not yet able to accurately identify species.

P068

Epidemiological Patterns of Viral Infections in the Caribbean Region of the Dutch Kingdom: Insights for Pandemic Preparedness

Charlene Maria[1], S van Roeden[2], M Tilanus[2], C Fleming[3], Dr. A Tromp[5], F Koene[5,6], Dr. J Kalpoe[7,8], Dr. P Klein Klouwenberg[9], D Souverein[8], J Juliet[2], M Euson[2], S Baboe-Kalpoe[10], R Plaisier[11], A Visser[6], W Luling[6], Dr. R Steingrover[2,3,4], Dr. L Verhagen[1,2]
[1]Radboud University Medical Center , [2]St Maarten Medical Center, [3]St. Maarten Laboratory Services, [4]Fundashon Mariadal, [5]Medical Laboratory Services, [6]Bonaire Laboratory, [7]Laboratory Horacio Oduber Hospital, [8]Steeklab Haarlem, [9]Analitisch Diagnotisch Centrum, [10]St. Eustatius Health Care Foundation, [11]Saba Cares Foundation

Introduction
The incidence of respiratory tract infections in the Caribbean is among the highest globally. The World Health Organization (WHO) recommends that preventive measures be based on local epidemiological data. In the absence of such data, the six Dutch Caribbean islands—Aruba, Bonaire, Curaçao, St. Maarten, Saba, and St. Eustatius—follow the timing of preventive measures in the European Netherlands for RSV and influenza. The local epidemiology, as well as the impact of location, climate, and tourism, remains poorly understood. This study aims to define the epidemiology of viral respiratory infections in the Caribbean by assessing seasonality to improve pandemic preparedness.

Methods
Viral respiratory data for 18 viruses were collected from eight laboratories across the six Dutch Caribbean islands from 2018 to 2024. Generalised Additive Models (GAM) were used to assess the association between viral infections and month, smoothing non-linear trends. Multivariate logistic regression was performed to analyse the effect of climate and tourism season on viral infections.

Results
This study included 34,075 tests, of which 9,124 (26.8%) were positive. The most frequently detected viruses were rhino-enterovirus (4,518 positive results, 54.3%), followed by influenza virus (1,298 positive results, 15.6%) and RSV (1,258 positive results, 15.1%). Influenza detection was significantly higher from November to March and notably less prevalent during the rainy season in St. Maarten and Bonaire (OR = 0.14 [0.05–0.34]; OR = 0.15 [0.05–0.33], respectively; $P < 0.001$). In contrast, RSV was significantly more prevalent from June to December, aligning significantly with the rainy season in the Caribbean (OR = 2.30 [1.69–3.19]; $P < 0.001$).

Conclusion
This study defines, for the first time, the seasonality of RSV and influenza across all six Caribbean islands, emphasising distinct regional patterns. These findings highlight the importance of tailored preventive measures based on local epidemiological patterns to enhance pandemic preparedness.

P074

Building Cristae from Scratch: Mitochondrial Dynamics During Malaria Parasite Differentiation

Dr. Irina Bregy[1], Silvia Tassan-Lugrezin[2], Dr. Taco Kooij[2], Prof. Laura van Niftrik[1]
[1]Radboud University, [2]Radboud UMC

Mitochondria are highly dynamic organelles essential for energy metabolism and cellular adaptation. A wide range of mitochondrial morphologies and functions have been described across species and even between cells within the same organism. Malaria parasites are an impressive example of these dynamic adaptations, as their mitochondrion undergoes significant transformations throughout the parasite's lifecycle.

During the asexual blood stage, malaria parasite mitochondria lack cristae. However, as the parasites prepare for life in the mosquito, mitochondrial morphology undergoes dramatic transformations. In mature gametocytes, mitochondria are packed with tubular cristae. This change is believed to be an essential part of the adaptation to the glucose-limited environment of the mosquito host.

Although these distinct mitochondrial states are well-established, how and when these transformations occur during sexual differentiation, remains poorly understood. We currently perform high-resolution three-dimensional electron microscopy to investigate the earliest stages of cristae formation and follow their progression along the parasite's differentiation timeline. Our research advances the understanding of mitochondrial dynamics in malaria parasites and contributes to broader knowledge of de novo cristae formation and mitochondrial dynamics in general.

P075

The antifungal activity of graphene quantum dots against Candida species

Laure van Hofwegen[1], M Hassnain[2], PPS Balraadjsing[1], K van Dijk[1], F Hagen[3], S Nizamoglu[4], SAJ Zaat[1]
[1]Amsterdam UMC, [2]KOC University, [3]Westerdijk Fungal Biodiversity Institute, [4]KOC University

Candida species are opportunistic fungi that can cause serious infections, for example catheter-related blood stream infections, which result in high morbidity and mortality rates. Traditional antifungals, such as azoles and echinocandins, are becoming less effective, especially against the emerging pathogen Candida auris, complicating the treatment of candidiasis. Prolonged hospital stay or a compromised immune system are risk factors for developing these infections. Graphene quantum dots (GQD) could be an alternative or addition to traditional antifungals. GQD consist of a single layer of carbon atoms arranged in a honeycomb-like structure with photo-activation properties. When activated by blue light, GQD can generate reactive oxygen species (ROS), which have broad microbicidal activity, including antifungal activity. We aimed to evaluate a newly developed carboxylated form of the GQD, colloidal GQD-COOH and a novel GQD-COOH thin film, for their fungicidal activity against Candida albicans and a panel of C. auris strains, spanning clades I to V. To assess 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 at a light intensity of 5 mW/cm2, the lowest concentration of colloidal GQD-COOH which killed 99.9% of fungi was 12.5 µg/ml for C. auris and 50 µg/ml for C. albicans. Additionally, we developed a novel thin film consisting of alternating layers of GQD-COOH and polymer applied on glass slides and we tested its surface fungicidal activity using the Japanese Industrial Standard assay. The GQD-COOH thin film showed promising fungicidal activity against both C. auris and C. albicans. Therefore, the GQD-COOH thin film shows potential for future application to for instance wound dressings or catheters.

P080

Impact of Host Factors and Invasive Meningococci on Bacterial Adhesion, Growth, Primary Nasal Epithelial Barrier Function, and Immune Response

Daan Arends[1,2], DM van Rooijen[1], E van Woudenbergh[1,2], JJ Veldman-Wolf[1], Dr. M Ohm[1], Prof. Dr. MI de Jonge[2], Dr. G den Hartog[1,2]
[1]National Institute for Public Health and the Environment (RIVM), [2]Radboud University Medical Center

Introduction
Neisseria meningitidis colonizes the human upper airway mucosa, which can progress into invasive meningococcal disease (IMD) upon breaching the epithelial barrier. Some serogroups and lineages are associated with IMD, whereas others are hardly ever isolated from patients. Although multiple virulence factors have been described, it is not entirely clear what makes some lineages hyperinvasive.

Methods
We examined the course of meningococcal infection in an air-to-liquid interface (ALI)-differentiated primary epithelial cell model (cells from 7 donors). Serogroup C and W reference and clonal complex 11 (cc11) strains, a well-described hyperinvasive lineage, were used, as well as a capsule null locus (cnl) strain. Transepithelial electrical resistance (TEER), epithelial cytokine response, and bacterial binding and growth on the epithelial layer were assessed to examine host- and strain-dependent differences.

Results
For most donor-strain combinations, meningococcal infection induced a drop in TEER, indicative of increased epithelial permeability, where higher bacterial loads were generally associated with lower TEER levels. Cc11 strains induced slightly lower TEER levels compared to other strains. Bacterial binding and growth were highly donor-strain dependent. The unencapsulated strain exhibited the highest binding levels, while MenW cc11 showed higher binding compared to the MenW reference strain. MenC strains showed no similar difference. We observed a universal cytokine response (CCL20, CXCL1, CXCL8, CXCL10, IL-18) upon infection, for all meningococcal strains, with only CCL20 levels being significantly higher 24 hours post infection for MenW cc11 compared to the other strains. Principal component analysis indicated that cytokine profiles were predominantly influenced by the epithelial cell donor rather than the meningococcal strain.

Conclusion
The results of our model suggests that the outcome of meningococcal infection of the epithelium is mostly dependent on specific donor-strain interactions. We also observed some subtle differences specific to the cc11 strains, which could contribute to the invasiveness of this lineage.

P081

Culturing Streptococcus pneumoniae in in vivo mimicking conditions affects pneumococcal phenotype and epithelial infection.

Daan Arends[1,2], Dr. K Surmann[3], Dr. LF van Beek[2], Dr. RS Jansen[4], Dr. M van Scherpenzeel[5], Prof. Dr. U Völker[3], Dr. G den Hartog[1,2], Prof. Dr. MI de Jonge[2]
[1]National Institute for Public Health and the Environment (RIVM), [2]Radboud University Medical Center, [3]Interfaculty Institute for Genetics and Functional Genomics, [4]Radboud University, [5]Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Center

Introduction

In most laboratories Streptococcus pneumoniae is cultured in nutrient-rich growth media. However, its natural niche, the upper respiratory tract, is nutrient poor, possibly influencing pneumococcal phenotype. We cultured pneumococci in a chemically defined medium mimicking nutrients available in the nasal cavity, to assess the effects on growth, proteome, extracellular metabolites, and polysaccharide capsule, as well as the impact on the infection of nasal epithelial layers.

Methods

We cultured carriage isolates BHN100 (serotype 19F) and BHN418 (serotype 6B) in a chemically defined medium (CDM) as described in literature. The medium was modified to include in vivo metal ion concentrations (CDM+MI) or further supplemented with in vivo monosaccharide concentrations and mucin (CDM+MI+MM). Pneumococcal growth was measured and expressed in colony forming units. The pneumococcal proteome and extracellular metabolites were determined via mass spectrometry. Polysaccharide capsule structure was assessed using EM. Nasal primary epithelial cells were differentiated in an air-to-liquid interface. IgM/CRP-pneumococcus binding was assessed by flow cytometry.

Results

Pneumococcal growth in CDM+MI+MM peaked at lower concentrations compared to other media. EM showed altered capsule macrostructure in the different media. Proteomics revealed three protein clusters expressed differently in CDM+MI+MM compared to CDM and CDM+MI: fatty acid biosynthesis (downregulated), galactose metabolism (upregulated) and sugar metabolism (upregulated). Virulence factors, including pneumolysin, were also differentially expressed. Metabolite analysis identified glycerophosphocholine to be specifically secreted in CDM+MI+MM, which inhibited IgM and CRP binding to pneumococci. During epithelial infection, CFU loads were generally higher when infected with CDM+MI+MM-grown pneumococci, especially for capsule deficient mutants. Cytokine responses were also affected.

Conclusion

Growth in our in vivo mimicking-CDM (CDM+MI+MM) altered metabolic pathways, virulence factor expression, capsule macrostructure, CRP and IgM binding and epithelial infection outcomes. These findings underscore the importance of mimicking upper respiratory tract conditions to better understand colonization and infection processes.

P082

Biomarker discovery for in situ detection of microbial biofilms on right-sided colorectal cancer precursor lesions

Floor Baas[1], F. Janssen[1], Dr. C.E. Bruggeling[1], R Cremers[1], A. Gusinac[1], Dr. Peter Siersema[2], Dr. I.D. Nagtegaal[1], Dr. A. Boleij[1]
[1]Radboud university medical center (Radboudumc), [2]Erasmus Medical Center

Introduction
Polymicrobial biofilms are often present on right-sided precursor lesions of colorectal cancer and are associated with cancer development. Precursor lesions are detected using colonoscopies, where right-sided lesions have a higher missing rate than left-sided lesions, warranting improved detection strategies. Identifying biomarkers for the in situ visualisation of biofilms, or their associated host (immune) response factors, could provide a novel avenue for detection of right-sided precursor lesions.

Methods
A targeted and untargeted approach was used to identify host and microbial protein biomarkers. Precursor lesions, advanced sessile serrated lesions (SSL) or conventional adenomas (CAA), of 23 prospectively included patients were classified as biofilm-positive or -negative and compared to paired matched control tissue. Additionally, a validation cohort of 9 SSLs and CAAs was used. For the targeted approach, immunohistochemistry on formalin-fixed paraffin-embedded tissue, was performed to quantify secretory immunoglobulin (SIg)A, SIgM and IgG in the biofilm. For the untargeted approach, label-free liquid chromatography-mass spectrometry (LC-MS) was applied to trypsin surface-shaved fresh-frozen colon biopsies, to detect differentially expressed proteins (DEPs).

Results
Targeted visualisation of SIgA, SIgM and IgG showed that all three markers could reliably detect colonic biofilms. Positive-pixel quantification of SIgA and SIgM enabled significant discrimination between biofilms on precursor lesions and paired normal tissue. Untargeted analysis of the surface proteome revealed 225 DEPs, mostly of human origin. Focusing on DEPs localised to the apical cell surface, we identified no DEPs in biofilm-positive precursor tissue. Yet, Na+/H+ exchange regulatory factor 2 (NHERF2) was downregulated in precursor lesions compared to normal tissue (p=0.002), and annexin A1 (ANXA1) was downregulated in biofilm-positive compared to -negative tissue (p=0.005).

Conclusion
Our study identified SIgA and SIgM, and possibly NHERF2 and ANXA1, as promising targets for in situ visualisation of microbial biofilms present on CRC precursor lesions, which could contribute to improved colonoscopic detection.

P084

Unraveling the gut microbiome's influence on colorectal cancer spread to the liver

ing Renske Cremers[1], Floor Baas[1], Esther Wagena[2], Alem Gusinac[1], Tom Ederveen[2], Prof. dr. Iris D. Nagtegaal[1], dr. Danielle Tauriello[3], Dr. Annemarie Boleij[1]
[1]Radboudumc, [2]Radboudumc, [3]Erasmus Medical Center

Introduction: The gut microbiome has emerged as important determinant in colorectal cancer (CRC), but their role in CRC liver metastasis remains unclear. This study investigated the microbial landscape in CRC and paired liver metastases using clinical samples. A syngeneic mouse model was used to provide a proof of concept for the functional relevance of either intestinal or hepatic bacteria on the development of CRC liver metastasis.

Methods: Human CRCs and paired liver metastases from 50 patients were analyzed via fluorescent in situ hybridization (FISH) and 16S rRNA sequencing to quantify and identify overlapping bacterial taxa. A syngeneic mouse model was used to explore the role of gut microbiota in CRC metastasis. Mouse tumor organoids (MTOs) were orthotopically implanted in the cecum or injected into the portal vein of 40 mice. Antibiotics were used to deplete gut and/or systemic bacteria, and liver metastatic burden was assessed.

Results: In human samples, FISH revealed higher bacterial density in liver metastases compared to adjacent healthy liver tissue ($p < 0.05$). 16S rRNA sequencing showed bacterial overlap between CRC and liver metastases, with Ralstonia, Acinetobacter, and Methylobacterium as common genera.
In mice, gut bacterial depletion reduced liver metastasis following orthotopic MTO implantation, suggesting pro-metastatic effects ($p < 0.05$). However, in the portal vein injection model (without a primary tumor), bacterial depletion increased liver metastases, indicating a protective role of bacteria ($p < 0.01$). These findings suggest that gut microbiota have context-dependent pro- and anti-metastatic effects.

Conclusions: The gut microbiota play a dual role in CRC liver metastasis, with pro-metastatic effects in early tumor dissemination and protective effects during liver colonization. Overlapping bacteria between CRC and metastases implies bacterial (co-)migration with tumor cells. These findings highlight the need to evaluate antibiotic use in CRC treatment and explore microbiome-targeted therapies to prevent or treat CRC liver metastases.

P085

The social and hygiene practices of the parents during COVID-19 pandemic influence the development of the infant's gut microbiota

MSc Evgenia Dikareva[1], Dr N van Best[1,2], Dr M Mommers[3], Ing C Driessen[1], Prof J Penders[1,4]
[1]Maastricht University, [2]RWTH Aachen University Hospital, [3]Maastricht University, [4]Maastricht University

Introduction: The infant gut microbiota (GM) has a lifelong impact on health. The roles of genetics, prenatal factors, and environmental influences in shaping the trajectory of microbiota maturation have been well-studied. The COVID-19 pandemic presented a unique opportunity to investigate how changes in behavior: social interactions, protective measures, and hygiene practices affect the GM. To explore these effects, we developed a specific index to assess their influence.

Methods: We collected fecal samples and questionnaire data from 139 infants as part of the Dutch longitudinal LucKi Birth Cohort Study, which explores microbiota development over the first 14 months of life. We used PERMANOVA to identify factors influencing infant GM development and conducted differential abundance analysis based on linear regression to examine the abundance of bacterial species. To investigate whether the behavior was associated with the microbiota, we used the constructed index to correlate it with the species abundance.

Results: We found that samples collected at the same age differed in microbiota composition depending on whether they were collected before or after the onset of the pandemic (PERMANOVA, 6 months, p-value: 0.022, R^2: 0.017). Several bacterial species showed differences in abundance in samples collected during the pandemic. Alpha diversity was significantly lower at 9 months of age in pre-pandemic samples. The index revealed that limited adherence to infection prevention and control measures was associated with lower abundances of Gordonibacter pamelaeae.

Conclusion: This study highlights the pandemic's impact on infant GM, with differences in profiles observed before and after its onset. These changes can be attributed to behavioral shifts, such as social distancing and hygiene practices, which may alter specific bacterial strains. Our findings emphasize the importance of considering how large-scale public health interventions, like those implemented during the pandemic, might unintentionally influence early-life GM development, potentially leading to downstream effects on health.

P086

Exploring the relation between flu and pneumococcal vaccine responsiveness and human gut microbiota

Dr. Prokopis Konstanti[1], Mrs. JK Kool[1], Dr. JB van Beek[1], Prof. DB van Baarle[1,2], Dr. Susana Fuentes[1]
[1]National Institute for Public Health and the Environment (RIVM), [2]University Medical Center Groningen

Background: Vaccination is one of the most effective strategies for preventing infectious diseases, however, with notable differences in individual responses. Many factors, such as age, genetics, immune status, and comorbidities contribute to variations in vaccine responses. Recent studies report associations between gut microbiota and vaccine responsiveness but data so far is limited.
Methods: We analyzed the microbiome of 253 individuals (20-100 years old) to explore potential associations with their responses against a booster quadrivalent influenza vaccine (QIV), and a primary 13-valent pneumococcal-conjugate vaccine (PCV13). Fecal samples were collected before the flu vaccination (n=253) and before the PCV13 vaccination (n=222), within a timeframe of 1 year. Samples were profiled using 16S rRNA gene sequencing, and stratified based on their response to the vaccines specific antigens and their age group (young adults=20-49 y.o., middle aged adults 50-59 y.o., and older adults 59+ y.o.).
Results: Beta diversity analyses showed significant association of the gut microbiota composition only for the QIV vaccine in older adults (PERMANOVA-R2 = 0.03, p-value = 0.02). Analysis of individual taxa showed that older adults with high responses against the QIV had higher abundances of the bacterial genera Faecalibacterium and Haemophilus. Moreover, older adults with high responses against the QIV had higher Prevotella/Bacteroides ratio. Response to both vaccines was not associated with a more stable microbiota composition.
Conclusions: This study demonstrates an association between the QIV vaccine response in older adults with the Prevotella/Bacteroides ratio and the presence of Faecalibacterium and Haemophilus.

P088

C-type lectin receptor sequencing is a novel platform to study glycan-mediated immune selection of bacterial species at host barrier sites

Jasper Mol[1], Dr. Rob van Dalen[1], Dr. Hilde Herrema[2], Dr. Franck Fieschi[3], Prof. Nina van Sorge[1,4]
[1]Amsterdam UMC, [2]Amsterdam UMC, [3]Institut de Biologie Structurale, [4]Amsterdam UMC

Bacterial microbiota located at host barrier sites harbor species essential for human health. Infection or vaccination induces antibody responses that confer durable immunity. Complementary, it has been demonstrated that select microbiota species induce local and systemic antibody responses that may contribute to host defense against opportunistic pathogens. However, the molecular mechanisms underlying selection of microbiota species from complex microbial communities and subsequent induction of immunity are currently unknown. Antigen-presenting cells (APCs) are local immune cells that sample the environment by extending their dendrites across epithelial barriers. Interestingly, these dendrites are densely covered with glycan-binding C-type lectin receptors (CLRs). Thereby, APCs are ideally positioned and equipped to probe bacteria-expressed glycans and subsequently induce both local and systemic immunity. To identify microbiota species that can be recognized by APC-expressed CLRs, we have developed CLR-SEQ. Soluble CLR receptors are used as probes to identify interacting microbiota species from human microbiota samples through bacterial cell sorting and subsequent metagenomic sequencing. We established and optimized our experimental pipeline using fluorescently-labeled CLRs on pooled microbiota from 24 different donors to cover a broad range of species. Additionally, we sorted and sequenced the IgA-bound population from this pool and compared this to the various-CLR bound populations. Beta-diversity analysis showed similarity between the IgA-bound and CLR-bound samples. Metagenomic sequencing identified bacteria to their species level and allowed subsequent validation of their binding as a monoculture of representative isolates. Our data provide a first snapshot of bacterial microbiota members that are likely to contribute to the protective antibody pool through CLR-mediated bacterial sampling. Using this pipeline as a blueprint, future research on clinically-relevant patient samples or CLRs with naturally-occurring mutations could reveal crucial disparities in glycan-induced immunity.

P089

A Root-to-Shoot Separation System to Study Plant Endophytes

María Negre Rodríguez[1], Prof. Dr. Á. T. Kovács[1]
[1]Leiden University

Over the past decades, the agricultural sector has emphasized the role of the plant microbiome providing a wide range of benefits to the plant, protection against pathogens, enhanced resilience to drought and nutrient stress, promotion of plant growth, and increased crop yields. When studying the plant microbiome, scientists have delved into the possibility of manipulating the rhizosphere, the thin layer of soil surrounding plant roots that acts as a communication network between plant roots, soil, and soil microbiota. Endophytes are a sub-population of plant-associated microorganisms that can penetrate the plant's tissues, like the roots, and adjust their metabolism to the host's environment. While many studies have focused on the dynamics of root colonization from the rhizosphere, there is still a missing gap understanding the endophytic lifestyle after penetrating the internal tissues. We use Bacillus subtilis, a Gram-positive ubiquitous soil bacterium, able to establish a mutually beneficial relationship with its plant host by utilizing nutrient rich root exudates while simultaneously providing growth-promoting traits. Our goal is to study the mechanisms enabling the plant colonization by this bacterium and its presence inside different plant tissues, including migration from roots to aerial structures, and reproductive organs. For this purpose, we developed a novel in vitro system, a petri plate with a barrier separating the inoculated rhizosphere and the non-inoculated phyllosphere. Using confocal fluorescence microscopy, we visually separate the outer and inner plant tissues, such as xylem vessels, and track the bacterial movement from roots to aerial parts. By using a library of GFP-labelled B. subtilis isolates, we explore and evaluate their colonization variability by visual identification and quantification in different Arabidopsis thaliana tissues. Understanding the mechanisms underlying the endophytic lifestyle of B. subtilis could emphasize its potential role in plant microbiome agricultural engineering.

P090

The Influence of Bifidobacteria-Derived Compounds on Intestinal Epithelial Cells

Ivonne Peugnet González[1,2], K.N Faber[1], H.J.M Harmsen[2]
[1]UMCG, [2]UMCG

Human milk oligosaccharides (HMOs) are indigestible by infants but selectively enrich bifidobacteria in the gut microbiota, in particular Bifidobacterium longum subsp. infantis (B. infantis), and Bifidobacterium breve, both of which are adapted to efficiently metabolize HMOs. In this study, we isolated B. breve and Bifidobacterium bifidum from healthy infants feces. Furthermore, we investigated the metabolic and functional impact of a mixture of five HMOs (5HMO-Mix):2'-fucosyllactose, 3-fucosyllactose, lacto-N-tetraose, 3'-sialyllactose, and 6'-sialyllactose on the growth of B. infantis, B. breve, and B. bifidum. We subsequently investigated how these bifidobacteria and different carbon sources affect CaCo-2 cells by assessing the impact of bacterial metabolites on the intestinal cells through changes in gene expression (qPCR), cell viability and proliferation (WST-1), and real-time cell analysis (IncuCyte). Our findings demonstrate the metabolic and functional differences among the bifidobacteria when metabolizing glucose or 5HMO-Mix. Growth profiles revealed that B.infantis thrived on glucose, whereas B. bifidum exhibited optimal growth with 5HMO-Mix. Distinct patterns of 5HMO consumption were observed, with B.infantis favoring LNT and 2FL, B. bifidum rapidly consuming LNT, 2FL, and 3SL, and B. breve displaying limited consumption of specific HMOs. Acetic acid production peaked at different time points depending on the strain and substrate. Functional assays using Caco-2 cells revealed significant strain -and substrate-specific effects on cell viability and proliferation, 5HMO-fed B. breve supernatants increased cell viability at concentrations of 1%($p < 0.05$) and 10%($p< 0.01$), while on the contrary, glucose-fed bifidobacteria showed varied impacts, including enhanced confluency with B. bifidum and bifidobacteria-mixtures at 5%($p< 0.001$). These findings underscore the distinct metabolic capabilities and functional outputs of bifidobacteria and highlight the potential role of 5HMO in modulating gut epithelial responses. Further investigation may confirm our observations that the effects on intestinal epithelial cells are not concentration-dependent, highlighting the complicated metabolic interactions between bifidobacteria and the intestinal epithelium.

P091

From root to microbiome members: Sorghum-derived flavonoids influence Bacillus growth

Adele Pioppi[1,2], Dr. Xinming Xu[1], Prof. Ákos T. Kovács[1,2]
[1]Leiden University, [2]Technical University of Denmark

The interactions between plants and their microbiome, particularly in the rhizosphere, has increasingly become the subject of investigation for sustainable agriculture. Plants naturally interact with their microbiome in a variety of ways which can result in enhanced or decreased colonization by certain microbial groups. For example, root exudates may contain substrates for microbial growth, signaling molecules, or antimicrobial compounds, which can be differentially produced depending on the plant's genotype and growth conditions. Sorghum plants exudes flavonoids including luteolinidin when experiencing salt stress or pathogen attack by fungi. This can be observed in vitro by the characteristic visible color of flavonoids like luteolinidin and apigeninidin. We analyzed the rhizosphere microbiome composition of two different sorghum varieties with distinct root exudation profiles, under normal conditions and under salt stress. Several bacterial strains including B. subtilis, which were isolated from the rhizosphere, exhibited decreased or delayed growth in vitro together with pure luteolinidin, while few showed increased growth. Therefore, we tested the growth of 27 genome-sequenced B. subtilis strains in the presence of luteolinidin to identify distinct growth patterns. We integrated these results into a genome wide association study (GWAS) to identify relevant genes potentially involved in the bacterial response to luteolinidin, and to provide insight into the growth of rhizobacteria when exposed to plant-exuded flavonoids. Moreover, we designed a simplified in-planta setup which enables the visualization of pigmented flavonoids formed on sorghum roots when in contact with the tested strains. The effect of root-exuded compounds on selected members of the microbiome, including potential plant growth-promoting rhizobacteria like Bacillus species, is a relevant aspect to consider in targeted microbial solutions towards sustainable agriculture.

This project is part of INTERACT within the Collaborative Crop Resiliency Program (NNF19SA0059360) funded by the Novo Nordisk Foundation.

P092

The ability of Anaerostips hadrus to degrade inositol contributes to the reduction of lipid and fat accumulation in Caenorhabditis elegans

Dr. Lei Liu[1], Miss J Qu[1], Dr. Eleni Tsompanidou[1], Prof.Dr. J.W.A. Rossen[1,2,3], Prof.Dr. J Fu[4,5], Prof. Dr. E.A.A Nollen[6], Prof. Dr. H.J.M. Harmsen[1]
[1]University Of Groningen, [2]University Of Groningen, [3]University Of Groningen, [4]University Of Groningen, [5]University Of Groningen, [6]University Of Groningen

Obesity is becoming a worldwide health issue, and its etiology has been associated with gut microbiota composition. An imbalanced gut microbiota has been reported to play a crucial role in obesity, which is also associated with lipid and glucose metabolism disorders, chronic inflammation, and an increase in developing metabolic diseases. The digestion of different carbohydrates by gut microbiota produces several
active substances, including short-chain fatty acids (SCFAs), that regulate gene expression and metabolic processes for maintaining human energy metabolism. A lower abundance of Anaerostipes hadrus has been linked to obesity and type 2 diabetes, and a ~31kb structural variation of A. hadrus encoding inositol catabolism is associated with human body mass index (BMI) and SCFA production. Nevertheless, the underlying mechanism remains unknown. Here, we investigate this relationship using a Caenorhabditis elegans model to understand better how A. hadrus strains that degrade inositol affect host energy accumulation. This
study showed the formation of propionate and acetate by inositol utilizing A. hadrus. Furthermore, we found that C. elegans fed with an A. hadrus strain with the inositol gene cluster grown on inositol had lower lipid and fat accumulation, which demonstrated the causal impact of inositol catabolism of A. hadrus on BMI. Here, the impact of gut microbiota utilizing different carbohydrates on host energy homeostasis is revealed at a strain level, suggesting a potential therapeutic method for metabolic disease treatment and prevention.

P093

Establishing optimized in vitro host-microbiota model systems

Freke Schaafsma[1], Dr. M.R. de Zoete[1]
[1]UMCU

The human intestine harbors a large variety of microorganisms, which comprises the gut microbiota. A close symbiotic relationship between the gut microbiota and the host exists, in which bacterial composition and function is tightly connected with human physiology and to health status. Research analyzing associations between microbiota composition and diseases has provided a wealth of information, but the underlying mechanisms largely remain to be elucidated. One of the limiting factors is the lack of appropriate complex in vitro model systems. Simplified in vitro models simulating the gut epithelium have been widely used to study bidirectional host-microbe effects and identify interactions between specific bacteria and host epithelial cells. However, the required co-culture of often strictly anaerobic bacteria on one hand and oxygen-demanding host cells one the other hand remains technically challenging. As a result, the culture and stimulation of human cells is usually done at ~21% oxygen, without considering the physiological low oxygen levels in the gut. Not only a large proportion of human gut bacterial species do not remain viable under these conditions, but also intestinal cells, which are adapted to naturally occurring low oxygen, exhibit altered, non-physiological host cell responses that may significantly impact host-microbe interaction research. We aim to optimize in vitro host-microbiota models that enable the co-culture of anaerobic gut bacteria with different human cells (e.g., Caco-2, small and large intestinal organoids) mimicking physiologically relevant conditions, with a focus on oxygen levels and mucus production. Using a hypoxia chamber in which oxygen levels can be tightly regulated, we will investigate bacterial and host cell growth, differentiation, metabolism and immunological responses to establish model systems to further unravel the complex interactions between the host and gut commensals.

P094

Human genetic determinants of invasive group A streptococcal infections

Dr. Gabriël Schut[1], Dr. B.W. Bardoel[1], Dr. M. van der Flier[2], Dr. H.L. Leavis[3], Dr. B. Boisson[4,5,6], Prof. JL. Casanova[4,5,6,7,8], Dr. A.N. Spaan[1]
[1]University Medical Center Utrecht, [2]Wilhelmina Children's Hospital, [3]University Medical Center Utrecht, [4]INSERM U1163, [5]Rockefeller University, [6]Paris Cité University, [7]Howard Hughes Medical Institute, [8]Necker Hospital for Sick Children

Introduction: Group A streptococcus (GAS) represents a considerable and worldwide cause of mortality and morbidity. The human-specific pathogen typically causes mild, superficial infections, but can also cause severe, invasive infections (iGAS) in a small subset of otherwise healthy individuals. The clinical variability and human specificity of GAS infections are poorly understood. We hypothesise that inborn errors of immunity (IEIs) underlie susceptibility to iGAS infections in some patients.

Methods: To decipher the human genetic determinants of iGAS infections, a prospective cohort comprising 85 otherwise healthy patients suffering from iGAS infections was assembled. Clinical data, whole exome sequencing (WES) data, and biological specimens were acquired for analysis. As a first step, we scrutinised the patients' WES data for the presence of established, but underrecognized IEIs. Variants in the set of 485 IEI-related genes were assessed by means of variant prioritization tools, and by fitting the data to known models of inheritance for these established IEIs.

Results: We identified candidate-variants in IEI-related genes in eight patients, suggesting an underrecognized IEI in ±10% of the cohort. Surprisingly, of these eight patients, three patients carried heterozygous ultra-rare and predicted deleterious missense variants in *TGFBR*1. All three patients suffered from iGAS infections, and one from a severe infection with *Staphylococcus aureus* at a later point in life. Autosomal dominant TGFBR1-deficiency underlies a connective tissue disorder with crosstalk to the Th_{17}-pathway, but without overt immunological disturbances.

Conclusion: To date, no IEIs are known to underlie iGAS infections in humans. Our findings indicate that autosomal dominant TGFBR1-deficiency is a human genetic etiology of iGAS infections. Functional characterizations of the patients' *TGFBR*1- alleles and immunological investigations are ongoing. Guided by our physiological-driven findings in *TGFBR*1, we will perform exploratory analyses of the remaining patients' WES data to discover and characterize new human genetic determinants of iGAS infections.

P095

Human milk oligosaccharides differentially modulate infant gut microbiota: a synthetic community approach

Yangwei Shan[1], Myrthe Beijnvoort[1], Prof. Stanley Brul[1], Dr. Jianbo Zhang[1]
[1]University of Amsterdam

Human milk oligosaccharides (HMOs) play an important role in the development of the gut microbiota in early life. The use of HMOs by individual infant gut 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 bacterial community (SynCom1) consisting of 8 representative infant gut species belonging to Bacillota, Bacteroidota, and Actinomycetota. SynCom1 was cultivated in media supplied with individual HMOs. As expected, we found that the total cell density of the community significantly increased and each individual HMO was completely consumed after 24h. Nevertheless, we observed divergent effects of HMOs in the bacterial composition and diversity, with sialylactoses dictate the dominance of Bacteroidota in the community. In contrast to sialyllactoses, neutral tetraoses maintained relatively higher diversity. The growth patterns of individual species in individual HMOs demonstrated that Bacteroides were the only one growing in sialyllactoses, supporting the dominance of Bacteroidota. Correspondingly, HMOs showed structure-dependent effects on the production of short chain fatty acids and organic acids such as acetate. Interestingly, butyrate was detected when SynCom1 grew in both sialyllactoses and tetraoses, despite the fact that the butyrate producer does not grow in all HMOs in monoculture, suggesting a potential cross-feeding. In summary, HMOs showed distinct effects on the infant gut community composition and metabolic function. The synthetic community could serve as a simplified model infant gut microbiota to test nutrients in the context of precision nutrition.

P096

Development of a Caco-2-based intestinal mucosal model to study intestinal barrier properties and bacteria-mucus interactions

Jinyi Su[1], Ms E Floor[2], Dr M Chatterjee[1], Msc E Kuipers[1], Dr N IJssennagger[2], Msc F Heidari[3], Msc L Giordano[3], Dr R Wubbolts[1], Dr S Mihăilă[3], Dr D Stapels[1], Dr Y Vercoulen[2], Dr K Strijbis[1]
[1]Utrecht University, [2]University Medical Center Utrecht, [3]Utrecht University

The intestinal mucosal barrier is a dynamic system that allows nutrient uptake, stimulates healthy microbe-host interactions, and prevents invasion by pathogens. The mucosa consists of epithelial cells connected by cellular junctions that regulate the passage of nutrients covered by a mucus layer that plays an important role in host-microbiome interactions. Mimicking the intestinal mucosa for in vitro assays, particularly the generation of a mucus layer, has proven to be challenging. The intestinal cell line Caco-2 is widely used in academic and industrial laboratories due to its capacity to polarize, form an apical brush border, and reproducibly grow into confluent cell layers in different culture systems. However, under normal culture conditions, Caco-2 cultures lack a mucus layer. Here, we demonstrate for the first time that Caco-2 cultures can form a robust mucus layer when cultured under air-liquid interface (ALI) conditions on Transwell inserts with addition of vasointestinal peptide (VIP) in the basolateral compartment. We demonstrate that unique gene clusters are regulated in response to ALI and VIP single stimuli, but the ALI-VIP combination treatment resulted in a significant upregulation of multiple mucin genes and proteins, including secreted MUC2 and transmembrane mucins MUC13 and MUC17. Expression of tight junction proteins was significantly altered in the ALI-VIP condition, leading to increased permeability to small molecules. Commensal Lactiplantibacillus plantarum bacteria closely associated with the Caco-2 mucus layer and differentially colonized the surface of the ALI cultures. Pathogenic Salmonella enterica were capable of invading beyond the mucus layer and brush border. In conclusion, Caco-2 ALI-VIP cultures provide an accessible and straightforward way to culture an in vitro intestinal mucosal model with improved biomimetic features. This novel in vitro intestinal model can facilitate studies into mucus and epithelial barrier functions and in-depth molecular characterization of pathogenic and commensal microbe-mucus interactions.

P097

Unlocking the microbiota's potential to improve immune checkpoint inhibitor therapy for cancer patients

Merel van Gogh[1], A. Celli[1], S. Gräve[1], R.J. Verheijden[2], M.J.M. van Eijs[2], E.J. van Dijk[2], K.P.M. Suijkerbuijk[2], J. Top[1], M.R. de Zoete[1]
[1]UMC Utrecht, [2]UMC Utrecht, Utrecht University

Introduction

For over a decade, immune checkpoint inhibitor (ICI) therapy is offered to cancer patients with a wide range of (advanced) cancers. Unfortunately, a large group of cancer patients do not respond to this therapy and/or develop (severe) side effects, such as colitis. What causes the varying response rate, and the occurrence of side effects remains largely unknown.

Recent years have implicated a crucial role for the gut microbiota in ICI response rate through its crosstalk with the immune system. We aim to further elucidate which members of the intestinal gut microbiota affect ICI therapy response and the occurrence of side effects, along with the underlying molecular mechanisms.

Methods & results

Firstly, we are using newly developed sequencing techniques (next-generation IgA-SEQ) to identify immunostimulatory bacteria which reveal (un)favorable bacterial strains. Besides these association studies we are interested in the underlying mechanisms; how do bacteria influence the immune system? We are using an in vitro reporter assay to screen the inflammatory potential of a wide range of bacterial strains from our human fecal-derived bacterial biobank. We have identified several known pro-inflammatory bacterial species, such as Akkermansia muciniphila and Fusobacterium spp., along with bacterial species with previously unknown inflammatory potential. On the contrary, we have also identified several bacterial species with anti-inflammatory potential. The underlying mechanisms are not fully resolved yet; however, this is our current focus of research.

Conclusion

In conclusion, we are investigating how the gut microbiota affects the immune system and thereby ICI therapy outcomes. In the future, we aim to identify new biomarkers for ICI therapy response and discover novel treatment strategies to improve ICI therapy outcome based on the inflammatory potential of members of the human gut microbiota.

P098

Characterizing Invasion Dynamics of Opportunistic Pathogens in Simplified Gut Microbiome Models

Pim Van Leeuwen[1], Dr. M.T. Wortel[1], Prof. S. Brul[1]
[1]University Of Amsterdam

The gut microbiome, a complex ecosystem of microorganisms, plays a vital role in host homeostasis, including nutrition, immune system development, metabolism, and defense against pathogens. Disruptions in its composition can lead to the invasion and overgrowth of opportunistic pathogens, jeopardizing host health. The intrinsic factors that determine whether these pathogens can invade and cause dysbiosis are not well understood. We aim to characterize the growth characteristics of opportunistic pathogens and their mode of invasion in established communities. We grew ten common gut microbiome members, of which two opportunistic pathogens, under anaerobic conditions to determine their growth characteristics and in the supernatant of all nine other members to determine their interactions. A mathematical model fitted on those interactions could predict relative abundance in co-cultures of up to five species. Next, we simulated invasions of the opportunistic pathogens in co-cultures of different sizes and validated four cases experimentally. This revealed that the two pathogens had different modes of invasion: (1) displacement of native species, resulting in a reduction or replacement of a consortium member, and (2) integration into the existing microbiome without displacing other species. We show that these modes can be explained by their differences in growth characteristics and interactions with the other microbiome members. We conclude that opportunistic pathogens can have at least two sets of properties to invade an established community. Future research is needed to reveal whether most opportunistic pathogens fall in one of those categories or whether there is a continuous spectrum of growth properties. This research underscores the importance of simplified community models in studying gut microbiome dynamics. Our insights may inform future therapeutic strategies aimed at preventing or mitigating pathogen invasions in the gut microbiome.

P100

Vancomycin-Induced Changes in Gut-Derived Bacterial Membrane Vesicles and Their Restoration with 2'-Fucosyllactose Supplementation

Drs. Jari Verbunt[1,2], Dr. Johan Jocken[2], Dr. Lars Vliex[2], Prof. Dr. John Penders[1], Prof. Dr. Ellen Blaak[2], Prof. Dr. Paul Savelkoul[1], Dr. Frank Stassen[1]
[1]Maastricht University Medical Centre +, [2]Maastricht University

Introduction
The microbiome plays a crucial role in human health. Gut-bacteria produce factors that travel to host organs. In animal models, bacterial membrane vesicles (bMVs) are shown to impact host metabolism. Antibiotic-induced disruption of microbiota can lead to abnormal bMVs, which may be modulated/restored using prebiotics. This study explores human gut-derived bMVs following microbiota disruption with vancomycin, and restoration through supplementation with prebiotic 2'-fucosyllactose (2FL).

Methods
Faeces were obtained from 37 participants with overweight/obesity (BMI 25-40 kg/m^2) and normal glucose-tolerance at (i) baseline, (ii) after a seven-day course of vancomycin, and (iii) after 8 weeks of 2FL supplementation or placebo. Gut-bMVs were isolated using (ultra)centrifugation and size-exclusion chromatography. Nanoparticle tracking analysis was used to assess bMV size and concentration. A human Toll-like receptor 4 (hTLR4) reporter was employed to measure bMV-associated endotoxin (Lipopolysaccharide/LPS).

Results
Post-vancomycin, mean bMV diameters decreased from 168 ± 5 nm to 144 ± 3 nm ($p < 0.0001$), while mean concentrations increased from 1.1×10^{10} bMVs/mL to 3.0×10^{10} bMVs/mL ($p < 0.0001$). Interestingly, only the placebo group showed bMV sizes similar to baseline after supplementation/recovery (161 ± 6 nm versus 146 ± 5 nm for the 2FL group). Additionally, post-vancomycin bMVs were more potent inducers of hTLR4 than baseline vesicles (OD600 1.31 ± 0.38 versus 0.48 ± 0.43, $p < 0.001$. No significant differences were observed between the placebo group and the 2FL group in terms of hTLR4 activation post-recovery.

Conclusion
This study is the first demonstrating that vancomycin alters human gut-derived bMVs. Antibiotic-induced overgrowth of endotoxin-producing bacteria appears associated with increased production of smaller bMVs. These vesicles are more effective at activating hTLR4 in-vitro. In further research on the properties and potential translocation of gut-derived bMVs we will deepen the knowledge on their roles in metabolic endotoxemia and disease.

P101

FalconSyn: a hydroponic growth system for high-throughput phenotyping of Arabidopsis thaliana with synthetic microbial communities

Dr. Xinming Xu[1], María Rodríguez[1], Prof. dr. Ákos Kovács[1]
[1]Leiden University

Soil and plants harbor an extensive, taxonomically diverse reservoir of microbes that are essential for plant development, fitness, and overall health. The vast diversity and complex interactions within plant-associated microbes pose challenges in deciphering the beneficial microbiota functions. Synthetic microbial communities (SynComs), that are designed to represent naturally occurring plant microbiomes, provide a powerful tool to reduce the complexity and establish causalities in plant-microbe interactions. Emerging research on SynComs has showcased its potential to translate mechanistic and ecological knowledge of plant-microbe interactions into sustainable crop management strategies. However, to effectively optimize SynComs in terms of size, composition, plant specificity, and multifunctionality, a high-throughput SynCom phenotyping system is essential. Such a phenotyping system should generate tractable data, including colonization efficiency, root morphology, plant biomass, stress resilience effects, and changes in root exudates, following the inoculation of diverse SynComs.

Here, we introduce FalconSyn, a novel hydroponic growth system for cultivating Arabidopsis thaliana with SynComs. FalconSyn employs standard 15ml Falcon tubes as growth vessels, ensuring cost-efficiency and adaptability. Using FalconSyn, we successfully cultivated Arabidopsis under sterile hydroponic conditions and demonstrated its versatility in applying various treatments, including stress assays, nutrient variation, and SynCom inoculation. Overall, this innovative platform provides a scalable and tractable solution for optimizing SynComs, with potential to advance research on plant-microbe interactions.

P102

Dephosphorylated tetra-acylated LPS is critical for immune evasion by Porphyromonas gingivalis

Phd candidate D. Xu[1], Phd candidate R.d.C Flores-Vallejo[1], Phd candidate Y. Ji[2], Dr. S. Jain[3], Pro. J.M. van Dijl[1]
[1]UMCG, [2]UMCG, [3]University of Washington

Periodontitis is a chronic inflammatory disease caused by the formation of dysbiotic bacterial biofilms in the gingival crevice. The Gram-negative bacterium Porphyromonas gingivalis is one of the main causative agents of periodontitis. In particular, P. gingivalis induces gingival inflammation, which attracts neutrophils to the site of infection. To avoid elimination by neutrophils, P. gingivalis employs the cysteine proteases RgpA, RgpB and Kgp, as well as a peptidyl-arginine deiminase (PPAD), which are secreted both in a soluble state and in association with so-called outer membrane vesicles (OMVs). It was previously shown that wild-type strains of P. gingivalis express heterogeneous lipopolysaccharide (LPS) forms that have either Toll-like receptor-4 (TLR4) antagonist activity or do not activate TLR4. The present study was aimed at investigating which LPS features allow P. gingivalis to avoid TLR4 activation and elimination by neutrophils. To this end, we employed mutant strains that produce phosphorylated and/or penta-acylated LPS forms instead of the dephosphorylated tetra-acylated LPS forms of wild-type strains. Our results show that especially penta-acylated LPS leads to TLR4 activation, enhanced expression of antimicrobial proteins in neutrophils, neutrophil degranulation and the formation of neutrophil extracellular traps. In addition, 4’ phosphorylated penta-acylated LPS leads to OMV aggregation and aberrant loading of OMVs with RgpA, RgpB and PPAD. Altogether, our findings show that in the formation of dephosphorylated tetra-acylated LPS is critical for immune evasion by P. gingivalis.

P107

Candida albicans Reduces Staphylococcus aureus Susceptibility to Antimicrobial Peptide SAAP-148

in Mixed Biofilms

Gizem Babuccu[1], full professor Martijn Riool[1,2], professor Dr Bastiaan P Krom[3], Assoc. prof. Sebastiaan A.J. Zaat[1]
[1]Amsterdam UMC, [2]Laboratory for Experimental Trauma Surgery, Department of Trauma Surgery, University Hospital Regensburg, [3]Academic Centre for Dentistry

Virulence, drug tolerance, and ultimately the outcome of polymicrobial biofilm infections are greatly influenced by microbial interspecies or even inter-kingdom interactions. This study investigates the synergistic interaction between Candida albicans and Staphylococcus aureus, focusing on the impact of excessive oxygen consumption of C.albicans on S.aureus. We assess how this interaction in planktonic and mixed biofilm forms affects S.aureus susceptibility to the Synthetic Antimicrobial and Antibiofilm Peptide, SAAP-148, which plays a key role in their polymicrobial biofilm infections.
The susceptibility of C.albicans SC5314 and S.aureus JAR060131 in planktonic and biofilm forms to SAAP-148 was assessed by quantitative culturing. Static mono-species biofilms were grown in RPMI-1640 medium with 2% glucose at 37°C for 24 hours in 96-well plates. For mixed biofilms, C.albicans was added first, allowed to adhere and form germ tubes for 90 minutes, then S.aureus was added and incubated for 24 hours at 37°C. Experiments were done under aerobic and anaerobic conditions using Oxoid™ CO2Gen™ sachets, with SAAP-148 treatment applied similarly.
As a result, planktonic S.aureus was 16-fold less susceptible to SAAP-148 when co-cultured with C.albicans compared to when grown as a single species. Mono-species S.aureus susceptibility increased only 2-fold in the absence of environmental O2. While 200 µM SAAP-148 completely killed mono S.aureus biofilms under aerobic conditions, the same concentration caused a 4.8-log reduction of mono S.aureus biofilms under anaerobic conditions. SAAP-148 did not cause a significant reduction in S.aureus when co-cultured with C.albicans in a biofilm.
S.aureus susceptibility to SAAP-148 is slightly reduced under anaerobic conditions in mono-species cultures, but further decreases when co-cultured with C.albicans in both planktonic and biofilm forms. The high oxygen consumption of C.albicans may limit oxygen availability, contributing to reduced AMP susceptibility in S.aureus. However, oxygen deprivation and C.albicans presence alone do not fully explain this tolerance, suggesting other mechanisms might be involved.

P108

Sink-associated outbreak of Verona integron-encoded metallo-β-lactamase-producing carbapenem-resistant Pseudomonas aeruginosa in a Dutch intensive care unit

MD PhD Sylvia Debast[1], Ing. MI Van den Bos-Kromhout[1], MSc. SV De Vries-van Rossum[1], none SEM Abma-Blatter[1], Ing. B Immeker[2], MD PhD JK Zuur[3], MD ML Hijmering[3], Prof. dr. JWA Rossen[1]
[1]Isala Hospital, [2]Isala Hospital, [3]Isala Hospital

Introduction: Moist environments, such as hospital sinks, are reservoirs for pathogens causing healthcare-associated infections (HAIs), particularly in ICUs. This study aimed to document an outbreak of a persistent carbapenem-resistant Pseudomonas aeruginosa (CRPA) strain linked to ICU sinks and to evaluate the efficacy of infection prevention measures.

Methods: This study was conducted in Isala's ICU. Selective culturing, PCR, and whole genome sequencing (WGS) were employed. Contact tracing and source investigation assessed colonization, carriage and infection rates.

Results: In November 2021, after repeated detection of a Verona integron-encoded metallo-β-lactamase-producing carbapenem-resistant P. aeruginosa (CRPA-VIM) in a siphon, the respective ICU sink was decommissioned. Two ICU patients tested positive for CRPA-VIM in December 2021 and April 2023, respectively. In October 2022, several interventions related to sinks were implemented, including installing splash- and aerosol-reducing inlets, replaced every three months. In December 2023, CRPA-VIM was detected in three patients. WGS revealed that the 2021 and 2023 isolates were genetically identical. Despite implementing a bundle of measures comprising contact precautions, intensified cleaning, improved hygiene procedures, retraining hygienic practices, monthly inlet replacements, and faucet modifications, a new cluster of five CRPA-VIM-positive patients emerged in March and April 2024. Patient and environmental sampling confirmed that the sinks remained the source. In response, all water fixtures in the ICU patient rooms were removed, establishing a water-restricted, drain-free environment. No new cases occurred thereafter.

Conclusion: CRPA-VIM was transmitted from sinks in patient rooms to patients, with no evidence of interpatient transmission. Comprehensive surveillance should encompass both patient and environmental sampling for effective infection control. WGS was critical in identifying a persistent strain linked to the outbreak. Transmission was halted after all water fixtures were removed from the ICU patient rooms. Hospital architecture should prioritize features that minimise microbial contamination from drains and sinks, reducing the HAI risk.

P109

Candida auris in Dutch hospitals, are we ready for it?

Dr. Laura Dix[1,2], Dr. Daan Notermans[2], Dr. Caroline Schneeberger[3], Dr. Karin van Dijk[4]
[1]RLM Dordrecht, [2]National Institute for Public Health and the Environment (RIVM), [3]Netherlands Institute for Health Services Research (Nivel), [4]Amsterdam University Medical Center

Introduction: Candida auris can cause nosocomial outbreaks and provides challenges concerning diagnosis, treatment, eradication and infection prevention. There are no Dutch standards or guidelines for C. auris and current hospital practices are unknown. Therefore, we assessed if Dutch hospitals are prepared for C. auris introduction.

Methods: An online questionnaire concerning screening, diagnostics, infection prevention and outbreaks was distributed amongst medical microbiologists and infection prevention practitioners in spring 2024.

Results: 52 were processed comprising 58 hospitals. Most participants (60%) did not screen for C. auris carriership and 51% did not have a protocol describing screening procedures. Healthcare workers were rarely screened. Screening sites and number of swabs varied. All respondents would place a patient with C. auris in isolation, 71% had a protocol describing isolation measures. Most hospitals took extra cleaning precautions after finding C. auris. None of the hospitals ever had a C. auris-outbreak, 29% had an outbreak protocol. Procedures to cease isolation were present in 31%, but 10% never declare a patient C. auris-free. A diagnostic protocol (available in 53%) was primarily based on culture, but the execution differed. Molecular diagnostics were rarely used (12%). The majority did not screen nor had a protocol describing MDR Candida outbreak coordination.

Conclusion: Screening, diagnostics, infection prevention, control and outbreak management of C. auris vary amongst Dutch hospitals, and most are not fully prepared for C. auris. As inadequate preparation for C. auris is an international concern, guidance documents could aid in fulfilling this need.

P110

Shining a Light on Mask Leakage: Using Fluorescent Tracers to Test Respiratory Protective Equipment Efficiency

Kirsten Lassing[1,2], L Eggenhuizen[1], Prof. dr. H. F. L. Wertheim[1], Associate Prof. P. T. J. Scheepers[2]
[1]Radboud University Medical Center, [2]Radboud University

The transmission of respiratory viruses through aerosols poses a significant public health risk, particularly in indoor environments. Effective respiratory protective equipment (RPE) is essential to reduce exposure to potentially infectious aerosols. Traditional testing methods often rely on pathogenic viruses or solid particles, which may not accurately represent aerogenic exposure. This study introduces a method utilizing a fluorescent tracer to quantitatively evaluate the protection efficiency and leakage of various masks.

A medical nebulizer was used to generate inhalable water droplets containing 1% fluorescein, with a particle size distribution of 6.5 - 14.8 µm. These droplets were sprayed in the direction of facemasks mounted on a mannequin head at distances of 10 cm and 30 cm. Fluorescein recovery was visualized and quantified from multiple materials, including a membrane filter placed in the mannequin's mouth, to assess the total inward leakage (filter penetration and face seal leakage). Various masks, including surgical masks and 3M Filtering Face Piece 2 (FFP2) respirators, were tested under controlled conditions.

The results aim to reveal differences in leakage and filtration efficiency across mask types. Preliminary data suggest that surgical masks exhibit higher leakage compared to 3M FFP2 respirators. Fluorescent tracer recovery demonstrates a direct correlation with facemask fit and filtration performance, highlighting the importance of proper fit for protection. The quantification of fluorescein enables calculation of the overall protection efficiency and total inward leakage.

This fluorescent tracer-based method provides a practical and quantitative approach for assessing RPE performance. We used a water droplet model which is closer to real-world aerogenic virus exposure compared to existing test standards that often based on solid particle testing. Future research will focus on refining the method in a human volunteer study, further advancing its application to further improve infection prevention strategies.

P111

Integrating patients' movement patterns into outbreak detection systems

Msc. Susanne Pinto[1], M.A.M. Harmelink-de Zoete[1], dr. M.S.M. van Mourik[1]
[1]UMC Utrecht

Timely detection of pathogen-related outbreaks in hospitals is essential for preventing onward transmission and ensuring effective infection control. Automated outbreak detection systems (AODS) have gained interest with the transition to electronic health record (EHR) systems, yet many existing methods overlook patient movements, focusing only on locations at the time of sampling. This limitation can delay or miss outbreaks involving patients who have transferred between wards.

We evaluated a newly designed AODS framework, based on the existing P75 and CLAR systems, incorporating patient movement data and ward clustering. Wards were grouped into communities based on shared medical services, hypothesizing that these clusters act as key hubs for pathogen transmission. Patient data, including prior ward visits and time spent on wards, were integrated to enrich the algorithm with additional contextual information. Using historical data (January 2014 to December 2021) from Utrecht University Medical Center's EHR system, the updated AODS systems were tested, and their alarms were reviewed for clinical relevance by infection prevention experts.

The base P75 system, which excludes movement history, performed well and was easy to interpret but likely missed critical spatial connections. Adding movement history improved detection by uncovering outbreaks that were previously undetected. However, this enhanced sensitivity also resulted in a higher number of false-positive alarms.

Incorporating patient movement data into AODS improves the sensitivity and accuracy of outbreak detection, addressing key gaps in current systems. However, further refinement is needed to balance detection accuracy with the burden of managing additional alarms. Future research should also focus on the careful investigation and optimization of parameters such as incubation periods and pathogen-specific transmission routes to ensure that the AODS performs effectively across all types of pathogens, minimizing the risk of missed outbreaks due to inaccurate assumptions.

P112

Impact of human milk oligosaccharides on the growth of early life pathogenic gut bacteria

Msc. Job Schlösser[1], Dr. M. M. S. M. Wösten[2], Prof. dr. G. Folkerts[1], Prof. dr. W. J. Unger[3], Drs. A. Groeneveld[4], Dr. S. Braber[1]
[1]Utrecht University, [2]Utrecht University, [3]Erasmus MC University Medical Center Rotterdam, [4]Friesland Campina N.V.

Background
Antibiotics are widely used to prevent and/or treat early life gut infections, but their overuse has raised global concerns about antibiotic resistance. Studies have shown that infants fed with breastmilk have a lower incidence of intestinal infections compared to those fed with infant formula. This observation stimulated scientific interest in the prebiotic and anti-pathogenic properties of breastmilk, specifically human milk oligosaccharides (HMOS). HMOS are non-digestible sugars that promote the growth of beneficial gut bacteria, such as Bifidobacterium and Bacteroides, while inhibiting the growth of pathogenic bacteria. Research has primarily examined the anti-pathogenic effects of pooled HMO mixtures. Our study will focus on the anti-pathogenic effects of individual HMOS, potentially enhancing the understanding of their specific roles and mechanisms.

Method
This study evaluates the growth-effects of individual HMOS on key early-life gut pathogens, Escherichia coli, Salmonella enterica, Yersinia enterocolitica, and Clostridium perfringens. Growth curves of these pathogens were generated by measuring the optical density over a 16-hour period. The area under the curve was used as an overall growth parameter to compare untreated versus HMO-treated pathogens. The most effective HMO-pathogen combination was further investigated by colony forming unit assays, testing in different growth media, and evaluation with clinical strains to confirm HMO-specific efficacy.

Results
S. enterica exhibited the most significant growth inhibition by HMOS and GOS, while C. perfringens demonstrated trends of both increased and decreased growth in response to the HMOS and GOS. Among the tested oligosaccharides, only 3'GL and GOS significantly impaired the pathogenic growth of all included Gram-negative facultative anaerobes. The overall screening revealed that slight structural differences in HMOs led to varying growth effects across the tested pathogens.

Conclusion
Overall, 3'GL and GOS emerged as the most promising candidates for studying mechanisms and early-life dietary applications due to their anti-pathogenic and structure-specific effects.

P113

First results of the implementation of the extended screening policy for patients admitted abroad as recommended by the Highly Resistant Microorganisms (HRMO) guideline.

Annelies Smilde[1], F. Stegeman-Heining[1], Dr. A.J.L. Weersink[2], Dr. R.H.T. Nijhuis[2]
[1]Meander Medical Centre, [2]Meander Medical Centre

Introduction

In October 2024, the revised guideline on highly resistant microorganisms (HRMO) became effective. This guideline states that in addition to screening for methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant Gram-negative bacteria (MDR-GNB), hospitals should also implement screening for vancomycin-resistant enterococci (VRE) and Candida auris for patients hospitalized abroad in the previous 12 months. Here, we present our findings of the first three months after implementation of the expanded screening policy.

Methods

An eSwab was collected from the nose, throat, rectum, axilla and groin of each hospitalized patient who should have been screened according to policy. MDR-GNB were screened using selective media, screening for MRSA and VRE was performed using an in-house real-time PCR assay after overnight broth enrichment, and Candida auris was screened using a commercially available real-time PCR assay (Altona). Positive real-time PCR results were followed by culture to isolate the specific HRMO.

Results

During a 3-month period, 66 patients had an admission abroad, of which 50 were admitted to our hospital and therefore screened for MRSA, MDR-GNB, VRE and Candida auris. Of these, 12 (24%) patients tested positive, including 9 (18%) for MDR-GNB and 3 (6%) for VRE. No MRSA or Candida auris were detected.
The MDR-GNB carrying patients were hospitalized in Turkey (4), Morocco, Germany, Hungary, Suriname (all 1) and 1 with the country unknown whereas the VRE positive patients had been hospitalized in Greece (2) and Turkey (1).

Conclusions

Focusing on the implementation of VRE and Candida auris diagnostics for the HRMO screening policy, our results show that three patients tested positive for VRE. By implementing the extended screening policy for patients hospitalized abroad, we were able to take the necessary isolation precautions and prevent possible further spread within the hospital.
Candida auris was not detected, probably due to its low prevalence and limited study period.

P114

Universal targeting of Staphylococcus aureus through antibodies binding wall teichoic acid

Dr. A. Robin Temming[1], A di Maggio[2], PMP van Bergen en Henegouwen[2], JDC Codeé[3], T Voskuilen[3], S Oliveira[2,4], NM van Sorge[1,5]
[1]Amsterdam Umc, [2]University of Utrecht, [3]Leiden University, [4]University of Utrecht, [5]Netherlands Reference Laboratory for Bacterial Meningitis

Staphylococcus aureus (S. aureus) is an opportunistic pathogen causing a diverse range of conditions like skin infections and sepsis. 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.

P115

Sinks at the ICU are reservoirs of antimicrobial resistance: a putative risk for critically ill patients.

MSc. Anne Verkerk[1], CAJ Rossel[2], Dr. SP van Mens[1], Prof. PHM Savelkoul[1], Dr. LB van Alphen[1]
[1]Maastricht University , [2]Zuyderland Medical Center

Antimicrobial resistance (AMR) threatens public health and healthcare worldwide. Extended-spectrum β-lactamase (ESBL) producing bacteria and carbapenemase producing Enterobacterales (CPE) complicate treatment significantly. AMR genes can disseminate clonally or via horizontal gene transfer (HGT). Patient and their bacteria may be exposed to bacterial reservoirs in the environment, possibly exchanging genetic material. To maintain effective healthcare and mitigate the spread of resistance, it is important to investigate the possible reservoirs in the patient environment and assess whether they pose a threat to vulnerable patient at the intensive care unit (ICU).

Taps (n = 41), sink drains (n = 44), and high-touch surfaces of patients and personnel (n = 57) at the ICU were sampled five times over 9 months, with an additional sampling of sinks at 12 months. Samples were plated on chromID ESBL-agar plates and isolates identified using MALDI-TOF. Resistance phenotype was determined via disc diffusion and genotypes via Illumina whole genome sequencing (WGS). The Gram-negative isolates of clinical samples underwent identical procedures.

Resistant bacteria able to grow on chromID ESBL-agar resided primarily in sink drains (688/735), with on average 2.8 isolates cultured from 88.4% of the sinks tested. Few isolates were recovered from the other surfaces (47/735). Of 735 isolates, 120 (16.3%) isolates were ESBL-producing Enterobacterales of the genera Citrobacter, Enterobacter, Escherichia, Klebsiella, and Pantoea. Additionally, 33 (4.5%) isolates contained carbapenemase of which 16 Pseudomonas sp. and 17 CPE. WGS revealed presence of NDM-7 in 9/15 (60%) environmental CPE. Two of these NDM-7 positive isolates were clonally related to three independent clinical isolates that had no link with the ICU.

These results indicate hospital sink drains as reservoirs of resistant Gram-negative bacteria, where genetic elements could be disseminated via HGT. Ongoing investigation with more patient samples will reveal whether a direct link between the environmental reservoir and patients exists.

P120

Extracellular electron transfer by ‘Candidatus Methanoperedens’: the role of multiheme cytochromes

Adrienn Groza[1], prof. dr. C.U. Welte[1], dr. P.E. Lopes Leão[1]
[1]Radboud Institute for Biological and Environmental Sciences

Anaerobic methanotrophic (ANME) archaea are important players in controlling the release of biogenic methane, a potent greenhouse gas. The genus ‘Candidatus Methanoperedens’ (ANME-2d) couples the oxidation of methane with the reduction of various electron acceptors such as nitrate, iron (Fe3+), manganese (Mn4+) or even electrodes. Since solid electron acceptors cannot be taken up by the cell, their reduction requires extracellular electron transfer (EET). Although 'Ca. Methanoperedens' can grow on bioanodes of bioelectrochemical systems by transferring intracellular electrons to the electrode, probably through the involvement of multiheme cytochromes (MHCs), the exact EET mechanism remains elusive. We hypothesise that different (sets of) MHCs are expressed depending on the properties of the solid electron acceptor. Here, we will investigate which MHCs are expressed by ‘Ca. Methanoperedens BLZ2’ during batch incubations with conductive particles of granular activated carbon of varying pore sizes, graphene oxide, and the metal oxides magnetite, birnessite, and ferrihydrite as the terminal electron acceptors. We will monitor the methane-oxidising activity through gas chromatography, assess gene expression using metatranscriptomics, and visualise the cells and the distribution of conductive particles/metals with scanning electron microscopy coupled with energy dispersive X-ray spectroscopy. The knowledge gained from this study will significantly contribute to the fundamental understanding of EET in archaea and can be used to support the development of biotechnological applications of ‘Ca. Methanoperedens’ to simultaneously reduce methane emissions and generate electricity during wastewater treatment.

P121

Getting out of the pH comfort zone: Adaptation potential of anaerobic methanotroph Ca. Methanoperedens

Vojtech Tlaskal[1,2], Cornelia U. Welte[1]
[1]Radboud University, [2]Biology Centre, CAS

Members of the archaeal genus Candidatus Methanoperedens are able to oxidise methane under anoxic conditions while utilising available electron acceptors such as nitrate. The most advanced laboratory enrichment cultures of Ca. Methanoperedens perform methane oxidation at near-neutral pH. However, 16S rRNA amplicon data from acidic peatlands show the presence of Methanoperedens also in acidic conditions with pH 4-5. Based on the presumed environmental diversity of the genus Ca. Methanoperedens, we investigated whether the available enrichment culture of Ca. Methanoperedens Vercelli Strain 1 is able to adapt to a stepwise decrease in pH in the cultivation bioreactor. Bioreactor batch activity assays utilising ^{13}C-CH_4 in the headspace were used to compare methane oxidation rates at individual pH checkpoints on the gradient from 7.25 to <6. The enrichment culture showed rather limited adaptation to pH changes. Biomass collection allowed determination of the proportion of Ca. Methanoperedens in the total bioreactor microbial community and per-dry mass rate correction. Analysis of archaeal membrane-spanning lipids from frozen biomass will allow to determine the extent to which Ca. Methanoperedens is able to modify its membrane composition in response to decreasing pH. This study shows that members of the genus Ca. Methanoperedens have optimised metabolism to their environmental niche with limited adaptation potential to pH changes. Such an observation can help understand their occurrence and estimate the activity of important anaerobic methane oxidisers in a range of potential habitats.

P126

Development of a new molecular test kit for quick and accurate detection of fungal pathogens

Alex Bonzi[1], CEO A.E. Budding[1], Senior Scientist AX. Van der Stel[1]
[1]Inbiome

Despite being largely overlooked in recent decades, fungal infections have been steadily increasing. Advances in modern medicine have led to the emergence of new opportunistic species causing infections, with invasive fungal infections presenting mortality rates as high as 80%, especially in immunocompromised patients. Early diagnosis is crucial, yet remains challenging, necessitating the collaboration of multiple experts to evaluate mycological, clinical, and radiological findings.

With the goal of improving long turnaround times, as well as difficulties faced when culturing certain species, Molecular Culture Fungi (MCFungi), a novel PCR-based diagnostic assay, has been introduced. By targeting the internal transcribed spacer (ITS) regions between the 18S and 28S genes, this assay allows for broad-range detection of fungal species. High-resolution melting (HRM) analysis is employed to identify and differentiate numerous (clinically relevant) fungi, with an average turnaround time of approximately four hours from sample to result, making it especially useful for the analysis of monomicrobial samples. In combination with fragment analysis, it also enables the analysis and identification of polymicrobial samples.

Critical aspects, including DNA extraction and the generation of non-specific products in the presence of human or bacterial DNA, were carefully examined and addressed during the assay's development. Additionally, a preliminary reference database of melt curve signatures, covering over 20 species, was established. Validation with a small set of clinical samples showed encouraging results, with most positive cases aligning with cultivation and species identification achieved in several instances using melt curve analysis alone

Although MCFungi is still in its early stages, the initial findings are promising. While there is room for improvement in overall sensitivity and the analysis of polymicrobial samples, the high specificity and rapidity of HRM present significant potential, with the possibility of improving patient outcomes in the future, all while maintaining cost-effectiveness.

Performance of 16S-23S interspace profiling for diagnosis of bacterial pericarditis

Dr. Martine Bos[1], Drs Maria Miguélez Sánchez[1], Drs Lauren Remijas[1], Dr Edgar J.G. Peters[3], dr Robin van Houdt[2], dr Dries Budding[1]
[1]InBiome, [2]Amsterdam UMC Location Vrije Universiteit Amsterdam, [3]Amsterdam UMC, location Vrije Universiteit Amsterdam

Introduction Bacterial pericarditis is a serious condition that can progress into purulent pericarditis, which is always fatal if left untreated. Diagnosis relies on culture of the pericardial fluid, which can remain negative in cases of difficult to grow organisms or prior antibiotic treatment. Molecular assays, based on DNA detection, have the potential to drastically improve diagnosis. We developed Molecular Culture® ID, a PCR-based pan-bacterial 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 (ISpro). Here, we evaluate the diagnostic accuracy of this novel test on pericardial effusions.

Methods Residual material of 42 pericardial effusion samples from 40 patients was subjected to DNA isolation followed by Molecular Culture® ID. Outcomes were compared to those of traditional culture.

Results Molecular Culture® ID identified 11 positive samples whereas culture detected 7. Four samples were concordant positive. Molecular Culture® ID identified 7 samples as positive where culture remained negative. These involved a.o. detections of Streptococcus pneumoniae/mitis group, Streptococcus pyogenes, Escherichia coli and Dermacoccus nishinomiyaensis . Three Cutibacterium acnes positive samples in traditional culture were not corroborated by Molecular Culture® ID. Two of these samples required enrichment culture to become positive, indicative of potential contamination. In contrast, the observed bacterial loads in all additional Molecular Culture® ID species identifications were medium or high. Three additional S. pneumoniae/mitis group-positive samples came from the same patient, supporting the true-positivity of these observations.

Conclusion Molecular Culture® ID detected bacteria in 1.6x as many samples as traditional culture (11 versus 7). This study demonstrates the enhanced potential of Molecular Culture® ID to diagnose bacterial pericarditis.

P128

Increased sensitivity using PCR detection of Salmonella species by adding an enrichment culture step

Dr. Sylvia Bruisten[1], Marenthe Fouraschen[1], Dr Brenda Westerhuis[1], Merel Admiraal[1], Fenna Bouwman[1], Dr Sacha Kuil[2]
[1]Public Health Laboratory GGD Amsterdam, [2]Amsterdam UMC

Introduction. Gastroenteritis (GE) is caused by various viruses, parasites and bacteria. When validating a multi-target commercial GE PCR we observed that 58% of Salmonella species were missed by direct PCR compared to bacterial culture by testing almost 700 samples. The aim of this study was to determine the sensitivity of Salmonella species detection and other infectious causes of gastro-enteritis when using a culture enrichment step.
Methods. Tenfold dilution series of pure bacterial culture suspensions or highly positive virus or parasite samples spiked in negative fecal suspensions were incubated overnight in triplicate using either selenite or GN broth after spiking them with 500 µL target solution. The Ct values of directly tested fecal suspensions were compared to those after the overnight enrichment step. Also 207 fecal samples were prospectively tested, comparing Ct values without and with a selenite enrichment step, according to instructions of the manufacturer (R-Biopharm): 50 µL selenite culture + 450 µL direct fecal suspension.
Results. The best PCR results in the spike experiments were obtained using selenite broth. There was a mean decrease of 12 Ct for Salmonella species. Other microorganisms could be detected more sensitive (Shigella and Yersinia) due to growth, or less sensitive (Campylobacter, all viruses and all parasites) due to dilution without growth in the broth.
In the prospective clinical study the Salmonella positive samples were detected with a mean of 14 Ct lower values. There were no extra Salmonella positives in the enriched samples, nor were any of the other targets missed using the selenite enrichment step as advised by the manufacturer.
Conclusion. Selenite enrichment of fecal suspensions substantially increases the sensitivity for detecting Salmonella species using PCR without affecting the detection of the other bacteria, viruses and parasites. This is a substantial improvement of GE diagnostics with only a minimal longer turn-around time.

P129

Molecular typing to discriminate between typhoid and nontyphoid Salmonella species

Dr. Sylvia Bruisten[1], Marenthe Fouraschen[1], Dr. Sacha Kuil[2], Fenna Bouwman[1], Merel Admiraal[1], Dr Brenda Westerhuis[1]
[1]Public Health Laboratory GGD Amsterdam, [2]Amsterdam UMC

Introduction. Salmonella enterica serovar typhi and para-typhi species are food-born enteral pathogens causing systemic enteral fever with a high morbidity. Complications arise in 30% of untreated typhoid Salmonella infections, whereas early treatment reduces the case-fatality rate to <1%. Contrarily, non-typhoidal Salmonella species cause mostly self-limiting gastroenteritis without antibiotic treatment. It is therefore imperative to quickly discriminate between typhoid and non-typhoid Salmonella infections.

Cultured Salmonella spp. are send to the RIVM for serovar typing with a turnaround time of several weeks. For faster results, a set of genes that discriminate between typhoid and nontyphoid Salmonella species was used to set up multiplex real time PCRs (Nair et al, JCM 2019). We aimed to validate these in house multiplex PCR results.

Methods. Pure cultures obtained after using selenite broth and Shigella/Salmonella agar plates were included with known outcome of serovar typing by multi-locus variable number of tandem repeat analysis and, in case of outbreaks, whole genome sequencing from RIVM. A set of genes was used to set up three multiplex real time PCRs. Control strains for each target gene were tested, with a Shigella flexneri strain as negative control.

Results. The targets for the different primer/probe sets for the real time PCRs were assessed for analytical sensitivity and specificity in multiplex combinations. During the study period of June to September 2024 a total of 10 Salmonella positive samples could be typed by serovar typing at the reference lab and by using the 3 multiplex PCRs. All samples showed fully concordant results, with 4 Salmonella enterica serovar enteritidis, one serovar Typhimurium and the other 5 typed as other nontyphoid.

Conclusion. A set of multiplex real time PCRs targeting specific genes can be used for fast discrimination between typhoid and nontyphoid Salmonella enabling fast treatment and preventive measures compared to centralized typing.

P130

Bordetella pertussis/parapertussis diagnostics, the performance comparison of in-house PCR to the Hologic assay

Drs. D. Hess[1], L.M. ter Horst[1], I. ten Dam[1], Dr. J.L. de Beer[1]
[1]Labmicta, Laboratory for Medical Microbiology and Public Health

Introduction After a few years of limited circulation due to the COVID-19 pandemic, the EU/EEA is currently in a high-incidence period for whooping cough caused by Bordetella pertussis (ref. ECDC). Especially in times of large epidemic perspectives of infectious diseases, it is of high importance to increase the awareness of health professionals and ensure public health capacity for early detection and diagnosis.

Methods A total of 243 samples from daily received samples in a diagnostic setting, nasopharyngeal/nose/throat swabs, were prospectively tested by an in-house PCR and the Panther Fusion Bordetella assay (Hologic). Both diagnostic platforms targeted the multicopy elements IS481 to detect Bordetella pertussis and IS1001 for the detection of Bordetella parapertussis. A commonly used conventional multiplex lab-developed test is compared to the CE-IVD assay on a high-throughput random access platform with just a few minutes of hands-on-time.

ResultsThe results show a high sensitivity for both assays, which makes them both suitable for diagnostic applications of whooping cough detection. As expected, only very small differences regarding the sensitivity were observed.

Conclusion Especially in times of high incidence, the molecular platform of Hologic with the Bordetella assay brings the benefits of random access and low hands-on time This allows a lab to perform fast and accurate diagnosis of whooping cough.

P131

Type III CRISPR-Cas for multi-input smart diagnostics

Sarah de Roode[1], Dr. H. van Leeuwen[2], Dr. B. Keijser[3], Dr. R.H.J. Staals[1]
[1]Laboratory of Microbiology, Wageningen University & Research, [2]TNO Defense Safety and Security, [3]TNO Healthy Living

Many applications require the simultaneous detection of multiple target molecules in a single reaction, which is challenging to achieve. Type III CRISPR-Cas systems are adaptive immune systems used by prokaryotes. They are promising in their use as diagnostic tools due to the stringency of cyclic oligoadenylate (cOA) signaling molecule production (which reduces off-target signals), the signal transduction achieved by ancillary effector protein activation (CARF and SAVED proteins), and inherent signal amplification upon target RNA binding. Type III systems have been integrated into diagnostic tools for the detection of viral diseases before, but the system's multiplexing potential has not been fully explored yet. Here, we aim to use the reactions of effector proteins (e.g. CARF and SAVED proteins) activated by cOA molecules to mimic basic Boolean logic operations with optical changes as output signals. We also aim to expand the repertoire of proteins activated by cOAs, allowing for multiplexed testing. Enzyme-based logic systems can embed multiple cues for a smart diagnostic output able to detect multiple targets at the same time, using the same tool.

P132

Promising novel molecular approaches compared to culture for detecting periprosthetic joint infections

Elizabeth Morreel[1], I.H.M. van Loo[1], R.M. Koeck[1], E. Beuken[1], P.H.M. Savelkoul[1], L. van Alphen[1]
[1]Maastricht Umc+

Introduction:
Periprosthetic joint infections (PJIs) are challenging to treat, as biofilm formation complicates pathogen detection using conventional culture. Molecular diagnostics like 16S rRNA sequencing and interspace-profiling (IS-Pro) potentially offer enhanced sensitivity and faster turnaround times.

Methods: We retrospectively analysed sonication fluid samples (n=21) from 18 patients. 16S rRNA sequencing was performed using Nanopore (MinION™) and analysed with in-house bioinformatics. IS-Pro (Molecular Culture®, inBiome B.V.) was analysed using proprietary Antoni software. Concordance with culture at genus and species levels was assessed, integrating results from non-sonication samples (tissues, synovial fluid), time-to-positivity, and contaminant/pathogen interpretation.

Results: The median culture positivity time was 4 days (range: 1–13), compared to 16S and IS-Pro turnaround times of 1–2 days and 4–8 hours, respectively. One sample was polymicrobial by culture. Concordance with culture at the genus level was 86.4% for 16S and 81.8% for IS-Pro, while species-level concordance was 77.2% and 81.8%, respectively. Negative 16S and IS-Pro results corroborated five contaminant culture interpretations.

In one sample, Proteus mirabilis was identified in culture (1/2 enriched liquid media) and deemed clinically relevant; however, it was not detected by either 16S or IS-Pro, suggesting contamination. In another sample, Pseudomonas aeruginosa and Staphylococcus aureus were identified by culture, but S. aureus was missed by 16S. A Staphylococcus epidermidis culture result was absent in IS-Pro, but detected as Staphylococcus species by 16S. In a culture-negative sample, IS-Pro detected Streptococcus dysgalactiae, corroborating findings from another sample of the same patient, thus enhancing pathogen detection.

Conclusion:
This first direct comparison of 16S rRNA sequencing and IS-Pro for PJI diagnostics highlights their potential to improve pathogen detection and distinguish pathogens from contaminants, with rapid turnaround times as a key advantage. This pilot study will expand to include approximately 180 additional samples, with further research needed to standardize thresholds and evaluate cost-effectiveness.

P133

Validation of targeted next-generation nanopore sequencing for routine diagnosis of gastroenteritis

Nicolas Vergauwe[1], Geert Meersseman[1], Silke Radoes[1], Margo Molhoek[2], Ray van der Veer[2], Annelies Riezebos-Brilman[3], Jeroen van de Bovenkamp[3]
[1]Impact Omics, [2]Diagnostiek voor U, [3]Laboratory for Medical Microbiology and Public Health (Labmicta)

We present a novel, targeted next-generation sequencing (NGS) methodology using the MinION device for routine clinical diagnostics of gastrointestinal infections in patients attending their primary care physician. This comprehensive diagnostic tool can detect all clinically relevant pathogens and currently comprises six bacterial species (with differentiation of four E. coli strains), four parasitic species, and five viral species. The NGS methodology is now validated for the bacterial and parasitic targets using a set of 214 retrospective and prospective clinical samples, and results were benchmarked against a qPCR reference method (Surefast GE panel) performed on the Roche FLOW system.

For samples with a Cq value lower than 30, the NGS methodology achieved 100% positive percent agreement with qPCR results. Samples with Cq values between 30 and 35 showed a 50% overall positive percent agreement. The negative percent agreement across all samples was 98.9% for the targets included in both tests. However, when considering all targets included in the NGS methodology, the negative percent agreement dropped to 77.2%, mainly caused by the presence of enteroaggregative E. coli and adenovirus.

This work marks the first application of targeted NGS for routine diagnosis of gastroenteritis outside the hospital setting. The NGS method can detect a broader range of pathogens with a low risk of false negatives, attributed to a reduced analytical sensitivity compared to conventional qPCR methods. Further, given the growing concern over antibiotic resistance, an expanded NGS panel with resistance markers could set a new standard for routine testing. Finally, the technique offers a significant economic advantage, as it is performed at the same cost—including manpower—as the qPCR method, making it a financially viable option for routine diagnostics.

P134

Development and evaluation of a rapid diagnostic assay for the screening of non-suppressible viremia in people with HIV-1

Ing Jolanda Voermans[1], Ing L.D. Schouwenburg[1], Ing D.G.J.C. Mulders[1], Dr. J.J.A. Kampen van[1]
[1]Erasmus MC

Introduction: Normally people with HIV-1 who receive antiretroviral therapy (ART) maintain an undetectable viral load. Yet in a minority of these people viral rebound occurs. Normally this is interpreted as active viral replication due to poor adherence to ART, other pharmacological issues or drug resistant HIV. Recently, this was challenged by the discovery that HIV proviruses with deletions in major splice donor (MSD) site can produce viral particles that are not replication competent but can lead to a detectable plasma viral load. This phenomenon is called non-suppressible viremia (NSV).

Methods: A double probed qPCR assay was developed for rapid detection of NSV. Analytical sensitivity was determined by serial dilution series tested in triplo. Retrospective clinical evaluation was performed on 85 stored plasma samples collected between January 2015 and June 2024: samples from people with HIV-1 suspected for NSV (n=57) and a control group (n=28) which consisted of baseline and ART resistance samples. Results were confirmed by Sanger sequencing.

Results: Analytical sensitivity was 4.38E2 c/ml, the viral load of the clinical evaluation samples was between 1.12E2 and 4.01E7. Linear regression lines were used to quantify the MSD qPCR. Log differences >0.8 between both probes resulted in changes in the MSD or ψ region, which were detected in 11/57 (19.3%) of the NSV suspected samples and in 4/28 (14.3%) samples of the control group. Mutations at the less conserved T742 position were found in both groups. A deletion confirming NSV and mutations at the highly conserved G744, T745 and G748 positions of the MSD and ψ site were only found in the group suspected for NSV.

Conclusions: We have developed a MSD qPCR which can be used for rapid screening of plasma samples for NSV. If the mutations at the highly conserved positions can also cause NSV has to be further investigated.

P139

Antibiotic resistance and gut microbiota dynamics during and following intercontinental travel

Jiyang Chan[1], Dr. Christian von Wintersdorff[1], Nathan Mills[1], Dr. Danyta Tedjo[1], Dr. Paul Savelkaul[1], Dr. Petra Wolffs[2], Dr. Niels van Best[1,3], Dr. John Penders[1]
[1]Maastricht University, [2]Maastricht University, [3]RWTH University Hospital Aachen

Introduction: With the rising accessibility of global travel, people regularly explore distinct environments. In this process, they expose themselves to new dietary habits, lifestyles, natural environments and infections which may affect their gut health and contribute to antimicrobial resistance genes (ARG) acquisition. Thus far, studies on the dynamics of the resistome and gut microbiota during travel are limited.

Methods: A cohort of 11 travellers were included with daily self-collected faecal swabs prior, during and after travel. Bacterial DNA was isolated from the collected samples and subsequently screened for the presence of several ARGs using qPCR and profiled by 16S rRNA gene amplicon sequencing to examine the microbial diversity, composition and community structure.

Results: The gut microbiota of the travellers displayed mostly stable diversity prior to travel, throughout travel and upon return. There were noticeable inter-individual differences as some individuals experienced a decrease in diversity following their arrival or during traveller's diarrhoea. Microbial community compositions highlighted inter-and intraindividual variation with distinct clustering of pre-travel, during travel and post-travel samples as projected in the Principal component analysis (PCA) plots of each individual's set of longitudinal samples. In addition, we found that ARGs encoding for quinolone resistance and/or for extended-spectrum beta-lactamases were acquired by participants who had visited South-Korea, India, China, Malaysia or the Philippines. The earliest detected ARG was on the 2nd day of travel and in a few cases, ARGs continued to be detected up to three months after travel.

Conclusions: Notable shifts in microbiota composition among travellers were detected, underscoring the gut microbiome's dynamic reaction to travel stressors. In addition, the acquisition of ARG harboring bacteria can occur within just several days, indicating that a relatively short exposure time to an antibiotic resistance prevalent environment is sufficient to contribute to the dissemination of ARGs around the world.

P140

PPE51 Modulates Membrane Integrity in Mycobacterium marinum

Vicky Charitou[1], Beatriz Izquierdo Lafuente[2], Eva Habjan[1], Joost Willemse[3], Wilbert Bitter[1], Alexander Speer[1]
[1]Amsterdam UMC, Location VUmc, [2]Vrije Universiteit Amsterdam, [3]Leiden University

PPE proteins, exclusive to mycobacteria and substrates of the Type VII secretion systems, have recently received attention for their association with nutrient uptake across the mycobacterial outer membrane and thus their potential as therapeutic targets. One of these nutrient transporters is PPE51, which is involved in the glycerol and glucose uptake in Mycobacterium tuberculosis. We investigated the role of PPE51 proteins in nutrient uptake and membrane integrity using the model organism Mycobacterium marinum, whose genome encodes four paralogs. We created single, double, and triple mutants of ppe51 and noted that a final ppe51 gene was essential for the bacteria. To assess whether PPE51 is also required for uptake of glycerol and glucose in the model organism M. marinum, despite having more copies, we performed growth experiments. It was observed that PPE51's function is conserved across these mycobacterial species. Further analysis of its secretion mechanism revealed that it is an ESX-5 substrate, part of the Type VII secretion system, which is consistent with its role in nutrient transport. Notably, the generated ppe51 mutants presented a substantially altered membrane permeability, as observed by increased EtBr influx and changes in membrane shape and cell cording, as shown by SEM analysis. Furthermore, the triple frameshift mutant demonstrated increased susceptibility to high molecular weight antibiotics, such as rifampicin and vancomycin. Infection experiments in murine macrophages showed that the triple mutant was attenuated, likely due to the observed loss of cell wall integrity. In conclusion, we have analyzed the characteristics of PPE51 in more detail, revealing a new role in membrane integrity and an indirect link with virulence.

P141

Interspecies induction of biofilm development in Bacillus subtilis

Jing Chen[1], K. Posthuma[1], N. V Machushynets[1], B. N.C. Bleichrodt[1], C Dinesen[1], G Wezel[1], Á. T. Kovács[1]
[1]Leiden University

Plants engage closely with a diverse community of microorganisms present in their vicinity. Among plant-associated bacteria, Bacillus subtilis is well-known for its ability to form biofilms that facilitate root colonization, promote plant growth, cycle nutrients, and defend against pathogens. The formation of these biofilms represents a complex biological system characterized by intricate processes and interactions that remain largely enigmatic, particularly regarding the interactions between different species that can either inhibit or promote biofilm development. Notably, B. subtilis biofilm formation is influenced by secondary metabolites produced by soil-derived bacterial isolates. Our research group has collected 123 spore-forming Bacillales strains from diverse habitats across the globe, a taxonomic group that is recognized for its capacity to produce various secondary metabolites. This study aims to identify chemical signaling molecules from these isolates that influence gene expression related to B. subtilis biofilm development, specifically focusing on eps and tasA genes that are required for essential extracellular matrix structure. For example, using LC-MS analyses, we discovered that Bacillus sp. (Ly60) produces pumilacidins, amicoumacins, and lipoamides, compounds associated with growth inhibition and potential biofilm modulation. Our next step is to identify peptide structures and to elucidate the gene regulatory pathways involved in signal perception in order to leverage this knowledge towards enhancing the plant-promoting properties of B. subtilis.

P143

Characterisation of Plasmid-Mediated Fluoroquinolone Resistance in E. coli Isolates from Broilers: A Retrospective Study

Sally Felle[1,2], MdB de Boer[2], MB Brouwer[2], AR Rebel[1,2], KV Veldman[2]
[1]Wageningen University & Research, [2]Wageningen Bioveterinary Research

Fluoroquinolones are potent, broad-spectrum antibiotics, which are important in human and animal health. Although veterinary fluoroquinolone sales in the Netherlands have decreased by 93.1% since 2011, fluoroquinolone resistance is still commonly found in indicator Escherichia coli from caecal samples of broilers (in 2023 25.6% were resistant). Resistance to fluoroquinolones is caused by genes found on plasmids and mutations in the chromosome. Plasmids play a pivotal role in the dissemination of antimicrobial resistance. The genetic determinants underlying this persistent fluoroquinolone resistance haven't been characterised. Understanding the epidemiology of fluoroquinolone resistance is essential for developing and implementing targeted interventions. The objective of this study is to determine the prevalence and genetic characteristics of plasmid-mediated fluoroquinolone resistance genes in E. coli from broilers in the last two decades.

E. coli isolates from the routine antimicrobial resistance monitoring of broilers in The Netherlands have been used in this study. EUCAST Epidemiological cutoff values were used to select isolates resistant to ciprofloxacin (>0.06 mg/L) from 2023 to 2004 (n=2568). These isolates were screened by PCR for the presence of qnrA, qnrB, qnrS, qepA and aac(6')-Ib-cr genes. PCR positive isolates were sequenced, using combined long read and short read methods.

The prevalence of plasmid-mediated resistance in isolates resistant to ciprofloxacin increased from 0.6% in 2009 to 10.8% in 2023. MLST analysis shows the isolates belong to diverse sequence types. qnrS and qnrB are the most frequently detected plasmid-mediated resistance determinants. qnrB genes are found on Col plasmids, while qnrS genes are found on a variety of plasmid types, with IncX and IncF the most common.

Fluoroquinolone resistance in broilers is mainly caused by mutations in the chromosome, which modifies the targets. However, plasmid-mediated resistance to fluoroquinolones has increased over the last 20 years. The genes that cause this resistance are found on mobilizable plasmids.

P144

Chitosan oligosaccharides as potential anti-infectives against antibiotic resistant respiratory pathogens.

Maria Blanca Fernandez-Ciruelos[1,2], S Vos[1], R. J. Pieters[4], W. J. Unger[3], G Folkerts[1], M. S. M. Wösten[2], S. Braber[1]

[1]Utrecht University, [2]Utrecht University, [3]Erasmus University Medical Centre, [4]Utrecht University

The healthcare and economic burden of antibiotic resistance (AR) infections is steadily increasing, while the discovery of novel antibiotics has stagnated. Thus, there is a need for the discovery of novel and alternative treatments. Lower respiratory infections are responsible for the largest number of deaths caused by AR. The main causative agents being Streptococcus pneumoniae in community settings and Staphylococcus aureus and Pseudomonas aeruginosa in hospital settings.

This project aims to evaluate a selection of nine natural occurring non-digestible oligo- and polysaccharides for their potential to inhibit growth, virulence and/or reduce AR of respiratory infection causing bacteria.

Of the nine selected oligo- and polysaccharides, chitosan oligosaccharides (COS) (derived from crab and shrimp shells, formed by deacetylated units of N-acetylglucosamine, and positively charged) were observed to reduce the growth of AR clinical isolates of Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus (MRSA). We then conducted checkerboard assays to explore the synergistic effect between COS and β-lactam antibiotics in β-lactam resistant Pseudomonas aeruginosa and MRSA and we calculated the Fractional Inhibitory Concentration Index (FICI) for the different combinations. We saw synergistic effects (FICI < 0.5) between COS and ampicillin and third-generation cephalosporines in P. aeruginosa (MICAMP and Cefotaxime from 100 to 6.25 µg/ml). In addition, we saw that COS reduced the MIC of the third-generation cephalosporin ceftriaxone from 100 to 6.25 µg/ml in MRSA. Preliminary mechanistical studies suggest that COS increases membrane permeability in both gram-positive and gram-negative bacteria, facilitating β-lactams in reaching their targets. Finally, we also saw that COS is able to reduce P. aeruginosa virulence traits such as swarming and motility.

In conclusion, COS shows a significant potential in combatting antimicrobial resistant infections by reducing bacterial growth, virulence and re-sensitizing resistant bacteria to β-lactam antibiotics. Our data suggest COS can be a promising compound for adjunctive therapies.

P145

IMPACT OF INFLUENZA-LIKE ILNESS ON THE HUMAN NASOPHARYNGEAL AND OROPHARYNGEAL MICROBIOTA IN THE AGING POPULATION

Jolanda Kool[1], H.H van Dijken, F Fuentes, M.L Chu, D. van Baarle, N.M Nanlohij, P. Konstanti, J. van Beek, E. van Woudenbergh
[1]Rivm

Introduction:
Influenza virus causes annual seasonal epidemics, resulting in millions of severe cases worldwide. Vulnerable populations, including elderly individuals, have the highest risk of complications and a severe illness. With the microbiota of the respiratory tract acting as a gatekeeper to provide resistance to colonization of respiratory pathogens, it is important to understand the role of the microbiome on the severity and sesceptibility of Influenza-like-illness (ILI). This study aims to investigate the impact of ILI on the microbiome within the oropharyngeal (OP) and nasopharyngeal (NP) niches in an aging population.

Methods:
A cohort study was performed in the Netherlands during 2 consecutive influenza seasons. Nasopharyngeal and oropharyngeal swabs were collected to perform quantitative analysis of the bacterial cells, determine the microbiome composition by sequencing the 16S rRNA gene and measuring the cytokine profiles of all individuals.

Results:
We demonstrated an increase in bacterial load in the individuals during acute ILI compared to a healthy situation. Although this effect did not result in a significant change in alpha diversity, we did observe a significant difference in the beta diversity for the NP samples, with a significantly higher amount of Escherichia shigella in the healthy participants and a higher Rhodococcus in infected participants. Both NP and OP samples have a more stable microbiome (SparCC) network in a healthy status compared to the ILI situation, although this effect is more profound in the NP samples.

Conclusions:
In conclusion, the NP niche appears to be influenced the most during acute ILI in an aging population and is therefore the most appropriate niche to investigate during Upper Respiratory Tract (URT) infections.

P146

Transforming Waste to Bioplastic, Butanol, and Blend

Christian Kröly[1]
[1]Wageningen University
We feed gasified waste streams (CO2, CO, H2) to anaerobic bacteria and produce an array of different products, that are traditionally synthesised from crude oil or sugars.

This has been done before on an industrial scale
with microbial mono-cultures for ethanol, and on a
laboratory scale for higher value products with synthetic microbial co-cultures (Two or more selected strains are being cultivated in the same reactor).

Here, we aim to increase the conversion efficiency of
these co-cultures by molecular engineering of acetogens (e.g., Eubacterium limosum ATCC 8486), and thereby enabling the bioconversion of gaseous waste streams to products such as "bioplastic" (polyhydroxyalkanoates), odors (esters), and jet fuel blend (butyl-butyrate).

P147

Plasmid acquisition related collateral effects in Klebsiella pneumoniae

Andreas Liaropoulos[1], PhD AL Liakopoulos[1]
[1]Utrecht University

Introduction:
The global spread of extended-spectrum cephalosporin-resistant (ESCR) Klebsiella pneumoniae represents a healthcare emergence requiring novel therapeutic strategies. The dissemination of ESCR is primarily driven by the horizontal transfer of epidemic plasmids carrying such resistance genes. Collateral sensitivity (CS)—where resistance to one antibiotic increases sensitivity towards other antibiotics—has emerged as a potential approach to reduce or reverse antibiotic resistance by designing effective antibiotic cycling protocols. In our study, we investigated the collateral effects associated with acquisition of plasmids encoding ESCR in K. pneumoniae.

Methods:
Using a panel of 40 diverse ESCR-encoding plasmids, we generated 40 ESCR K. pneumoniae ATCC 43816 mutants via conjugation. Minimum inhibitory concentrations (MICs) for 12 commonly used antibiotics were determined for each strain via broth microdilution in triplicate. CS was determined by comparing the MICs of each resistant strain to the isogenic wild type strain, with their magnitude defined as the log2-fold change. Conserved collateral effects were assessed based on the CS_{50} and CR_{50} threshold, defined as effects occurring in more than 50% of the tested mutants.

Results:
Each of the 40 generated K. pneumoniae mutants harbored a unique ESCR-encoding plasmid, representing 11 different plasmid replicon types (X, I1, F, A/C, N, K, B/O, Y, HI1, HI2, L/M) and encoding 10 different resistance genes, including blaCTX-M, blaTEM, blaSHV, and blaCMY variants. We observed highly conserved CS to rifampicin (87.5%) and fosfomycin (60%) with a median fold-decrease in MICs of 2, thus highlighting these antibiotics for CS-based treatment protocols. Conversely, conserved collateral resistance (CR) to ciprofloxacin (78.1%) and trimethoprim-sulfamethoxazole (50%) was evident.

Conclusions:
This study represents the first detailed mapping of CS/CR networks in ESCR K. pneumoniae, providing a foundation for novel therapeutic strategies to combat ESCR K. pneumoniae infections.

P148

Two Conserved Lipoproteins Play Unexpected Key Roles in Mycobacterial Cell Envelope Integrity

Robin Lissner[1], P Moynihan[2], W Bitter[1], C Kuijl[3]
[1]Vrije Universiteit Amsterdam, [2]University of Birmingham, [3]Amsterdam UMC

Being the most deadly bacterial pathogen, Mycobacterium tuberculosis (Mtb) poses a significant global health threat. A key factor contributing to Mtb's virulence is its unique cell envelope, which acts as a protective barrier and mediates direct interactions with the host. Among the components of this envelope, lipoproteins represent a critical but understudied group of proteins. Although a small subset of these proteins has been linked to lipid transport, nutrient acquisition and virulence, the functions of most Mtb lipoproteins remain unknown. In this study, we focused on 79 conserved putative lipoproteins shared between Mtb and the closely related model organism M. marinum. Leveraging the recently developed CRISPR1-Cas9 (Sth1Cas9) gene editing system for Mycobacteria, we generated frameshift mutations in each conserved non-essential lipoprotein-coding gene, targeting one gene at a time. The resulting library of validated lipoprotein mutants was then evaluated for various phenotypes, including antibiotic susceptibility. We identified two mutants, lpqZ and fecB, that exhibited increased susceptibility to all tested antibiotics, suggesting essential roles in cell envelope biogenesis. Interestingly, despite belonging to the class of periplasmic binding proteins, neither protein is associated to any cytoplasmic transporter complex. Instead, co-immunoprecipitation experiments revealed that these lipoproteins interact with the key enzymes AftA and AftB, which are involved in lipoarabinomannan (LAM) and arabinogalactan synthesis. These interactions were validated through reciprocal pulldowns, accompanied by observed alterations in LAM production in lpqZ and fecB out-of-frame mutants. Alphafold predictions and conserved residue analyses further supported a direct interaction between the lipoproteins and AftA or AftB. Together, these findings show that periplasmic binding proteins can have alternative functions in chaperoning key enzymes involved in arabinogalactan biosynthesis.

P150

Overexpression of Outer Membrane Proteins (OMPs) in Outer Membrane Vesicles: A Model for Studying Vesiculation and OMPs in native form

S. S Sahu[1], G K Koningstein[1], Dr PvU Ulsen[1], Dr J. L Luirink[1]
[1]Alife Vrije Universiteit

Outer membrane vesicles (OMVs) are nanoparticles that shed form the outer membrane (OM) of Gram-negative bacteria, playing roles in virulence, stress and intercellular communication. Conceptually, OMVs constitute an ideal biological scaffold for the expression of homologous or heterologous outer membrane proteins (OMPs) in their native environment for instance for structural studies.

This study investigates OMV formation in the protein expression strain Escherichia coli BL21(DE3) Gold ΔABCF that lacks the major OMPs (OmpA, LamB, OmpC, OmpF). We show that overexpression of OMPs in this strain leads to extreme hypervesiculation. The expressed OMPs are efficiently included in the OMVs in a folded and assembled conformation. Furthermore, the integrity and size of the OMVs appears to be on par with natural OMVs. Taken together, the OMVs from this strain are ideally suited for expression of OMPs in their native environment. Importantly, the expressed OMPs are by far the most abundant proteins in these OMVs.

Methods

OMVs were isolated by ultracentrifugation and analysed for size distribution and particle count using nanoparticle tracking analysis. Protein folding, topology, and assembly were assessed using heat-modifiability, protease accessibility and protein complex analysis.

Results

The ΔABCF strain was found to produce ten times more OMVs than its parental strain, with production dramatically enhanced upon overexpression of the Bam complex or PhoE. OMP expression was found to correlate with the number of OMVs produced. The OMVs appeared structurally intact, with a uniform size distribution. The Bam complex and PhoE were properly folded and assembled within the OMVs in their correct orientation. Interestingly, a higher proportion of folded BamA was detected in OMVs compared to the cell envelopes of whole bacteria, suggesting selective recruitment of folded OMP during OMV formation.

Conclusion

The E. coli BL21(DE3) Gold ΔABCF strain is ideal for native OMP overexpression in OMVs, enabling structural and functional studies.

P151

Investigating mirA: A PPE38-Independent ESX-5 Substrate and Its Interaction With N-WASP in Mycobacterium marinum

Arbaaz Sait[1], dr. C Kuijl[1], prof. dr. W Bitter[1,2]
[1]Amsterdam UMC, [2]Vrije Universiteit Amsterdam

Introduction:
The ESX-5 secretion system is essential for the virulence of Mycobacterium marinum, mediating the secretion of PE-PGRS proteins that facilitate host-pathogen interactions. One such substrate, mirA, promotes actin tail formation, a process critical for bacterial motility. Unlike other ESX-5 substrates, mirA secretion occurs independently of PPE38, a known chaperone for ESX-5 substrates. Interestingly, PPE38 mutants exhibit enhanced actin tail formation. MirA also does not seem to require processing by PecA, a PE-PGRS protease known to cleave itself as well as other PE-PGRS proteins. Given the similarity between mirA and IcsA, a Shigella protein that binds N-WASP to drive actin polymerization, we hypothesize that mirA interacts similarly with specific N-WASP domains to regulate actin dynamics.

Methods:
Actin tail formation was assessed by infecting U2-OS cells expressing F-tractin-mGFP with the wild-type, ESX-5, and PPE38 mutant strains of M. marinum. Additionally, we analyzed the role of PecA, an ESX-5-associated protease, in processing and activating mirA by comparing actin tail formation in wild-type and PecA/B/C mutant strains.

Results:
Our results confirm that mirA secretion and activity were independent of PPE38, with PPE38 mutants forming even more actin tails than the wild type. Furthermore, mirA functions without processing by PecA. Preliminary binding studies suggest that mirA interacts with specific N-WASP domains, supporting its functional similarity to IcsA.

Conclusion:
MirA is a unique ESX-5 substrate that bypasses canonical secretion and processing requirements while facilitating actin tail formation in M. marinum. These findings expand our understanding of the diverse mechanisms utilized by ESX-5 substrates and highlight mirA's role as a key player in host-pathogen interactions.

P152

Diversification of blaOXA-48-harbouring plasmids among carbapenemase-producing Enterobacterales, 11 years after a large outbreak in a general hospital in the Netherlands

Dr. Pieter Smit[1], Dr C Tienen[1], F Landman[2], S Zager[1], M Drijver[1], A Burggraaf[1], D.W Notermans[2], M Damen, A.P Hendrickx[2], C Jamin[2]
[1]Maasstad Ziekenhuis, [2]RIVM

After a large blaOXA-48 plasmid mediated outbreak in 2011, routine screening of patients at risk of CPE carriage on admission and every seven days during hospitalization was implemented in a large hospital in the Netherlands. The objective of this study was to investigate the dynamics of the hospitals' 2011 outbreak associated blaOXA-48 plasmid among CPE collected from 2011-2021.

A selection of 86 blaOXA-48 carrying CPE isolates was made from 374 isolates collected over an 11-year study period. Species included Escherichia coli (Eco), Klebsiella pneumoniae (Kpn), Enterobacter cloacae complex (Ecl), Citrobacter freundii (Cfr), Citrobacter koseri (Cko) and Morganella morgani (Mmo). Short-read sequencing was combined with long-read sequencing for all isolates to reconstruct blaOXA-48-like plasmids and chromosomes of CPE. pOXA-48 plasmids were compared to plasmids sequences that were sequenced for the Dutch CPE surveillance.
In total for the 86 CPE, 2 failed genomic assembly, 78 blaOXA-48-encoding plasmids were reconstructed and 6 blaOXA-48 genes were located chromosomally. The 2011 outbreak associated blaOXA-48 plasmid of 63.6 kb with IncL replicon was found in Cfr, Ecl, Eco, Kpn, Mmo and primarily between 2011-2014 and, indicated as LR025105 as MASH nearest neighbor. From 2014 onwards, eleven other types of blaOXA-48 carrying plasmids with different antibiotic resistance genes and replicons were discovered, representing the earlier defined distinct pOXA-48 plasmid groups found in the Netherlands. Furthermore, on a national level the LR025105 plasmid was found after 2015 in many different bacterial backgrounds, highlighting the promiscuous nature of this pOXA-48 plasmid.

After a large blaOXA-48 outbreak in a large hospital in Netherlands, the composition of the blaOXA-48 plasmid population in this hospital diversified over time and is in line with national surveillance data. Plasmid sequencing provided valuable insight into transmission dynamics of OXA-48-containing plasmids and showed no indication of persistence of the 2011 blaOXA-48 plasmid in the hospital environment.

P153

Proximity versus activity: the molecular uncoupling of OTULIN recruitment to LUBAC from its enzymatic function.

Barathram Swaminathan[1], L. Scheepmaker[1], Dr. G.M Baltodano[2], Dr. B. Boisson[3,5], Prof. Dr. J. Bustamante[3,5], Prof. Dr. J. Casanova[3,6], Dr. B. Bardoel[1], Dr. A.N. Spaan[1]
[1]UMC Utrecht, [2]Hospital Infantil "Manuel de Jesús Rivera", [3]St. Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, [4]Necker Hospital for Sick Children, [5]Paris Cité University, [6]Necker Hospital for Sick Children, [7]The Rockefeller University

Introduction:
M-1 ubiquitin chains regulate cellular responses to inflammation and infection. Their homeostasis is controlled by the Linear Ubiquitin Chain Assembly Complex (LUBAC) and the deubiquitinase OTULIN. Mutations in the genes encoding LUBAC components or OTULIN, particularly in its catalytic domain, increase susceptibility to bacterial infections. OTULIN contains a PUB-Interacting Motif (PIM) domain, enabling it to bind LUBAC. The nature and consequence of this interaction in human physiology remain unclear. Whole exome sequencing in a patient with severe hyperinflammation identified a homozygous, predicted deleterious, missense mutation in the OTULIN-PIM domain. We hypothesize this mutation underlies the patient's disease by disrupting OTULIN-LUBAC binding, adversely affecting M-1 ubiquitin chain regulation.

Methods:
The patient's allele (p.R57C) was characterized in overexpression by assessing the capacity to inhibit NF-kB signalling. OTULIN-LUBAC interactions in patient-derived primary dermal fibroblasts (PDF) were assessed by immunoprecipitating LUBAC components. Using inhibitors targeting checkpoints of the NF-κB pathway, the response of the PDFs was measured using cellular viability assays.

Results :
In isolation, the allele was normally expressed and functionally active, indicating that the patient's disease cannot be explained by a loss of catalytic activity. In the patient's PDFs, OTULIN expression was normal compared to controls, but M1 ubiquitination was markedly increased. Immunoprecipitation revealed a reduced binding of OTULIN to LUBAC. The patient's PDFs were sensitive to TNF-induced cell death under stress conditions. Caspase inhibition improved viability, while RIPK1 inhibition showed no effect, suggesting an alternate mechanism underlying apoptosis in patient PDFs compared with the catalytically inactive variants.

Conclusion:
The patient's mutation disrupts the recruitment of OTULIN to LUBAC and causes dysregulation of M-1 ubiquitin-mediated inflammatory signalling. The molecular uncoupling of the catalytic activity of OTULIN from its role in recruitment to the LUBAC complex explains differential cellular response to TNF when compared with OTULIN variants with catalytic deficiency.

P154

GENDARM: GENe-Directed re-sensitization of Antibiotic Resistant Microorganisms within complex communities

Dr. Thaysa Tagliaferri[1,2], Dr Damien Tortuel[3], Tanja Schwab[2], Nicolas Charbonnel[3], Daniel Kaplan[2], Sylvine Batista[3,4], Alina Viehof-Beckmann[2], Ousmane Traoré[4], Racha Beyrouthy[4], Richard Bonnet[4], Geneviève Bricheux[3], Thomas Clavel[2], Christiane Forestier[3], Hans-Peter Horz[2]
[1]Maastricht University Medical Center, [2]RWTH Aachen University Hospital, [3]Université Clermont Auvergne, [4]CHU de Clermont Ferrand

Introduction: CRISPR-Cas-derived tools have widened gene editing possibilities within bacterial genomes. Yet, the applicability of the system strongly depends on the development of efficient and specific delivery systems. Here, we constructed two CRISPR-Cas9 delivery systems based on conjugation and transduction to perform in situ gene editing. We aimed at promoting plasmid clearance, while keeping the CRISPR-Cas9 recipient cells alive in order to maintain the microbial community unchanged. Methods: For the conjugation delivery system, an auxotrophic mutant of E. coli K12 MG1655 was used and the CRISPR-Cas9 was delivered via the highly conjugative IncP-1β plasmid pB10. Phage delivery was performed with a priori developed non-propagative mutant version of the T7 phage, packed with either a conjugative or a non-conjugative CRISPR-Cas9 carrying plasmid. As CRISPR-Cas9 target, the plasmid-borne resistance gene blaOXA-48 was selected and inserted into the E. coli TG1 strain. Delivery, editing and plasmid clearance rates were evaluated in planktonic cultures, biofilms and under gut microbiome conditions using ddPCR and colony forming units counting. Results: Regarding conjugation delivery, higher conjugation rates were achieved (10^{0} to 10^{-1}) in planktonic cells when compared to biofilms (10^{-2} to 10^{-4}). For phage delivery, transduction rates reached similar levels in planktonic cells and biofilms ($<10^{-2}$). As opposed to conjugation, cell killing was observed in the host TG1 cells as a side effect when transduction was performed in both planktonic cells and biofilms. Similar levels of conjugation and transduction rates were achieved when CRISPR-Cas9 was delivered in the intestinal tract of Oligo-MM12 mice. Re-sensitization (>90%) was achieved in all scenarios, resulting in blaOXA-48 plasmid clearance in the CRISPR-Cas9 treated cells. Microbiome composition showed no change following both conjugative- or phage-delivery of CRISPR-Cas9. Conclusion: Both phage and conjugation systems were able to deliver CRISPR-Cas9 at different efficiencies, achieving plasmid clearance in planktonic cultures, biofilms and gut microbiome conditions.

P155

Cross-domain plasmid mediated AMR transmission

C Jamin[1], RM Koeck[1], MFQ Kluytmans - van den Bergh[2,3], JA Stegeman[4], JAJW Kluytmans[4], CJPA Hoebe[1,5,6], PHM Savelkoul[1], Dr LB Van Alphen[1], i-4-1-Health Study Group
[1]Maastricht University Medical Center (MUMC+), [2]University Medical Centre Utrecht, [3]Amphia Hospital , [4]Utrecht University, [5]Public Health Service South Limburg, [6]Maastricht University

Introduction

Extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE) significantly contribute to the disease burden among hospitalized patients. A One-Health approach is essential to identify the spread of antimicrobial resistance between different domains (humans and animals) and identify potential reservoirs. The recently conducted i-4-1-health project collected swabs from pigs and chickens, children in daycare centers and patients in care facilities in the Dutch-Belgian border region. Sporadic transmission of clonal resistant isolates among humans and between humans and animals was observed. Here, we aimed to determine the relative contribution of plasmid dissemination to the overall spread of antibiotic resistance genes between domains.

Methods

450 Enterobacteriaceae isolates were subjected to long-read whole-genome sequencing using Oxford Nanopore technology on R9.4 flow cells. Plasmids were reconstructed and clustered using Unicycler and MOB-typer.

Results

In total 1,139 plasmids were identified in 450 isolates. The median number of antimicrobial resistance (AMR)-encoding plasmids per domain was 1 for humans, 1 for broilers, and 2 for pigs. One potential transmission cluster of bacterial dissemination between humans and broilers was identified. However, 94 potential AMR plasmid clusters were found. Of these, 20 clusters (21%) contained plasmids present in both humans and animals. In nine of these 20 clusters, plasmids were highly related and classified as One-Health plasmids. The largest cluster of these coded for blaCTX-M-1 on an IncI plasmid.

Conclusion

The i-4-1-health project revealed limited clonal transmission of antibiotic-resistant bacteria. Long-read sequencing demonstrated plasmid size and diversity varied across domains, but clusters could be identified. These findings highlight the critical role of plasmids and mobile genetic elements in the spread of antibiotic resistance and suggest cross-domain AMR transmission between humans and animals at the plasmid level. Addressing AMR within a One-Health framework, with a focus on mobile genetic elements, is crucial for effective control and prevention of antimicrobial resistance.

P156

Diversity and taxonomic classification of Campylobacterota plasmids and development of a Campylobacter plasmid typing scheme

Dr Linda van der Graaf[1,2], S Bloomfield[3], A Mather[3], Depict Consortium, A Schürch[4], B Duim[1,2], J. Wagenaar[1,2,5], A. Zomer[1,2]
[1]Utrecht University, [2]WHO Collaborating Centre for Reference and Research on Campylobacter and Antimicrobial Resistance from a One Health Perspective / WOAH Reference Laboratory for Campylobacteriosis, [3]Quadram Institute, [4]University Medical Center Utrecht , [5]Wageningen Bioveterinary Research

Introduction

Campylobacteriosis is the most common bacterial foodborne infection in Europe and poultry meat is a major source of human infection. The coexistence of Campylobacter spp., like C. jejuni and C. coli, in the same (animal) host and under the same selective pressure can lead to an extensive increase in interspecies horizontal gene transfer (HGT). The aim of this study is to describe the network and diversity of plasmids from phylum Campylobacterota, to be able to classify them in plasmid taxonomy units (pTUs), followed by the development of a typing scheme for Campylobacter spp. plasmids based on the plasmid-backbone.

Methods

For the taxonomic classification, a set of 1,145 plasmid sequences from phylum Campylobacterota hosts was compiled from public databases, in-house generated sequences, and predicted binned sequences using ECPlasmid/gplas2. We constructed an ACcNet bipartite network and classified the plasmid taxonomy as described by Redondo-Salvo et al, 2020. For the development of the Campylobacter plasmid typing scheme, a set of 1,185 plasmid sequences from the genera Campylobacter was compiled from public databases, in-house generated sequences and predicted plasmidomes using RFPlasmid. The Campylobacter plasmid typing database consisted of four marker genes: replication genes, mobilization proteins, single-stranding binding proteins, and virD4 genes of the type IV secretion system, and can easily be used to screen WGS data using the tool Abricate.

Results and conclusion

The bipartite networks showed that plasmid-encoded proteins cluster separately according to the Campylobacterota family. The taxonomic classification based on Average Nucleotide Identity scores resulted in 13 pTUs, of which eight were specific for Campylobacteraceae and five for Helicobacteraceae plasmids. The developed typing scheme can be used to classify Campylobacter plasmids based on four plasmid-backbone genes. Inter- en intraspecies transmission of plasmids between Campylobacter spp. and within the phylum Campylobacterota indicated HGT and plasmid mediated exchange of AMR genes.

P157

Staphylococcus aureus α-Toxin Displays a pH-Dependent Activity in a Cell-Type Specific Manner

Tristan van der Linden[1], Lisette Scheepmaker[1], Dr. Bart Bardoel[1], Dr. András Spaan[1]
[1]University Medical Center Utrecht

Introduction: Staphylococcus aureus is a major cause of infection-associated morbidity and mortality in humans. An important S. aureus virulence factor is the pore-forming toxin α-toxin (AT). Despite being one of the best studied S. aureus virulence factors, the molecular determinants defining AT cytotoxicity in the human host are incompletely understood. In particular, the role of environmental factors on AT cytotoxicity is understudied. Here we study the effect of the pH on AT cytotoxicity.
Methods: We used a panel of human nonhematopoietic (A549 and HaCaT) and hematopoietic (U937 and THP-1) cell lines to demonstrate the effect of culture medium pH on AT cytotoxicity. Culture medium pH was calibrated and measured under live-cell conditions. AT cytotoxicity was evaluated using cell viability. Surface expression of the AT receptor A Disintegrin and Metalloprotease 10 (ADAM10), AT binding, heptamerization and pore-formation were measured in A549 cells. The role of ADAM10 metalloprotease activity was investigated in A549 cells expressing wild-type and catalytically inactive ADAM10 variants.
Results: Acidification of the culture medium conditions enhanced AT cytotoxicity in nonhematopoietic, but not in hematopoietic cells lines. In the human alveolar epithelial cell line A549, pH-driven changes in cytotoxicity were independent of ADAM10 expression, activity, or AT binding. Instead, culture medium acidification led to greater increases in membrane permeability through enhanced pore formation.
Conclusion: S. aureus AT displays a cell type-specific, pH-dependent potentiation of activity in non-hematopoietic cell lines. As such, our work reveals the local pH as a molecular determinant of AT cytotoxicity by mediating the process of pore formation. Testing the effect of pH on cytotoxicity induced by other pore forming toxins will reveal the specificity of AT as a pH-dependent cytotoxin. These findings provide an understanding of the effects of the acidification of infected tissues and biofilms on S. aureus virulence.

P158

Phage Pool Party: Developing a High-Throughput Assay to Determine Phage Activity

Baltus Adrianus van der Steen[1], dr. Yuval Mulla[1]
[1]Vrije Universiteit Amsterdam

The firm rise of antibiotic-resistant bacterial infections and stagnation in the discovery of new antibiotics has become a major worldwide concern. Phage therapy is considered one of the most potent alternatives to antibiotic resistance. However, determining the right combination of phages to put into a cocktail for treatment is a laborious and time-intensive process that relies on century-old poorly scalable technology. Therefore, we aim to develop a novel high-throughput method to quickly analyse and characterize a large number of phages to determine which are the most potent for treatment. In brief, we expose many phages simultaneously to a single bacterial isolate. As the bacterium replicates, active phages multiply on their host. Next, by sequencing the metagenomic pool of phages present at the end, we can detect which phages have replicated and by comparing the number of reads per phage before and after infection we can quantify a multiplication factor for each phage. Our initial findings are promising to build further upon and show that phages with the fastest replication cycle take over the phage pool. Furthermore, due to the high accuracy for assigning DNA sequence reads and low false positivity rate (<1/1000), phages present at very low densities can be detected.

P159

Identifying viable Neisseria gonorrhoeae through validation and application of viability RT-PCR

Drs. Sem Vellema[1,2], M Lucchesi[1,2], PHM Savelkoul[1], CJPA Hoebe[1,2,3,4], PFG Wolffs[1,2]
[1]Department of Medical Microbiology, Infectious Diseases and Infection Prevention, [2]Care and Public Health Research Institute (CAPHRI), [3]Department of Sexual Health, Infectious Diseases, and Environmental Health, [4]Department of Social Medicine

INTRODUCTION The incidence of gonorrhoea, caused by the bacterial pathogen Neisseria gonorrhoeae (NG), is increasing globally, accompanied by rising antimicrobial resistance, including resistance to first-line treatment. Current molecular diagnostic methods, such as nucleic acid amplification tests, cannot differentiate between viable and non-viable bacteria. Bacterial culture—an inherent viability assessment—is challenged by the organism's fastidious nature and sensitivity to environmental factors. Molecular viability assays have been developed, but to our knowledge not yet applied to NG. This study aims to validate and apply a viability PCR (V-PCR) method for NG, while correlating the results to successful culture recovery in clinical samples.

METHODS Viability was assessed using PMAxx, a membrane-impermeable dye that selectively binds to nucleic acids in non-viable cells, allowing for discrimination between viable and non-viable cells. Technical validation employed dilution series of viable NG mixed with heat-killed NG, treated or untreated with PMAxx, followed by PCR targeting the porA gene to determine delta Ct-values based on PMAxx status. Clinical samples (anorectal, oropharyngeal, and urogenital) were subsequently analysed using V-PCR, and results were compared with standard diagnostic culture results.

RESULTS Validation demonstrated that V-PCR eliminated 99.97% of DNA from non-viable NG, with strong correlation in viability detection (R^2=0.973) across dilution series. Clinical samples showed a significantly higher median log viable load of 3.69 log_{10} copies/mL (IQR: 0.872–7.39) in culture-positive samples than culture-negative samples (10.7 log_{10} copies/mL; IQR: 6.05–13.1, p=0.0003). Median viability percentage was significantly higher in culture-positive samples (16.4%; IQR: 0.887–37.7%) compared to culture-negative samples (0.31%; IQR: 0.01–11.9%, p=0.0003).

CONCLUSION V-PCR effectively discriminates viable NG from non-viable cells and performs well across varying bacterial concentrations. V-PCR reliably assesses viability in clinical specimens, with higher absolute and relative viability shown in culture-positive samples. V-PCR holds potential for improving diagnostic stewardship and provides insights into factors affecting culture recovery and infection dynamics.

P160

Gene amplifcation as a consequence of de novo acquisition of antibiotic resistance

xinyu Wang[1]
[1]uva

Amplification of chromosomal fragments surrounding resistance genes is a common phenomenon in bacteria exposed to sublethal concentrations of antibiotics. Six bacterial species were evolved to acquire resistance to high concentrations of six antibiotics, with each tested in duplicate. Among the 72 resistant strains obtained, gene duplication and amplification (GDA) were identified in 17 strains, along with 5,582 SNPs. The length of repetitive sequences at the junctions may suggest the mechanism triggering gene amplification. Specifically, repetitive sequences of <3 bp (19/24), 7–8 bp (2/24), and 30–40 bp (3/24) were documented at the junctions of amplified fragments, correlating the level of GA with the fragment size. A read-splitting mapper was used to investigate whether GA had integrated into the other chromosomal locations. Additionally, long-read sequencing was performed to determine whether GA occurred within or outside the chromosome. We conclude that gene amplification is a common way for bacteria to develop antimicrobial resistance.

P161

White-box Machine Learning Identifies a Novel Mechanism of Action for Celastrol

Msc Jurian Wijnheijmer[1]
[1]University Of Amsterdam

The rising prevalence of antibiotic resistant bacterial infections is threatening human health. This highlights the need for the development of antimicrobial compounds with novel mechanisms of action. In the postgenomic era, transcriptome profiling has become a useful tool in mechanism of action prediction, but has not yielded the expected results in identifying novel mechanisms. In this study we employed machine learning to predict the mechanism of action of an antimicrobial compound found in thunder god vine, namely celastrol, and identify it as a potentially novel mechanism. We then followed up with an extensive mechanism of action study on the compound using a Bacillus subtilis model. We showed the previously proposed mechanism of ftsZ binding and subsequent cell division impairment to be an unlikely killing mechanisms of celastrol. Instead, we found celastrol to affect membrane structure and fluidity without causing depolarization of invagination of the cell membrane. In this, we demonstrated the potential for using transcriptomic profiling and machine learning in identifying novel mechanisms of action from the natural world and facilitate mechanistic knowledge on celastrol, facilitating future drug development efforts.

P162

Breaking barriers: differences in permeability and resistance between various M. avium strains

Sanne Peters[1], M Thomas[2], M Oudejans[1], Dr. J van Ingen[2], Dr. E.N.G Houben[1]
[1]Vrije Universiteit Amsterdam, [2]Radboud Medical Centre

The genus Mycobacterium consists of over 200 species, including the notorious pathogens Mycobacterium tuberculosis and Mycobacterium leprae. In addition, there are numerous opportunistic mycobacterial pathogens, often referred to as nontuberculous mycobacteria (NTM), that roam free in the environment. Over the past decades, the numbers of infections caused by NTM species has increased drastically, with the most important species belonging to the Mycobacterium avium complex. These infections are difficult to treat due to high intrinsic resistance to many antibiotics, resulting in poor treatment outcomes. In this project, we aim to get insight into the underlying mechanisms of intrinsic resistance by linking resistance profiles of different M. avium strains, including 3 lab strains and 10 clinical isolates, to cell wall permeability, efflux pump activities and metabolic preferences. Here, we show variability in cell wall permeability, suggesting differences in cell wall architectures between M. avium strains, and a correlation of low permeability with high resistance against antibiotics that target intracellular structures. Genomic comparison suggests the involvement of an energy conversion gene cluster potentially linking lower permeability to increased lipid production. These insights into mycobacterial cell wall composition and its connection to antibiotic resistance will contribute to the design of novel therapeutic agents to treat NTM infections.

P171

Subjective Vs. Objective Health:

A Comparative Study Between Self-Rated Health and a Frailty-Like Health Index.

David Boverhoff[1,2], J. Kool[1], R. Pijnacker[1], Q.R. Ducarmon[3], G. Zeller[3], S. Shetty[1], F. van der Klis[1], L. Mughini-Gras[1,4], D. van Baarle[1,2], S. Fuentes[1]
[1]RIVM, [2]University Medical Center Groningen, [3]European Molecular Biology Laboratory, [4]Utrecht University

Introduction: this study examines the relationship between the gut microbiome and health in individuals aged 15–87 years.
Methods: participants provided fecal samples (n = 2,867) and completed health and lifestyle questionnaires. We used two health indices, including a self-rated health (SRH) and a composite health index (CHI), created by combining 25 variables of disease history, medication use, and other health-related factors.
Results: Shannon diversity was significantly higher among individuals self-rating their health as "Very good" or "Good" compared to those rating it as "Fair" or "(Very) poor". Similarly, the CHI showed a negative correlation with diversity, with individuals scoring zero (total absence of disease) showing the highest diversity. Analysis of microbiome composition revealed significant differences within age groups, with Bifidobacterium and Prevotella among the most influential taxa. Multivariate analyses linked health indices to specific bacterial genera. Genera associated with better health, such as Christensellaceae R-7, were positively correlated with health indices, while potentially pathogenic taxa like Streptococcus were negatively correlated. Functional predictions using PICRUSt2 indicated reduced sugar degradation pathways in individuals with better self-rated health. Network analysis further demonstrated clustering of butyrate-producing bacteria for both health indices, suggesting functional bacterial interdependencies.
Conclusion: our findings highlight complementary insights provided by a subjective self-rated health score and an objective health index. Both indices aligned in identifying higher diversity and health-associated taxa in healthier individuals. However, they also provided distinct microbiome signatures, emphasizing their combined utility in defining microbiome characteristics indicative of health. This research underscores the potential of integrating subjective and objective measures to elucidate gut microbiome associations with health. These findings contribute to defining the characteristics of a healthy gut microbiome and underscore its potential clinical relevance for disease prevention and early detection in the future.

P172

Keep the hospital clean; diagnostic performance of ten different molecular and culture-based methods to detect Candidozyma auris.

Dr. Koos Korsten[1], B. Gerrits van den Ende[2], R.D. Pique[1], Prof. F. Hagen[2], Dr. K. van Dijk[1]
[1]Amsterdam UMC, [2]Westerdijk Fungal Biodiversity Institute

Rationale: Candidozyma auris (formerly Candida auris) is a globally emerging multi-drug resistant human pathogenic yeast. To detect C. auris we aimed to compare different culture-, and molecular-based methods.
Methods: Rectal swabs routinely collected in clinical care were spiked with different concentrations of C. auris. Co-infection/colonization was mimicked by spiking part of these samples with other pathogenic Candida species. Spiked materials were cultured at 37°C or 42°C using two different CHROMagar Candida plates. In parallel, samples were incubated in a dulcitol salt enrichment broth. Additionally, we compared seven in-house and commercial molecular tests on the direct material and from the broth one day after inoculation.
Results: Culture-based methods showed sensitivities up to 100% within 48 hours of incubation, although sensitivity decreased as low as 44% at lower concentrations (≤50 CFU per inoculum), in the presence of an abundance of other species and at higher temperature (42°C). Incubation at 42°C made visual identification possible since other species with similar colony morphologies did not grow at this temperature. No added value of using the dulcitol salt enrichment broth was found. qPCR on direct materials was highly sensitive and specific (both up to 100%) but major differences between various molecular tests were observed.
Conclusion: We showed that both culture-based and molecular methods are sensitive for diagnosing C. auris. The clinical setting (routine screening versus an outbreak), local prevalence and the load in those that carry or are infected by C. auris are important factors to consider when determining which diagnostic tests should be employed.

P173

Diagnostic Stewardship using the Antibiotic Treatment Modeling Tool: A Semi-Automated Evaluation of Diagnostic Practices for Bacterial Infections

Msc Martijn Siepel[1,2], Dr Kim Sigalof[1], Dr Martijn Schut[3], Dr Jan Prins[1], Dr Rogier Schade[2]
[1]Amsterdam UMC, [2]Amsterdam UMC, [3]Amsterdam UMC

Diagnostic stewardship ensures accurate infection diagnosis before antibiotics are prescribed. Traditional analyses often focus on single prescriptions, limiting insights into entire treatment courses. To address this, we developed the Antibiotic Treatment Modeling Tool (ATMT) to combine consecutive prescriptions into courses, including details like indications, cultures, and practitioner information. Using the ATMT, we evaluated the appropriateness of culturing practices related to antibiotic prescriptions.

Using the ATMT, we analyzed data from inpatient, outpatient, and emergency settings at Amsterdam UMC (Jan–Jul 2022), grouping consecutive prescriptions per admission into 6,457 courses with associated indications and culture data. An appropriate culture was collected from minimally required specimen types per indication within 72 hours before to 24 hours after antibiotic initiation (up to 48 hours after for intra-abdominal infections). We calculated the proportion of first prescriptions with at least one appropriate culture per indication, stratified by practitioner groups: surgical, emergency, and others.

Compliance, defined as ordering at least one appropriate culture per indication, varied by indication and practitioner group. For "unknown/sepsis of unclear origin," compliance was high: emergency practitioners at 92.9%, surgical at 90.6%, others at 88.1%. For urinary tract infections (UTI) and respiratory tract infections (RTI), compliance was lower. In UTIs, "others" had higher compliance at 81.4% compared to surgical and emergency groups at 71.7%. For RTIs, compliance varied: emergency practitioners highest at 76.1%, others at 70.5%, surgical lowest at 60.3%.

The ATMT revealed significant differences in culture-taking practices across indications and practitioner groups. High compliance occurred when infection sources were unknown, but gaps exist for UTIs and RTIs where culture-taking is equally important. Insufficient material for RTI cultures partly explains this; further investigation is needed to assess its extent. The ATMT integrates diagnostic and prescription data, enabling targeted interventions to enhance diagnostic accuracy and optimize antibiotic use, strengthening antimicrobial stewardship in other settings.

P178

Herpesvirus reactivation, including EBV, is associated with COVID-19 disease severity.

Naomi Berkeveld[1], Dr. Wouter L. Smit[2], Dr. Michiel Heron[1], Dr. Steven F.T. Thijsen[1,3]
[1]Diakonessenhuis, [2]University Medical Center Utrecht, [3]Erasmus University Medical Center

To date, it remains unclear why Corona Virus Disease 2019 (COVID-19) causes different disease progression in individuals. While most people experience mild symptoms, some individuals require hospitalization or even admission to the Intensive Care Unit (ICU). Reactivation of herpes viruses has been suggested as a possible cause of COVID-19 disease severity (e.g. persistent symptomatology, long COVID). Therefore, we aimed to investigate whether the severity of COVID-19 is associated with a different extent of herpesvirus reactivation.

In total, 198 hospitalized COVID-19 patients were included in the study of whom 171 serum samples, 183 nasopharyngeal swabs, and 89 saliva samples were collected at the time of admission. Hospitalized patients were categorized into two groups: severe disease (ICU, 45%; 89/198) and moderate disease (hospitalized, 55%; 109/198). Mild or asymptomatic cases comprised 61 non-hospitalized SARS-CoV-2-infected hospital care workers of whom a serum sample was collected. All samples were analyzed for EBV, CMV, HSV-1, and HHV-6 DNA using real-time PCR.

EBV and HHV-6 DNA were detected significantly more often in serum of severe disease than in that of moderate disease 59.8% vs. 39.3% (p= 0.007) and 26.8% vs 13.5% (p= 0.025). Detection of CMV DNA was not significant different (8.5% vs 2.2%) and HSV-1 DNA was not detected. In serum of mild or asymptomatic cases the herpesviruses were not detected. Samples of nasopharyngeal swabs and saliva showed no significant differences between COVID-19 disease groups. Whether herpes virus reactivation was linked to persistent symptomatology or long COVID could not be investigated within this cohort.

EBV DNA was detected in serum of more than half of patients with severe COVID-19 disease, while a significantly lower percentage was detected in patients with moderate disease. EBV DNA was not found in mild or asymptomatic cases. These findings may suggest an association between EBV and severity of COVID-19.

P179

Respiratory syncytial virus NS1 protein prevents interferon and chemokine secretion by differentiated primary epithelial cells in vitro.

Rosanne Koutstaal[1,2], A.J. Lakerveld[1], A. Munoz Garcia[1], N Ebert[3], M.F. Licheri[4], H Hamstra[1], A.T. Gelderloos[1], R Dijkman[4], V Thiel[3], C.A.C.M. Van Els[1,2], P.B. Van Kasteren[1]
[1]Rijksinstituut voor Volksgezondheid en Milieu (RIVM), [2]Utrecht University, [3]University of Bern, [4]University of Bern

Respiratory syncytial virus (RSV) is a major cause of severe respiratory infections in children, older adults and immunocompromised individuals. To this date, the immunopathology of severe RSV disease is not fully understood. Nonstructural protein 1 (NS1) has been found to modulate the host immune response by repression of interferon (IFN) production, thereby also interfering with downstream antiviral pathways. Characterization of NS1 was mainly performed in immortalized cell lines, which do not necessarily represent the in vivo situation.

In this study, we assessed the effect of inactivating NS1 mutations on replication and host responses in well-differentiated human primary airway epithelial cells (AEC) cultured at air-liquid interface. We hypothesized that NS1 mutants induce increased interferon production in AEC, leading to reduced viral replication. The NS1 mutations used in this study have been described before [1] and were incorporated in a 1998 RSV-A strain using a newly developed RSV reverse genetics system based on previous work by Thi Nhu Thao et al. [2].

In AEC, infection with NS1-mutated strains results in enhanced cytokine production compared to wild-type virus, negatively impacting viral replication. Bulk RNA-sequencing analysis of AEC infected with wild-type or NS1-mutant RSV not only showed an upregulation of genes involved in the antiviral response, but also of chemokines that are known to attract cells of both the innate and adaptive immune system. These findings were confirmed using a Legendplex assay, in which increased chemokine levels were detected in basolateral supernatants of cultures that were infected with NS1-mutant compared to wild-type RSV. Further experiments are ongoing to confirm an effect on immune cell recruitment in vitro.

In conclusion, our data suggest that the RSV NS1 protein supports viral replication not only via inhibition of IFN production, but also by reducing chemokine secretion by infected epithelia, likely resulting in decreased immune cell recruitment.

P180

Determinants and their relative contribution to SARS-CoV-2 intrahost evolution in immunocompromised patients

Kees Mourik[1], drs. N.A. Rogowski[1], dr. N. Kuttiyarthu Veetil[1], dr. S.A Boers[1], dr. A.H.E. Roukens[1], dr. S.P. Jochems[1], prof. A.C.M. Kroes[1], dr. I. Sidorov[1], prof. J. Goeman[2], dr. J.J.C. de Vries[1]
[1]LUMC, [2]LUMC

Previous studies have associated the evolution of SARS-CoV-2 genetic variants with prolonged infections and subsequent transmission. However, the underlying determinants remain incompletely understood. Here, we describe SARS-CoV-2 intrahost evolutionary signatures in immunocompromised patients (N=10) and immunocompetent controls (n=5), in relation to host, virus, and treatment determinants. Opposed to observations in immunocompetent patients, genomic evolutionary signatures were highly dynamic in solid organ transplant and haematological patients. Distinct dynamic patterns were observed for both non-synonymous and synonymous mutations. Convalescent plasma treatment was associated with significantly higher mutation rates, predominantly in the spike genomic region. Ranking the determinants by their relative contribution resulted in the largest dependence being immunocompromised status, treatment duration, followed by antibody treatment. In addition, novel geometric sequence profiling assisted prediction of determinants for intra-host evolution. These findings suggest that accumulation of mutations over time in prolonged infections is a major factor driving SARS-CoV-2 intrahost evolution. This provides a rationale for treatment of prolonged infections as a preventive measure to hamper the development of novel virus variants.