P001

Prevalence and clinical outcome of routine treatment of patients with suspected pelvic inflammatory disease in the presence of Mycoplasma genitalium infection

Miss Nikki Adriaens[1], Dr. Joyce Braam[1], Dr. Sylvia Bruisten[1], Dr. Clarissa Vergunst[2,3], Mr. Jeffrey Bosch[1], Mrs. Luann Noordpool[2], Dr. Paul Van Kesteren[4], Drs. Paul Gruteke[5], Miss Nienke Van Dekken[4], Miss Fatima Makhkoufi[4], Dr. Roel Nijhuis[6], Drs. Arien Bukman[7], Drs. Merel Van Doorenmaalen[8], Mrs. Chantal Esveld[8], Dr. Alje Van Dam[1,9]
[1]GGD Amsterdam, Amsterdam, The Netherlands, [2]GGD Amsterdam, Amsterdam, The Netherlands, [3]NWZ Den Helder, Den Helder, The Netherlands, [4]OLVG, Amsterdam, The Netherlands, [5]OLVG, Amsterdam, The Netherlands , [6]Meander Medical Centre, Amersfoort, The Netherlands, [7]Meander Medical Centre, Amersfoort, The Netherlands , [8]Flevo Hospital, Almere, The Netherlands , [9]Amsterdam UMC, Amsterdam, The Netherlands

Background. Women of reproductive age may suffer from pelvic inflammatory disease (PID), resulting from the ascension of microorganisms. Although Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are recognized to cause PID, up to 70% of the cases have an unidentified etiology. Evidence on the role of Mycoplasma genitalium (MG) in PID has been conflicting. Whether the current regime to treat women suspected for PID improves the outcome in case of a MG infection is unknown. We aimed to investigate the prevalence of MG in suspected PID and clinical effectivity of current standard treatment for PID in patients infected with MG.

Methods. We conducted a prospective multicenter cohort study at the hospitals OLVG, Meander Medical Centre, Flevo Hospital, and the Amsterdam Centre for Sexual Health. Women suspected for PID were asked to participate in this study. Vaginal swabs were obtained for testing of CT, NG and MG infection at inclusion and only MG at days 14 and 28. Patients were asked to keep a diary to report clinical symptoms. C-reactive protein (CRP) was tested at inclusion and day 28. Patients were routinely treated for PID according to Dutch guidelines.

Results. From 2021 to 2023, a total of 61 women suspected for PID were enrolled. Preliminary data showed that 3/61 (4.9%) tested positive for CT, 5/61 (8.2%) for NG and 5/61 (8.2%) for MG at inclusion. For 52/61 (85.3%) patients, an increased CRP-value of >10 was measured, indicating an inflammatory event. Follow-up data at day 28 was collected for 39/61 (63.9%) patients.

Conclusion. We showed that the prevalence of infection with MG among suspected PID patients is similar to the prevalence of NG, which is a frequently recognized causative agent of PID. Clinical effectiveness of the current standard treatment in PID patients infected with MG will be evaluated in future analyses.

P002

Treatment of human metapneumovirus with remdesivir in an immunocompromised patient. A clinical case report.

Drs. MCM Aerts[1], Drs. SA de Jager[1], Drs. JW Bosma[2], Dr. W Ang[1]
[1]Comicro, Hoorn, The Netherlands, [2]Zaans Medisch Centrum, Zaandam, The Netherlands

Introduction

Viral respiratory infections are usually mild, but in older or immunocompromised patients the clinical course can be quite severe. Standard treatment is mostly supportive. During the SARS-CoV-2 pandemic several known anti-viral drugs have re-emerged as possible treatments. One of those drugs is remdesivir, which is a prodrug of a nucleoside analog. It has been shown to have a broad-spectrum in vitro inhibition of viral replication by targeting the RNA-dependent RNA polymerase of multiple virus families. Here, a case is described of a hematological patient with a SARS-CoV-2 and human metapneumovirus (hMPV) coinfection treated with remdesivir.

Case description

A 66-year old male with a history of an autologous stem-cell transplant and maintenance rituximab therapy for mantle cell lymphoma was admitted due to fever and dyspnea. A month prior to presentation, he was admitted with similar symptoms due to persistent SARS-CoV-2 infection for which he was successfully treated with remdesivir. Radiological examination revealed increased ground-glass opacification. Nasal/throat swab tested positive for SARS-CoV-2 again with a Ct-value of 23.7, earlier value after remdesivir 32.7 and 10 days prior to presentation 27. A day later, sputum tested positive for hMPV, no Ct-value available, using a broader viral diagnostic platform. No pathogenic bacterial micro-organisms were isolated. In addition to antimicrobial treatment, remdesivir was started, initially directed at the persistent SARS-CoV-2. Symptoms resolved within 48-72h. A repeat nasal/throat swab, performed on day 7, showed a positive signal for SARS-CoV-2, Ct-value 36.7, but no positive signal for hMPV.

Conclusion

The clinical picture and microbiological findings were consistent with an infection with hMPV superimposed on a non-resolving chronic SARS-CoV-2 infection. Remdesivir treatment had an effect on the viral load of both viruses. The broad acting anti-viral action of remdesivir can be advantageous in treating patients with multiple respiratory viruses.

P004

Common drugs alter bacterial protein expression in fecal cultures from Crohn's disease patients

Heike Becker[1,2], R Mohren[3], Prof. Dr. DMAE Jonkers[2], Dr. J Penders[1]
[1]Maastricht University, Maastricht, The Netherlands, [2]Maastricht University, Maastricht, The Netherlands, [3]Maastricht University, Maastricht, The Netherlands

Introduction: Crohn's disease (CD) is a chronic inflammatory gastro-intestinal condition with a variable disease course. Medical drugs are the most important treatment regimen to induce and maintain remission. However, a substantial number of patients experience side-effects and non-response. As the intestinal microbiota can interact with xenobiotics as well as with the host, the aim of this study is to explore the effect of common CD drugs on the patient's microbiome in vitro.
Methods: Five CD patients donated a fecal sample, which was anaerobically collected and processed for the experiments on the same day. The individual fecal microbiota was exposed to budesonide, 6-mercaptopurine, tofacitinib, or DMSO-control for 24 hours. Subsequently, DNA and proteins were isolated and subjected to 16 rRNA gene amplicon sequencing and HPLC-MS proteomic analysis, respectively.
Results: Metagenomic and metaproteomic analyses revealed larger differences between donors than between drug exposures. Metaproteomic analyses could discriminate between 6-MP, tofacitinib, and the control condition, but not between budesonide and control. Applying a highly stringent selection, 18 proteins were overrepresented and 21 underrepresented in all 6-MP cultures and could thereby discriminate clearly between 6-MP and control. In contrast to metaproteomic analyses, metagenomic analyses could not detect drug-related shifts in microbiota composition and diversity.
Conclusion: Tofacitinib and especially 6-MP clearly affect microbial function, but not microbial composition in vitro. These drug-induced functional changes may subsequently influence host physiology and potentially inflammation in CD. Our findings emphasize the relevance to include functional microbial studies when investigating drug-microbiota interactions. Further research needs to elucidate the impact of 6-MP-induced microbial alterations on intestinal physiology and inflammation in CD.

P005

Development of a dual-target Kingella qPCR leads to the identification of Kingella negevensis in a Dutch patient from 2006

Dr. Lucas Bernts[1], Dr. Theun de Groot[1], Dr. Erik van Hannen[2], Dr. Bert Mulder[1]
[1]Canisius Wilhelmina Ziekenhuis, Nijmegen, The Netherlands, [2]St. Antonius Ziekenhuis, Nieuwegein, The Netherlands

Introduction: Kingella kingae is an important cause of osteoarticular infections in young children. The newly described Kingella negevensis has been implicated in septic arthritis, keratitis and bacterial vaginosis. It may be misclassified as K. kingae with standard qPCR or MALDI-TOF. We aimed to develop a diagnostic qPCR that is able to accurately detect and distinguish these Kingella species.

Methods: MEDLINE was searched for PCR-targets. Primers/probes were designed with Primer3Plus 3.2.6. Analytical sensitivity and specificity were tested in quadruplicate. Five positive and ten negative synovial fluid samples, six cultured Kingella strains, and K. negevensis Sch538T were used. Limit of detection was assessed at 0.3 – 100 genome copies/reaction. Kingella carriage was measured in routine clinical materials, such as sexually-transmitted diseases (STD) screening.

Results: Two targets were chosen: rtxA (positive in K. kingae and negevensis), and mdh (positive in K. kingae only). Sensitivity and specificity were 100% in synovial fluid. K. negevensis Sch538T was rtxA-positive and mdh-negative. Limit of detection was 3 copies/reaction for K. kingae and K. negevensis. Surprisingly, a Kingella species strain from 2006, was rtxA-positive (Ct 17.9), but mdh-negative; 16S-sequencing confirmed the identification of K. negevensis. The strain had been cultured from a genital ulcer of a 45-year old male, which was negative for other pathogens. Subsequently, urogenital samples of 181 patients (66% female; mean age 31.4; 17.1% STD-positive) all tested negative for Kingella. In 171 other samples, one pharyngeal swab was positive for K. kingae.

Conclusion: We developed a sensitive diagnostic qPCR-test to detect Kingella kingae and Kingella negevensis. To our knowledge, this K. negevensis strain isolated in 2006 is the earliest identification of this species in Europe. K. negevensis carriage appears uncommon in adults, but may contribute to disease. Widespread use of dual-target qPCR could reveal the epidemiological significance of this novel species.

P006

Selective enrichment broth versus non-selective enrichment broth for the detection of extended-spectrum-beta-lactamase-producing Enterobacterales.

Drs. M.J.M. Boderie[1], Dr. N.L.A. Arents[2], M.J.T.W. Nijs[3], Dr. I.T.M.A. Overdevest[4]
[1]Maastricht University Medical Centre, Maastricht, The Netherlands, [2]VieCuri Medical Centre, Venlo, The Netherlands, [3]Eurofins PAMM, Veldhoven, The Netherlands, [4]Rijnstate hospital, Arnhem, Arnhem, The Netherlands

Introduction:
The Dutch guidelines advise the use of an enrichment broth prior to the use of a selective agar plate for the detection of extended-spectrum beta-lactamase Enterobacterales (ESBL-E). We investigated if a selective or a non-selective enrichment broth yields the best results.

Methods:
Using 215 rectal ESwab (Copan diagnostics Inc, Murrieta, California, USA) samples from a routine screenings survey for ESBL-E carriage in hospitalized patients, we compared the use of a non-selective tryptic soy broth (TSB) to a TSB containing vancomycin 8 mg/liter and cefotaxime 0.25 mg/liter (TSB-VC). After incubation a selective ESBL screening agar plate (EbSA agar, Cepheid Benelux, Apeldoorn, The Netherlands) was inoculated with 10 µl of the TSB and TSB-VC broth. Growth of Enterobacterales isolates on any of the selective ESBL screening agar plates were phenotypically tested for the production of ESBL using the combination disk diffusion method.

Results:
Eighteen (18) patients were found to be ESBL-E carriers (prevalence 8.4%). Twelve (12) patients were detected by both the TSB and TSB-VC groups. Four ESBL-E carriers were detected only in the TSB group and two ESBL-E were detected only in the TSB-VC group. The sensitivities in the TSB and TSB-VC groups were 88.9% and 77.8% respectively (p=0.68).

Some samples showed growth of non-fermenting gram-negative bacilli or growth of Enterobacterales without the ESBL-phenotype. The specificities for the TSB and TSB-VC group were 61.1% versus 65.2% (p=0.59) respectively when considering any growth of gram-negative bacilli as relevant, and 73.1% versus 88.9% (p<0.001) when only considering growth of Enterobacterales without the ESBL-phenotype as relevant.

Conclusion:
Although the sensitivity of the non-selective enrichment broth was higher than the selective enrichment broth, no significant difference was found. However, the non-selective enrichment broth showed significantly more growth of Enterobacterales without ESBL-phenotype, which in practice leads to a higher workload and costs.

P007

Antimicrobial activity of medical-grade honey against Pseudomonas aeruginosa in biofilm and in wound models

PhD Bouke Boekema[1], A Elgersma[1], M Vlig[1], CCF Pleeging[2,3,4], Prof. T Coenye[5], D Chrysostomou[6], PhD NAJ Cremers[7]
[1]Association of Dutch Burn Centres, Beverwijk, The Netherlands, [2]Ghent University, Ghent, Belgium, [3]Radboud University Medical Center, Nijmegen, The Netherlands, [4]Dierenkliniek Parkstad, Heerlen, The Netherlands, [5]Ghent University, Ghent , Belgium, [6]Wound Clinic Health@45, Johannesburg, South Africa, [7]Triticum Exploitatie BV, Maastricht, The Netherlands

Medical-grade honey (MGH) is a promising therapy because of its broad-spectrum antimicrobial activity and the lack of risk for resistance development. The aim of this study was to investigate the antibacterial activity of different established wound care products.
The inhibitory and eradicative activity of different MGH-based wound care formulations (Medihoney, Revamil, Mebo, Melladerm, L-Mesitran Soft) against multidrug-resistant Pseudomonas aeruginosa (strain PAO1) biofilms were investigated on artificial dermis. In addition, the effects of L-Mesitran Soft, Medihoney, silver sulphadiazine and silver nitrate on the inhibition of PAO1 and the reepithelialization of the skin were investigated in an ex vivo burn wound model with human skin.
L-Mesitran Soft demonstrated the most potent antimicrobial activity against PAO1 biofilms on artificial dermis (6.1-log inhibition and 3.2-log eradication) followed by Melladerm (4.8-log inhibition and 1.7-log eradication). Other MGH formulations ranged between 0.9-log and 2.49-log inhibition and 0.7-log and 1.3-log eradication. In the burn wound model, the highest bacterial reduction (7.9-log) was found for L-Mesitran Soft and silver sulphadiazine, Medihoney was slightly less effective (4.7-log reduction). The high activity in L-Mesitran Soft lead us to investigate the contribution of the different ingredients of L-Mesitran Soft. The individual activity of raw MGH, vitamins C and E, and all ingredients except MGH clearly supported the synergistic activity within the L-Mesitran Soft formulation against PAO1 in biofilms and in burn wound models. L-Mesitran Soft and silver sulphadiazine slightly reduced epidermal regeneration in burn wound models, while Medihoney completely inhibited epidermal regeneration likely due to the presence of methylglyoxal. The clinical antimicrobial efficacy of L-Mesitran Soft against Pseudomonas aeruginosa biofilms was also illustrated in several clinical cases.
MGH is a potent treatment for biofilms and infected wounds. L-Mesitran Soft has the strongest antimicrobial activity, which is likely due to the synergistic activity mediated by its supplements.

P008

Rapid diagnostics of pleural infections by Molecular Culture

Dr Martine Bos[1], dr Robin van Houdt[2], BSc Linda Poort[1], dr Anne-Xander van der Stel[1], Prof dr Paul Savelkoul[3], dr Dries Budding[1]
[1]inbiome, Amsterdam, The Netherlands, [2]Department of Medical Microbiology and Infection Control, Amsterdam Institute for Infection and Immunity, Amsterdam UMC, VU University Medical Center, Amsterdam, The Netherlands, [3]Department of Medical Microbiology, School of Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University Medical Center, Maastricht, The Netherlands

Introduction: Diagnosis of pleural infections relies on culture, however the yield from culture-based pathogen detection is often low due to previous receipt of antimicrobials or to nutritionally fastidious microorganisms. This low yield severely hampers rapid and tailored antibiotic treatment. Molecular assays, based on DNA detection, have the potential to drastically improve diagnosis. We developed the Molecular Culture test, a PCR based assay that combines length polymorphisms of the 16S-23S interspace rDNA region with phylum-specific fluorescently labelled primers to identify bacteria to species level. This assay takes approximately four hours, thus offering a much faster turn-around time than culture. In this study, we evaluated the diagnostic accuracy of this novel test on pleural effusions.
Methods: A total of 430 pleural effusion samples (residual material from routine diagnostics) were subjected to DNA isolation followed by Molecular Culture. Outcome of Molecular Culture was compared to routine culture results.
Results: 55 out of 430 samples were positive in routine culture (13%) whereas 130 samples (30%) were positive in the Molecular Culture assay. Forty-six samples were concordant positive. The 9 culture-positive/Molecular Culture-negative samples were mostly low-load skin bacteria. Molecular Culture detected 75 different species, whereas routine culture detected only 35. The most frequently identified bacteria were different species of Streptococci: 17x in routine culture and 70x in Molecular Culture. The Molecular Culture assay detected an additional 5 cases of Staphylococcus aureus, 4 of Staphylococcus epidermidis, and 9 of Escherichia coli.
Conclusions: Molecular Culture showed a sensitivity significantly exceeding that of culture, the current gold standard. Combined with its fast turnaround time, Molecular Culture may provide a much-needed option for faster diagnosis and treatment of patients suffering from pleural infections.

P009

Technical feasibility of automated surveillance of hospital onset bacteraemia

Manon Brekelmans[1,2], Dr Maaike S.M. van Mourik[1]
[1]University Medical Centre Utrecht, Utrecht,, the Netherlands, [2]National Institute for Public Health and the Environment, Bilthoven,, The Netherlands

Background
Automated surveillance of hospital acquired infections (HAI) is emerging to replace manual surveillance. Hospital-onset bacteraemia (HOB) is currently under study as a hospital-wide HAI target to include in (fully automated) surveillance. In general terms, HOB is defined as a positive blood culture, more than two days after admission. However, there is discussion about the detailed definition such as handling of common skin commensal, polymicrobial episodes and ward attribution. We aimed to study the technical feasibility of an automated HOB surveillance. We aligned our definitions as much as possible with the draft definitions formulated by the PRAISE (providing a roadmap for automated infection surveillance in Europe) Network.

Methods
Data on hospital admissions and blood cultures in the period 2017-2021 were collected in our hospital. An algorithm was developed to identify HOBs. Furthermore, HOB rates (number of HOBs/1000 patient-days) were calculated.

Results
It was technically feasible to develop the algorithm and identify HOBs in a fully automated fashion. The hospital-wide incidence of HOB was between 1.8 and 2.2 HOBs per 1000 patient days. Highest HOB-rates were found at the intensive care unit (ICU) (range 6.1 and 12.3 in five years), haematology department (range 4.5-8.0) and paediatric ICU (range 4.2-5.3). Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis were the most frequent causes of HOB in all years.

Conclusion
It is technically feasible to develop an algorithm that identifies HOBs. Since the surveillance of HOB was fully automated in this study, some choices were made on HOB episode duration, how to handle common skin commensals and polymicrobial HOBs. Further research should focus on the acceptance and interpretability of surveillance results, including comparability between hospitals and different countries and associations with other patient outcomes.

P010

Laboratory-acquired Salmonella Typhi, confirmed by core genome multi-locus sequence typing

MD MSc J Broertjes[1], dr. R. R. Jansen[2], dr. I. R. N. Verly[1], MSc K van der Zwaluw[3], dr A. P. van Dam[1]
[1]Amsterdam Umc, Amsterdam, Nederlands, [2]OLVG, Amsterdam, Netherlands, [3]RIVM, Bilthoven, Netherlands

Introduction: At clinical microbiology laboratories, personnel routinely works with specimens containing infectious agents. Despite ongoing improvements in biosafety, laboratory-acquired infections do occur incidentally. Potential causes of laboratory-acquired infections are Brucella spp., Francisella tularensis, Neisseria meningitidis, Mycobacterium tuberculosis, Shigella spp., Salmonella spp., as well as several viruses. Salmonella Typhi is a known but rare cause of laboratory infections. Here we describe a laboratory-acquired infection with Salmonella Typhi, due to which an employee was hospitalized.

Methods: Salmonella Typhi isolates were sequenced for whole genome sequencing using the Illumina platform. A core genome multilocus sequence typing (cgMLST) analysis was done, based on 3002 alleles.

Results: The employee was admitted to the department of neurology with fever and headache. Surprisingly, blood culture and subsequently feces diagnostics were positive for S. Typhi, while cerebrospinal fluid culture remained negative. The employee had no history of recent travel to an endemic country or contact with patients with typhoid fever. However, a few days before the onset of clinical symptoms, the employee was working with a Salmonella stain, which at that time was typed as Salmonella Paratyphi A. This laboratory isolate and the two clinical isolates were send to the RIVM, sequenced and shown to be S. Typhi, with an identical cgMLST profile. The isolates could be clearly differentiated from two unrelated S. Typhi isolates. The post-hoc analysis of the laboratory procedures did not reveal any breach in biosafety protocols. The employee fully recovered after appropriate antibiotic therapy.

Conclusion: Salmonella Typhi is a highly infectious and virulent pathogen. Working with a clinical strain resulted in a serious infection. The incident occurred despite good laboratory precautions and handling Salmonella cultures in a biosafety cabinet.

P011

Development of a micro-Focus Reduction Neutralization Test for measuring West Nile and Usutu neutralizing antibodies

BSc FD Chandler[1], BSc K Wijnans[1], Dr. N Mumtaz[1], Dr. MD CH Geurts van Kessel[1], prof. MD L Barzon[2], prof. dr. H.L. Zaaijer[3], Prof. MPG Koopmans[1], MSc RS Sikkema[1]
[1]Erasmus Medical Centre, Rotterdam, The Netherlands, [2]Padova University Hospital, Padua, Italy, [3]Sanquin Blood Supply Foundation and Amsterdam University Medical Centre, Amsterdam, The Netherlands

Usutu virus (USUV) and West Nile virus (WNV) are emerging mosquito-borne flaviviruses that belong to the Japanese encephalitis serocomplex. Current serological assays require confirmation using classical and time-consuming virus neutralization assays. The micro-Focus Reduction Neutralization Test (mFRNT) is a fast and reliable assay to determine titers of neutralizing antibodies. Here, we describe the sensitivity and specificity of the mFRNT for USUV and WNV.

In this study, we cultured and titrated WNV (lineage 2) and USUV (Africa-3 strain) on Vero cells to determine the tissue culture infectious dose 50 (TCID 50) and a suitable focus-forming units (FFU) concentration number. To determine the cutoffs for WNV and USUV neutralizing antibody detection, we tested human serum panels of patients with USUV and WNV infection and other flavi- and Cytomegaloviruses confirmed by PCR, enzyme-linked immunosorbent assay (ELISA) and/or virus neutralization test (VNT). The sera were diluted and mixed with an equal volume of titrated virus stocks. After incubation, the serum-virus mixture was added onto Vero cells and incubated for 24 hours, followed by fixation and staining with USUV and WNV primary mouse-antibodies and a horseradish peroxidase goat-anti-mouse secondary antibody. After staining with TrueBlue, foci were counted with the ImmunoSpot Analyzer.

Based on a cut off of ≥80 (FRNT70), a sensitivity of 80% and a specificity of 92% were calculated for USUV. For WNV, based on a cut off of ≥160 (FRNT90), a sensitivity of 95% and a specificity of 100% was determined. For final confirmation of USUV or WNV positives, a four-fold or greater difference between WNV and USUV titers is used.

We observed that the WNV mFRNT is a specific, sensitive rapid assay to detect antibodies in serum samples compared to VNT. USUV mFRNT results needs to be interpreted with caution.

P012

Uncovering the spread of drug-resistant bacteria through cutting-edge sequencing techniques: a study of extended-spectrum β-lactamase-producing Enterobacterales transmitted by a contaminated duodenoscope

Dr. Mariëtte Lokate[1,2], M.D., PhD Erik Bathoorn[1], Dr. ir. Arjo Jozef Loeve[3], M.D., PhD Monika Fliss[1], PhD Matthijs Berends[1,4], Prof. Dr. Axel Hamprecht[2], Prof. Dr. Andreas Voss[1], PhD Mariëtte Lokate[1]
[1]Department of Medical Microbiology and Infection Prevention, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, , , [2]Institute for Medical Microbiology and Virology, University of Oldenburg, Oldenburg, Germany, , , [3]Department of Biomechanical Engineering, Faculty of Mechanical, Maritime and Materials Engineering, Delft University of Technology, Delft, The Netherlands, , , [4]Certe Medical Diagnostics and Advice Foundation, Department of Medical Epidemiology, Groningen, the Netherlands, ,

Introduction
Transmission of multidrug-resistant organisms (MDRO) by duodenoscopes is a possible complication of endoscopic retrograde cholangiopancreatography (ERCP). Several outbreaks have been described worldwide, commonly caused by contaminated scopes. Here, we present the role of routine-based next-generation sequencing (NGS) in the detection of transmission of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales by a contaminated ERCP duodenoscope.

Methods
ESBL-producing isolates per patient are routinely typed by NGS and stored at the University Medical Center Groningen. In July 2020, two genetically closely related blaCTX-M-15 encoding Citrobacter freundii isolates had been detected: one from an adult patient in the internal medicine service causing a bloodstream infection (BSI), the other from a pediatric patient causing a urinary tract infection. The same duodenoscope had recently been used in both patients. Additionally, a patient from the gastroenterology service developed a BSI caused by blaSHV-12 encoding Klebsiella pneumoniae after ERCP with the same duodenoscope.
This duodenoscope had been cultured eight times and was dismantled for inspection and additional culturing. Throat and rectal swabs were obtained from all patients who had undergone ERCP with the same duodenoscope in the previous six months.

Results
Among screened patients (n=39), three additional patients with ESBLs were found matching the initial cases by cgMLST: one with blaCTX-M-15 encoding Citrobacter freundii, and two with blaSHV-12 encoding Klebsiella pneumoniae. No microorganisms were detected in eight routine antegrade and retrograde culture samples retrieved from the duodenoscope, using nationally advised methods of sampling. Only after destructive dismantling of the duodenoscope, the forceps elevator was positive for blaSHV-12 encoding Klebsiella pneumonia which was identical to the isolates detected in patients.

Conclusion
Our findings demonstrate the value of using NGS for monitoring MDRO transmission and show that standard scope cultures might fail to detect contaminated duodenoscopes.

P013

Detection of MRSA on the utility channel of the new cobas® 5800 platform

BS F.B. Verhees[1], BS E. Meijers[1], Dr. S.A. Boers[1], Dr. E. Wessels[1], Dr. E.C.J. Claas[1]
[1]Leiden University Medical Center, LEIDEN, The Netherlands

Introduction

Molecular amplification methods have become a major tool in diagnosing microbial infections. With the increased application, automation of the workflow has become a requirement to ensure reliable results. All major diagnostic companies now market such fully automated sample-in, result-out platforms, exemplified by the Roche cobas® 6800 and 8800, Hologic Panther®, Abbott Alinity m, Qiagen NeuMoDxTM 288 and the Becton Dickinson BD-CORTM system. Most companies have CE-IVD assays available for the high volume parameters as the Blood Borne Viruses, Sexually Transmitted Diseases, Transplant associated infections, Human Papillomavirus, and Respiratory infections (Flu/RSV/SARS-CoV2). In addition, the Hologic Panther® Fusion module enables detection of non CE-IVD parameters as well. Recently Roche launched a new, smaller automated platform: the cobas® 5800. This platform also has a so called Utility Chanel (or Open Channel), that enables laboratories to run non CE-IVD assays using the same automated workflow.

Methods

An MRSA assay, specifically designed by IDTTM for use on the Utility Channel of the cobas® 5800 was applied for detection of MRSA in 133 Brain Heart Infusion (BHI) culture broths. The cobas® 5800 Utility Channel results were compared to culture and the BD MaxTM StaphSR assay.

Results

All culture positive MRSA were also detected by the cobas® 5800 assay and the StaphSR assay. Both assays were multiplex assay detecting a S. aureus specific gene and the mecA/C genes. In addition, the StaphSR detects the chromosomal cassette and orfX gene junction, whereas the IDTTM assay on the cobas® detects the chromosomal cassette (SCCmec). The latter generates additional positive results, for which the specificity is unclear.

Conclusion

The cobas® 5800 Utility Channel provides an interesting alternative for testing parameters not provided by the company in a CE-IVD approved workflow. However, the specific requirements in the design of the assay may hamper broad implementation.

P014

Candida khanbhai sp. nov., a new clinically relevant yeast within the Candida haemulonii species complex

Msc. Auke de Jong[1,2], MD K Al-Obaid[3], MD R Mohd Tap[4], B Gerrits van den Ende[1], dr. M Groenewald[1], L Joseph[5], S Ahmad[5], Prof. dr. F Hagen[1,2,6]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, [2]University of Amsterdam, Amsterdam, The Netherlands, [3]Mubarak Al-Kabir Hospital, Jabriya, Kuwait, [4]National Institutes of Health, Ministry of Health Malaysia, Shah Alam, Malaysia, [5]Kuwait University, Jabriya, Kuwait, [6]University Medical Center Utrecht, Utrecht, The Netherlands

Invasive fungal infections caused by non-albicans Candida species are increasingly reported. Recent advances in diagnostic and molecular tools enabled better identification and detection of emerging pathogenic yeasts. The Candida haemulonii species complex accommodates several rare and recently described pathogenic species, Candida duobushaemulonii, Candida pseudohaemulonii, Candida vulturna, and the most notorious example is the outbreak causing multi-drug resistant member Candida auris. Here, we describe a new clinically relevant yeast isolated from geographically distinct regions, representing the proposed novel species Candida khanbhai, a new member of the C. haemulonii species complex.

Two clinical strains of this new species were isolated from patients at distinct geographical locations (Kuwait and Malaysia). Standard biochemical identification methods resulted in misidentification. To this end, the strains were identified by sequencing the internal transcribed spacer ITS1-5.8S-ITS2 region. Next, strains were phylogenetically analyzed by Amplified Fragment Length Polymorphism (AFLP) fingerprint analysis and amplification of the D1/D2 region of the large subunit (LSU) ribosomal RNA gene. Finally, Phenotypic and physiological characterization was carried out together with antifungal susceptibility testing.

The phylogenetic analyses showed both strains cluster tightly together within the C. haemulonii complex and form a distinct lineage. Also phenotypically, the strains have unique characteristics with growth up to 42°C and fermentation of maltose. Notably, both strains also displayed elevated minimum inhibitory concentrations for most of the azoles and amphotericin B.

Taken together with the description of C. khanbhai we add an important clinical Candida species to the growing list of emerging fungal pathogens. The isolation from clinical sources at geographically distinct locations together with a worrisome multi-drug resistance pattern and misidentification by commonly used laboratory methods shows many similarities with the story of C. auris. Therefore, monitoring and correct identification of this species is essential.

P015

Acute bacterial arthritis caused by Legionella sainthelensi in an immunocompromised patient

Dr Jasmijn De Rooij-Rademakers[1], MM Waltmans-den Breejen[1], Dr CHW Klaassen[1], Dr BCGC Mason-Slingerland[1]
[1]Erasmus MC, Rotterdam, the Netherlands

Introduction

Acute bacterial arthritis after intra-articular injection with corticosteroids is a well-known complication, with Staphylococccus aureus as common causative pathogen. Sporadically, unexpected pathogens are cultured. We describe a 63-year old immunocompromised patient with rheumatoid arthritis, presenting with an acute arthritis of the left wrist, two weeks after an intra-articular triamcinolone injection as treatment for a chronic arthritis. Intravenous cefuroxime was administered awaiting the results of microbiological testing.

Methods

Gram staining and cultures on synovial fluid and pus of the wrist were performed to detect and identify the pathogen. Identification methods used were MALDI-TOF mass spectrometry and 16S-sequencing.

Results

Gram staining of synovial fluid and pus samples showed gram negative rods. Initial cultures did not show any bacterial growth after 48 hours. To identify the pathogen, despite the negative cultures on the standard used agar plates, a specific culture for Legionella spp. was performed and turned positive after 2 days, identifying Legionella sainthelensi as pathogen.

Etest gradient diffusion showed a MIC of 0.25ug/ml for levofloxacin and a MIC of 2ug/mL for doxycycline. No clinical breakpoints are available.

Therapy was switched to levofloxacin for the duration of 6 weeks. Two days after starting this therapy symptoms diminished and ultimately vanished within the treatment period.

The intra-articular injection is most likely the cause of infection. Less likely is contact with contaminated water with a prolonged skin barrier disruption caused by delayed wound healing due to the immunocompromised status of the patient. In this case, no nosocomial or other source for this infection could be identified.

Conclusion

Awareness of uncommon pathogens as Legionella sainthelensi causing bacterial arthritis is needed. Risk factors are immunocompromised status and skin barrier defects such as after intra-articular injections. In case cultures remain negative despite gram staining showing negative rods, specific Legionella cultures can be helpful awaiting molecular diagnostic testing.

P016

Rapid increase of parasitaemia in a patient with Plasmodium falciparum infection

Dr Jasmijn De Rooij-Rademakers[1,2], Dr WSN Lekkerkerk[2], Dr AJM de Graan[3], R Koelewijn[1], Dr E van Nood[1], TA Khartabil[4], Dr JJ van Hellemond[1]
[1]Erasmus MC, Rotterdam, the Netherlands, [2]Maasstad hospital, Rotterdam, the Netherlands, [3]Van Weel Bethesda hospital, Dirksland, the Netherlands, [4]Erasmus MC, Rotterdam, the Netherlands

Introduction

Mild Plasmodium falciparum infections can be treated with oral medication, where severe infections require intravenous treatment with artesunate. Severe Plasmodium falciparum infections are diagnosed by parasitaemia >5%, presence of schizonts, or clinical symptoms such as vomiting, neurologic symptoms and/or organ failure. Hereby we describe a rapid rise of parasitaemia in a patient with a recent travel history to central Africa to show that microscopic diagnosis alone can not be used to rule out severe Plasmodium falciparum infections.

Method

Patient characteristics and clinical presentation were described. Patient samples were taken and analyzed at time of presentation and ten hours later for blood cell count and liver enzyme tests. For diagnosis of malaria antigen testing, microscopy of Giemsa stained thick and thin blood smear, quantitative buffy coat (QBC) analysis and flow cytometry using the Sysmex XN-31.

Results

A 38-year old female presented at the emergency unit three weeks after returning from central Africa with fever, nausea and diarrhoea. Her clinical condition had worsened despite being treated with ciprofloxacin prescribed by the general practitioner. Anemia, thrombocytopenia and elevated liver enzymes were diagnosed. Rapid antigen testing and QBC were positive for malaria. Microscopy and flow cytometry showed an infection with Plasmodium falciparum with a parasitaemia of 1.2% in absence of schizonts. PCR was positive for Plasmodium falciparum. Awaitening the results, intravenous treatment of artesunate was started. Due to clinical deterioration with the presence of neurological symptoms, 4 hours after starting treatment and 10 hours after the first sample collection, microscopy and flow cytometry was repeated and showed a parasitaemia of 12% with schizonts in the blood smear.

Conclusion

In this case we show that parasitaemia may rapidly increase, demonstrating the necessity of combining clinical findings with laboratory diagnosis in order to assess the need for intravenous treatment.

P017

Additional value of IgM serology to standard serology and Treponema pallidum PCR for diagnosing early syphilis

Dr. Md. Anne Dirks[1,2], Dr. J. Flipse[1,2,3], Dr. AM Niekamp[4], Dr. NHTM Dukers-Muijrers[4,5], Prof. MD. CJPA Hoebe[4,5], Dr. PFG Wolffs[1,2,5], Dr. IHM van Loo[1,2]
[1]Maastricht UMC+, Maastricht, The Netherlands, [2]Maastricht University, Maastricht, The Netherlands, [3]Rijnstate, Velp, The Netherlands, [4]Public Health Service South Limburg, Heerlen, The Netherlands, [5]Care and Public Health Research Institute (CAPHRI), Maastricht University, Maastricht, The Netherlands

Introduction
Syphilis diagnoses and staging are based on clinical findings and serology and determine the treatment and partner notification. However, it is known that up to 30% of the cases with primary syphilis present with negative serology. Early cases of syphilis can be detected by PCR or IgM serology. Here we investigated the benefit of supplementing conventional serology with these tests to enhance earlier detection of syphilitic cases.

Methods
171 patients (182 samples: 77 positive) with a suspected syphilis ulcer were included from our STI clinic in South Limburg, the Netherlands from 2012 – 2018. Syphilis diagnosis is based on clinical symptoms, serology (reversed algorithm) and PCR (polA gene). . Mikrogen IgM-immunoblot was used for IgM detection in a subset of patients. Review of clinical data determined the additional value of PCR and IgM-immunoblot per patient.

Results
23.1% of consults benefitted from having a T.pallidum PCR (33 aiding diagnosis, 9 staging). The sensitivity of the PCR compared to only the clinical diagnosis and serology is 89.0%(65/73), specificity 99.1%(88/109), PPV 98.5%(65/66) and NPV 93.1%(108/116). For IgM-immunoblot: 87.0%(40/46), 94.9%(37/39), 95.2%(40/42) and 86.0%(37/43). Best performance is in primary infections; 93.8%(30/32), 94.3%(33/35), 93.8%(30/32) and 94.3%(33/35). Agreement between the PCR and IgM-immunoblot is 85% (71/84). IgM-immunoblot detected active syphilis in 3/11 cases (27%) with an inconclusive diagnosis based on clinical assessment and serology alone. However, the IgM-immunoblot also had 14.3% false-positives.

Conclusion
PCR and IgM-immunoblot can aid diagnosing early syphilis in approximately ~25%. PCR has best sensitivity and can be applied to primary infections and re-infections, but requires and ulcer. The IgM-immunoblot does not require an ulcer but can only reliably be applied to suspected primary infections (93.8% vs 71.4%), and false-positives are a concern. The population, testing algorithm, time and costs should determine if either test will be implemented in clinical practice.

P018

Accuracy of disks containing either cloxacillin or cefepime in the detection of extended-spectrum β-lactamases in Enterobacterales from clinical samples

Dr Laura Doornekamp[1], Dr. C.H.W. Klaassen[1], Dr. W.H.F. Goessens[1], Dr. L.G.M. Bode[1]
[1]Erasmus MC, Rotterdam, The Netherlands

Introduction: Reliable detection of extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases is needed to prevent spread of plasmid-encoded β-lactamases. Guidelines recommend the use of a combination disk method (CDM) including cefepime and clavulanic acid as a confirmation method for ESBL detection. However, an alternative CDM - combining ceftazidime and cefotaxime with clavulanic acid and/or cloxacillin - is not only able to confirm ESBL presence, but also capable of providing information about the presence and expression of AmpC β-lactamases. We hypothesize that this cloxacillin-CDM is non-inferior to cefepime-CDM in the detection of ESBL production.
Methods: Between April and August 2022, we collected 102 unique clinical Enterobacterales isolates that were designated as possible ESBL producers by the Advanced Expert System of the VITEK 2 system. 69 were known chromosomal AmpC producers. Phenotypical confirmation was performed with cefepime-CDM and cloxacillin-CDM (Rosco®). As a gold standard, a multiplex ESBL PCR was performed targeting blaSHV, blaTEM and blaCTX-M, followed by sequence analysis if only blaSHV or blaTEM β-lactamases were found.
Results: 28% of the isolates were ESBL positive according to the molecular assays. The results of cefepime-CDM and cloxacillin-CDM were concordant with these results in 99 and 94%, respectively. Cefepime-CDM showed a sensitivity of 100% (95% confidence interval (CI) 88,1-100) and specificity of 98,6% (95% CI 92,6-100) and cloxacillin-CDM of 96,6 (95% CI 82,2-99,9) and 93,2% (95% CI 84,7-97,7), respectively.
Conclusion: Comparison of cefepime-CDM and cloxacillin-CDM as ESBL confirmation method showed no significant difference between diagnostic performance of the two tests and a high sensitivity and specificity for both. Therefore, we suggest to add the cloxacillin-CDM as an alternative ESBL confirmation method to AMR detection guidelines in clinical settings, as it gives additional information regarding presence of AmpC β-lactamases. In view of future developments, we expect that the detection of plasmid-encoded AmpC production will gain importance.

P020

A new machine learning-based approach to classifying patients with invasive fungal disease

Mehmet Ergün[1,2], RJM Brüggemann[1,3], A Alanio[4,5], RG Bentvelsen[6,7], K van Dijk[8], M Ergün[9], K Lagrou[10], JA Schouten[11,12], J Wauters[13], PL White[14], PE Verweij[1,2]
[1]Radboud University Medical Center, Nijmegen, The Netherlands, [2]Radboud University Medical Center, Nijmegen, The Netherlands, [3]Radboud University Medical Center, Nijmegen, The Netherlands, [4]Hôpital Saint-Louis, Assistance Publique Hôpitaux de Paris, Paris, France, [5]National Reference Center for Invasive Mycoses and Antifungals, Institut Pasteur, Université de Paris, Paris, France, [6]Amphia Hospital, Breda, The Netherlands, [7]Leiden University Medical Center, Leiden, The Netherlands, [8]Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, [9]Technical University Eindhoven, Eindhoven, The Netherlands, [10]University Hospitals Leuven, Leuven, Belgium, [11]Radboud University Medical Center, Nijmegen, The Netherlands, [12]Radboud University Medical Center, Nijmegen, The Netherlands, [13]University Hospitals Leuven, Leuven, Belgium, [14]University Hospital of Wales, Heath Park, Cardiff, The United Kingdom

Introduction: Over the past decades new risk groups have emerged for developing invasive fungal disease (IFD), including influenza and COVID-19. It has been difficult to classify these patients based on existing IFD case definitions due to absence of host factors and specific radiological findings, resulting in new case definitions for influenza-associated pulmonary aspergillosis and COVID-19-associated pulmonary aspergillosis (CAPA). The increasing number of case definitions and reliance on fungal biomarkers prompted us to investigate if a machine learning (ML) approach would be applicable to classify patients with IFDs.
Methods: A data set of 219 critically-ill COVID-19 cases was used, including one proven, 38 probable, 19 possible, and 161 without (sufficient) evidence of CAPA. Six international mycology experts were provided with diagnostic information, antifungal therapy and outcome, and asked to determine if CAPA was present. These results were then used to train a ML-based logistic regression model, and the calculated likelihood of CAPA was compared with the ECMM/ISHAM definitions.
Results: Our results indicate that experts weigh diagnostic information differently including: type of sample (bronchoalveolar lavage versus non-bronchoscopic lavage), test (galactomannan versus Aspergillus culture), and number of positive tests in their decision making. Furthermore, negative test results proved critical to reject IFD. Our ML algorithm was able to predict the likelihood of CAPA cases, differentiating patients with likely CAPA (median 87%, IQR 76-98%) from unlikely cases (median 2%, IQR 2-2%).
Conclusion: Using ML we were able to account for factors that are critical for experts to determe the presence or absence of CAPA. Further exploration of ML algorithms through incorporating host factors and clinical symptoms may provide a uniform approach to classifying IFD in various risk groups. Given the ever-increasing population “at-risk” of IFD, such an endeavor should be considered a worthwhile investment by the mycology community.

P021

INFLUENCE OF MATERNAL IMMUNIZATION AND PRIMARY VACCINATION WITH ACELLULAR OR WHOLE-CELL PERTUSSIS VACCINE ON THE MUCOSAL ANTIBODY RESPONSE IN INFANTS

Janeri Froberg[1,2], Anja Saso[3,5], Haddijatou Jobe[5], Marc Eleveld[1], Beate Kampmann[3,4,5], Dimitri Diavatopoulos[1,2]

[1]Radboudumc, Nijmegen, Netherlands, [2]Radboudumc, Nijmegen, Netherlands, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]Charite Universitatsmedizin , Berlin, Germany, [5]MRC Unit The Gambia, Fajara, The Gambia

Introduction

Pertussis is an acute respiratory tract infection caused by the bacterium Bordetella pertussis (Bp). Although maternal vaccination programs protect against disease in early life, their effect on the infant's subsequent response to primary pertussis vaccination is not fully understood.

Methods

The Gambian Pertussis Study (GaPS), performed as part of the PERISCOPE consortium, is a randomised, double-blind controlled clinical trial in pregnant women, exploring the impact of aP vaccination in pregnancy on the immunogenicity in infants randomized to receive either an acellular (aP) or whole-cell (wP) pertussis vaccine. In an immunological sub study, we analyzed vaccine-induced nasal antibody responses to Bp, measuring antibody binding to wildtype Bp bacteria (Bp_wt) and an isogenic Bp deletion mutant that lacks all aP vaccine antigens (Bp_mut).

Results

We show that infants born to mothers vaccinated with aP during pregnancy have significantly higher nasal IgG binding levels to Bp_wt prior to primary vaccination compared to infants born to mothers vaccinated with a control (tetanus-toxoid) vaccine. Subsequent primary vaccination of infants with wP results in significantly higher nasal IgG binding compared to aP, both at 5 and 9 months of age. At 9 months of age, aP-vaccinated infants born to mothers vaccinated with aP during pregnancy had significantly lower nasal IgG levels compared to the three other groups.

Conclusion

Our study demonstrates that maternal vaccination results in increased nasal antibody levels in infants, and furthermore identifies clear differences in nasal antibodies induced after primary aP versus wP vaccination. Clinical significance of the findings remains to be established.

P022

Cellular immune responses against SARS-CoV-2 during the first two waves of infections in the southern region of The Netherlands.

Drs. Daniëlle Hanssen[1,2], K. Arts, N.N.B. Sweelssen[1], W.H.V. Nix, T.T.J. Welbers, C. de Theije[3], D.M.E. Pagen[4,5], S. Brinkhues[8], J. Penders[1,2,6], N.H.T.M. Dukers-Muijrers[2,4,7], C.J.P.A. Hoebe[1,4,5], P.H.M. Savelkoul[1,2], I.H.M. van Loo[1,2]
[1]MUMC+, Maastricht, The Netherlands, [2]CAPHRI, Maastricht, The Netherlands, [3]BioBank Maastricht UMC+, Maastricht, The Netherlands, [4]Public Health Service (GGD) South Limburg, Maastricht, The Netherlands, [5]CAPHRI, Maastricht, The Netherlands, [6]NUTRIM, Maastricht, The Netherlands, [7]Maastricht University, Maastricht, The Netherlands, [8]Public Health Service (GGD) South Limburg, Maastricht, The Netherlands

Introduction

Seroprevalence studies solely based on antibody responses might underestimate the extent of immune responses against SARS-CoV-2 in a population. This cross-sectional study determined humoral and cellular immune responses against SARS-CoV-2 in a large Dutch cohort.

Methods

From October 28th to November 30th, 2020, a blood sample and questionnaire were collected from 10.001 inhabitants of a southern province of The Netherlands. Total Ig and anti-S-RBD responses were examined. In a randomly selected subset of samples cellular immune responses were analysed using IFN-γ ELISpot.

Results

In total 1.948/10.001 (19.5%) of participants showed an total Ig response and 143/272 (52.6%) had a positive IFN-γ ELISpot. Among the seropositive participants 129/182 (70.9%) had a positive IFN-γ ELISpot result, compared to 14/90 (15.6%) of seronegative participants. A moderate correlation was found between the anti-S-RBD results and the IFN-γ results, $r(179) = 0.377$, $p<0.01$.

Conclusions

In the present study, SARS-CoV-2-specific immune responses were detected in a considerable part of total Ig seronegative participants. Testing of both humoral and cellular immune responses can contribute to a more thorough estimate of the extent of immune responses against SARS-Cov-2 in a population.

P023

Fungal-bacterial microbiota interactions in Clostridioides difficile carriage and infection

Dr. Henderickx J.G.E.[1,2], Crobach M.J.T.[2], Dr. Terveer E.M.[2,3], Dr. Smits W.K.[2], Prof. Dr. Kuijper E.J.[1,2,3], Dr. Zwittink R.D.[2]
[1]Center for Microbiome Analyses and Therapeutics, Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands, [2]Department of Medical Microbiology and Leiden University Center of Infectious Diseases (LU-CID), Leiden University Medical Center, Leiden, The Netherlands, [3]Netherlands Donor Feces Bank, Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands
The composition and function of the bacterial microbiota is well-recognized for its role in C. difficile infection. Conversely, fungi and yeasts are understudied in C. difficile carriage and infection. The aim of this study was to analyze the mycobiota and its interactions with the bacterial microbiota in light of C. difficile colonization and infection.
The mycobiota was profiled by ITS2 sequencing of fecal DNA from infected patients (CDI; n = 29), asymptomatically colonized patients (CDC; n = 38) and healthy controls with C. difficile negative stool culture (n = 38). Previously published 16S rRNA gene sequencing data of the same cohort were used for fungal-bacterial network analysis and machine learning.
The most abundant fungal genera comprised Candida, Saccharomyces, Penicillium and Aspergillus. CDI patients were characterized by a significant increase in Candida spp. (MD 0.28 ± 0.09, p = 0.02) and Candida albicans (MD 0.17 ± 0.08, p = 0.002) compared to healthy controls. Additionally, they were deprived of Aspergillus spp. (MD -0.07 ± 0.02, p = 0.0002) and Penicillium spp. (MD -0.13 ± 0.04, p = 0.0004) compared to CDC patients. Network analysis revealed positive associations between fungi and bacteria in CDI and CDC, although Clostridioides spp. did not have a direct association with fungi. Furthermore, the mycobiota and 16S-ITS2 combined classifier were outperformed by the microbiota classifier, which successfully distinguished CDI from CDC (AUROC = 0.872) and CDI from healthy status (AUROC = 0.911). Collinsella, Blautia and Bifidobacterium were marker genera associated with healthy status and C. difficile carriage.
The gut mycobiota differs between CDI, CDC and healthy controls, and may affect Clostridioides spp. through indirect interactions. The identification of bacterial marker genera associated with healthy status and carriage warrants further investigation. Although the mycobiota's predictive value was low, fungal-bacterial interactions should be considered when treating C. difficile infection.

P024

Optimization of the ARTIC network primers for SARS-CoV-2 long-read sequencing

BSc Noud T.H. Hermans[1], BSc K.H. Stoffers[1], BSc L Manrho[1], MSc D Eiloz[1,2], BSc L Hogenkamp[1], J Beverdam-Vincent[1], Dr. R jansen[1], Dr. A Riezebos-Brilman[1], Dr. J Bovenkamp[1], Dr. K Kremer[3], Dr. A van der Zanden[1], Dr. J de Beer[1]
[1]Labmicta , Hengelo, The Netherlands, [2]Mozand BV , Eindhoven, The Netherlands, [3]KNCV Tuberculosis Foundation, The Hague, The Netherlands

Introduction

The ARTIC protocol, with corresponding primers, is extensively applied for genomic surveillance of SARS-CoV-2. This protocol uses a tiled PCR approach to amplify the entire SARS-CoV-2 genome before sequencing. Initially, the protocol was introduced for short-read sequencing on the MiniSeq sequencing system (Illumina, San Diego, California, USA). Subsequent demand for methods applicable to the MinION (Oxford Nanopore Technologies (ONT), Oxford, UK) resulted in the development of ARTIC protocols and primers for long-read sequencing. However, long-read sequencing remains challenging, due to insufficient sequencing coverage. The ARTIC primers were optimized to solve this problem.

Methods

Initially, RNA was extracted using the Kingfisher Apex System (ThermoFisher Scientific, Waltham, Massachusetts, USA), and cDNA synthesis was performed using LunaScript RT Mix (New England Biolabs, Ipswich, Massachusetts, USA) with a VeritiPro 96-Well Thermal Cycler (ThermoFisher Scientific). Sequencing data analysis was performed with the EPI2ME Desktop Agent (ONT). PCR analysis before sequencing on a MinION was first executed by using the original ARTIC primers resulting in short fragments. Then, a set of fewer ARTIC primers was selected to amplify longer fragments. Finally, primer concentrations were optimized.

Results

Analysis of the sequencing data of the short fragments showed primer drop-offs and insufficient coverage (less than 100 depth, threshold value) (max depth 400). Using only approximately half of the primers, the coverage increased; however, it was still insufficient. After primer optimization coverage increased significantly and was more equally distributed. With the final long-read sequencing protocol, all sequenced regions showed coverage of approximately 100 depth or higher.

Conclusion

An optimized approach was developed for long-read sequencing of SARS-CoV-2 using MinION devices and achieved sufficient and more equally distributed coverage of the SARS-CoV-2 genome. This approach enables more efficient SARS-CoV-2 sequencing at lower cost, which is needed especially in low-resource settings.

P025

A multi-angle study on antimicrobial resistance in Dutch residential care facilities for people with intellectual and/or developmental disabilities

Soemeja Hidad[1], Ing. Dr. S.C. de Greeff[1], Dr. C. Schneeberger[1], Dr. G.L. Leusink[2], Dr A. Timen[2]
[1]National Institute for Public Health and the Environment, Bilthoven, The Netherlands, [2]Radboud University Medical Center, Nijmegen, The Netherlands

Introduction
As of today, bacterial antimicrobial resistance (AMR) has become one of the leading public health threats worldwide. In institutional care settings for people with intellectual and/or developmental disabilities (IDD), the presence of AMR could have a significant impact on healthcare.
However, little is known about AMR prevalence, knowledge and control measures in these settings, as surveillance systems are limited and data is scarce. The aim of this multi-angle study is to gain insight into the occurrence and impact of AMR in Dutch residential care facilities for people with IDD, and their (informal) caregivers.

Methods
In the first part of the project, we will use quantitative research methods to gain insight in antibiotic prescribing patterns and prevalence of AMR. Furthermore, we will use qualitative research methods to investigate measures taken to control AMR and explore the impact of AMR on clients and health care professionals. During the course of the project we will reflect and include relevant stakeholders. Based on findings from the first part and other available and relevant information, we finally aim to develop tailored interventions to optimize AMR control in Dutch residential care facilities for people with IDD.

Result
The multi-angle study will gain insight into existing bottlenecks and will provide new strategies which will be aligned with daily practice. Ultimately, findings from this study will result in tailored interventions, such as practical recommendations and tools, either for further in-depth research, or for clinical practice and for instance, training for healthcare professionals, infection prevention practices or clinical guideline development.

Conclusion
The multi-angle study will provide a first in-depth insight into occurrence and impact of AMR in Dutch residential care facilities for people with IDD, and will raise awareness to AMR and adequate infection prevention practices in this setting. Additionally, this project advocates further research for inclusive healthcare.

P026

Bacterial β-glucuronidase activity in postmenopausal breast cancer patients: a pilot study

Lars Hillege[1,2], drs. Janneke Waelen[2], Millou Stevens[2], drs. Janine Ziemons[1,2], dr. Romy Aarnoutse[1,2], dr. Judith de Vos-Geelen[1,3], dr. Maaike de Boer[1,3], Yvonne van Riet[4], drs. Jeroen Vincent[5], Prof. dr. Koen Venema[6,7,8], dr. Joshua Simpson[9], Prof. dr. Matthew Redinbo[9], dr. John Penders[6,7,10], Prof. dr. Marjolein Smidt[1,2]
[1]GROW - School for Oncology and Developmental Biology, , Maastricht, The Netherlands, [2]Maastricht University Medical Centre, , Maastricht, The Netherlands, [3]Maastricht University Medical Centre, , Maastricht, The Netherlands, [4]Catharina Hospital, , Eindhoven, The Netherlands, [5]Elkerliek Hospital, , Helmond, The Netherlands, [6]NUTRIM - School of Nutrition and Translational research In Metabolism, , Maastricht, The Netherlands, [7]Euregional Microbiome Center, , Maastricht, The Netherlands, [8]Maastricht University, , Venlo, The Netherlands, [9]University of North Carolina, , Chapel Hill, NC, United States, [10]Maastricht University Medical Centre, Maastricht, The Netherlands

Introduction: Long-lifetime exposure to elevated estrogen levels is associated with a higher risk of developing estrogen receptor-positive (ER+) breast cancer in postmenopausal women. A factor that can influence estrogen levels is the intestinal bacterial enzyme β-glucuronidase (GUS), which can de-conjugate estrogens, leading to their reabsorption into the circulation. In this pilot study, we aimed to investigate if there was a significant difference in fecal GUS activity between postmenopausal ER+ breast cancer patients and postmenopausal controls without breast cancer.

Methods: For this cross-sectional study, postmenopausal ER+/HER2- breast cancer patients were included in an ongoing cohort study in the Netherlands. Patients collected a fecal sample before starting neoadjuvant chemotherapy. Controls, enrolled from the National Dutch Breast Cancer Screening Programme, without signs of breast cancer on the mammogram also collected a fecal sample. The GUS activity was measured using a fluorescence-based activity assay with 4-MUG as substrate and quantified as the amount of 4MUG that was converted to 4MU over time.

Results: GUS activity was analyzed in samples derived from fifteen breast cancer patients and fifteen postmenopausal controls. The results from these experiments will follow shortly. Previous pilot experiments in nine breast cancer patients and nine postmenopausal controls showed that GUS activity could successfully be quantified in human fecal samples, but was not significantly different between breast cancer patients and controls.

Conclusion: To the best of our knowledge, this is the first study investigating GUS activity in fecal samples derived from breast cancer patients. As a next step, proteomics-based identification of specific GUS that affects estrogen metabolism should be considered to investigate estrogen-specific GUS activity. More knowledge concerning the role of GUS in breast cancer will significantly contribute to the prevention of the development or recurrence of ER+ breast cancer.

P027

Malaria-screening in a non-endemic setting

Dr. Jorrit Hofstra[1]
[1]Certe, Groningen, The Netherlands

Timely and accurate diagnosis of malaria infections prevents complications and reduces mortality. In a non-endemic setting, with only a few cases per year per laboratory, the 24/7 availability of adequately trained and experienced staff may be a challenge. A study by Boonstra et al. in 2019 demonstrated a high degree of variation in strategies to diagnose malaria in clinical laboratories in the Netherlands. The new guideline provides a framework of test-properties for screening and confirmation of malaria. The guideline is in essence agnostic to the specific test used for screening as long as it meets the stated requirements (i.e. available 24/7, result within 90 minutes, highly sensitive and with a high negative predictive value). Although the guideline does provide examples of appropriate (combinations of) tests that can be used, new appropriate tests can be implemented as they become available.

Molecular screening for the presence of Plasmodium spp. using Loop-mediated isothermal amplification (LAMP), a nucleic acid amplification technique (NAAT), has been extensively evaluated both in endemic as well as in non-endemic settings and has found its way into an increasing number of clinical microbiology laboratories worldwide. However various other methods are being developed and evaluated, including methods using flow cytometry and (semi)automated microscopy using artificial intelligence tools.

The subject of this presentation is to summarize the available data on using LAMP for malaria screening in a non-endemic setting, and to elaborate on new screening strategies currently under development.

P028

Chronic follicular conjunctivitis associated with Chlamydia felis acquired from a domestic cat; a case report.

Dr Laura Hughes[1], Dr S Visser[1], Dr E Heddema[2], Dr R-J Wijdh[1], Dr R Huis in 't Veld[1]
[1]University Medical Centre Groningen, Groningen, Netherlands, [2]Zuyderland Medisch Center, Sittard-Geleen/Heerlen, Netherlands

Introduction

Chlamydia felis is predominantly a pathogen of domestic cats and is rarely reported as a cause of follicular conjunctivitis in humans. We report a case of zoonotic transmission from a domestic cat and describe how the initial and definitive identification of the pathogen was made.

Methods

Real-Time PCRs (RT-PCR) for Chlamydia pneumonia/psittaci and Chlamydia trachomatis were performed on conjunctival swabs followed by sequence analysis for further species characterisation. Chlamydial serology was also performed.

Case & Results

A 38-year-old female was referred to a tertiary hospital in the Netherlands with a 3-month history of red, painful eyes. She had received a single 1g oral dose of azithromycin and topical antibiotic and corticosteroid eye drops with no good effect. The patient's cat had sneezed into her face prior to symptom onset.

Ophthalmological examination revealed reduced visual acuity in the left eye, papillary and follicular conjunctivitis in both eyes and substantial superficial keratitis of the right cornea.

RT-PCR (OmpA gene) of conjunctival swabs tested positive for Chlamydia psittaci (Ct 39). Subsequent PCR and sequence analysis (23s gene) identified the isolate as C. felis. Serology indicated a recent infection.

The patient was treated with doxycycline 100mg twice a day for 28 days. After treatment, the patient's visual acuity returned to baseline and the conjunctivitis resolved.

Conclusion

Because C. felis is a rare cause of conjunctivitis in humans, most microbiology laboratories do not have specific diagnostic tests available. However, RT-PCR based on the 23s gene followed by sequence analysis was useful in the identification of this pathogen.

Treatment with a single 1g dose of oral azithromycin was clinically unsuccessful. A recent treatment with azithromycin should be evaluated carefully before ruling out persistent chlamydial infection. Treatment of C. felis conjunctivitis is unknown, consider doxycycline 100mg twice a day for at least 14 days.

P030

Development and worldwide implementation of the simple one step stool processing method for Xpert testing to detect Mycobacterium tuberculosis and rifampicin resistance

P de Haas[1], A Slyzkyi[1], Dr. Kristin Kremer[1], Dr. E Tiemersma[1], Dr. E Klinkenberg[2]
[1]KNCV Tuberculosis Foundation, Den Haag, Netherlands, [2]TB Connect, , Netherlands

Introduction
The diagnosis of pulmonary tuberculosis (TB) in children and people living with HIV is hampered because they frequently have nonspecific signs and symptoms, and laboratory confirmation of their disease is difficult due to its paucibacillary nature and the difficulty to obtain sputum. Since 2020, with an update in 2022, the World Health Organization (WHO) recommends the use of Xpert MTB/RIF, and the more sensitive Xpert MTB/RIF Ultra, on stool as an initial diagnostic test for the detection of TB and rifampicin resistance in children with signs and symptoms of pulmonary TB. Various methods to process stool for Xpert testing are described, but most methods are too complex to perform near point of care.

Methods
We developed the simple one step (SOS) stool processing method for the detection of TB and rifampicin resistance that uses the same supplies and equipment as used for Xpert testing of sputum. Pilot implementation studies and head-to-head comparative studies for stool testing in children were performed in seven countries. In addition, a modelling study was performed to determine the cost-effectiveness of the method.

Results
In head-to-head comparisons, the SOS stool processing method showed similar sensitivity and specificity as other methods, and the method was evaluated to be the easiest to implement in routine settings at low cost. Subsequently, the SOS stool method was selected by WHO as one of the two processing method best to be used. A modelling study showed that stool testing close to the point of care is cost-effective, expected to increase access to bacteriological confirmation of TB diagnoses in children and reduce mortality.

Conclusion
SOS stool processing enables painless, easy, and cost-effective TB diagnoses close to point of care in resource-poor settings. Currently, it has been rolled out successfully in three countries and many other countries started or are planning implementation.

P031

Validation of a bacterial β-glucuronidase activity test for the gastrointestinal toxicity in colorectal cancer patients receiving irinotecan-based combination treatment – a study protocol

Paulien Kristen[1,2], Milou Stevens[1,2], Eva de Jong[2,4], Lars E. Hillege[1,2], Janine Ziemons[1,2], Prof. dr. Matthew R. Redinbo[7], dr. John Penders[5,6,8], Prof. dr. Marjolein L. Smidt[1,2]
[1]GROW - School for Oncology and Reproduction, , Maastricht, The Netherlands, [2]Maastricht University Medical Centre, , Maastricht, The Netherlands, [3]Maastricht University Medical Centre, , Maastricht, The Netherlands, [4]Catharina Hospital, , Maastricht, The Netherlands, [5]NUTRIM - School of Nutrition and Translational Research in Metabolism, , Maastricht, The Netherlands, [6]Euregional Microbiome Center, , Maastricht, The Netherlands, [7]University of North Carolina, , Chapel Hill, NC, United States, [8]Maastricht University Medical Centre, , Maastricht, The Netherlands

a. Irinotecan-based systemic therapy is a treatment used in metastatic colorectal cancer (mCRC) patients. One of the major disadvantages of this therapy is an unpredictable late-onset gastrointestinal toxicity, mainly diarrhoea. Pre-clinical research suggests that high activity of the bacterial enzyme β-glucuronidase (β-GUS) is a possible indicator for this irinotecan-induced toxicity. Normally, irinotecan is metabolized to the toxic SN-38 and subsequently, conjugated in the liver to generate the inactive metabolite SN-38G for excretion via the bile. Research demonstrates that gut microbiome-derived β-GUS activity in the gut leads to re-activation of SN-38G into the cytotoxic SN-38, which might cause severe mucosal damage and diarrhoea. Additionally, only certain microbial β-GUS enzyme variants exert SN-38G-specific activity and presence of these variants differs between individuals. Therefore, we aim to validate a β-GUS enzyme activity assay which can be used to quantify SN-38G-specific β-GUS activity in a clinical setting and relate β-GUS activity to diversity, composition and functional capacity of the gut microbiome in mCRC patients.

b. We will include 182 stool samples from mCRC patients which were already collected during previous studies. We will apply liquid chromatography-mass spectrometry (LC-MS/MS) for validation of the β-GUS activity assay. Metagenomic shotgun sequencing will be performed to identify the different GUS variants present in faecal samples and to map the gut microbiome composition and functional capacity.

c. A validated β-GUS enzyme activity assay will be selected if the results of this assay resemble the results from the LC-MS/MS method. Furthermore, gut microbiome composition will be mapped, annotation of GUS variants will be achieved and a non-redundant catalogue of β-GUS proteins will be generated.

d. If the assay can quantify β-GUS activity in a clinical setting, it will be used for a clinical study investigating the link between β-GUS activity and late-onset gastrointestinal toxicity in patients during irinotecan-based combination therapy.

P032

Vaginome abnormalities are associated with hrHPV infection in women attending the Dutch cervical cancer screening program

Dr. Anne Loonen[1,2], BSc Femke Verhagen[1], Bsc Ilse Luijten- de Vrije[1], Dr. Marjolein Lentjes- Beer[1], Dr. Ronald Huijsmans[1], Dr. Adriaan van den Brule[1]
[1]Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands, [2]Fontys University of Applied Sciences, Eindhoven, The Netherlands

HPV is a sexually transmitted virus, which infects about 80% of all men and women at some time in their lives. Usually, the infection is dealt with successfully by the body's immune system. The risk of developing cancer is relatively low (<1%). Persistent infection with hrHPV is necessary but not sufficient for cervical cancer development and additional factors are involved in disease establishment and progression. The aim of this study is to investigate whether certain pathogens which can be present in the vaginome or vaginome imbalances are possible co-factors for hr-HPV infection and HPV-induced cervical dysplasia.
In this study, 492 hrHPV positive and 500 hrHPV negative cervical smears from women attending the Dutch cervical cancer screening program were included (biobank Jeroen Bosch hospital). Age and cytology were known for the hrHPV positive samples. All cervical smears (PreservCyt medium) were directly tested with Aptima assays (Hologic) targeting Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma genitalium (MG), Candida spp. (CS), C. glabrata (CG), Trichomonas vaginalis (TV) and bacterial vaginosis (BV). Data were analysed by using SPSS.
The Aptima assays were performed on PreservCyt medium instead of Aptima collection medium (swabs). The prevalences of CT, NG, MG, CS, CG, TV and BV in this cohort were determined and found to be 1,9%, 0,0%, 1,7%, 5,4%, 1,4%, 0,1% and 27,2% respectively. When comparing HPV groups it was found that CT, MG and BV had a significantly higher prevalence in hrHPV positive smears as compared to hrHPV negative samples (for all $p<0,001$). No significant differences were found when comparing different age groups and cytology outcomes.
In conclusion, vaginome abnormalities are associated with hrHPV infection in women attending the Dutch cervical cancer screening program. Currently, a subgroup of this cohort is being analysed with MinION to characterize the complete (bacterial) vaginome.

P033

Relevant once again: diphtheria surveillance and outbreak response in the Netherlands

Dr. Rob Mariman[1], Dr. D. Notermans[1], Dr. T. Kortbeek[1], Dr. B. Voordouw[1], J. Van Veldhuizen[1], Dr. E. Heikens[2], Dr. M. De Graaf[3], Dr. N. Van Maarseveen[3], Dr. H. Ruijs[1], Dr. T. Woudenberg[1], D. Van Meijeren[1], Dr. M Van Den Beld
[1]RIVM, Bilthoven, Netherlands, [2]St Jansdal, Harderwijk , Netherlands, [3]Saltro Diagnostics Centre, Utrecht , Netherlands

Diphtheria is a notifiable disease in The Netherlands. Between 1990-2021, 22 cases of diphtheria with PCR confirmed toxigenic strains have been reported to the RIVM. As many European countries, we observed an increase of diphtheria caused by Corynebacterium diphtheriae in 2022. Here, we describe the implementation of pathogen surveillance based on whole genome sequencing (WGS), and the application of a molecular assay for rapid detection of diphtheria.

In the current diagnostic practice, throat or wound swabs from patients are analyzed by regional medical microbial laboratories using culture based methods. MALDI-TOF confirmed relevant Corynebacterium spp. are sent to the Reference Laboratory for Diphtheria for confirmation and determination of the toxigenic status. This procedure generally takes at least 3-4 days. Therefore, we invested in the implementation and validation of a multiplex qPCR for rapid detection of Corynebacteria that can cause disease in combination with the toxin-gene. Preliminary results indicate that direct testing of patient material can reduce the turnaround time to 24 hours.

We routinely apply WGS for pathogen surveillance of a broad panel of bacteria and viruses. Illumina sequencing technology coupled to automatic data transfer to high performance computing clusters allows us to generate sequence type information and cgMLST profiles between 48-72 hours upon arrival of the clinical isolate. An analysis of isolates from five cases of cutaneous diphtheria caused by a C. diphtheriae in 2022 revealed two different sequence types, ST384 (4) and ST377 (1). In depth cgMLST analysis of these cases, all Syrian refugees, showed clustering with previously identified outbreak clusters among refugees in Europe.

Rapid detection of diphtheria in combination with pathogen surveillance for outbreak detection supports regional and national public health authorities in optimizing control measures. These include treatment and isolation of the case, throat sampling of close contacts, prophylactic antibiotic treatment and/or vaccination where needed.

P034

Complement-resistance of multi-drug resistant Klebsiella pneumoniae clinical isolates

Frerich Masson[1,2], B W Bardoel[1], S H M Rooijakkers[1]
[1]UMC Utrecht, Utrecht, The Netherlands, [2]Lund University, Malmö, Sweden

Introduction: The Gram-negative bacterium Klebsiella pneumoniae is an opportunistic pathogen associated with diseases like pneumonia and sepsis. Due to the rising emergence of multi-drug resistance in these bacteria, new treatment options are needed. As part of the human innate immune response, the complement system can kill Klebsiella via insertion of the membrane attack complex (MAC) into the bacterial cell envelope. However, Klebsiella can withstand killing in human serum. The aim of this study is to assess complement deposition and MAC formation on different clinical K. pneumoniae isolates.

Methods: Several clinical K. pneumoniae strains were collected. Complement-mediated killing was assessed using a membrane impermeable DNA-dye that becomes fluorescent upon damage of the bacterial inner membrane. The antimicrobial peptide nisin was used to assess occurrence of outer membrane damage. Bacteria were treated with normal human serum, followed by detection of specific human complement proteins. Analysis of MAC-formation was performed with either fluorescence-labelled complement components or specific antibodies using flow cytometry.

Results: Serum-susceptibility was assessed by colony forming units and membrane damage upon serum exposure. 10 out of 23 strains were determined to be resistant. Strains presenting LPS O1-antigen were more serum resistant, O2-antigen presenting strains were susceptible to serum. Of the serum-resistant strains, all showed deposition of C5b-9 on their surface, while circumventing killing via the MAC by unknown mechanisms. Five isolates showed damage of the inner membrane when serum and nisin – a membrane impermeable antimicrobial peptide – was added, while five isolates remained resistant.

Conclusion: Our data show that Klebsiella presenting the O1-antigen, are more serum-resistant, whereas Klebsiella presenting O2-antigen are serum-susceptible. All resistant strains deposit C5b-9 on their surface, even though functional MAC might not be formed as indicated by survival in serum. Understanding the complement evasion mechanisms of these strains is important to explore novel antimicrobial strategies.

P035

Evaluation of Alinity m cytomegalovirus (CMV) and Epstein-Barr virus (EBV) Assay Performance in Plasma

Ing./b As Erin Meijers[1], Dr. E. Wessels[1], BS T. Vreeswijk[1], Dr. E.C.J. Claas[1]
[1]Leiden University Medical Center (LUMC), Leiden, Netherlands

Introduction
Viral load monitoring of cytomegalovirus (CMV) and Epstein-Barr virus (EBV) is critical for transplant patients. The analytical and clinical performance of the Alinity m CMV and EBV PCR assays on the high-throughput Alinity m system with random and continuous access was evaluated in comparison to our routine laboratory developed PCR tests (LDT).

Methods
Fifteen commercial CMV and EBV plasma panels with concentration levels of 2.3-6.6 log IU/ml and 2.7-6.7 log IU/ml, respectively, were tested across 5 days to evaluate accuracy and precision of the Alinity m CMV and EBV assays. Furthermore, 153 CMV and 152 EBV patient plasma specimens were retrospectively tested with Alinity m. Values were compared to LDT results.

Results
Panel testing of Alinity m CMV and EBV revealed good accuracy (differences to target values: 0.01-0.22 and 0.24-0.28 log IU/ml for CMV and EBV, respectively) and high precision (standard deviation: 0.05-0.17 [CMV] and 0.04-0.06 log IU/ml [EBV]). Good correlations (R^2=0.922 [CMV] and R^2=0.831 [EBV]) and low biases (0.34 [CMV] and -0.19 log IU/ml [EBV]) were observed between Alinity m and LDT with positive and negative percent agreements of 99% and 84% for CMV and 98% and 81% for EBV. Discrepancies were limited to specimens with low viral loads. Longitudinal patient courses were very similar across all 26 CMV and 24 EBV patients with results of ≥ 2 days. Alinity m median onboard and processing turnaround times were <3 hours and ≤116 min, respectively.

Conclusions
Alinity m CMV and EBV provided fast, accurate and precise results that compared well with our LDT assays.

P037

A Spitting Image: Molecular Diagnostics Applied to Saliva Enhance Detection of Streptococcus pneumoniae and Pneumococcal Serotype Carriage

Willem Miellet[1,2], J van Veldhuizen[2], D Litt[3], R Mariman[2], A.J. Wijmenga-Monsuur[2], T. Nieuwenhuijsen[1,2], R. Thombre[3], S. Eletu[3], T. Bosch[2], N.Y. Rots[2], M.A. van Houten[4], E. Miller[5], N.K. Fry[5], E.A. Sanders[1,2], K. Trzciński[1]
[1]University Medical Center Utrecht, Utrecht, The Netherlands, [2]National Institute for Public Health and the Environment, Bilthoven, The Netherlands, [3]United Kingdom Health Security Agency, London, United Kingdom, [4]Spaarne Gasthuis, Hoofddorp, The Netherlands, [5]United Kingdom Health Security Agency, London, United Kingdom

Introduction: Despite strong historical records on high accuracy of saliva testing, oral fluids are considered poorly suited for pneumococcal carriage detection. We propose an approach for carriage surveillance and vaccine impact studies that increases the sensitivity and specificity of pneumococci and pneumococcal serotypes detection in saliva samples.

Methods: qPCR-based methods were applied to detect pneumococcus and pneumococcal serotypes in 971 saliva samples collected from 653 toddlers and 318 adults. Results were compared with culture-based and qPCR-based detection in nasopharyngeal samples collected from children and in nasopharyngeal and oropharyngeal samples collected from adults. Optimal Cq cut-offs for positivity in qPCRs were determined via receiver operating characteristic curve analysis and accuracy of different approaches was assessed using a composite reference for pneumococcal and for serotype carriage based on isolation of live pneumococcus from the person or positivity of saliva samples determined with qPCR.

Results: We identified 51.5% and 31.8% saliva samples from children and from adults, respectively, as positive for pneumococcus. Detection of pneumococcus by qPCR in culture-enriched saliva exhibited enhanced sensitivity and higher agreement compared with diagnostic culture of nasopharyngeal samples from children (Cohen's κ:0.69-0.79 vs 0.61-0.73) and from adults (κ:0.84-0.95 vs 0.04-0.33) and culture of oropharyngeal samples from adults (κ:0.73-0.82 vs -0.12-0.19). Similarly, detection of serotypes with qPCR in culture-enriched saliva exhibited enhanced sensitivity and higher agreement compared with nasopharyngeal culture in children (κ:0.73-0.82 vs 0.61-0.73) and adults (κ:0.073-0.82 vs 0.00-0.30) and oropharyngeal culture in adults (κ:0.90-0.96 vs -0.13-0.30). However, results of qPCRs targeting serotypes 4, 5, 17F and serogroups 9, 12, 35 were excluded due to assays' lack of specificity.

Conclusion: We show that molecular testing of culture-enriched saliva samples improves the sensitivity of overall surveillance of pneumococcal carriage in children and adults, but we also emphasize limitations of qPCR-based approaches for pneumococcal serotypes carriage detection.

P038

Longitudinal Dynamics of Streptococcus pneumoniae Carriage and SARS-CoV-2 Infection in Households with Children and Non-elderly Adults.

Willem Miellet[1,2], R. Mariman[2], D. Eggink[2], M.A. Nicolaie[3], J. van Veldhuizen[2], G. Pluister[2], L.M. Kolodziej[4], S.F.L. van Lelyveld[5], S.M. Euser[6], E.A.M. Sanders[1,2], M.A. van Houten[7], K. Trzciński[1]
[1]University Medical Center Utrecht, Utrecht, The Netherlands, [2]National Institute for Public Health and the Environment , Bilthoven, The Netherlands, [3]National Institute for Public Health and the Environment , Bilthoven, The Netherlands, [4]Amsterdam University Medical Centres, , Amsterdam, The Netherlands, [5]Spaarne Gasthuis, Hoofddorp, The Netherlands, [6]Regional Public Health Laboratory Kennemerland, Haarlem, The Netherlands, [7]Spaarne Academy, Hoofddorp, The Netherlands

Introduction: To characterize interferences between Streptococcus pneumoniae and SARS-CoV-2 we investigated the longitudinal patterns of viral infection and pneumococcal carriage in households infected with SARS-CoV-2.

Methods: SARS-CoV-2 and pneumococcus were detected with quantitative molecular methods in saliva from members of eighty households. Samples were collected between October 2020 and January 2021 from n=197 adults and n=118 children of which n=176 adults and n=98 children have a complete set of ten samples collected within 42 days since enrollment. Time-dependent Cox models were used to assess the time to SARS-CoV-2 infection and time to SARS-CoV-2 infection clearance in association with pneumococcal carriage and correcting for other factors.

Results: In the entire cohort, cumulative pneumococcal carriage and SARS-CoV-2 infection rates were 58% and 65%, respectively. Increased viral loads were observed among pneumococcal carriers and individuals with high 16S abundances. Pneumococcal abundances were not associated with an altered risk of SARS-CoV-2 infection (HR 0.89, 95% CI, 0.79 – 1.00, P=0.08) or clearance of SARS-CoV-2 infection (HR 1.15, 95% CI, 1.00 – 1.31, P=0.05) and there were no longitudinal differences in viral loads in linear mixed effects models. Individuals with high 16S abundances displayed delayed viral clearance (HR 0.66, 95% CI 0.53-0.82, P<0.0001).

Conclusions: Despite high within-household pneumococcal carriage prevalence rates among SARS-CoV-2 infected individuals, the synergistic interactions between pneumococcus and SARS-CoV-2 were limited. However, we observed a relationship between high overall bacterial abundances and the probability of SARS-CoV-2 infection clearance, suggesting a link between SARS-CoV-2 and the upper respiratory tract microbiome, not unique for pneumococcus.

P040

Comparison of the performance of two targeted metagenomic virus capture probe-based methods using synthesized viral sequences and clinical samples.

Drs. Kees Mourik[1], Drs. Ellen C. Carbo[1], Dr. David van der Meer[2], Dr. Arnoud Boot[2], Prof. Aloys C.M. Kroes[1], Dr. Eric C.J. Claas[1], Dr. Jutte J.C. de Vries[1]
[1]Leiden University Medical Center, Leiden, The Netherlands, [2]GenomeScan B.V., Leiden, The Netherlands

Introduction: Viral metagenomic sequencing enables pathogen-agnostic detection of viruses in clinical samples, however accurate sensitivity and specificity remains a challenge. In this study, the performance of two virus capture probe-based methods was compared.

Method: The following hybridization capture methods were evaluated: the SeqCap EZ HyperCap workflow (Roche) and the Twist Comprehensive Viral Research Panel. In order to mimic the complexity of clinical samples while reducing the number of environmental sequences, a panel was prepared consisting of synthesized respiratory virus sequences (SARS-CoV-2, influenza A virus, measles, enterovirus D68, bocavirus) in varying concentrations, and combined with 90-99% human cell free DNA (cfDNA) background sequences. In addition, ATCC Virome Virus Mix of human mastadenovirus F, human herpesvirus 5, human respiratory syncytial virus, influenza B virus , reovirus 3, and zika virus, each 16.7%, was analyzed and nine clinical samples with viral loads of 500, 5,000 and 50,000 IU/ml of adenovirus, Epstein Barr virus and hepatis B virus were included. Sequencing was performed on the NovaSeq6000 (Illumina), and metagenomic pipeline Genome Detective was used to classify the detected sequences .

Results: The virus capture probe-based methods showed comparable sensitivity for detection of the synthesized sequences over the different concentrations with varying proportions of human background sequences. The clinical samples were challenging with regard to some of the lower viral loads. Additional findings were detected in a minority of the samples, usually by both hybridization capture methods.

Conclusion: Though the SeqCap EZ HyperCap workflow and Twist Comprehensive Viral Research Panel are composed of unidentical probe sets, they performed corresponding in this comparison, and are applicable for the detection of a broad range of RNA and DNA viruses in clinical samples.

P041

Management of vancomycin-resistant Enterococcus faecium (VRE) in Dutch hospitals: a nation-wide survey

Dr. Marlies Mulder[1,2], Karuna E.W. Vendrik[2], dr. Daan W. Notermans[2], Sophie A.M. van Kessel[2], dr. Annelot F. Schoffelen[2], Cathalijne M. Dodemont - van Breen[2], dr. Antoni P.A. Hendrickx[2], dr. Wil van der Zwet[1], dr. Caroline Schneeberger-van der Linden[2]
[1]Maastricht University Medical Center+ , , The Netherlands, [2]National Institute for Public Health and the Environment, Bilthoven, The Netherlands

Background
Although the incidence of vancomycin-resistant Enterococcus faecium (VRE) in the Netherlands is low, outbreaks in Dutch hospitals do occur. There are several national guidelines and guidance documents on different aspects of VRE-management available, but these do not cover all aspects on VRE-management and the level of compliance to these guidelines is unknown. It is expected that variation in infection prevention control measures between hospitals exists. The aim of this study was to get insight into the VRE-policies in Dutch hospitals with regard to screening, diagnostics and infection prevention control measures.

Materials and methods
An extensive online questionnaire was sent out to all Dutch hospitals in December 2022 (n=69) via the Infection Prevention and Control Working Group (HIP Working Group), which is part of the Dutch Society of Medical Microbiology (NVMM) and via the Dutch Society of Infection Prevention and Control (VHIG). The questionnaire included question regarding VRE-screening, sample collection, diagnostics, patient isolation, cleaning procedures, VRE-outbreaks and investigation of VRE clearance. A first rough exploratory analysis, regarding VRE-policies, was performed.

Results
To date, the questionnaire was completed by 60 respondents, which included 56 hospitals of 53 different hospital groups (response rate 75.7%) and four were other types of health care facilities. All 56 hospitals isolate VRE-positive patients, but only 48 (85.7%) perform some sort of screening for VRE. Furthermore, 36 (64.3%) hospitals answered they had one or more VRE-outbreaks in the previous ten years (18 (32.1%) no outbreaks; 2 (3.6%) unknown). In 52 (92.9%) hospitals, extra cleaning measures are performed after discharge of a VRE-carrier (2 (3.6%) never; 2 (3.6%) only during an outbreak).

Conclusion
This study has shown that (almost) all hospitals in the Netherlands have some kind of VRE-management, although the comprehensiveness and details of the implementation measures differ per hospital.

P042

No evidence for an environmental source of vancomycin-resistant Enterococcus faecium in a low-grade hospital outbreak

Dr. Marlies Mulder, Ella Klomp-Berens, Dr. Anne Dirks, Dr. Lieke van Alphen, Prof. Paul Savelkoul, Dr. Wil van der Zwet
[1]Maastricht University Medical Center+, , The Netherlands

Background
Although the incidence of Vancomycin-resistant Enterococcus faecium (VRE) in the Netherlands is low, outbreaks in Dutch hospitals do occur. Recently, the Maastricht UMC+ had an ongoing small VRE-outbreak with the unusual MLST type 787 in the period November 2021 – June 2022 confirmed by WGS. However, an epidemiological link could not be established for several patients. Possible explanations could be unknown VRE-carriers or an environmental source. Nowadays, we use H2O2 fogging for the disinfection of rooms of VRE carriers, but this method does not disinfect wet environmental locations. Therefore, we investigated whether these locations could explain the ongoing transmission of this VRE-clone.

Materials and methods
We swabbed all patient sanitation sites (toilets, drains of sinks and showers) of six hospital departments where VRE-carriers had been identified during the outbreak in July and August 2022 and tested them for the presence of VRE. The sampling of VRE-positive locations VRE was repeated after 4-6 weeks to study persistence. All environmental VRE-isolates were genotyped by MLST to determine whether they were identical to the outbreak strain.

Results
We performed 391 cultures of patient sanitation sites. Of these cultures were 9 (2.3%) positive for VRE of 3 different clonal types, of which only one isolate was MLST type 787, which could be explained by a patient who was readmitted in that room . One (11.1%) of these positive cultures could be explained by a known VRE-carrier in that room. Of these locations, 2/9 (22%) of locations were still positive after 4-6 weeks without extra cleaning measures.

Conclusion
We demonstrated a low percentage of VRE in patient sanitation sites. H2O2 fogging does not disinfect these locations and additional cleaning measures are needed. However, we could not show that these wet environmental locations were the source for our small VRE MLST 787 outbreak.

P043

Malaria diagnostic in non-endemic settings: current practice, challenges & outlook

PD Dr. med. Andreas Neumayr[1]
[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]James Cook University, College of Public Health, Medical and Veterinary Sciences, Townsville, Australia
Invited key note lecture in the session:
"New guideline 'Malaria diagnostics for Dutch laboratories'"

Considering the format, the demanded abstract structure (intro, methods, results, conclusion) is not really feasible and I propose the following abstract text:

Malaria diagnostics is and remains one of the most important indications of emergency microbiological diagnostics in everyday clinical practice. In the age of large global travel and migration flows, this particularly concerns physicians and microbiological diagnosticians in non-endemic areas.
On the medical side, the differential diagnosis of malaria is usually made quickly today, although there is often a lack of knowledge about the available diagnostic methods and their rational use.
On the diagnostic side, highly sensitive molecular diagnostic methods are increasingly available, especially in non-endemic areas, while microscopic diagnostics, still considered the gold standard, often lack expertise.
This presentation will attempt to summarise and comment on the current practice of malaria diagnostics in non-endemic areas.

P044

Analysis of a current vancomycin resistant Enterococcus faecium (VREfm) outbreak implies the need for an adapted diagnostic algorithm

Dr. Roel Nijhuis[1], Annelies Smilde[2], Frouke Stegeman[2], Dr. Annemarie Weersink[1], Ada Gigengack[2]
[1]Meander Medical Centre, Amersfoort, Netherlands, [2]Meander Medical Centre, Amersfoort, Netherlands

Introduction: In the last quarter of 2022, we were challenged with an outbreak of vancomycin resistant Enterococcus faecium (VREfm) including both VanA and VanB containing isolates, introduced into the respective department at almost the same time. Remarkably, it was observed that screening by use of VanA and VanB PCR sometimes tested positive for VanB with low Ct-values, whereas VREfm-specific culture remained negative. Here, we describe the analysis of the results of our diagnostic algorithm leading to adaptation of this algorithm.

Methods: Per specimen of each patient, obtained up to 5 months prior to the first identification of VREfm, Ct-values of the VREfm screening PCR were collected, as well as the amount of detected colonies in the VREfm-specific culture. The relationship of the identified VREfm isolates was established by WGS and subsequent cgMLST analysis. A second VREfm-specific culture medium was introduced and compared.

Results: Thirty-six patients were identified as VREfm-carrier, including 4 with VanA, 28 with VanB, 3 with two isolates carrying either VanA and VanB and 1 also including a single isolate carrying VanA and VanB. cgMLST appointed all VanA isolates as STnew-CT7088, whereas VanB were ST80-CT1065, ST117-CT7117 or ST117-CT7118. VREfm ST80-CT1065 was correctly detected by culture in each first PCR positive specimen, whereas it could take up to 12 positive PCR results (Ct-values <30) before ST117 was cultured. If ST117 was cultured, only minimal growth on the specific medium was observed. ST117 was identified in 4 specimens when evaluating the second VREfm-specific medium, of which 3 remained negative on the original medium.

Conclusion: Our data showed that these VanB ST117 isolates were insufficiently detected by the VREfm-specific culture media, possibly contributing to unnoticed spread of VREfm. For this reason, the diagnostic algorithm was adapted by taking PCR positivity (Ct<30) into account and most likely introducing another VREfm-specific medium.

P045

Antibiotic prophylaxis in European intensive care units does not affect the pangenome composition of ESBL E. coli

Mr. Julian Paganini[1], Mr. J. Scharringa[1], Prof. Dr. R. J. L. Willems[1], Dr. A. C. Schurch[1], Dr. N. L. Plantinga[1]
[1]Umc Utrecht, Utrecht, The Netherlands

Introduction

In Dutch intensive care units (ICUs) patients routinely receive selective digestive decontamination (SDD) as a prophylactic treatment to prevent infections. SDD consists of a mix of antimicrobial agents targeting aerobic Gram-negative pathogens, S. aureus and yeast of the gastro-intestinal tract and oropharynx. SDD has been associated with improved patient outcome, but its effects on the resistome and pangenome composition of opportunistic pathogens have not been extensively tested. Consequently, in this work we compared 130 genomes of ESBL E.coli isolates from patients that received SDD or baseline treatment in five ICUs located across three European countries.

Methods

E. coli isolates were obtained by inoculating rectal swabs in ESBL-selective media.
Genomes were sequenced with Illumina pair-end technology. Population structure was evaluated with MLST(v2.16) and PopPUNK(v2.3). Antibiotic resistance genes (ARG) were identified using AMRFinderPlus(v3.11). Pangenome composition was studied using BAKTA(v1.6) and Panaroo(v1.3). Plasmidome was predicted using plasmidEC(v1.3) and gplas(v2.0).
Effects of treatments in the pangenome composition were evaluated using PERMANOVA, based on gene presence/absence distances.

Results

ST131 was the predominant clone in both treatment groups (baseline=24%, SDD=22%). Simpson's diversity indexes calculated using STs (baseline=0.91, SDD=0.92) and PopPUNK clusters (baseline=0.94, SDD=0.94) were similar.
Isolates from baseline contained a similar number of ARGs (median=12, IQR=8-14) than those from SDD (median=11, IQR=7-13) (p-value=0.27, Wilcoxon rank sum test). Similar results were observed when sub-classifying ARGs according to their target.
Variations in the accessory genome were not explained by treatment (p-value=0.359, R2=0.008), but rather by ST (p-value=0.001, R2=0.44). Additionally, treatment did not have an effect in plasmidome composition (p-value=0.236, R2=0.0085), while ST explained a smaller fraction of the observed variation (p-value=0.001, R2=0.12).

Conclusion

SDD does not seem to impact the resistome or pangenome of ESBL E. coli isolates from ICU admitted patients.

P046

ClonalTracker: A new pipeline to survey vancomycin-resistant Enterococcus faecium outbreaks

Victoria Pascal[1], Janetta Top[1], Rob Willems[1], Anita C. Schürch[1]
[1]University Medical Center Utrecht, Utrecht, The Netherlands

Background
The rise of reported vancomycin-resistant Enterococcus faecium (VRE) infections worldwide highlights the importance of detecting outbreaks in a timely fashion. Whole-genome sequencing data can be very useful to understand dissemination modes of vancomycin-resistance genes and provide insight into transmission networks. We have built ClonalTracker (also available as a web server at www.clonaltracker.nl) and used it to easily compare any two given VRE genomes at the level of whole-genome, transposon and van type using short-read sequencing data.

Methods
The pipeline uses Blastn to detect the van gene, Blastn, RagTag, ISEScan and Clinker for transposon typing, Mash for whole genome comparison and PopPUNK to contextualize the genomes within a larger VRE collection. Pairs of isolates with dissimilar genomic background but identical van gene and transposon are considered candidates for horizontal gene transfer (HGT) of vancomycin resistance. The results and selected thresholds were validated using two previously analysed datasets: 39 vanB VRE isolates (dataset 1, Zhou et al, 2018) and 314 vanB VRE genomes (dataset 2, Arredondo-Alonso et al, 2021).

Results
Pairwise comparisons between isolates from dataset 1 revealed 18 different transposons, including 11 singletons and 5 types among 23 isolates. ClonalTracker identified more transposon types (differences at SNP or IS/gene content level) than reported by Zhou et al., but agrees with cgMLST groups.

The analysis of dataset 2 showed that there are 134 different transposons, mostly spread clonally. HGT candidates were frequently isolated in the same year (80/349 cases), but more rarely in the same hospital (24/349 cases).

Conclusions
ClonalTracker provides a combination of high-resolution transposon- and genome typing in the absence of long read data, which could be very useful for outbreak management in hospitals. In addition, the genomes are clustered with a larger VRE dataset to obtain insight in the dissemination modes and dynamics of van-carrying transposons.

P047

The pharmacodynamics and synergy testing of currently recommended antibiotics against Nocardia nova.

Msc Jelmer Raaijmakers[1], Bsc I Tabak[1], M.D., PhD J van Ingen[1]
[1]Radboudumc, Nijmegen, The Netherlands

Background:
Nocardia species cause severe opportunistic infections. The currently recommended multi-drug antibiotic regimens lack a firm evidence base, both in vitro and in vivo. Here, we study the in vitro pharmacodynamics of currently recommended antibiotics.

Methods:
We determined MICs (minimum inhibitory concentration) of cotrimoxazole (SXT), imipenem (IPM), linezolid (LZD), amikacin (AMK) and clarithromycin (CLR) by broth microdilution using SensiTitre Nocardia plates (ThermoFisher).
We performed 72-hour time-kill kinetics experiments of all single drugs and 2- and 3-drug combinations, in cation-adjusted Mueller-Hinton broth with 0.05% Tween and antibiotic concentrations of 0.25xMIC to 32xMIC, except for IPM (0.015 to 32x MIC), SXT (0.25 to 512x MIC) and LZD (0.03 to 32x MIC); combinations were tested at 2xMIC concentrations, except SXT which was added in 8xMIC concentration. Bottles were inoculated with a 0.5 McFarland suspension of N. nova ATCC BAA-2227, incubated at 37°C and shaken at 100rpm;we sampled at 0, 12, 24, 36, 48 and 72 hours of incubation for cfu counting.

Results:
We measured the following MICs: AMK 0.5 mg/l, CLR 0.06 mg/l, IPM 0.25 mg/l, LZD 0.5 mg/l, SXT 0.12/2.4 mg/l.
IPM and AMK showed bactericidal activity, whereas SXT, LNZ and CLR had bacteriostatic activity.
In 2-drug combinations, IPM-containing combinations were most active and IPM was the driver of the effect. In the 3-drug combinations, IPM was also the main driver of the effect and combinations featuring IPM, SXT, AMK and LNZ were most effective. In all 2- and 3-drug combinations, adding CLR decreased the efficacy.

Conclusions:
IPM is the driver of the efficacy of antibiotic combination in vitro against N. nova. Combinations of IPM with AMK, SXT or LNZ proved most potent, in line with current treatment recommendations. The exact added value of a third drug to regimens requires further in vitro studies, preferably in dynamic models, and in vivo confirmation.

P048

Bioactive Glass S53P4 as a non-antibiotic antimicrobial strategy to treat Biomaterial Associated Infections

Ms Deeksha Rajkumar[1]
[1]Amsterdam University Medical Center, Amsterdam, The Netherlands, [2]Bonalive, Turku, Finland

Biomaterial associated infection (BAI) is a frequent complication in the use of medical implants. The bacteria that most frequently cause these infections are staphylococci. BAI is generally challenging to treat due to tolerance or resistance to antibiotics. Biofilm formation on the implant surface contributes to phenotypic tolerance, and possibly to antimicrobial resistance (AMR) and persistent infection, which raises the danger of unsuccessful treatment and recurrent infection.
Goal: In order to prevent BAI, we employed a non-antibiotic antimicrobial strategy. We investigated Bioactive Glass (BAG) S53P4. BAG granules are currently used as bone cavity fillers with proven osteostimulative and antimicrobial properties. Application of BAG as a coating on biomaterial surfaces may therefore provide a way to prevent BAI. A novel BAG cream has the potential to be applied on implant material surfaces. In this study we compare the antimicrobial activity of different BAG formulations against Staphylococcus aureus. Method: The antibacterial activity of BAG cream, granules and powder against S. aureus was analysed after 24h using the minimum bactericidal activity (MBC) assay. Similarly, the antibacterial activity of novel BAG cream applied on Titanium Aluminum Niobium (TAN) and Polyether Ether Ketone (PEEK) discs was analysed against S. aureus either directly, or after pre-incubation of the discs in medium for 24h. Furthermore, the antibacterial activity of the media containing eluted ions (eluate) from the BAG cream was analysed. Results: BAG cream showed to have better antibacterial activity than BAG granules and powder, and at lower concentrations. Additionally, when used as a coating on TAN and PEEK implant materials, BAG cream as well as its eluate exhibits bactericidal activity. Conclusion: With these encouraging results, we intend to investigate whether this BAG-cream coating is antibacterial in a mouse biomaterial-associated infection model.

P049

Dissemination of an extensively drug-resistant resistance island within Enterobacteriaceae in two ICU's in South-Limburg

Drs Connor Rossel[1], Dr. E Heddema[1], Dr. C Linssen[1], Drs. L van Alphen[2], Drs. C Jamin[2], Dr. H van Westreenen[3], Dr. T Dormans[3], Prof. P Savelkoul[2]
[1]Zuyderland Medisch Centrum, Sittard-Geleen, The Netherlands, [2]Maastricht University Medical Center, Maastricht, The Netherlands, [3]Zuyderland Medisch Centrum, Sittard-Geleen, The Netherlands
Introduction. Polyclonal and diverse plasmid K.pneumoniae and E.cloacae complex strains resistant to aminoglycosides, chloramphenicol, extended spectrum beta-lactam, quinolones, sulfonamides, tetracycline and trimethoprim have been isolated in our hospital. Next-Generation Sequencing (NGS) showed an antimicrobial resistance island (ARI) located on the chromosome or plasmids (IncF/IncH). It contains 12 resistance genes against seven classes of antibiotics and 16 Tn3-family insertion elements, making it a mobilizable element. Most isolates were derived from two intensive care units (ICU). The objective is to map dissemination of this ARI from environmental and patient-derived samples from the ICU's.

Methods. The sink, aerator, medical equipment and bedside were sampled within 24 and 10 ICU rooms at Zuyderland medical Center. Suspected patient isolates were screened retrospectively and prospectively via rectum-swabs for the ARI. Isolates were derived by ESBL-screening and standard antimicrobial susceptibility testing. The ARI was detected by a three-target multiplex PCR (Tn-qnrB1; Tn-CTX-M-15; OXA-1–AAC(6')-Ib-cr). Core-genome multilocus sequence typing (1928 diagnostics, Sweden) was used to identify clusters.

Results. The ARI has been detected within the sinks of the two ICU 22/24 and 1/10, respectively. E.cloacae complex appeared predominately (22/24; 1/10), followed by C.freundii complex (8/24), E.coli (7/24), K.oxytoca complex (6/24), K.pneumoniae (6/24), and four other Enterobacteriaceae spp. (8/24). Aerators and bedside appeared culture-negative, while one E.cloacae isolate was medical equipment derived. Retrospectively, isolates of 66/121 suspected patients were PCR positive. Prospectively, 13/320 patient samples were PCR-positive, of which 9/13 were culture-positive. Six patient-sink clusters containing the ARI were found: E.cloacae complex (Sequence Type (ST)93, ST133, ST190 ST790, ST1200); K.michiganensis ST50.

Conclusion. The ICU sinks provide a reservoir for the ARI which appears to be incorporated into nine Enterobacteriaceae spp, from where transmission to patients may have occurred. The dissemination of ARI is often an overlooked phenomenon of antimicrobial resistance spread, possibly limiting the effectiveness of infection prevention measures.

P050

Has the human scabies mite become resistant to permethrin? A critically appraised topic.

S Vos[2,3], Md Phd Esmée Ruizendaal[1], MD T A M Hekker[4]
[1]Radboudumc, Nijmegen, The Netherlands, [2]NSPOH (Netherlands School of Public & Occupational Health), , The Netherlands, [3]GGD Gelderland-Midden, , The Netherlands, [4]Amsterdam UMC Universitair Medische Centra, Amsterdam, the Netherlands

Introduction

Scabies in humans is a parasitic skin disease caused by Sarcoptes scabiei var. hominis. A significant increase in scabies cases has been reported in the Netherlands over the last years. Simultaneously, public health services are increasingly consulted for treatment failures with permethrin, even without observed issues of non-compliance with the therapeutic and hygienic regimen. Therefore, the question arises if permethrin resistance exists in human scabies mites.

Methods

A systematic search was performed in PubMed and the Cochrane library retrieving 87 articles. After title and abstract screening 25 articles remained. Full text screening resulted in three articles for analysis.

Results

Two phenotypical in vitro studies and one molecular study were included. Yurekli et al. collected scabies mites suspected of resistance and exposed these mites in vitro to varying concentrations of permethrin. All mites died within several hours except for the mites in the control solution. The authors concluded that there was no resistance against permethrin. Unfortunately a control group of mites not suspected of resistance was not included.

Mounsey et al. describe a population of mites from one scabies crustosa patient that showed mild extended survival time after in vitro permethrin exposure compared to a historical cohort of S. scabiei var. suis mites. The comparison with non-human mites limits the validity of these data.

Lastly, Andriantsoanirina et al. tested 40 human scabies mites for the presence of single nucleotide polymorphisms (SNPs) that have been associated with permethrin resistance in S. scabiei var. canis. None of the isolates harbored any of the SNPs. However, it is unknown if these are the relevant SNPs in human scabies mites.

Conclusion

There is insufficient evidence to confirm true resistance to permethrin in human scabies mites. The high burden of disease and the reports of clinical therapeutic failure however warrant further high quality research.

P051

Validation of a PCR panel to diagnose patients with suspected postoperative endophthalmitis

Dr Tim Schuurman[1], Michele Manzulli[2], Jan van Meurs[2], Adriaan Heineken[2], Dr Ruud Jansen[1]
[1]Regionaal Laboratorium Medische Microbiologie Dordrecht-Gorinchem, Dordrecht, The Netherlands, [2]Eye Hospital, Rotterdam, The Netherlands

Introduction

In recent years, ocular surgery has increased dramatically, especially Intra vitreal injection with Anti VEGF for macular degeneration. Although rare (0.03-0.7%), endophthalmitis, an infection of the inner eye, is a serious postoperative complication that can lead to functional loss of the eye. When endophthalmitis is suspected immediate action is required, starting with injection of broad-spectrum antibiotics into the eye, since rapid intervention strongly increases the chance for a successfully therapy. At present, we have to delay antibiotic injection until after a biopsy of the vitreous fluid is obtained for identification of the infectious agent. To circumvent this problem we have developed a series of PCRs that can identify the bacterial species regardless of prior antibiotic treatment. The series of PCR's consists of 24 monoplex PCRs and is referred to as multi-mono PCR (mmPCR).

Material and methods

The mmPCR identifies seven Streptococcus species, five Staphylococcus species, Pseudomonas spp., Candida spp., Haemophilus influenzae, Enterococcus faecium, Proteus mirabilis, Cutibacterium acnes and control PCRs for generic 16S rRNA, the human beta-globin gene and PhHV. To evaluate the mmPCR, 27 vitreous biopsies of patients with suspected endophthalmitis, were obtained at the Eye Hospital Rotterdam, and subjected to culture and mmPCR. Antibiotic treatment within this cohort was started after obtaining the vitreous biopsy to prevent inhibition of the cultures.

Results

The mmPCR and the culture identified the same species in 24 of the 27 samples. One sample had a misinterpretation on the Streptococcus pneumoniae-mitis-oralis group. The other two samples had low bacterial loads and therefore stochastic variables might explain these two discrepancies.

Conclusion

The mmPCR can be used to replace the bacterial culture. The introduction of the mmPCR allows the immediate injection of antibiotics in endophthalmitis suspected patients, preventing treatment delay and gaining successful treatment.

P052

Structural changes of the chemoreceptor array in response to clinically relevant conditions

Msc Adam Sidi Mabrouk[1], AR Muok[1], A Briegel[1], YAH Matser[2], GAM Tytgat[2]
[1]Leiden University, Leiden, Netherlands, [2]UMC, Utrecht, Netherlands
Motile bacteria detect changes in nutrient concentrations in the environment and can control their movement in response. This system is dependent on chemoreceptors that are responsible for the detection of different types of ligands. In Escherichia coli, the chemoreceptor Tsr is well known to respond to the amino acid L-serine. Furthermore, it has been shown to also respond to 3,4 Dihydroxymandelic acid (DHMA), a metabolite of norepinephrine. Here we show that Vanillymandelic acid (VMA), another norepinephrine metabolite, is also an attractant for Tsr. We are currently characterizing the structural response of this system at the nanoscale level in an E. coli mini cell system using cryo-electron tomography and subtomogram averaging. Furthermore, both metabolites are highly enriched in the urine of neuroblastoma cancer patients. Here we show that the sensitivity of the Tsr chemoreceptor toward DHMA and VMA makes it suitable to detect these molecules in cancer patient samples in a high-throughput screening platform. This provides the basis to develop a low-cost, easy to use biosensor system for clinical applications.

P053

Diversification of blaOXA-48 plasmids among carbapenemase-producing Enterobacterales after a large outbreak in a general hospital in the Netherlands, 2011 - 2021

Dr Pieter Smit[1], Dr C van Tienen[1], F Landman[2], S Zagers[1], M den Drijver[1], A Burggraaf[1], V.A. Ganesh[2], M Damen[1], A.P.A Hendrickx[2]
[1]Maasstad ziekenhuis, Rotterdam, The Netherlands, [2]RIVM, Bilthoven, The Netherlands
After a large blaOXA-48plasmid mediated outbreak in 2011, routine screening of patients at risk of CPE carriage on admission and every seven days during hospitalization was installed at a large hospital in the Netherlands. The objective of this study was to investigate the dynamics of outbreak associated blaOXA-48plasmid among CPE collected from 2011-2021 in a large hospital in the Netherlands.

A collection of 86 blaOXA-48 carrying CPE isolates were selected over an 11-year study period. CPE 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.

In total, 78 blaOXA-48-encoding plasmids were reconstructed for 86 CPE and 8 blaOXA-48 genes were located chromosomally. The 2011 outbreak associated blaOXA-48plasmid of 63.6 kb with the IncL replicon was found in Cfr, Ecl, Eco, Kpn, Mmo and primarily between 2011-2014. SNP analysis indicated 2 variants of this 63.6 kb plasmid of which variant 1 was spread among Kpn, Eco and Cfr isolates, while variant 2 was spread among Kpn, Eco, Ecl and Mmo. From 2014 onwards, 11 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 and globally. Chromosomally localized blaOXA-48 alleles were located on variable genetic elements comprising distinct regions of blaOXA-48 plasmids.

After a large blaOXA-48 mediated outbreak in a large hospital in Netherlands, the composition of the blaOXA-48 plasmid population diversified over time and in is line with national and global compositions. Plasmid sequencing provided valuable insight into transmission dynamics of OXA-48-containing plasmids and showed no indication of persistent the 2011 blaOXA-48 plasmid in the hospital environment, highlighting the potential of strict infection prevention.

P055

What have we learned from two years of performing SARS-CoV-2 PCR in a diarrhea PCR panel?

Drs. K.L. Stegeman[1], Dr. L. Gard[1], Dr. K.J. von Eije[1], Dr. X. Zhou[1], Drs. A.T. Klak[1], Professor A. Voss[1], Dr. C.C. van Leer[1], Dr. M. Knoester[1]
[1]University Medical Center Groningen, Groningen, The Netherlands
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections can present with a variety of symptoms and organ localizations, but the incidence of gastrointestinal (GI) symptoms and fecal shedding are a matter of debate. The aim of this study was to evaluate the presence of SARS-CoV-2 by polymerase chain reaction (PCR) in feces and to determine the added value as compared to flocked nasopharyngeal swab (FLN). A SARS-CoV-2 PCR was performed on fecal samples as part of standard patient care in our diagnostic PCR panel for GI pathogens, since the start of the pandemic until December 2022. Data were extracted from the laboratory information system (LIMS) and matched with the nearest available FLN PCR.

Among 5312 fecal samples tested by PCR, 221 (4.2%) were found to be SARS-CoV-2 positive. A FLN PCR performed within 7 days was available in 2681 fecal samples (50.5%). Overall agreement between fecal and FLN PCR within 7 days was 97.3% (143 positive/positive and 2466 negative/negative). Of the 72 (2.7%) samples with discrepancies, 47 feces were negative/FLN positive and 25 were feces positive/FLN negative (1.8% and 0.9%). Median time between tests for the feces positive/FLN negative samples was one day [IQR 0-2]. Fecal PCR Ct values were not statistically significantly higher in cases with negative FLN than with positive FLN, median Ct 33.2 [IQR 31.0-36.0] compared to 28.0 [24.0-31.0].

Possible explanations for feces positive/FLN-negative cases could be found in clinical data. Based on information available, six cases were under the age of 3, and six patients were hematology patients. Theoretically, it could be a matter of sample error, prolonged shedding in immunocompromised patients, or local shedding in patients presenting with GI symptoms.
In conclusion, the yield of SARS-CoV-2 PCR on feces in patients with GI-symptoms is low and the added value compared to FLN is limited.

P056

Comparison of three approaches for targeted sequencing of SARS-CoV-2

BSc. K.H. Stoffers[1], BSc. L. Manrho[1], MSc. D. Eiloz[1,2], BSc. L. Hogenkamp[1], BSc. J. Beverdam-Vincent[1], Dr. R. Jansen[1], Dr. J. Van de Bovenkamp[1], Dr. A. Riezebos-Brilman[1], Drs. D. Hess[1], Dr. K. Kremer[3], Dr. A.G.M. Van der Zanden[1], Dr. J.L. De Beer[1]
[1]Labmicta, Hengelo, The Netherlands, [3]KNCV tuberculosis foundation, The Hague, The Netherlands

Background:
Genomic surveillance of SARS-CoV-2 is essential to gain information on circulating variants, detect new variants, and potentially indicate the impact of control measures. We compared the performance of three widely used protocols for targeted next-generation sequencing of SARS-CoV-2.

Methods:
Fourteen SARS-CoV-2 positive samples of nasopharyngeal swabs were randomly selected to be sequenced with three protocols. Firstly, the EasySeq Reverse complement (RC)-PCR Library Preparation method for sequencing the entire SARS-CoV-2 genome (Nimagen, Nijmegen, Netherlands) with short-read sequencing using a MiniSeq (Illumina, San Diego, California, USA). Secondly, the Midnight kit (Oxford Nanopore Technologies (ONT), Oxford, UK), which uses a multiplexed tiled amplicon approach that creates amplicons of 1200 base pair (bp) that cover the whole genome and are sequenced on a MinION (ONT). Thirdly, the Midnight Amplicon panel (Integrated DNA Technologies (IDT, Coralville, Iowa, USA), combined with the rapid barcoding kit (ONT), followed by MinION sequencing, creates also 1200 bp amplicons covering the whole genome.

Results:
The number of mutations detected per sample, compared to the reference SARS-CoV-2 sequence (Wuhan) was comparable for all three methods. Sequence data quality depends on the sequencing depth, genome coverage, and base-calling quality. Higher sequence read coverage and base-calling quality were achieved with the Nimagen kit on the MiniSeq compared to the other two methods on the MinION. Using both the IDT and the ONT Midnight kit, one amplicon was missed due to primer drop-off.

Conclusion
All three sequencing protocols can be used for typing of SARS-CoV-2. The coverage of the Nimagen EasySeq kit was higher than that of the IDT and Midnight kits. The newer versions of the latter two kits will overcome the primer drop-off problem. All three approaches generate reliable sequencing data, enabling the identification of circulating SARS-CoV-2 variants. The choice of the assay will depend on the specific aim and setting.

P057

Generation of a database to study the spread of resistance plasmids of highly resistant microorganisms between One Health domains in the Netherlands

Mr. Gijs Teunis[1], dr. AC Schürch[2], dr. E van Duijkeren[1], dr. KT Veldman[3], dr. J Hordijk[1], F Landman[1], dr. MSM Brouwer[3], dr. H Schmitt[1], dr. L Mughini-Gras[1,4], dr. APA Hendrickx[1], the Dutch CPE and MRSA surveillance study groups
[1]RIVM, Bilthoven, Netherlands, [2]University Medical Center Utrecht, Utrecht, Netherlands, [3]Wageningen Bioveterinary Research, Lelystad, Netherlands, [4]Utrecht University, Utrecht, Netherlands

Introduction. Extra-chromosomal elements such as plasmids encode genes that confer resistance to last-resort antibiotics, including carbapenems and colistin, and can transfer between highly resistant micro-organisms (HRMO; CPE/CPPA/MRSA/ESBL+/Salmonella). A database will be generated of HRMO plasmids to analyse the spread of resistance plasmids in the Netherlands in humans, animals and the environment.
Methods. Fifty medical microbiological laboratories throughout the Netherlands submit suspected HRMO from humans to the RIVM as part of the national HRMO surveillance program. Resistance surveillance in animals is conducted at WBVR. Additionally, both perform HRMO surveillance in livestock, while the RIVM also monitors wastewater. 10,972 HRMO were sequenced using short-read sequencing (Illumina) and an additional 1,364 HRMO by short- and long-read sequencing (Nanopore) between 2009-2022. Additional HRMO plasmid sequences will be reconstructed using gplas and PlasmidEC, two new bioinformatics tools capable of reconstructing plasmids from short-read sequencing data of a variety of bacterial species.
Results. A total of 12,417 plasmids were reconstructed using hybrid assemblies from human-retrieved HRMO. These plasmids can be used to assess the accuracy and polish those plasmids reconstructed using short-read data. Furthermore, a pipeline will be developed that can be used for reconstruction and analysis of resistance plasmid sequences from additional short-read sequencing data sets. Finally, the reconstructed plasmids will be clustered using a variety of methods for plasmid classification such as Mash, COPLA, and mge-cluster, which allows for identification of plasmids that can spread among humans, animals and the environment.
Conclusion. A national database of reconstructed resistance plasmid sequences will allow for in-depth analysis of the plasmid-mediated spread of resistance genes in the Netherlands across the One Health domains.

P058

Comparison of typing methods for regional data sharing of nosocomial bacterial clusters

Lieke van Alphen[1], I Maas[1], E Beuken[1], E Heddema[2], J de Vries[3], T Schülin[4], R Baggen[5], W. van der Zwet[1], P Savelkoul[1]
[1]Maastricht University Medical Center, Maastricht, the Netherlands, [2]Zuyderland Medical Centre , Sittard-Geleen, the Netherlands, [3]VieCuri Medical Centre, Venlo, the Netherlands, [4]Laurentius Ziekenhuis Roermond, Roermond, the Netherlands, [5]Limburgs Infectiepreventie en ABR zorgNetwerK (LINK), Maastricht, the Netherlands

Introduction
The identification of clonal clusters of nosocomial pathogens is essential to support the epidemiological investigation of outbreaks in hospitals. As different typing methods can be employed, a trade-off between time to result and high resolution, highly consistent typing is important. In addition, patients are often transferred between hospitals within a region, putatively spreading an outbreak. Data sharing on (potential) outbreak isolates between hospitals is therefore crucial. We compared three typing methods for cluster identification and the possibility of data sharing within a regional setting.

Methods
E. coli (n=91), K. pneumoniae (n=41) and E. cloacae (n=27) isolated from 178 patients >48h after admittance to the hospital were sent to the central typing laboratory. Typing was performed with: IR-biotyping (Biotyper, Bruker), IC-typing (InBiome) and Whole-genome-sequencing (WGS) (Illumina MiSeq) with subsequent cgMLST analysis on the cloud-based platform of 1928 Diagnostics.
Cluster analysis was performed per method against a reference threshold and compared across the platforms. In addition, capability of data sharing with the participating hospitals was assessed.

Results
All three typing methods showed a high resolution, with significant discrepancies in the number of clusters: IR-biotyping 22, IC-typing 9, WGS 11. In addition, the number of isolates per cluster varied per method. Although fast (<48h) typing results could be obtained using IR-biotyping and IC-typing, data sharing with these platforms was non-existent (IR-biotyping) or difficult to work with (IC-typing). Typing by WGS was more time-consuming, but data sharing proved user-friendly for regional partners.

Conclusion
Cluster comparison showed discrepancies between methods in such a way that a two-tier approach of a fast method with subsequent slower, but higher resolution typing would not be efficient. Data sharing of WGS data was feasible and resistance gene information and cluster analysis was available for each regional hospital. Shortening the turn-around time of WGS could optimize the regional surveillance.

P059

Same day diagnostics of bacterial infections using ultra-fast PCR and real-time Nanopore full length 16S sequencing

C Jamin[1], E Beuken[1], J Louwerse[2], R Struyk[2], A Beijers[3,4], M Kaijen – Lambers[3,4], E Schrauwen[3,4], Lieke van Alphen[1]
[1]MUMC+, Maastricht, the Netherlands, [2]Molecular Biology Systems B.V, Goes, the Netherlands, [3]Avans University of Applied Science, Breda, the Netherlands, [4]Avans University of Applied Sciences, the Netherlands, the Netherlands

Introduction

Sterile site infections require timely and appropriate treatment to mitigate negative outcomes for patients such as increased hospital stay, morbidity and mortality.
Conventional culturing takes days and can lack sensitivity for low bacterial loads and difficult to culture microorganisms. DNA sequencing approaches can improve the diagnostic performance for the identification of the causative agent, but are time-consuming. Therefore, we aimed to implement a single day molecular diagnostics workflow for the identification of bacteria in sterile site infections using an ultra-fast PCR and real time Nanopore 16S sequencing.

Methods

DNA from clinical samples were spiked using the ZymoBIOMICS Spike-in Control II, and were isolated using blood and tissue kit (QIAGEN). 16S PCR was conducted using NanoporeTech 16S kit, according to the manufacturer's protocol, using Q5 polymerase (NEB). Ultrafast PCR was conducted on the NextGenPCR™ Thermal Cycler platform (MBS, The Netherlands). 16S data analysis was performed using the Maastricht Bacterial 16S (MABA16S) pipeline (https://github.com/MUMC-MEDMIC/MABA16S).

Results

When using NanoporeTech 16S sequencing, sensitivity compared to Sanger 16S sequencing improved 10-fold. With Nanopore 16S sequencing, almost all samples (15/17) were typable compared to Sanger 16S sequencing (8/17), as sufficient reads from internal control were identified in these samples. Borrelia miyamotoi, a rare tick-borne pathogen was identified in a liquor sample, previously not typable by Sanger sequencing. In addition, the overall time required for 16S amplicon generation by PCR was lowered to merely 20 minutes (versus 2,5 hours) using the ultrafast PCR chemistry of MBS.

Conclusion

Implementing Nanopore 16S sequencing vastly improved bacterial identification in sterile site infections due to improved sensitivity and the ability to identify mixed infections compared to conventional Sanger 16S sequencing. Acceleration of bacterial identification based on this novel ultrafast 16S nanopore typing vastly improve time-to-result and may ultimately contribute to better patient care in diagnostic settings.

P060

Development of a long-read sequencing assay for the detection of drug resistance in Mycobacterium tuberculosis in sputum

A.R. van den Bout, N.T.H. Hermans[1], L. Manrho[1], Dr. R. Jansen[1], Dr. R.M. Anthony[2], Dr. K. Kremer[3], Dr. A.G.M. van der Zanden[1], Dr. J.L. de Beer[1]
[1]Labmicta, Hengelo, The Netherlands, [2]National Institute for Public Heath and the Environment (RIVM), Bilthoven, The Netherlands, [3]KNCV Tuberculosis Foundation, The Hague, The Netherlands

Introduction:
Detection of mutations in Mycobacterium tuberculosis associated with drug resistance can directly influence the selection of appropriate treatment. Testing directly from clinical specimens shortens the turnaround time significantly when compared to culture, but genome sequencing from clinical samples is challenging. Therefore, diagnostics able to reliably detect multiple resistance-associated genes close to the point of care are needed. Nanopore sequencing is a technical development that fits this need.

Methods:
To detect mutations associated with four first-line anti-tuberculosis drugs, a sequencing assay was designed targeting: fabG1-inhA, katG (isoniazid), rpoB (rifampicin), embB (ethambutol), and pncA (pyrazinamide). Primers were designed with target sequence lengths varying between 800-1100 base pairs. Optimization of the multiplex PCR was challenging due to the low load of target DNA, inhibiting factors in sputum, GC-rich nature of the Mycobacterial DNA, relatively long PCR products, and the need to obtain a balanced yield of PCR products for each target. Nanopore sequencing was performed on the MinION (Oxford Nanopore Technologies (ONT), Oxford, UK). Data analysis was performed on the CLC Genomics Workbench (Qiagen, Hilden, Germany) and using EPI2ME wf-tb-amr (ONT).

Results:
An optimal combination of PCR and sequencing conditions was identified that generated high-quality sequence data. With the assay, the yield of reads per target was equally distributed amongst the different strains and sputa. The coverage of the sequenced data was >2000 reads. The no-template control showed ≤0.02% mapped reads.

Conclusion:
A test was successfully developed to sequence five resistance-associated genes from M. tuberculosis directly in sputum. Patients could benefit significantly from the rapid prediction of drug resistance to four first-line anti-tuberculosis drugs, with high confidence due to screening of relatively long fragments. Ideally, the sequencing assay should be expanded to include the prediction of drug resistance to second-line anti-tuberculosis drugs to increase its utility with respect to informing treatment decisions.

P061

Comparison of three assays for Mycobacterium tuberculosis drug resistance testing in sputum with Nanopore sequencing

A.R. van den Bout[1], N.T.H. Hermans[1], L. Manrho[1], Dr. R. Jansen[1], Drs. R. Klont[1], Dr. R.M. Anthony[2], Dr. K. Kremer[3], Dr. A.G.M van der Zanden[1], Dr. J.L. de Beer[1]
[1]Labmicta, Hengelo, Netherlands, [2]National Institute for Public Heath and Environment (RIVM), Bilthoven, The Netherlands, [3]KNCV Tuberculosis Foundation, The Hague, The Netherlands

Introduction:
Currently, two commercial assays are available for the prediction of Mycobacterium tuberculosis drug resistance using Illumina sequencing; Deeplex Myc-TB (GenoScreen, Lille, France) and Deepchek 13-plex KB Drug Susceptibility Testing (Advanced Biological Laboratories, Luxembourg). However, Illumina sequencing is costly and less suitable for low-income countries, where most M. tuberculosis infections occur. Nanopore sequencing is less demanding, but a commercial test for this platform is unavailable. Therefore, a Lab Developed Test (LDT) was designed and compared with the two commercial tests by MinION Nanopore sequencing (Oxford Nanopore Technologies (ONT), Oxford, UK).

Methods:
DNA was extracted from strains and sputa using cetyltrimethylammonium bromide and NucliSENS EasyMag (Biomerieux, Marcy-l'Étoile, France) respectively. An LDT was developed targeting fabG1-inhA and rpoB to detect resistance to isoniazid and rifampicin. The performance of the LDT was compared with the Deeplex and Deepchek assays on a MinION. The PCRs were performed according to the manufacturer's protocols. Sequencing data were analysed by using the CLC Genomics Workbench (Qiagen, Hilden, Germany) and EPI2ME wf-tb-amr (ONT).

Results:
After sequencing, the distribution of mapped reads from amplicons was visualized. The mapped reads from strains showed an equal distribution in all assays. For sputum specimens, only the LDT showed an equal distribution with a coverage of ≥400 mapped reads.

Conclusion:
All three assays performed well on strains, but the two commercial assays showed low coverage of specific resistance genes in sputum due to inefficient amplification. The LDT successfully detected mutations in sputum specimens, making this test promising for use in clinical settings. A limitation of the LTD is that it covers only two genes. Future research will expand the number of resistance-associated genes.

P062

Functional and mass spectrometry-based characterization of antibody responses during Klebsiella pneumoniae infection in kidney transplant patients

Sjors Van Der Lans[1], Maurits den Boer[2,3], Hadia Hamdaoui[1], Maartje Ruyken[1], dr. Albert Bondt[2,3], dr. Pieter-Jan A. Haas[1], dr. Arjan D. Van Zuilen[4], dr. Bart Bardoel[1], prof. Albert J. R. Heck[2,3], prof. Suzan H. M. Rooijakkers[1]
[1]University Medical Center Utrecht, Utrecht, The Netherlands, [2]Bijvoet Center for Biomolecular Research, Utrecht, The Netherlands, [3]Utrecht University, Utrecht, The Netherlands, [4]University Medical Center Utrecht, Utrecht, The Netherlands

Bacterial infections are a risk for immunocompromised patients, such as kidney transplant (KTx) patients receiving immunosuppressing medication. To better treat infections, it is crucial to understand the naturally immune response in these patients. Infections in KTx patients are often caused by the Gram-negative bacterium Klebsiella pneumoniae. Here we combined flow cytometry and mass spectrometry approaches to investigate longitudinal anti-bacterial antibody responses in two KTx patients who developed a K. pneumoniae bacteraemia post-transplantation.

Using flow cytometry, we quantified the presence of anti-bacterial antibodies in plasma of KTx patients pre-and post-infection. In both patients, we detected a specific antibody response (IgG, IgA and IgM) against the clinical K. pneumoniae isolate causing the infection. After the infectious episodes, the antibodies levels against the infectious strain remained constant for the rest of the study period (>70 days). Anti-Klebsiella antibodies from both patients were functional, since post-infection plasma contained antibodies capable of inducing Fc-mediated effector functions such as complement activation and phagocytosis.
Using LC-MS clonal profiling, we assessed the dynamics of the antibody repertoires, aiming to identify strain-specific clones. A 1518 unique IgG clones and 851 unique IgA1 clones were identified that could be followed in concentration over time. By relating longitudinal antibody binding to the clinical isolates to the concentrations of the identified clones, we identified at least five different strain-specific IgG and three strain-specific IgA clones that were produced by the patients.

Our work gives a first indication that it is possible for KTx patients to mount a specific polyclonal antibody response against K. pneumoniae, and that the antibodies produced can activate the innate immune system.

P063

ISO 15189 accreditation of Juno pipelines for whole-genome sequencing analysis of bacterial foodborne pathogens

Dr. Boas Van Der Putten[1], A Hernandez Segura[1], R Wolthuis[1], K Hajji[1], S Kuiling[1], R Mariman[1], R Verhagen[2], E Franz[1], T Bosch[1], K van der Zwaluw[1], M van den Beld[1]
[1]Rijksinstituut Voor Volksgezondheid En Milieu (RIVM), Bilthoven, the Netherlands, [2]Rijksinstituut Voor Volksgezondheid En Milieu (RIVM), Bilthoven, the Netherlands

Introduction
While whole-genome sequencing (WGS) has become an integral part of modern medical microbiology, few WGS pipelines in medical microbiology have been accredited under ISO15189. This might be due to fast-paced developments or unclear application of ISO15189 to genomics. Here, we report the accreditation of three pipelines developed and maintained by the RIVM which perform assembly, typing and antimicrobial resistance gene identification and are part of the Juno pipelines used for diagnostics and surveillance.
Methods
We validated the Juno-assembly, Juno-typing and Juno-AMR pipelines according to ISO15189:2012 standards based on measurement trueness, accuracy and measurement repeatability. For Juno-assembly, we assessed seven parameters such as N50 and genome completeness for 34 well-characterized isolates of foodborne pathogens, using predefined criteria. Juno-typing was assessed based on multi-locus sequence typing (MLST) of 111 diverse isolates for which MLST schemes were available. For Juno-AMR, resistance was predicted for 9 antibiotics using 21 ATCC reference strains and 817 foodborne bacteria with phenotypic resistance profiles.
Results
Juno-assembly scored 100% accurate on four parameters namely number of contigs, average coverage depth, completeness and contamination, while scores of 97% on GC content and N50 and 88% on assembly size were found. Measurement repeatability was 100% for de novo genome assemblies. Juno-typing scored 100% on all parameters for foodborne bacteria and 97% of accuracy for rare bacteria. Juno-AMR achieved satisfactory results (≥95% sensitivity and specificity) for eighteen of the twenty-four combinations of antibiotic and pathogen.
Conclusion
All three Juno pipelines described here were accredited successfully and are freely available to enable uniform data analysis. Inclusion of bioinformatics workflows in accreditation procedures promotes transparent and consistent WGS analysis. This is of high importance for subsequent interpretation and action. However, there remains a need for a clear and broadly supported extension of ISO15189 to microbial bioinformatics analyses, potentially driven by the bioinformatics community.

P064

An outbreak of Shigella sonnei among travelers returning from Cape Verde

Kim van der Zwaluw[1], Roan Pijnacker[1], Ilse Zutt[1], Sjoerd Kuiling[1], Airien Harpal[1], Eelco Franz[1], Thijs Bosch[1], Maaike van den Beld[1]
[1]RIVM, Bilthoven, Netherlands

Introduction

Since 2022, the National Institute of Public Health and the Environment (RIVM) has implemented a national genomic surveillance for Shigella spp., in addition to mandatory notification of cases to health authorities. From August 2022, we noticed a rise in Shigella isolates from patients with a travel history to Cape Verde and used whole genome sequencing (WGS) to investigate this possible outbreak.

Methods

We used Illumina sequencing data from the submitted Shigella isolates, along with data on shigellosis cases that were notified to the RIVM. The Shigella serotype was determined in silico with ShigaTyper and relatedness was assessed with core genome Multi-Locus Sequence Typing (cgMLST) using the EnteroBase scheme with 2,513 targets and a cluster cut-off of ≤5 alleles.

Results

In 2022, we received a total of 242 isolates for our national surveillance. Among these, a cluster of 35 S. sonnei cases was identified based on cgMLST (median allelic difference: 2), of which 30 reported travel to Cape Verde and five had unknown travel history. Based on case notifications since August, there were twelve more cases of shigellosis among travelers from Cape Verde for which no Shigella isolate was available. Further epidemiological investigation into the source of this outbreak showed that out of eighteen cases for which place of stay was known, seventeen patients (94%) reported stay at the same hotel chain on the same Cape Verdean island.

Conclusion

Genomic surveillance for Shigella proved its added value to epidemiological investigation; it helped to identify and delineate an outbreak of 35 confirmed and twelve probable S. sonnei cases among travelers returning from Cape Verde. Local authorities on Cape Verde were alerted so appropriate actions could be taken and other European countries were notified through the European Centre for Infectious Disease Control (ECDC), which included sharing of sequence data for better comparison.

P065

Azole resistance in veterinary clinical Aspergillus fumigatus isolates from the Netherlands

Marloes Van Dijk[1], J.B. Buil[2], M. Tehupeiory-Kooreman[2], M.J. Broekhuizen[1], Dr. E.M. Broens[1], Prof. Dr. J.A. Wagenaar[1,3], Prof. Dr. P.E. Verweij[2,4]
[1]Utrecht University Faculty of Veterinary Medicine, Utrecht, The Netherlands, [2]Radboud University Medical Center, Institute for Molecular Life Sciences, Nijmegen, The Netherlands, [3]Wageningen Bioveterinary Research, Lelystad, The Netherlands, [4]Radboudumc-CWZ Center of Expertise for Mycology, Nijmegen, The Netherlands

Introduction
Aspergillus fumigatus (Af) is a saprophytic fungal pathogen causing opportunistic infections in animals and humans. Azole resistance has been reported globally in human Af isolates, but the resistance prevalence in isolates from animals is largely unknown.

Methods
A retrospective surveillance study was performed in a collection of clinical Af isolates from various animal species collected in 2015-2020. Agar based azole resistance screening of all isolates was followed by in vitro antifungal susceptibility testing (AST) and Cyp51A gene sequencing of azole-resistant isolates.

Results
Over the five year period 16 (11.2%) of 143 culture-positive animals harbored an azole-resistant Af isolate. Resistance frequencies varied from 0% (95% CI 0.0 – 15.5%) to 26.1% (95% CI 11.1 – 48.7%) per year without a clear trend. Resistant isolates were found in birds (15%; 2/13), cats (21%; 6/28), dogs (8%; 6/75) and free-ranging harbor porpoise (33%; 2/6). Azole-resistance was Cyp51A mediated in most isolates: 81.3% (T-67G/)TR34/L98H, 12.5% TR46/Y121F/T289A. In one azole-resistant Af isolate a combination of C(-70)T/F46Y/C(intron7)T/C(intron66)T/M172V/E427K single-nucleotide polymorphisms in the CYP51A gene was found. Of animals with an azole-resistant isolate and known azole exposure status 71.4% (10/14) were azole naive.

Conclusion
Azole resistance frequency in Af isolates from animals is similar to that found in humans and was predominantly Cyp51A TR-mediated. Our data support the need for including veterinary isolates in resistance surveillance programs and azole resistance should be considered as a reason for therapy failure when treating aspergillosis in animals.

P066

From information, to insight, to intervention in antimicrobial resistance control by analyzing combined data from various data sources on institutional level

Sophie van Kessel[1], dr. CCH Wielders[1], dr. C Schneeberger-Van der Linden[1], Prof A Verbon[2], dr. AF Schoffelen[1]
[1]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, , The Netherlands, [2]Department of Internal Medicine, University Medical Center Utrecht, , The Netherlands

Introduction: Knowledge on antibiotic use, the epidemiology of antimicrobial resistance (AMR) and the epidemiology of hospital acquired infections is essential to carry out antibiotic stewardship and to set proper goals for infection prevention and control in healthcare institutes. Currently, different determinants of AMR are captured and monitored in separate databases, for example for national surveillance systems. However, these databases are not integrated on institutional level, although the determinants are inextricably linked to each other. The aim of this project is to determine the feasibility and added potential of integrated analysis, combining AMR data from various sources on the level of healthcare institute, with the ultimate goal to increase the impact of data monitoring.
Methods: The first step of the project will be an exploration of existing AMR surveillance systems and other available data sources. Second, pilots answering specific research questions will be performed by combining relevant data on institutional level. Depending on the focus of the pilots and the availability of relevant information, additional data will be collected.
Results: The results of the data exploration will show the possibilities of the readily available data: which data is missing, and which governance issues and challenge are in place. The pilots of the proposed methodology will give insight into correlations of different AMR aspects. Ultimately, results of the pilots will consist of practical deliveries and advice, either for further in-depth research, or for clinical practice, for instance in infection prevention or clinical guideline development.
Conclusion: This project will create a foundation for research concerning integration of data from different surveillance systems and data sources on AMR-determinants. By creating this foundation, and a first exploration by several pilot research questions, this project defines possibilities and challenges of the proposed methodology and may increase the impact of future research and for clinical care.

P067

Humoral and cellular Immune responses to three doses of the COVID-19 vaccination in Lung transplantation recipients

MD. R.A.S. Hoek[2], MD. PhD. E.A.M. Verschuuren[3], PhD. R.D. de Vries[4], Msc. S. Liu[1], PhD. J.M. Vonk[5], PhD D. van Baarle[1,6], MD. PhD. C.H. GeurtsvanKessel[4], MD.PhD. Coretta Van Leer Buter[1]
[1]University Of Groningen, University Medical Center Groningen, Groningen, The Netherlands, [2]Erasmus Medical Center, Rotterdam, The Netherlands, [3]University Medical Center Groningen, Groningen, The Netherlands, [4]Erasmus Medical Center, Rotterdam, The Netherlands, [5]University Medical Center Groningen, Groningen, The Netherlands, [6]Center for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Introduction
Lung transplant recipients (LTRs) have a high risk of severe COVID-19. Inducing a strong and durable immune response by vaccination in this group has been difficult, as the majority of patients did not respond to the first two vaccination doses. To investigate the humoral and cellular responses to third dose, we studied a group of 70 previously vaccinated LTRs.
Methods:
Seventy LTRs were initially vaccinated with the Moderna vaccine twice, with a 28 day interval. Humoral and cellular responses were determined. Patients were classified according to their response to these first two doses, as non-responders (n-44, 63%), low-responders (n=19, 27%), and responders (n=7, 10%). Six months after the second dose, a third dose (Pfizer) was given. Antibodies measured using automated platforms, and T-cell activity was measured using ELIspot.
Results
Seven responders to the first vaccination round also responded to the third dose, all but one achieving higher titers. Of the 19 initial low-responders, 15 showed increased titers following the booster, placing ten within the responder range. Of 44 initial non-responders, 25 developed measurable antibodies, whereas 19 remained non-responders. Following the three vaccinations, 48 (69%) were (low)-responders, and 22 (31%) were non-responders; compared to 26 (37%) (low)-responders and 44 (63%) non-responders before
Cellular responses correlated poorly with humoral responses after the initial two vaccinations. A group of 21 LTRs initially had a T-cell response, but no detectable antibodies. Following the booster, 18 of these 21 had a serological response.
Conclusions:
Although the immunological responses of LTRs are well below other populations, this study shows that booster vaccinations in this group are effective. Following a third vaccination, the majority of LTRs developed antibodies, contrary to what was seen after the initial two vaccinations. LTRs developing T-cells but no antibodies after two vaccinations, were prone to develop antibodies after a third dose.

P068

Using a new device for self-collection of capillary blood for HIV and syphilis testing among PrEP using men who have sex with men

Dr. Inge Van Loo[1], Drs C Goense, Dr. Y Evers, R Heuts, Prof. dr. C Hoebe, C Cannon, Dr. N Dukers-Muijrers
[1]Maastricht Umc, Maastricht, The Netherlands

Introduction. In recent years, home-based opportunities for sexually transmitted infections (STI) testing have become available. Blood collection is required for reliable testing for syphilis and HIV. The Tasso+ blood collection device has the potential to collect larger blood volumes compared to finger-prick blood and therefore may be suitable for home-based sampling. This study assessed acceptability and feasibility of this device in terms of blood volume and test results.
Methods. Between August 2022 and January 2023, we recruited 47 men who have sex with men (MSM) during their routine pre-exposure prophylaxis (PrEP) consultation at two Dutch STI Clinics. Participants tested the Tasso+ device directly after their consultation. An online questionnaire evaluated acceptability and feasibility of the device. Volumes were measured after sampling and centrifugation. Sera were tested with routine screening-tests for HIV and syphilis (Elecsys, Roche). Test results of the Tasso+ samples were compared with test results of the sera obtained by intravenous sampling.
Results. Based on direct experience, 41 (87%) of participants had positive attitude towards use of the device and 36 (77%) would use it again for home-sampling. The device was considered easy to use (96%) and participants were confident to collect blood at home (94%; self-efficacy). The average collected serum volume was 244µl. In 43 (91%) routine screenings tests could be used for HIV and syphilis. For HIV 34 and for syphilis 32 serum pairs were available for comparison. For HIV, all sera tested negative and showed no discrepancies. For syphilis, 30 (94%) serum-pairs showed agreement.
Conclusion. Use of the device showed high acceptability and has been largely feasible for collecting sufficient blood volumes to perform screening-tests for syphilis and HIV. Test results also showed high agreement. Therefore, home-based testing including blood sampling with the Tasso+ device may be a valuable tool for STI testing in PrEP-using MSM.

P069

Dominance of the toxicogenic M1UK variant among group A streptococcal meningitis

PhD Boas CL van der Putten[2], MD, PhD Bart JM Vlaminckx[3], PhD Brechje de Gier[2], MD, PhD Wieke Freudenburg-de Graaf[1], Dr. Nina van Sorge[1]
[1]Amsterdam Umc Location University Of Amsterdam, Amsterdam, Netherlands, [2]Netherlands Institute for Health and the Environment, Bilthoven, Netherlands, [3]St. Jansdal Hospital, Nieuwegein, Netherlands

Introduction

Streptococcus pyogenes is the causative agent of a wide range of invasive infections, collectively referred to as invasive group A streptococcal (iGAS) infections. Several European countries, including the Netherlands, are experiencing a highly increased prevalence of iGAS infections. Here we report an increase in the observed cases of GAS meningitis based on national bacteriological surveillance data. GAS meningitis is a rare but very severe disease manifestation of iGAS infections with an estimated incidence in adults of 2 per 10,000,000 persons per year.

Methods

S. pyogenes isolates from patients with (suspected) meningitis have been submitted to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) since 1974. We considered GAS meningitis cases based on submitted isolates cultured from cerebrospinal fluid (CSF) between 1982-2022. For molecular epidemiological purposes, sequence variation in 180 bp of the emm gene has been routinely performed since 2013. Whole genome sequencing was performed using short-read Illumina sequencing (Core Facility Genomics, Amsterdam UMC) and analysis was performed using the NRLBM pipeline.

Results

Between 1982 and 2021, an average of 5 (range 1-15) S. pyogenes CSF isolates was submitted annually. In contrast, the NRLBM received 19 S. pyogenes CSF isolates in 2022. Moreover, emm1.0 represented 84% (16 out of 19) of the submitted isolates, which is significantly higher its contribution to GAS meningitis between 2013-2021 (35% emm1.0). Whole-genome sequencing showed that 11 out-of-14 emm1.0 CSF isolates (78%; 2 emm1.0 isolates not included) belonged to the toxicogenic M1UK variant, with all isolates received after May 2022 exclusively consisting of this variant.

Conclusion

The NRLBM has received the highest number of S. pyogenes CSF isolates in 40 years of sustained surveillance. During this period of unprecedented cases of iGAS, vigilance is warranted for the rare clinical manifestation of meningitis, which may have a higher than expected occurrence with the M1UK variant.

P070

Bacterial Membrane Vesicles in Human Metabolic Health

Msc Jari Verbunt[1,2], PhD J. Jocken[2], PhD E. Blaak[2], PhD P. Savelkoul[1,3], PhD F. Stassen[1]
[1]Maastricht University Medical Center, Maastricht, Netherlands, [2]Maastricht University, Maastricht, Netherlands, [3]Amsterdam University Medical Centers, Amsterdam, Netherlands

Introduction: The intestinal microbiota play a pivotal role in human health. In diseases such as type 2 diabetes (T2D) and obesity, the composition/functionality of the gut microbiota is often altered, with profound implications for the human host. Importantly, gut bacteria produce bacterial membrane vesicles (bMVs) containing bacterial metabolites, nucleic acids and toxins. Effects of bMVs on host in the context of metabolic disease are uncharacterized. In this work, we present an initial analysis of the fecal bacterial composition and bMV repertoire produced by these bacteria in lean and overweight/obese subjects.

Methods: Fecal samples (12 lean, 12 overweight/obese) were collected and DNA was obtained from feces-derived bacteria and from purified bMVs. Purified bMVs were obtained through consecutive (ultra)centrifugation and size exclusion chromatography steps. Column-purified DNA was subjected to 16S rRNA variable region amplification and Illumina sequencing.

Results: Within the lean group, the mean Firmicutes/Bacteroidetes (FB) ratio was 50.7 (SD=44.9) in bacterial fraction and 3.7 (SD=7.4) in bMVs. In the overweight/obese group, these ratios were 46.3 (SD=29.5) and 0.69 (SD=0.65), respectively. Furthermore, on average Actinobacteriota accounted for approximately 9% of ASVs (amplicon sequence variants) detected in bacterial analyses whereas <0.01% of ASVs in bMVs is attributed to this phylum. In both lean and overweight/obese subjects, there was significantly decreased ASV richness and alpha diversity at the species level for bMV DNA compared to bacterial DNA. When comparing lean versus overweight/obese subjects, no significant differences in richness, diversity or bacterial abundance could be distinguished in vesicles or bacteria.

Conclusions: In both lean and overweight/obese subjects, the FB ratio in bacteria is diametrically opposed to the FB ratio in bMVs. The paramount finding is thus that the most prevalent bacteria in the intestine seem not to be the most profuse vesicle producers, indicating an additional layer of complexity in gut-host interaction warranting further investigation.

P071

Evaluation of antimicrobial susceptibility testing performance of the RevealAST® system on cerebral spinal fluid containing Escherichia coli

Dr. Iedan Verly[1], I. Messoussi[1], Dr. H. Siegel[2], E. Seznec[2], Dr. A.P. van Dam[1], Dr. C.E. Visser[1]
[1]Amsterdam UMC, Amsterdam, the Netherlands, [2]Specific Diagnostics & BioMérieux, Paris, France

Introduction: Escherichia coli meningitis is a neonatal emergency associated with high mortality, which requires adequate antibiotic therapy. Worldwide multiresistant strains are on the rise, complicating empirical antibiotic therapy and making rapid antimicrobial susceptibility testing (AST) even more relevant. Previous studies showed that the RevealAST® system is capable of rapid AST directly on positive blood cultures with Gram negative rods without the necessity of subcultures. In this study, the RevealAST® system was evaluated directly on positive cerebral spinal fluid (CSF) cultures. AST performance was compared to the Vitek®2 AST system.
Methods: Clinical CSF samples in pediatric flasks (1 clinical and 50 negative spiked with different Escherichia coli strains) were tested in the RevealAST® system. Bacteria with various resistance patterns were included: wildtype, ESBL-, AmpC- and carbapenemase-producing. Time-to-result was compared between the RevealAST® and the Vitek®2 AST systems. Influence of CSF composition on AST results was investigated by comparing CSF composition between samples and repeating measurements with the same Escherichia coli strain in different CSF samples.
Results: AST performance on CSF showed an average categorical agreement (CA) of 96% between the RevealAST® system and the Vitek®2 AST system. CA for most antibiotics was above 90%, except for cefoxitin (83%). In CSF, complete AST results by the RevealAST® system were available in 6.5 hours on average with a range of 3-6.5 hours depending on the antibiotic. Analysis of the composition of CSF showed no correlation between concentration of composites (erythrocytes, leucocytes, glucose, protein) and the AST results. AST analysis of identical Escherichia coli strains in multiple different CSF samples showed similar categorical results.
Conclusion: Our findings suggest that the RevealAST® system can perform reliable AST directly on positive CSF with Escherichia coli regardless of its composition. Further investigations including more clinical CSF samples, preferably with other bacterial causes of meningitis are warranted.

P072

Evaluation of sepsityper pretreatment for direct MALDI-TOF of positive blood cultures

Dr. Iedan Verly[1], L. Ibrahim[1], Dr. C.E. Visser[1]
[1]Amsterdam UMC, ,

Introduction: Bacterial sepsis is associated with high mortality, which increases by the minute if adequate antibiotic therapy is delayed. Empirical antibiotic therapy is usually not covering bacteria such as group 2 enetrobacterales, non-fermenters, entroccoci, Staphylococcus aureus and coagulase negative staphylococci. Therefore, rapid identification of the bacteria is necessary for adequate adjustment of therapy. In this study, the sepsityper kit (Bruker) was evaluated for direct identification of positive blood cultures.
Methods: 1 ml of positive blood cultures (n = 200, preferably the aerobic flask) was pretreated with the sepsityper kit according to the manufacture's instruction, plated in duplicates on the MALDI-TOF chip, formic acid applied and subsequently analyzed. The highest score of the duplicates was chosen. MALDI scores were compared to routine diagnostics obtained from subcultures. Genus and species levels were identified when MALDI score in sepsityper setting was >1.6 and ≥1.8, respectively.
Results: Gram positive cocci in cluster (n = 100), Gram positive cocci in diplo/chains (n = 38), Gram positive rods (n = 5) and Gram negative rods (n = 56) were identified at their genus (92%, 92%, 60% and 95%, respectively) and species (75%, 76%, 40% and 86%, respectively) level. All S. aureus (n = 8) and non-fermenters (n = 3) were identified at species level. In polymicrobial blood cultures (n = 23), the dominant bacterium was identified at genus and species level in 96% and 87%, respectively. In one sample, also the 2nd bacterium was identified at species level. Time gap between positive blood culture identification was reduced by 4-24 hours.
Conclusion: Sepsityper pretreatment can help reduce diagnostic delay, however, further fine-tuning is needed before sepsityper could replace standard diagnostics with subculture. Therefore, currently it should be used as an additional tool, mainly in cases that require rapid identification.

P073

A workflow-pipeline to follow-up patients at risk of developing SARS-CoV-2 escape variants

Dr Babs Verstrepen[1], Dr Bas Oude Munnink[1], Djenolan van Mourik[1], Suzanne van Efferen[1], Susanne Bogers[1], Prof Bart Rijnders[2], Dr Bart Haagmans[1], Prof Marion Koopmans[1], Dr Corine GeurtsvanKessel[1]
[1]Erasmus Medical Centre, Rotterdam, Netherlands, [2]Erasmus Medical Centre, Rotterdam, Netherlands

Immunocompromised patients with no measurable immune responses against SARS CoV-2, are often treated with antivirals or antibodies, upon infection with SARS CoV-2. The aim of this treatment is to prevent ongoing virus replication, which may result in viral evolution. In a minority of patients, treatment is not successful with high virus loads throughout the treatment period, prolonged shedding of SARS-CoV-2 and an increased risk of development of escape variants.

Variant specific virus neutralization assays measure functional antibodies to different variants. We developed a workflow-pipeline to investigate the effect of prolonged virus evolution on neutralization-sensitivity that combines sequence analysis with live virus neutralization assays before and after treatment.

Nasopharyngeal swabs in which SARS-CoV-2 viral genome was detectable, were sequenced by using an amplicon–based approach to identify potential amino acid substitutions in the spike protein that are characteristic for escape variants. Simultaneously, in vitro expanded virus from the same time points was subjected to a neutralization assay against a panel of reference sera with known neutralizing capacity. Reference sera included sera obtained from individuals that were either vaccinated two times with the wild-type vaccine, vaccinated two times plus boosted with the wild-type vaccine or vaccinated two times plus boosted with the wild-type vaccine and had an omicron breakthrough infection

The reference sera which were obtained from individuals after a full vaccination series and subsequent omicron breakthrough infection, showed most efficient neutralization of isolates pre- and post- treatment. So, in this set of patients the identified evolution of SARS CoV-2 did not lead to increased immune escape.

The described workflow is a useful tool in the follow-up of patients at risk of developing SARS-CoV-2 escape variants.

P074

Development of a serological assay to detect and characterize intrathecal antibodies against enterovirus species

Merijn Verwijmeren[1], drs. P. van Dooren-van Beek[1], dr. JL. Murk[2], drs. K. Couderé[2]
[1]Microvida, Tilburg, The Netherlands, [2]ETZ, Tilburg, The Netherlands

Introduction

Infections with enteroviruses (EV) are common and although most are mild, severe disease occurs. Reverse transcriptase polymerase chain reaction (RT-PCR) is the most common method to detect EV infections. However, RT-PCR cannot always detect EV in late complications including encephalitis and acute flaccid myelitis (AFM), while antibodies can. Therefore, we aimed to validate an EV serological assay to detect intrathecal antibody synthesis.

Methods

In this study the viral capsid 1 proteins (VP1) from coxsackievirus (CV)A4, CVB1, echovirus (E)30, EV-D68, poliovirus (PV)1, and acute phase EV proteases 2A and 3C were coupled to MagPlex Avidin microspheres (Luminex Corporation, Austin USA) as targets. Intrathecal antibody synthesis was determined by comparing the ratio of enterovirus specific immunoglobulin G (IgG) in cerebrospinal fluid (CSF) and serum with the total IgG ratio, measured with the Atellica NEPH 630. The Luminex results were compared with a commercially available enzyme-linked immunosorbent assay (ELISA) from Serion/Virion. 15 CSF/serum pairs that were EV negative with RT-PCR were included to determine the clinical cut-off and 35 CSF/serum pairs were tested for validation of the xMAP method.

Results

The xMAP method showed a sensitivity of 71% and a specificity of 93% in CSF and a sensitivity of 59% and a specificity of 67% in serum compared with the Serion ELISA. Besides one patient with a positive EV-antibody index in both assays, our assay found two additional patients with intrathecal EV-antibodies. The antibodies were directed against EV protease 2A, not included in the Serion ELISA, and CVB1.

Conclusion

Differences in results can be explained by inclusion of different assay targets. Although our assay has potential as a tool for CSF diagnostics, further clinical validation is necessary.

P075

The Bruker IR Biotyper® can successfully distinguish Salmonella species, serotype and serogroup on polysaccharide level using Fourier Transform Infrared spectroscopy

Marit Visser[1], Dr S de Jager[1], S Bruinsma[1]
[1]Comicro Bv., Hoorn, The Netherlands

Salmonella subspecies enterica is an important cause of gastroenteritis. There is extensive genetic diversity within the Salmonella species which have been separated by using serogroups and serotypes. Salmonella spp. is the fourth detected by Matrix-assisted laser-desorption-ionisation time-of-flight (MALDI-TOF) due to it analysing proteins, not polysaccharides. Bruker IR Biotyper® (IRBT) can identify Salmonella serotypes using Fourier Transform Infrared spectroscopy (FTIR). This provides a characteristic absorption spectrum of biomolecules (e.g. polysaccharides) which represent a bacterial fingerprint. This research is focussed on whether the IRBT can identify the serotypes of Salmonella spp. and whether it can distinguish between serotype, serogroup and species, with the prospect of replacing or augmenting Salmonella agglutination tests.

In this research, 38 different Salmonella spp. isolates were used, which have 13 different species and 5 different serogroups. We measured 252 spectra in total and isolates were prepared following the manufacturer's instructions. All isolates were cultivated on Tryptic soy agar (TSA) for 24 ±0,5 hours at 37°C.

Results show a clear separation of serogroups, serotypes and species for Salmonella. Even Salmonella spp. with the same serotype could still be distinguished from one another with FTIR. Salmonella serotypes were identified by the IRBT, which was highly accurate, and all the isolates had an iso score >80.0, which is the highest accuracy in serotyping with the IRBT. The most clinically important species, like S. typhi and S. paratyphi, were distinguishable from other species of their serogroup.

In conclusion, the IR biotyper proved dependable and accurate in identification of Salmonella spp., even outperforming the current agglutination used in our laboratory for Salmonella typing. For typing of difficult strains or a more precise identification, the IR Biotyper was superior to agglutination.

P076

Primary MenACWY-TT vaccination induces a serum antibody response in older adults one month post vaccination

Maxime Visser[1], J. Wolf[1], D. van Rooijen[1], A. Buisman[1], G. den Hartog[1]
[1]National Institute for Public Health and the Environment, RIVM, Bilthoven, Netherlands

Introduction: Although the incidence of Invasive Meningococcal Disease (IMD) caused by Neisseria meningitidis is low, a high case fatality rate is observed in older adults. Hence, protecting the increasing older population against IMD is important to support healthy aging. Studies evaluating the immunogenicity of the tetravalent meningococcal serogroups A-, C-, W-135 and Y- conjugate vaccine (MenACWY-TT) in terms of serum circulatory antibodies in the senior population are therefore essential, yet lacking. We measured the serum antibody response in older adults (aged between 65-85 years) pre- and one month after primary immunization with the MenACWY-TT vaccine.
Methods: In a phase IV single-center and open-label study, participants (65-74 years of age (N=139) and 75-85 years of age (N=84)) have received a MenACWY-TT vaccine. Blood samples were collected both pre- and 28 days after vaccination. Serum meningococcal polysaccharide-specific IgG, IgM and IgA antibody levels were measured using a fluorescent bead-based multiplex immunoassay (MIA), after which geometric mean concentrations (GMCs) were determined for both age groups.
Results: Upon MenACWY-TT vaccination, a significant increase in meningococcal-specific serum IgG antibodies was observed after 28 days ($p=<0.0001$ for all four serogroups) in both age groups. Similarly, this increase was also witnessed in case of meningococcal-specific IgA as well as IgM ($p=<0.0001$ for all four serogroups). Interestingly, persons aged between 75 and 85 were found to have higher MenC-specific IgG serum concentrations 28 days post vaccination (GMC= 11.15 µg/mL)) in comparison to the group aged 65-74 (GMC=5.46 µg/mL, $p=0.006$). This difference was not observed in case of IgA and IgM. Yet, to confirm whether these vaccine-induced antibody levels are effectively resulting in killing of meningococci, serum bactericidal assays will be performed.
Conclusion: These data suggest that primary immunization with a tetravalent MenACWY-TT vaccine induces systemic serogroup-specific IgG, IgA and IgM responses older adults at one month post vaccination.

P077

In vitro effects of the chemotherapeutic compound 5-Fluorouracil (5-FU) on gut microbiota composition and SCFA concentrations

Janine Ziemons[1,2], L. Hillege[1,2], Dr. R. Aarnoutse[1,2], Dr. J. de Vos-Geelen[1,3], Dr. L. Valkenburg - van Iersel[1,3], Dr. S. Rensen[2,4], Dr. A. van Helvoort[4,5], Dr. G. Guus Roeselers[5], Dr. J. Penders[4,6,7], Prof. Dr. M. Smidt[1,2], Prof. Dr. K. Venema[7,8]
[1]Maastricht University, Maastricht, The Netherlands, [2]Maastricht University Medical Centre, Maastricht, The Netherlands, [3]Maastricht University Medical Centre, Maastricht, The Netherlands, [4]Maastricht University, Maastricht, The Netherlands, [5]Danone Nutricia Research, Utrecht, The Netherlands, [6]Maastricht University Medical Centre, Maastricht, The Netherlands, [7]Euregional Microbiome Center, Maastricht, The Netherlands, [8]Maastricht University – Campus Venlo, Venlo, The Netherlands

Introduction: 5-Fluorouracil (5-FU) is an antimetabolite of uracil and is converted to metabolites which cause RNA and DNA damage and inhibition of thymidylate synthase. Since tumor tissues use uracil more rapidly than normal tissues, 5-FU has anti-neoplastic effects and is used for the treatment of various solid cancers. Previous studies indicated bi-directional interactions between 5-FU and the gut microbiota and that the gut microbiota might influence 5-FU-induced toxicity and tumor response. However, available data are inconsistent and often obtained using isolated bacterial species. Therefore, this study aimed to investigate the effects of 5-FU on a whole human-derived gut microbial consortium, using the validated TNO in vitro model of the colon (TIM-2).

Methods: Fecal samples were collected from ten healthy women (56-72 years) and processed under anaerobic conditions. Subsequently, samples were pooled and inoculated in the TIM-2 model. Samples were left untreated or were treated with 26.4mg 5-FU two times a day for three consecutive days. Samples from the lumen (reflecting the colonic microenvironment) as well as the dialysate (reflecting the circulation) were analyzed. Changes in luminal gut microbiota composition were assessed by 16S rRNA gene amplicon sequencing. Cumulative levels of short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) in lumen and dialysate were quantified using liquid chromatography-mass spectrometry (LC-MS).

Results: During the intervention period, 5-FU induced shifts in overall gut microbiota composition as well as microbial diversity, when compared to a control condition without treatment. In addition, cumulative levels of acetate, iso-valerate and iso-butyrate increased more during 5-FU compared to control.

Conclusion: In-vitro 5-FU treatment had a considerable impact on gut microbiota composition, diversity as well as levels of some SCFA and BCFA. Consequently, 5-FU might induce or deteriorate microbial dysbiosis in cancer patients, with potential negative consequences for chemotherapy toxicity and tumor response.

P100

Nutritional requirements of the human vaginal isolate Lactobacillus crispatus in a chemically defined medium.

Puck Achterberg[1], I. van Vondelen[2], D. Molenaar[2], M. van Loosdrecht[1], R. Kort[2], R.Y. Hertzberger[2]
[1]Technische Universiteit Delft, Delft, The Netherlands , [2]Vrije Universiteit Amsterdam , Amsterdam , The Netherlands

Introduction: Lactobacillus crispatus is a gram-positive, homofermentative lactic-acid bacterium, commonly found in the human vagina (France, et al., 2022). L. crispatus dominance in the vaginal microbiome is associated with reduced risks for unfavourable reproductive and sexual health outcomes, such as human immunodeficiency virus (HIV) infection (Gosmann, et al., 2017). In this study, the nutrient requirements and metabolic capabilities of L. crispatus RL10 (van der Veer et al., 2019) have been studied by means of working towards a chemically defined minimal growth medium.

Methods: Single omission experiments were performed for each component of a Lactobacillus chemical defined medium. Growth and acidification were determined, by measuring the optical density at 600nm and pH in multi-well plates under anaerobic conditions. Metabolites were analysed by high-performance liquid chromatography (HPLC).

Results: L. crispatus RL10 could proliferate on a chemically defined medium and acidified the medium (pH ~ 4.0), producing predominantly lactic acid. Omission of iron or manganese did not affect growth, whereas omission of magnesium completely abolished growth. Furthermore, L. crispatus RL10 showed reduced growth without adenine despite the presence of other purines (xanthine, guanine and inosine). It showed growth on medium without guanine and uracil. It is auxotrophic for most amino acids, vitamins as well as Tween80. The latter requirement indicates a growth dependency on unsaturated (long chain) fatty acids.

Conclusion: Lactobacillus crispatus has a fastidious requirement for nutrients, suggesting to be highly dependent on the human host(ess). Understanding the need for certain nutrients adds to fundamental knowledge concerning commensal lactobacilli and could aid in the development of therapeutics promoting their growth in the vaginal environment.

P101

Defining the impact of microorganisms on subsurface hydrogen storage

Dr Anne-Catherine Ahn[1], Adrian Hidalgo-Ulloa[1], Dr Yehor Pererva[1], Dr Bart Lomans[1,2], Prof Diana Z. Sousa[1]

[1]Wageningen University & Research, Wageningen, The Netherlands, [2]Shell Global Solutions International B.V., Amsterdam, The Netherlands

Subsurface hydrogen (H_2) storage in salt caverns, porous reservoirs and aquifers is proposed as a potential solution for balancing fluctuations between the production and the usage of sustainable energy. However, H_2 is also an excellent energy source for diverse anaerobic microbial metabolisms striving in subsurface environments. Methanogens, sulfate reducers and acetogens could exert a significant detrimental impact on H_2 storage. Through their activities, these microbes could cause a loss of the stored H_2, but also the production of unwanted products like H_2S (leading to the corrosion of the equipment) and the loss of injectivity through accumulation of bio-based solids as biofilms and extracellular polymeric substances. Yet, the extreme conditions of subsurface environments can limit the microbial impact on H_2 storage. In our project, we assess the potential impact of microorganisms on H_2 storage, related to H_2 depletion and formation of unwanted products. Formation water samples from two salt caverns and ten porous reservoirs collected across Europe were incubated in batch lab microcosms. Tests were done at four different temperatures (35, 50, 65 and 80°C, reflecting common temperature range of potential storage sites), at a total pressure of 1.7 bar with 20%CO_2/80%H_2 or 100%H_2, and with or without trace elements and nutrients amendments. Microbial activities were observed in the majority of the tests for at least one of the tested conditions, with the exception of tests with samples from one of the salt caverns and three porous reservoirs. Acetogenesis was observed up to 50°C and sulfate reduction with sulfide production took place up to 65°C, whereas methanogens showed activity even at 80°C.

P102

Overflow metabolism at the thermodynamic limit of life: how carboxydotrophic acetogens mitigate carbon monoxide toxicity

Maximilienne Allaart[1], M Diender[2], DZ Sousa[2], R Kleerebezem[1]
[1]Delft University Of Technology, Delft, The Netherlands, [2]Wageningen University & Research, Wageningen, The Netherlands

Introduction. Overflow metabolism is the exploitation of a short, energetically inefficient metabolic route even when excess nutrients are available. This mechanism is used to optimally use cellular resources when sustaining increasing metabolic rates. Carbon monoxide (CO)-rich syngas fermentation is an upcoming avenue for waste-to-product conversions. We propose that ethanol production is the overflow branch of CO metabolism, and that the product spectrum of syngas fermentation can be controlled by controlling the metabolic CO conversion rate.

Methods. We address the theoretical case study of Clostridium autoethanogenum grown on CO and syngas mixtures to assess the relation between growth rate and ethanol formation based on literature data. Thermodynamic pathway analysis is used to generate a deeper understanding of this relation. Furthermore, pulse feeding experiments in a chemostat cultivation of C. autoethanogenum (DSM10061) are performed to establish the relationship between CO feeding rate and metabolic strategy.

Results. Literature data shows that elevated ethanol production occurs at increasing biomass-specific growth rates, both when CO is the only carbon-and electron source and when syngas mixtures are fed. Pulse feeding experiments confirm that increased CO feeding rates lead to increased ethanol formation rates at the expense of acetate. Thermodynamic pathway analysis reveals that more Gibbs free energy is dissipated when reducing acetate to ethanol than when converting CO to acetate. Conversion of CO to acetate has a higher ATP yield than acetate reduction to ethanol. Yet, at high CO fluxes the less energy-efficient pathway with higher energy dissipation is preferred.

Discussion. Overflow metabolism can thus be recognized even in anaerobic syngas fermentation. This phenomenon is recognized in different species of the acetogen ecological niche and is likely exploited to cope with CO toxicity. Fundamental understanding of the drivers of cellular metabolism can provide new strategies for industrial process control and optimization.

P103

Unravelling the microbial ecology of slow sand filters

Valentina Attiani[1]
[1]Wageningen University & Research, Wageningen, Netherlands

Introduction: Slow sand filtration is a robust and reliable drinking water treatment technology for the removal of microorganisms, biodegradable organic carbon (BDOC) and particulate matter, and the production of biologically stable water, which prevents the growth of undesired pathogens. The role of physical-chemical processes in this treatment step have been clarified in the past, but the (micro)biological processes still remain largely unknown.
The main objective of this work is to unravel the microbial ecology of different slow sand filters (SSFs) in the Netherlands by comparing variables such as sand depth and sampling points. A subsequent objective is to identify the key microbes in BDOC degradation, the bioorthogonal non-canonical amino acid tagging (BONCAT) technique coupled with fluorescence-activated cell sorting (FACS).
Methods: Full-scale SSFs from three drinking water treatment plants (DWTPs) were sampled at different points at increased distance from the influent water inlet, and from different depths of the sand bed. The DNA was isolated and then used for 16s rRNA gene-amplicon sequencing.

Results: The most abundant taxa observed in all SSFs were Nitrospiraceae, Gemmataceae, Pierllulaceae, Nitrosomonadaceae, A4b families, and the PLTA13 order.
The SSFs depth and the treatment plant sampled significantly influenced the bacterial community composition ($p < 0.05$), whereas the spatial sampling point did not significantly influence the microbial community.
The use of BONCAT-FACS on SSFs sample showed that was possible to successfully detach cells from the sand and to distinguish fluorescent/active cells.

Conclusions: We can conclude that there was probably an even distribution of the nutrients coming from the water influent, and that the conditions were similar across the whole filter area. The most abundant taxa were similar in all SSFs. Additionally, the BONCAT-FACS technique is being tested and the results obtained until now are promising for the identification of the BDOC-degrading microbes in SSFs.

P106

Establishment of a synthetic mucin-degrading community that can be used to model the ecological interactions that occur in the mucosal layer of the human gut

Ms. Maryse Berkhout[1], Dr. C. M. Plugge[1], Dr. C. Belzer[1]
[1]Wageningen University & Research, Wageningen, The Netherlands

Introduction

A mucus layer protects the intestinal epithelium from contact with microbes. The outer mucus layer attracts specific gut microbiota of which several bacteria can degrade the mucin glycoproteins that constitute the mucus. The degradation of mucus by commensal gut bacteria is part of the normal turnover and results in production of beneficial short-chain fatty acids (SCFAs) near the host epithelium. Mucin glycan degradation by microbes is a complex process that requires a range of extracellular glycan degrading enzymes, as mucin glycans are intricate and diverse. Consequently, it is hypothesised that microbial mucin breakdown requires concerted action of various enzymes in a network of multiple mucosal residents and that this leads to cross-feeding.

The objective of this study was to assemble and study an in vitro synthetic mucin-degrading community in anaerobic bioreactors.

Methods

We designed a synthetic community consisting of 15 reported residents of the human mucosal layer. We selected mucin degraders, butyrate producers and hydrogen consumers. This community was grown in an anaerobic bioreactor with continuous mucin supply. Community dynamics, enzyme expression and metabolite production were monitored.

Results

All members of the selected synthetic mucin degrading community grew and were active in our experiments. The community was dominated by mucin degraders Akkermansia muciniphila, Bacteroides spp. and Ruminococcus spp. While butyrate producing bacteria and hydrogen consumers were able to cross-feed on the products of mucin degradation. The main metabolites produced by the synthetic community where acetate, propionate and butyrate.

Conclusion

Overall, we established a synthetic mucin-degrading community that can be used to model the ecological interactions that occur in the mucosal layer. This will lead to new insights in microbial dynamics in the human gut and host-microbial interactions at the mucosal layer.

P107

Biodegradation potential of gabapentin and sotalol in Biological Oxygen-dosed Activated Carbon (BODAC) filters

Miss Olga Bernadet[1,2], Dr M.C. Gagliano[1], Prof. dr. G.J.W. Euverink[2]
[1]Wetsus, Leeuwarden , The Netherlands, [2]University of Groningen, Groningen, The Netherlands

At Ultrapure Water Factory, Biological Oxygen-dosed Activated Carbon (BODAC) filters comprising BODAC 1 and 2 have been successfully implemented to prevent biofouling of reverse osmosis units for >12 years without carbon replacement or ex-situ regeneration. Periodic backwashing is carried out to prevent biofilm overgrowth in the filters. Interestingly, in BODAC filters, a wide range of organic micropollutants (OMPs) are efficiently removed.

The microorganisms forming BODAC's biofilms were thought to contribute strongly to the remarkable and persistent performance of BODAC filters. A two-year longitudinal microbial community analyses of both carbon granules (before and after backwashing) and backwash water (BW) samples from BODAC filters was performed by means of 16S rRNA gene next generation sequencing. The results showed that manganese-oxidizing and nitrifying bacteria were relatively abundant in granule samples rather than in BW samples, suggesting that backwashing hardly affected the structure of the resident microbial communities in the biofilms. Manganese oxidation and nitrification can contribute to OMPs removal via co-metabolism pathways.

To test the biodegradation potential of OMPs in BODAC filters, batch experiments were carried out by feeding the real influent of BODAC filters spiked with Gabapentin (GAB) and Sotalol (SOT) to BW sampled at two different time points (2020 and 2021). Autoclave-inactivated BWs were used as control. Allylthiourea (ATU), an unspecific inhibitor for Cu-dependent enzymes (e.g. ammonia monooxygenase), was added to assess the role of such enzymes in OMPs removal.

The removal of GAB and SOT varied between samples from 2020 and 2021, a result likely connected to the variability of microbial community composition, which differed between filters and between sampling dates. The addition of ATU hindered SOT removal, suggesting that Cu2+-dependent enzymes might facilitate the biodegradation of this OMP.

P108

An energetically advantageous relationship between the butyrate oxidizer Syntrophomonas wolfei and the acetoclastic methanogen Methanothrix soehngenii

MSc Maaike Besteman[1], dr. A Doloman[1], prof. dr. DZ Sousa[1]
[1]WUR, Wageningen, The Netherlands

Introduction: Anaerobic digestion (AD) is used to convert organic waste into biogas by the activity of a complex microbial community. Microbial interactions are vital to the functioning of the community, including the obligatory thermodynamic dependence between fatty-acid oxidizers and methanogens, termed syntrophy. Most studies so far focus on hydrogenotrophic methanogens as syntrophic partners during fatty-acids oxidation. However, knowledge is lacking on the role for acetoclastic methanogens on the energy conservation mechanisms of fatty-acid oxidizers.

Methodology: We studied syntrophic interactions in predefined bi-and tri-cultures containing Syntrophomonas wolfei as a butyrate degrading syntroph, Methanospirillum hungatei as a hydrogenotrophic methanogen, and only in tricultures Methanothrix soehngenii as an acetoclastic methanogen. Cultures' performance was evaluated by measuring butyrate consumption and production of acetate and methane. Further on, the gene expression patterns of S. wolfei in the absence and presence of the methanogenic acetate scavenger were compared. Analysis was performed using DESeq2, including low-count filtering as factor in data normalization.

Results & conclusion: Rates of butyrate consumption by S. wolfei were similar between the two studied conditions. Methane production and acetate production and consumption matched stoichiometric predictions. Compared to bi-cultures, 223 genes were significantly less expressed in tri-cultures ($p<0.05$). Kegg orthology analysis showed most of these genes have a function in environmental/genetic information processing, carbohydrate metabolism or energy metabolism. In tri-cultures, 201 genes were significantly higher expressed ($p<0.05$). Most of these genes have a function in environmental/genetic information processing, signalling and cellular processing, or amino acid metabolism. Interestingly, while most genes in the main energy metabolism were less expressed in tri-cultures, a formate dehydrogenase complex (FDH3) of S. wolfei was significantly higher expressed ($p<0.001$). Overall, although kinetics of butyrate conversion was not affected by the presence of acetoclastic methanogens, transcriptomic results do show its' influence on the expression of the syntrophic energy metabolism.

P109

Culturing novel nitrifiers through targeted cell sorting

Pieter Blom[1], Pascal Huizing[1], Joao P. Requiao Correa de Monlevad[1], prof. dr. Mike S.M. Jetten[1], dr. Maartje. A.H.J. van Kessel[1], dr. Sebastian Luecker[1]
[1]Radboud University, Nijmegen, Netherlands

Nitrification was long regarded as a two-step process that is performed by separate guilds of ammonia and nitrite oxidizing microorganisms. The recent discovery of complete ammonia oxidizing (comammox) bacteria resulted in a paradigm shift. The to date only available comammox pure culture (Nitrospira inopinata) yielded interesting physiological insights, which were partially corroborated by the physiological characterization of the enrichment culture of Candidatus Nitrospira kreftii. More isolates are needed to test whether the observed traits are common to all comammox bacteria, such that their contribution to nitrogen cycling in natural and engineered ecosystems is better understood. However, classical cultivation methods have for a long time overlooked the existence of comammox bacteria, which not only illustrates the recalcitrance of these fastidious bacteria to cultivation, but also our still incomplete knowledge about nitrifying microorganisms. Here, we present a workflow for the targeted enrichment and isolation of novel ammonia oxidizing microorganisms, including comammox bacteria, from complex environmental samples. Specific in vivo fluorescent labelling of ammonia monooxygenase, the key enzyme required for ammonia oxidation, was combined with fluorescence-activated cell sorting (FACS) into 96-well plates containing mineral medium amended with ammonium and nitrite. All wells were regularly screened for the production of nitrite and nitrate, with nitrate production distinguishing complete nitrifiers from canonical ammonia oxidizers. Wells containing nitrifiers were selected for subcultivation for physiological characterization. Applying this workflow to various biomass sources, we managed to obtain six highly enriched cultures containing comammox Nitrospira and two apparently pure Nitrosomonas-related ammonia oxidizing bacteria. In conclusion, we demonstrated that our approach is well-suited to isolate novel ammonia oxidizers from complex environmental samples, circumventing the biases involved in classical cultivation techniques that hindered the isolation of many relevant nitrifying microorganisms. This cultivation approach will greatly advance our understanding of the environmental role and biotechnological potential of these intriguing microorganisms.

P110

Effects of UV radiation on virus infectivity and virus-host interactions

Charlotte Eich[1,2], Prof Corina Brussaard[1,2]
[1]Nioz Royal Netherlands Institute for Sea Research, Texel, The Netherlands, [2]Univ Amsterdam, Amsterdam, The Netherlands

During the summer season, polar seas are exposed to relatively high UV radiation. Moreover, global warming induced ice-melt is expected to result in strengthening of the vertical stratification and consequently enhanced UV exposure for the polar algae. Sea-ice melt will also lead to increased shipping in the Arctic Seas. Ballast water from ships are a major cause for the distribution of invasive species and viruses, which are considered a major threat to biodiversity. To reduce/prevent the spread of invasive species, ships need to treat the ballast water before discharging it and UV-C is the most commonly used method. We examined therefore the effects of (1) ecologically relevant UV-AB doses (6 -48h exposure, 16:8 h light:dark cycle) on the Arctic alga Micromonas polaris and its virus MpoV-45T, and (2) UV-C radiation (doses 25–800 mJ cm-2) on MpoV-45T and other temperate algal viruses. Virus production rates and burst sizes were reduced by more than half after 28h exposure (compard to non-UV controls). The UV-C dose needed to obtain the desired log-4 reduction was at least 400 mJ cm-2, which is higher than the commonly used dose of 300 mJ cm-2 in ballast water treatment.

P111

In situ imaging of negatively charged polymers in the extracellular matrix of biofilms using FT-IR microscopy

Ir. Stefan De Bruin[1], C. Hof[1], Prof. Dr. D.Z. Sousa[2], Prof. Dr. M.C.M. Van Loosdrecht[1], Dr. Y. Lin[1]
[1]Delft University Of Technology, Delft, Netherlands, [2]Wageningen University & Research, Wageningen, Netherlands

Introduction: The extracellular matrix (ECM) encases the biofilm inhabitants and acts as a scaffold that protects the micro-organisms against environmental stresses. Solubilization of the ECM yields extracellular polymeric substances (EPS) that are enriched in negatively charged polymers. Sialic acids and sulfated polymers are among the most negatively charged polymers found in various granular sludge biofilms. Sialic acids are present in the high molecular weight polymers of EPS, whereas sulfated polymers are found across all molecular weight ranges, indicating a potentially different role. Sialic acids and sulfated polymers have been imaged separately but have not been imaged simultaneously. Therefore, FT-IR microscopy was performed on sliced biofilm samples to image a broad range of polymers in the ECM. Differences in polymer locations would show that sialic acids and sulfated polymers might have different roles in the biofilm.

Methods: Granular sludge was collected from an anaerobic digestion reactor treating paper mill effluent. Samples were cut in 20 µm slices using a cryotome. FT-IR microscopy was performed with the Spotlight 400 FTIR microscope, capable of imaging a 500 x 500 µm area. Absorbance was recorded in the 4000 cm-1 to 600 cm-1 range with 4 accumulations, a spatial resolution of 1.56 µm and a spectral resolution of 4 cm-1. Spectral data processing was executed in MATLAB.

Results: The visualization of the height of specific absorbance bands attributed to protein, polysaccharide, lipid, sialic acid and sulfated compound regions, resulted in a polymer distribution image across the measured area. Interestingly, a negative correlation coefficient between sialic acid and sulfated absorbance bands (-0.82) was measured.

Conclusion: FT-IR imaging is a useful technique for imaging the polymer distribution of a broad range of polymers. The difference in location of sialic acid and sulfated polymers shows that these negatively charged polymers have different roles in the biofilm.

P112

The membrane of Caldalkalibacillus thermarum TA2.A1 integrates an acyl-phosphatidylglycerol at high pH

Ir. Samuel De Jong[1], Dr. Nicole Bale[2], Dr. Laura Villanueva[2,3], Prof. dr. ir. Mark van Loosdrecht[1], Dr. Duncan McMillan[1]
[1]Delft University Of Technology, Delft, The Netherlands, [2]NIOZ Royal Institute for Sea Research, Texel, The Netherlands, [3]Utrecht University, Utrecht, The Netherlands

An alkaline environment is defined by a lack of protons. Alkaliphiles maintain their internal pH close to neutral, resulting in a proton gradient oriented in an opposite manner compared to all other life on earth. Yet, aerobic alkaliphiles prefer to generate energy via respiration using those scarce protons, which has a profound implication. Even for normal bacteria, leakage of protons over the membrane is inefficient, and therefore all bacteria have mechanisms to limit proton leakage. Alkaliphiles have to be even more frugal with their protons – few are available outside of the cell to replenish those lost. Thus, the implication of using a proton-based respiratory chain is that alkaliphiles require a membrane with additional mechanisms to combat proton leakage. Alkaliphiles preferentially use apolar lipids such a squalene, which reside in the hydrophobic region of the lipid bilayer, perpendicular to the polar lipids that constitute the lipids bilayer. Due to its orientation, squalene increases the barrier function of the membrane against unwanted diffusion of ions/protons over the membrane.

Caldalkalibacillus thermarum TA2.A1 is incapable of producing squalenes, yet can replicate at a rate of $\mu = 0.1$ h-1 at pH 10.5. We grew this microorganism in a chemostat at a dilution rate of $D = 0.1$ h-1 and 65 °C, at pH 7.5 and pH 10.5, and analyzed the lipid fraction of the cells. The membrane at pH 7.5 consisted of 34.2 ± 0.2% cardiolipins and 65.0 ± 0.2% phospholipids as lipid head groups. At pH 10.5, the fraction of cardiolipin decreased to 29.4 ± 2.8%, while an unexpected acylated lipid, pentadecanoyl-phosphatidylglycerol (C15-acyl-PG) was detected at 5.3 ± 0.1%. The question is whether the C15-acyl-PG, being more apolar than regular phosphatidylglycerols, resides in the hydrophobic region of the lipid bilayer, or whether it aids in combating proton leakage in another manner.

P113

Anaerobic cell extraction of live biomass from marine sediments

Ir. Gerben De Zwaan[1], MSc YM Kaspareit[1], Dr.rer.nat. Burak Avcı[1], Dr.rer.nat. Patricia Geesink[1], Prof.dr.ir. TJG Ettema[1]
[1]Wageningen University & Research, Wageningen, The Netherlands

About 90% of all archaeal and bacterial biomass is found in the deep subsurface sediments, with a genetic diversity as great as that on the surface. While the vast majority of these microorganisms have not been cultured in a laboratory yet, studying them is essential to understand their ecological roles and physiology and is potentially informative about major evolutionary transitions. New developments in state-of-the-art cultivation methods such as microfluidics and anaerobic fluorescence activated cell sorting (FACS) as well as microscopy techniques such as correlative light and electron microscopy (CLEM) and anaerobic live cell fluorescence microscopy will improve our ability to isolate and study yet uncultured organisms. However, these methods are often sensitive and hampered by sediments or other particles. Therefore, an important step in sample preparation is separating the cells from the sediment. Current cell extraction methods for soil and sediment are mostly focused on enumerating as much biomass as possible, without regarding the viability of these cells for further cultivation or microscopy purposes. In this project, we compare and optimize different cell extraction methods for the extraction of live biomass in an anaerobic setting. We employ viability assays such as Syto9/propidium Iodide (PI), Redox sensor green/PI and propidium mono azide (PMA) in combination with flow cytometry, microscopy and Illumina amplicon sequencing to assess and optimize different extraction methods. Using this data we are able to select the best extraction methods for live biomass of our organisms of interest. The obtained results allow us to generate a flexible, yet effective cell extraction protocol that can be adapted to different sample types and organisms of interest. While highlighting the bottlenecks of live cell extractions, our assay paves the way from sediment cultures to sophisticated sorting and microscopy methods of viable cells.

P114

Microbial nitrogen cycle in marine Lake Grevelingen

Nicky Dotsios[1], Dr M.A.H.J. Van Kessel[1], A.J. Wallenius[1], J. Venetz[1], G.H.L. Nuijten[1], Dr. N.A.G.M. Van Helmond[1,2], Dr W.K. Lenstra[2], O.M. Zygadlowska[1], R. Klomp[1], prof. dr. ir. C.P. Slomp[1,2], prof. dr. ir. M.S.M. Jetten[1], Dr. S. Lücker[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]Utrecht University, Utrecht, The Netherlands

The microbial nitrogen (N) cycle is one of the major biogeochemical cycles on earth. Due to anthropogenic activities, nitrogen cycling in many coastal ecosystems is out of balance, leading to severe environmental problems. Microbial conversions of ammonium, nitrite and nitrate eventually lead to production of N2O and N2. This ultimately leads to loss of bioavailable nitrogen, but the processes controlling N loss from oxygen-limited coastal ecosystems are poorly understood.

For several decades, marine Lake Grevelingen in The Netherlands increasingly suffered from oxygen depletion in the lower part of the water column. During summer months, the oxycline (the transition zone from oxic to anoxic water) moves upwards in the water column. This increased anoxia leads to a lack of electron acceptors and the consequences of these changes on microbial N cycling processes and their adaptation are not well known. Besides increased denitrification rates, it is hypothesized that the role of nitrifying microorganisms is expected to increase. However, the putative nitrification pathways under oxygen limitation remain unclear.
Methods. To investigate nitrogen cycling in the water column of Lake Grevelingen, we measured oxygen and nitrogen compounds at a high resolution in the water column, and determined ammonium and nitrite-oxidation rates at several depths. Furthermore, we analysed the microbial diversity by 16S rRNA amplicon sequenicing.
The results indicate that nitrification was mostly of minor importance. The biogeochemical data of the water column showed that nitrite concentrations peaked at the oxycline in late summer. This corresponded to an increase in abundance of detectable 16S rRNA genes of ammonia- and nitrite-oxidizing bacteria at the same depth in the lake.
Conclusion. This study provides valuable insight into the microbial N cycle network and its spatiotemporal dynamics in an eutrophic basin, and thereby contributes to a better understanding of the impact of human activity on oxygen-limited coastal ecosystems.

P115

Cell-cell contacts during archaeal/bacterial syntrophy and their role to unravel the origin of eukaryotes

Dr. Kerstin Fiege[1], Laura Villanueva[1]
[1]Royal Netherlands Institute for Sea Research, 't Horntje (Texel), The Netherlands

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

P117

Isolation and characterisation of a new CO-utilizing Moorella sp. from a terrestrial hot spring

Mrs Anastasia Galani[1], Ben Tumulero[1], Detmer Sipkema[1], Diana Zita Sousa[1]
[1]Wageningen University And Research, Wageningen, The Netherlands

Anaerobic microbes with the potential of using carbon monoxide (CO) as energy or carbon source are often found in hydrothermal environments. Thermophilc microbes in these environments are promising bio-catalysts for syngas fermentation to chemicals. Here we report the isolation of a new thermophilic CO-utilizing bacterium, strain AZ24.1, from a terrestrial hot spring in the island of São Miguel, Azores, Portugal. Strain AZ24.1 is an anaerobic, gram-positive, endospore forming bacterium that grows in a temperature range of 45-70 °C with an optimum at 60-65 °C. It is capable of growing fermentatively on several sugars (e.g. fructose, maltose and xylose) and organic acids (e.g. fumarate and lactate). It can also respire these compounds in the presence of some external electron acceptors (e.g. thiosulfate and perchlorate). Based on genome-based analyses strain AZ24.1 is a species of Moorella humiferrea (average nucleotide identity >95%). However, it is notably different from Moorella humiferrea type strain because of its ability to utilize CO. Carbon monoxide is utilized by strain AZ24.1 as sole carbon and energy source producing acetate as an end product, whereas M. humiferrea type strain was not capable of utilizing CO (10%, 1.7 atm) under laboratory conditions. This isolate expands the list of known thermophilic CO-utilizing acetogens and can further be studied for its potential application for syngas fermentation.

P118

Enriching Bathyarchaeota from freshwater sediments using genome-guided cultivation

Dr. Patricia Geesink[1], Prof. Dr. Thijs JG Ettema[1]
[1]Wageningen University and Research, Wageningen, Niederlande

Archaea represent one of the two primary domains of life and have been shown to inhabit almost every environment on Earth. Sequencing-based studies have uncovered a wide diversity of previously unknown archaeal lineages in a variety of habitats. However, the majority of archaeal phyla, including the ubiquitous Bathyarchaeota, lack cultivated representatives and information on their potential role in the environment is solely predicted based on genomic information. Here, we sampled ten sediment cores from Lake Erken (Sweden) for metagenomic sequencing and cultivation work in order to elucidate the diversity and lifestyle of Bathyarchaeota in freshwater sediments. With a relative abundance of up to 23% members of the Bathyarchaeota are dominating the microbial community in Lake Erken sediments. The analysis of 17 high-quality metagenome assembled genomes (MAGs) that were reconstructed from Lake Erken indicates a widespread potential of Bathyarchaeota to break down different carbohydrates including chitin, cellulose and mannan. Enrichment cultures of Bathyarchaeota were set up and monitored over a period of over two years. With Bathyarchaeota increasing up to a relative abundance of 29%, the response of Bathyarchaeota to different carbon sources allows to draw conclusions on the metabolic potential of these elusive archaea in freshwater sediments. Using a subsequent dilution to extinction approach the overall diversity of the enrichments could be further reduced, resulting in low-complexity enrichments of several Bathyarchaeota representatives. Their high abundance, diversity and ability to degrade a wide range of complex carbon sources highlights the importance of Bathyarchaeota in lake sediments and potentially global carbon turnover. The combination of genome-resolved metagenomics and classical cultivation work lay the foundation for further attempts to isolate and study Bathyarchaeota using high-throughput cultivation techniques.

P119

Nitrate-dependent anaerobic methane oxidation (N-DAMO) as a bioremediation strategy for agriculture-affected waters

Dr. Martyna Glodowska[1], A. Legierse[1], Q. Struik[2], Dr. G. Smith[1], M. Echeveste[1], Dr. S. Stefan Weideveld[3], Dr. G. van Dijk[2,3], Prof. M. Jetten[1], Dr. A. Veraart[2], Dr. C.U. Welte[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]Radboud University, Nijmegen, The Netherlands, [3]B-WARE Research Centre, Nijmegen, The Netherlands

Agricultural drainage ditches are subjected to high anthropogenic nitrogen input leading to eutrophication and greenhouse gas emission. This methane (CH4) and nitrate (NO3-) rich environment creates suitable conditions for nitrate-dependent anaerobic methane oxidation (N-DAMO) offering a promising water bioremediation strategy. In this study we aimed to evaluate the potential of N-DAMO to remove excess NO3- and decrease CH4 release from agricultural drainage ditches. A microcosm experiment was conducted using sediment and surface water collected from three different sites: a sandy-clay ditch (SCD), a freshwater-fed peatland ditch (FPD), and a brackish peatland ditch (BPD). The microcosms were inoculated with an N-DAMO enrichment culture dominated by Candidatus Methanoperedens and Candidatus Methylomirabilis and supplemented with 13CH4 and 15NO3-. A significant decrease in CH4 and NO3- concentration was only observed in the BPD sediment. In freshwater sediments (FPD and SCD) the effect of N-DAMO inoculation on NO3- removal was negligible, likely because N-DAMO microorganisms were outcompeted by heterotrophic denitrifiers consuming NO3- more quickly. Overall, our results suggest that bioaugmentation with N-DAMO might be a potential strategy to decrease CH4 emission and eutrophication in drainage ditches subjected to increased agricultural activities where the native microbial community is incapable of efficient denitrification.

P120

Metaproteomics analysis reveals microbial and functional differences related to cow's milk tolerance and nutritional interventions

Dr. Diana Hendrickx[1], Dr. Ran An[1], Sjef Boeren[2], Dr. Sumanth Mutte[2], Dr. Jolanda Lambert[3], Dr. Clara Belzer[1]

[1]Wageningen University, Wageningen, The Netherlands, [2]Wageningen University, Wageningen, The Netherlands, [3]Danone Nutricia Research, Utrecht, The Netherlands

Introduction

Development of the gut microbiome occurs in early life. Previous studies provide evidence for an association between gut microbiota modifications and the development of food allergies. Nutritional interventions have been proposed to support food tolerance development by altering the gut microbiome. We aim to gain insight into microbial and functional differences related to outgrowth of cow's milk allergy (CMA) and nutritional interventions.

Methods

This study included 120 faecal samples of the PRESTO clinical trial (NTR3725), where 40 infants with CMA were monitored after diagnosis, as well as 6 and 12 months after a nutritional intervention with standardized amino acid formula or similar formula with a synbiotic blend (oligosaccharides (oligofructose, inulin) + Bifidobacterium breve). Twenty-four out of forty infants showed outgrowth of CMA after 12 months. We performed 16S rRNA sequencing and metaproteomics analysis of the bacterial gut microbiome.

Results

Outgrowth of CMA was characterized by significantly higher levels of proteins produced by some members of the core gut microbiota. Significantly higher levels of Bifidobacteriaceae and several microbial proteins were observed after treatment with synbiotics. The synbiotic group also had higher levels of several bifidobacterial carbohydrate-active enzymes, known to metabolize oligosaccharides.

Conclusions

Apart from studying the microbiome, metaproteomics also allows to study protein expression. By combining metaproteomics and 16S rRNA sequencing, several microbial and functional differences related to outgrowth of CMA and nutritional interventions could be revealed.

P121

Culturing the uncultured: Towards a microfluidic-based droplet cultivation method for the long-term enrichment of novel prokaryotes

Mr. Yassin Mokhtar Kaspareit[1], Dr. Aigars Piruska[2], Dr. Eric R. Hester[1], Prof. Dr. Wilhelm T.S. Huck[2], Prof. Dr. Thijs J.G. Ettema[1]
[1]Wageningen University & Research, Wageningen, The Netherlands, [2]Radboud University Nijmegen, Nijmegen, The Netherlands

The isolation and cultivation of novel prokaryotes from environmental microbial communities is a challenging task due to their unknown physiology, biotic interactions and abiotic dependencies. Traditional cultivation methods, while important and successful in isolating uncultured microorganisms, can be time-consuming and require careful testing of media and conditions. A novel approach to addressing their cultivation is the use of microfluidic droplet cultivation. Microfluidic droplet cultivation is a high-throughput method to generate subpopulations of microbial communities by encapsulating microorganisms within micro-scaled droplets in a fluorocarbon oil phase supplemented with surfactant.
Here, we present an adapted microfluidic-based workflow for the incubation of slow-growing microorganisms from environmental samples to enrich and sort out actively growing microorganisms and study their physiology and biotic dependencies.
This study shows that microfluidic droplet cultivation methods can be used to separate and incubate environmental samples over prolonged time periods in dependence of the surfactant concentration. Furthermore, the setup is designed to be operated under anoxic conditions, striving to culture uncultured microorganisms, which thrive in anoxic environments. Regulating the initial cell concentration and flow velocities, microorganisms can be separated in droplets ranging from a few nanoliters to a microliter. An algorithm to approximate growth within the microfluidic droplets has been applied to sort out droplets containing growing microbial communities using an electrical pulse. The microbial composition of the droplet communities can also be screened using PCR-based sequencing methods.
We have developed an initial framework for the cultivation, screening and sorting of slow-growing microorganisms using microfluidic droplets. This high-throughput method will allow us to enrich and study members of the uncultured majority from various environments.

P122

Understanding the influence of Human Milk Oligosaccharides and mucin on infant gut microbiota interactions

Yuvashankar Kavanal Jayaprakash[1], Ms Athanasia Ioannou[1], Ms Maryse Berkhout[1], Dr. Clara Belzer[1]
[1]Wageningen University & Research, Wageningen, The Netherlands

Background: The early infant gut microbiota development is influenced by indigestible carbohydrates present in breast milk or infant formula. Especially, the indigestible human milk oligosaccharides (HMOs) present in breast milk are made up of glycans that have structural similarities to those present in intestinal mucin. Previous studies have established that certain gut bacteria such as Bifidobacterium bifidum, Phocaeicola vulgatus, Akkermansia muciniphila and Ruminococcus gnavus are able to degrade mucin as well as HMOs. Furthermore, the extracellular degradation and consumption of HMOs and mucin release monosaccharides and short-chain fatty acids (SCFAs). These monosaccharides, organic acids along with CO2 and H2 gas enable the growth of cross-feeding SCFA-producing bacteria. Altogether the networks of bacteria formed can have beneficial effects on the infant. Therefore, we aim to elucidate the influence of the glycan structural similarity between HMOs and mucin on the ecology infant gut microbiota.

Methods: In this study, the effects of structural similarity of HMOs and mucin glycans were tested through sequential batch fermentation of glycan molecules using a synthetic bacterial community. Different glycan molecules were used to create several nutrient conditions that simulate the breastfed and formula-fed infant gut. Purified pig gastric mucin was used to simulate the microbial mucosal niche, while HMOs and Galacto-/Fructo-oligosaccharides (GOS/FOS) were used to simulate breast milk and infant formula respectively.

Results and Conclusions: We have successfully created a synthetic community resembling infant gut microbiome comprising of five mucin- and HMO-degraders along with two cross-feeders. This community was able to grow in vitro in carbon sources that simulated breastfed and formula-fed infant gut. Our results further demonstrate that certain bacteria thrive on both milk and mucin glycans and they can be used to explore avenues for understanding the effects of glycan structural similarity in the developing infant gut microbiome.

P123

Beyond nitrite oxidation: metabolic versatility in the phylum Nitrospinota

Hanna Koch[1], L.F.M. Kop[1], M.S.M. Jetten[1], H. Daims[2], S. Lücker[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]University of Vienna, Vienna, Austria

Nitrite-oxidizing bacteria (NOB) play a key role in the marine nitrogen cycle. Nitrate produced by nitrification is the main bioavailable form of nitrogen in the ocean and represents a growth limiting factor for marine organisms. The most abundant marine NOB belong to the phylum Nitrospinota. Despite their importance in nitrogen and carbon cycling, there are only few cultured representatives that all belong to the class Nitrospinia. Metagenomic sequencing has led to the discovery of several other classes of Nitrospinota, which remain uncultivated and have not been analyzed in detail. While Nitrospinota were previously thought to be restricted to marine environments, metagenome-assembled genomes have also been recovered from the subsurface.
To understand the role of these novel Nitrospinota in biogeochemical element cycling, we analyzed a non-redundant set of nearly 100 Nitrospinota genomes. Based on phylogenomic analysis and average amino acid identities, the highly diverse phylum Nitrospinota can be phylogenetically divided into over 30 different genera that differ in their metabolic capacities. Our analyses show that members of this phylum are more widespread and metabolically versatile than previously recognized, as not all Nitrospinota are nitrite oxidizers. Potential metabolic traits of non-Nitrospinia Nitrospinota include the oxidation of sulfide, hydrogen or formate coupled to oxygen or nitrate reduction for energy conservation. Furthermore, some Nitrospinota-bacteria possess the enzymatic repertoire for nitrogen fixation encoded by the nif genes. Thus, in addition to its known role in the global nitrogen and carbon cycles, Nitrospinota might be involved in nitrate reduction, nitrogen fixation and sulfur cycling. Overall, this study expands our knowledge of the potential ecophysiology and phylogeny of the phylum Nitrospinota and shows that the nitrite-oxidizing metabolic capability for which this group is well known evolved later than previously assumed in this phylum.

P124

The application of cold-adapted anammox on treating reverse osmosis concentrate

Ms Shiyi Liu[1], Dr. P.H.A Timmers[2], Dr. M.A.H.J van Kessel[1], Dr. S Lücker[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]KWR Water Research Institute, Nieuwegein, The Netherlands

Reverse osmosis (RO) is an effective way to remove contaminants from different types of water and is investigated to be used in the future for drinking water production. Before discharge, pollutants enriched in the RO concentrate must be removed. For the removal of ammonium (NH4+), anaerobic ammonium oxidation (anammox) would be a sustainable and cost-effective option. However, the relatively low temperature (below 15 °C) and low NH4+ concentration (less than 20 mg/L) are not ideal for the application of anammox. In this project, we intend to selectively enrich cold-adapted anammox bacteria to test if the anammox process can be used to remove nitrogen from RO concentrate in a laboratory culture. For this, a bioreactor operating at 18 °C was inoculated and fed synthetic RO concentrate supplemented with ammonium and nitrite. We will slowly adapt this culture to operate at 12 °C and monitor anammox activity to validate and quantify their contribution to nitrogen removal. Quantitative and structural analysis of the microbial community will be obtained by 16S rRNA gene amplicon sequencing and fluorescence in situ hybridization. In conclusion, this research contributes to the development of new technologies for safe and responsible drinking water production by investigating the application potential of cold-adapted anammox for the sustainable treatment of RO concentrate.

P125

Nitrogen cycle symbiont transmission pathways revealed using gnotobiotic zebrafish (Danio rerio)

Wouter Mes[1,2], Prof. dr. ir. M.S.M. Jetten[1], Prof. H. Siepel[2], Dr. S. Lücker[1], Dr. M. Gorissen[2], Dr. M.A.H.J. van Kessel[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]Radboud University, Nijmegen, The Netherlands
Introduction. Aquaculture is one of the fastest growing food producing sectors. Due to the high fish density in aquaculture systems in combination with protein-rich diets; the main nitrogenous waste product of fish, ammonia, can reach high concentrations. Ammonia is toxic, so reducing water ammonia concentrations is of key interest in aquaculture.
Recently, it was shown that ammonia, excreted via the gills, is partly converted into dinitrogen gas in carp and zebrafish gills through the combined activity of ammonia-oxidizing and denitrifying bacteria. Interestingly, these bacteria have an intracellular localization, making it the first known instance of intracellular symbiosis in a vertebrate host. At the moment, the transmission pathway of these bacterial symbionts is unknown.
Methods and results.
We investigated the transmission pathways of ammonia-oxidizing symbionts in fish, making use of a gnotobiotic zebrafish model. 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 at multiple developmental stages using PCR targeting ammonia monooxygenase subunit A (amoA). We were able to determine the likely transmission pathway as being vertical, through deposition of Nitrosomonas on fertilized eggs, possibly through the water. We confirmed this finding by showing that adult zebrafish can release these bacteria into the water. Additionally, we investigated the egg microbiome using 16S rRNA amplicon sequencing and studied the localization of ammonia-oxidizing bacteria in adult gills using fluorescent in situ hybridization.
Conclusion. The transmission pathway of symbiotic nitrogen cycle bacteria in zebrafish was determined to be via deposition of the bacteria into either the water or onto the eggs. It is possible to remove the symbionts using gnotobiotic derivation and at 3 dpf re-colonization can be achieved. Future studies should determine the interval of colonization and how the immune system is involved in establishing this symbiosis.

P126

Virus competition for same algal host

Claudia Meyer[1,2], Victoria Jackson[3], Dr Adam Monier[3], Dr Henk Bolhuis[1], Prof. Dr. Corina Brussaard[1,2]
[1]NIOZ Royal Netherlands Institute for Sea Research, Den Burg, Texel, The Netherlands, [2]University of Amsterdam, Amsterdam, The Netherlands, [3]University of Exeter, Exeter, United Kingdom

With about half of the world's primary production taking place in the ocean, marine photoautotrophs play a dominant role in the world's biogeochemical cycles. Small phytoplankton are the main primary producers in the ocean and form the base of most marine food webs. Lately, viral lysis has been shown to be an important mortality factor and subsequently a significant factor promoting biodiversity of phytoplankton species and strains. Multiple viruses infecting the same host strains can coexist in a stable manner, however, knowledge regarding marine virus competition hardly exists. Most mechanistic studies examining marine virus-host interactions have focussed on one host – one virus strain model systems or used bacterial hosts. We examined virus competition in a polar picoeukaryotic alga Micromonas polaris and the dsDNA MpoV-45T and MpoV-46T viruses. These viruses resemble each other in their particle and genome size, as well as in latent period and burst size. Despite their similarities, one-step infection experiments pairing these two viruses infecting the same host strain show distinctly different virus proliferation dynamics (compared to single infection). Using flow cytometry, qPCR assays and full genome sequencing, we show that both viruses were affected by the presence of the other to varying degrees. Our study illustrates that there is still much insight to gain into the ecological importance of virus competition from studying the infection dynamics with multiple viruses.

P127

Reversible bacteriophage resistance by shedding of the bacterial cell wall and characterization of cell wall-revertants

Véronique Ongenae[1,2], Adam Sidi Mabrouk[1,2], Marjolein Crooijmans[1,2], Daniel Rozen[1], Ariane Briegel[1,2], Dennis Claessen[1,2]
[1]Molecular Biotechnology, Institute of Biology, Leiden University; P.O. Box 9505, 2300 RA Leiden, The Netherlands, Leiden, Netherlands, [2]Centre for Microbial Cell Biology, Leiden University, Leiden, The Netherlands, Leiden, The Netherlands

The cell wall plays a central role in protecting bacteria from some environmental stresses, but not against all. In fact, an elaborate cell envelope may even render the cell more vulnerable, since it contains molecules and structures that bacteriophages recognize as the first step of host invasion. Therefore, bacteria have evolved sophisticated defense systems to withstand phage attacks, like CRISPR/Cas, restriction-modification or abortive infection. However, some bacteria are known to be able to shed the bacterial cell wall in response to several environmental stressors. We hypothesized that wall-deficient bacteria may be temporarily protected against phages, since they lack the essential entities that are necessary for phage binding and infection. To test this hypothesis, three model organisms (Streptomyces, Escherichia coli and Bacillus subtilis) were inoculated in osmoprotective medium and infected with bacteriophages at MOI=1. Cryo-electron tomography was used to give a detailed overview of the interaction between phages and wall-deficient bacteria. We have uncovered a previously unknown mechanism by which mono- and diderm bacteria survive infection with diverse phages by shedding the cell wall. In addition, the wall-deficient cells caused by phage infection can become resistant against infection with the same lysogenic phage when switching to a walled lifestyle. Could phages influence the behavioral programming of bacteria?

Altogether, these results show that the formation of cell wall-deficient cells prevents complete eradication of the bacterial population and suggest that wall-deficiency may limit the efficacy of phage therapy, especially in highly osmotic environments or when used together with antibiotics that target the cell wall.

P129

Thermodynamics strongly limit the phenotype of anaerobic polymer conversions in polyphosphate accumulating organisms

Ir Timothy Paez Watson[1], M. C. M. van Loosdrecht[1], S. A. Whal[2]
[1]Delft University of Technology, Delft, The Netherlands, [2]Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Dynamic environments are almost the norm in ecology. One of the main microbial metabolic strategies to buffer this dynamism relies on storage polymers, a strategy strongly applied by polyphosphate-accumulating organisms (PAOs) of the genus “Candidatus Accumulibacter”. PAOs thrive in dynamic environments by continuously cycling a range of polymers, including glycogen, polyphosphates, and polyhydroxyalkanoates (PHAs). Understanding how these organisms control their metabolism will deliver insights on the fundamentals of storage polymers in life and our understanding of ecosystems.

Current research on this topic is hindered by the difference between the slow growth and the comparably fast dynamic changes. The limited time frame for responding to rapid dynamic changes results in minimal changes in the genome or proteome. Rather, responses will occur at the metabolome of these organisms. Here we use metabolic flux analysis and pathway thermodynamics to study the adaptation of microorganisms to dynamic environments.

We applied Elementary Flux Modes (EFMs) analysis to identify all the possible metabolic pathways that “Candidatus Accumulibacter” could use anaerobically. Not all EFMs are feasible, hence we applied Max-min Driving Force (MDF) to each to identify thermodynamic bottlenecks and unfeasible solutions in these pathways.

We identified 917 flux distributions that describe unique metabolic fluxes in the anaerobic uptake of substrate (acetate) by cycling different polymers. Important relations between specific pathways such as glycogen degradation and type of PHA generated, CO2 fixation, polyphosphate use, etc. were evident. We found that only 21 EFMs were thermodynamically feasible when applying MDF. Several of the identified flux distributions had not been previously described. Our results revealed previously unknown pathway combinations that could shape the metabolism of “Candidatus Accumulibacter” and its control over the use of polymers.

Our results contribute to a deeper understanding of the robust phenotype of these organisms and the role of storage polymers in microbial ecology.

P130

Building a phylogenomic framework to decipher the evolutionary histories of PVC bacteria

Kassiani Panagiotou[1], Stephan Köstlbacher[1], Daniel Tamarit[2], Jennah Dharamshi[3], Thijs Ettema[1]
[1]Wageningen University & Research, Wageningen, The Netherlands, [2]Utrecht University, Utrecht , The Netherlands, [3]Stockholm University, Stockholm, Sweden

The Planctomycetes-Verrucomicrobiae-Chlamydiae (PVC) superphylum is a group of bacterial phyla, the members of which often exhibit unique cell biological attributes and a well-supported monophyly. In recent years, however, the PVC superphylum has experienced a major expansion concerning the microbial diversity it comprises owing to the advent of next-generation sequencing and metagenomics. This has led to the accumulation of novel candidate lineages that, depending on the phylogenomic datasets and methods used, either form a monophyletic relationship with traditional PVC members or appear as sister lineages. Since this has resulted in phylogenetic inconsistencies, there have been conflicting descriptions of the phyla comprising PVC. As such identifying the true members of PVC bacteria and their evolutionary relationships is of great importance. Thus, the aim of this study is to develop and employ large-scale phylogenomics that will account for sources of error from different key steps of phylogenetic analyses as well as strategies to mitigate these. This will not only contribute to inferring more accurate phylogenetic relationships among PVC members, but also to building a comprehensive evolutionary framework where we can reliably investigate the evolution of their genome content underlying their unique cellular biology.

P131

Synthetic microbial co-cultures for syngas fermentation to odd-chain carboxylic acids and alcohols

Ivette Parera Olm[1,2], C Dubaere[1], Prof. DZ Sousa[1,2]
[1]Laboratory of Microbiology, Wageningen University & Research, Wageningen, The Netherlands, [2]Centre for Living Technologies - Alliance TU/e, WUR, UU, UMC, Utrecht, The Netherlands

Co-cultivation of chain-elongating microorganisms (e.g., Clostridium kluyveri) with acetogens is a promising platform for the production of medium-chain carboxylic acids (MCCAs) from C1 feedstocks. Acetogens use the Wood-Ljungdahl pathway to fix CO2 and produce acetate and ethanol as end products, which are typical substrates for chain-elongating bacteria. In our research group, we have established several synthetic co-cultures for the production of MCCAs from syngas (CO, H2 and CO2), with ethanol as intermediate.

Previously, we showed that co-cultures of the acetogen Clostridium autoethanogenum with C. kluyveri were able to convert CO/syngas into butyrate, caproate and octanoate. Here, we investigated the production of odd-chain carboxylates (OCCAs; i.e., valerate, heptanoate) by introducing a third partner, Anaerotignum neopropionicum, which grows on ethanol producing propionate in the presence of CO2. First, we show that a co-culture of A. neopropionicum and C. kluyveri fed solely on ethanol and CO2 can produce C5-C7 MCCAs. The co-culture is robust and tolerates ethanol concentrations of at least 14 g L-1. In chemostat cultivation, concentrations up to 0.9 g L-1 valerate and 0.5 g L-1 heptanoate were obtained, with OCCAs representing about 40 % of the products. Next, we established a tri-culture composed of C. kluyveri, A. neopropionicum and the acetogen Acetobacterium wieringae strain JM, previously isolated in our lab and able to grow at pH 7. In fed-batch reactor cultivation, the tri-culture converted CO into a mixture of odd- and even-chain MCCAs. Upon accumulation of acids, the respective alcohols were produced, including 0.4 g L-1 pentanol. To our knowledge, this is the first time that this alcohol is reported in a syngas-fermenting culture.

Overall, the results of this work demonstrate that MCCAs and their respective alcohols can be produced from syngas by using synthetic clostridial co-cultures, which are as robust as mixed cultures, yet simpler and modular.

P132

Elucidating acidic ammonia oxidation by Candidatus “Nitrosacidococcus tergens”

Msc Ida Peterse[1], Dr. Sebastian Lücker[1], Dr. Annelies Veraart[2], Prof. dr. ir. Mike Jetten[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]Radboud University, Nijmegen, The Netherlands

Although acidic nitrification has been observed in soils over a century ago, ammonia oxidation was supposed to be impossible in highly acidic environments. Protonation to ammonium was thought to lead to lower substrate availability for ammonia oxidizing microorganisms. However, recently different acidophilic and acid-tolerant microbes have been isolated and are now available in (pure) culture, among which the chemolithoautotrophic “Candidatus (Ca.) Nitrosacidococcus (Na.) tergens” sp. RJ19. This gammaproteobacterial ammonia oxidizer still shows growth on ammonium at pH 2.5 in a continuous culture. At a pH of 3.5, NO and nitrate both constitute a significant share of total produced nitrogen in this system. However, the exact biological nitrogen transformations catalyzed by Ca. Na. tergens at acidic conditions remain uncertain due to chemical instability of nitrogen species like nitrite and NO. Moreover, it is unclear how these nitrogen transformations mediated by Ca. Na. tergens react to pH changes.

Here, we investigate the nitrogen metabolism of Ca. Na. tergens in a continuous bioreactor enrichment culture under changing pH conditions by accurately following dynamics of various nitrogenous compounds (ammonium, nitrite, nitrate, NO and N_2O), protein content, transcriptome and community changes. A continuous membrane bioreactor was set up and subjected to the stepwise increase of pH from 2.5 to 7.5. Our data shows stoichiometric conversion of ammonia to nitrite at pH 6, transient production of N_2O peaking at pH 4.4, and furthermore indicates chemical production of NO and nitrate only at acidic pH. In a next step, we will combine these findings with transcriptomic data to elucidate the exact mechanisms enabling Ca. Na. tergens to perform ammonia oxidation at extremely acidic conditions, and to obtain a better understanding of the contribution of nitrification in acidic environments to the production of harmful greenhouse gasses.

P133

Seasonal dynamics of Eukaryotic virus diversity in coastal Antarctic waters

Gonçalo Piedade[1,3], ME Schön[2], TEG Biggs[1], H Bolhuis[1], N Yutin[4], M Fischer[2], BE Dutilh[5], CPD Brussaard[1,3]

[1]NIOZ Royal Netherlands Institute for Sea Research, Texel, The Netherlands, [2]Max Planck Institute for Medical Research, Heidelberg, Germany, [3]Institute for Biodiversity and Ecosystem Dynamics (IBED), Amsterdam, The Netherlands, [4]National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, USA, [5]Friedrich Schiller University Jena, Jena, Germany

Viruses that infect microorganisms play a crucial role in shaping their host population dynamics and biodiversity in the oceans, impacting ecosystem functioning and biogeochemical cycles. In the Southern Ocean, ongoing global warming-induced changes are expected to have a profound impact on marine productivity and microbial community composition. Despite their ecological importance, there have been only a few publications on the diversity and seasonal dynamics of these viruses in the Antarctic, and no seasonal studies. This study provides new insights into the temporal diversity and dynamics of dsDNA viruses that infect microbial eukaryotes in the Southern Ocean. Using metagenomics, we recovered 51 genomes of NCLDV viruses, including 27 from the Imitervirales family 9, 1 from Imitervirales family 12, 11 from the Mesomimiviridae family, 10 from the Prasinoviridae family, 1 from the Pithoviridae family, and 1 from the Pimascovirales family 1. These were connected to their co-occurring hosts using shared gene homology and the host of the closest relative. We also identified 24 virophages and 405 polinton-like viruses (PLVs) which we connected to their potential NCLDV partner using the promoter motif. The most abundant virus was a Mesomimivirus, predicted to infect the ecologically significant phytoplankton species Phaeocystis antarctica.We connect the increase in this virus with the decrease in abundance of a specific P. antarctica genotype and with two PLVs, closely related to P. globosa PLV. The virus-to-host ratio dynamics suggest a temperate infection of P. antarctica by the PLVs, which are activated during the Mesomimivirus infection. Our results provide new insights into the ecology of Antarctic eukaryotic viruses and their hosts, emphasizing the importance of studying virus-host interactions in the Southern Ocean for a better understanding of the ecosystem, especially in the face of climate change.

P134

Microbial nitrogen cycling potential in sediment from a seasonally hypoxic lake

Isabel Rigutto[1], Prof. Dr. Ir. Caroline P. Slomp[1], Prof. Dr. Ir. Mike S. M. Jetten[1]
[1]Radboud University, Nijmegen, The Netherlands

Introduction: Coastal ecosystems experience increased eutrophication and deoxygenation due to anthropogenic activities. Such shifts in environmental conditions can change the redox zonation of an ecosystem, which can impact the system's biogeochemical functioning. Nitrous oxide (N_2O), an extremely potent greenhouse gas, is an intermediate of various reactions in the nitrogen cycle. Therefore, a predictive understanding of the impact of eutrophication and deoxygenation on microbial nitrogen cycling and the formation and removal of N_2O in coastal waters is crucial. At present, the microbial population dynamics and metabolic pathways underlying N_2O formation and degradation are not yet well explored.

Methods: Sediment was collected from the seasonally hypoxic, saline Lake Grevelingen in September 2021 when the bottom waters were depleted in oxygen. Porewater was analyzed for oxygen, pH, organic carbon, trace elements, and nitrogen compounds. Denitrification and N_2O removal potential of the sediment were studied using batch incubations with sediment from 5-10 cm depth. Nitrite or N_2O were added as electron acceptor, and when endogenous sources were depleted, sodium acetate or monomethylamine were supplied as electron donor and carbon source. The microbial diversity was assessed by 16S rRNA amplicon sequencing.

Results: Chemical profiling of the sediment showed diffusion of ammonium to the water column with nitrate and nitrite depleted within the first cm. In the batch incubations, nitrite was consumed over time, with significant N_2O formation, followed by N_2O removal. Sequencing of the V3-V4 region of 16S rRNA genes will reveal the shifts in the microbial community over time, and possibly identify the responsible microorganisms in dilution series that have been started.

Conclusion: These findings indicate that there is potential for microbial nitrite and N_2O removal during hypoxia in sediments of Lake Grevelingen. This is important information to make better predictive models of the future state of our coastal waters and their N_2O emissions.

P135

Microbial paracetamol degradation involves a high diversity of novel amidase enzyme candidates

Phd Ana Rios Miguel[1], GJ Smith, G Cremers, T van Alen, N Elezi, MSM Jetten, HJM Op den Camp, CU Welte

[1]Radboud University, Nijmegen, The Netherlands

Pharmaceuticals are relatively new to nature and often not completely removed in wastewater treatment plants (WWTPs). Consequently, these micropollutants end up in worldwide water bodies posing a great environmental risk. Paracetamol full degradation has been linked to several microorganisms. However, the genes and corresponding proteins involved in microbial paracetamol degradation are still elusive. To improve our knowledge of the microbial paracetamol degradation pathway, we inoculated a bioreactor with sludge of a hospital WWTP (Pharmafilter, Delft, NL) and fed it with paracetamol as the sole carbon source. Paracetamol was fully degraded without any lag phase and the enriched microbial community was investigated by metagenomic and metatranscriptomic analyses, which demonstrated that it was very diverse. Dilution and plating on paracetamol-amended agar plates yielded two Pseudomonas sp. isolates: a fast-growing isolate that degraded 200 mg/L of paracetamol in approximately 10 h while excreting 4-aminophenol, and a slow-growing isolate that degraded paracetamol without obvious intermediates in more than 90 days. Each Pseudomonas sp. contained a different highly-expressed amidase (31% identity to each other). E. coli BL21 cells were transformed with a plasmid containing the genes encoding for these two amidases and cells were able to transform paracetamol at different rates compared to the no transformation of BL21 cells. These amidase genes were not detected in the bioreactor metagenome suggesting that other as-yet uncharacterized amidases may be responsible for the first biodegradation step of paracetamol. Uncharacterized deaminase genes and genes encoding dioxygenase enzymes involved in the catabolism of aromatic compounds and amino acids were the most likely candidates responsible for the degradation of paracetamol intermediates. This study increases our knowledge about the ongoing microbial evolution towards biodegradation of pharmaceuticals and points to a large diversity of (amidase) enzymes that are likely involved in paracetamol metabolism in WWTPs.

P136

Microbiome of the pest insect southern green shield bug Nezara viridula aids its host with digestion, nutrient provision and detoxification

Msc Magda Rogowska-van der Molen, Dr. S. Coolen, Dr. J.L. Postma, Dr. R.S. Jansen, Dr. C.U. Welte
[1]Radboud University, Nijmegen, the Netherlands

Introduction: There is an increasing demand for food production, which requires taking actions to decrease crop losses in the future. Shield bugs are major pest insects causing damage on soybean, potato and cabbage, among many agriculturally important crop species. Insect-associated microorganisms provide a target for pest control strategies since they harbour detoxifying properties that contribute to insect resistance against plant toxins. In our study, we investigated the detoxifying capacity of insect microbiome from a newly emerging threat in the Netherlands: the southern green shield bug Nezara viridula to ultimately develop pest control strategies.

Methods: We characterized the gut and salivary glands microbiome with an emphasis on the microbial functions in Nezara viridula. To determine the core microbiome, using 16S rRNA sequencing, we compared the microbial composition of insects obtained from different locations within the Netherlands. Furthermore, using metagenomics we analysed the metabolic potential of metagenome-assembled genomes and identified the genes involved in the production of essential nutrients, digestion and detoxification. Subsequently, using specific probes, we performed fluorescence in situ hybridization on salivary glands and guts to localize and visualize bacterial symbionts.

Results: The microbiome analysis revealed that insect gut is mainly populated with Pantoea sp. symbionts and salivary glands are dominated by Sodalis sp. In line with that, fluorescence in situ hybridization showed that salivary glands are colonized with Sodalis sp. and that the M4 section of the gut harbours Gammaproteobacteria. Moreover, using metagenomics, we found that the microbiome has the potential to provide the host with amino acids and vitamins, aid in digestion of plant matter, suppress plant defences and detoxify plant metabolites solanine and nitropropionic acid.

Conclusions: Our study provides insights into the microbiome of Nezara viridula and its role in insect-microbiome interactions. We advocate that targeting bacterial symbionts contributes to the development of novel pest control strategies.

P137

Proteome-based wastewater microbiome metabolism dynamics underlying nitrous oxide emissions

Nina Roothans[1], M. Pabst[1], M. van Diemen[1], C. Herrera Mexicano[1], M. Zandvoort[2], T. Abeel[1,3], M. van Loosdrecht[1], M. Laureni[1]
[1]Delft University Of Technology, Delft, Netherlands, [2]Waternet, Amsterdam, Netherlands, [3]Broad Institute of MIT and Harvard, Cambridge, United States of America
Globally, microbial communities in managed and engineered ecosystems are the main sources of the potent greenhouse gas nitrous oxide (N2O). Yet, despite decades of research, the complex network of biological processes underlying N2O emissions remains largely unsolved. We use microbiomes from full-scale wastewater treatment plants as model ecosystems, and combine metagenomic and metaproteomic analysis to simultaneously resolve the identity and functional role of the dominant community members. To the best of our knowledge, this is the first multi-year proteomic profile focused on elucidating labour-division in dynamic microbiomes. Therefore, we also discuss the broader challenges and opportunities of our approach.

As for any metabolic intermediate, N2O emissions are the result of unbalanced substrate fluxes among and within organisms producing and consuming it. Yet, resolving these dynamic (un)balances remains a challenge, leaving most often the underlying mechanisms at the hypothesis level. By quantifying the expressed proteins, used here as proxy for activity, we revealed marked opposite seasonal profiles of nitrite and N2O reductases, with individual expression levels varying up to 10-fold. Importantly, these observations confirm the often hypothesized association between temperature, unbalanced denitrification and subsequent net N2O accumulation. Furthermore, the asynchronous profiles of the core denitrifying proteins illuminates the actual metabolic labour division in microbiomes, inherently overlooked when solely characterized at the genetic-level.

In conclusion, open challenges do of course remain, such as ensuring sufficient metabolic coverage of process-relevant yet low-abundant species. Nevertheless, long-term metaproteomic profiling brings us a step closer to linking genotype and metabolic phenotypes in widespread dynamic ecosystems.

P138

New leads on the mechanism of suppressive soils: The addition of keratin-rich amendments leads to functional shifts of the soil communities

Lina Russ[1], E Nijhuis[1], B Andreo Jimenzez[1], J Postma[1]
[1]Wageningen UR, Wageningen, Netherlands

Organic soil amendments can improve soil quality and plant health in agricultural systems. Recently it has been shown that keratin-rich soil amendments can increase the suppressiveness of beetroot seedlings to damping off disease caused by the soilborne pathogen Rhizoctonia solani. This effect is mediated via the soil microbiome, but the mechanism behind this observation is still unknown. The objective of the present work was to gain a deeper knowledge on the shifts of the taxonomic and functional microbial profiles three weeks after the addition of a keratin-rich amendment to two different Dutch soils (Lisse and Vredepeel) by high throughput metagenomic sequencing. The amount of reads that could be classified using a customized Kraken database was generally low and depended on the soil as well as the treatment it had received. The percentage of classified reads in Lisse soils supplemented with keratin increased from 24.70±0.46 to 36.92±0.89, whereas classified reads in Vredepeel soil dropped after having received keratin-rich treatment from 26.98±0.40 to 21.01±0.35. Differential abundance analysis of classified taxa on family level showed a similar shift in both soils upon the addition of the keratin-rich product. Both soils increased in Flavobacteriaceae and Sphingobacteriaceae, Boseaceae, Phyllobacteriaceae and Caulobacteraceae, Oxalobacteraceae and Comamonadaceae, Rhodanobacteraceae and Steroidobacteraceae. These families also did show a very high occurrence of proteases belonging to MEROPS families of known keratinases, implying that they are stimulated by the substrate added to the soils. We could link additional attributes to these families by doing a Pfam enrichment analysis on protein-based assemblies including increased abundance of the type VI secretion system, cell appendages, chitinase C and metallopeptidases and bacteriocins. We propose that the addition of keratinaceous compounds (and possibly organic materials) leads to an enrichment of functions that each by themselves have been shown to play a role in disease suppression.

P140

Large outbreak of typhoid fever on a river cruise ship used as a shelter location for asylum seekers in the Netherlands

MD, MPH D Ooms[1], MD JMC de Vries[2], MSc F Koedijk[3,4], PhD E. Generaal[3,5], PhD IHM Friesema[6], MD M Rouvroye[7], MD, PhD S van Lelyveld[7], PhD M van den Beld[8], MD, PhD D Notermans[8], MD P Schelven[9], MD J de Brink[10], T Hartog[2], T Veenstra[1], MD, PhD S Slavenburg[11], MD J Sinnige[11], MD, PhD WLM Ruijs[1]
[1]National Institute for Public Health and the Environment (RIVM) , Bilthoven, The Netherlands, [2]Municipal Health Service Kennemerland , Haarlem, The Netherlands, [3]National Institute for Public Health and the Environment (RIVM) , Bilthoven, The Netherlands, [4]Municipal Health Service Twente , Enschede, The Netherlands, [5]Municipal Health Centre Amsterdam, Amsterdam, The Netherlands, [6]National Institute for Public Health and the Environment (RIVM) , Bilthoven, The Netherlands, [7]Spaarne Gasthuis, Haarlem, The Netherlands, [8]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, [9]Municipal Health Service Gelderland-Midden , Arnhem, The Netherlands, [10]Municipal Health Service IJsselland , Zwolle, The Netherlands, [11]Regional Public Health Laboratory Kennemerland, Haarlem, The Netherlands

Introduction:
April 6 th 2022, the Municipal Health Service was notified about an outbreak of fever and abdominal complaints among asylum seekers, living on an old river cruise ship, used as shelter. Meals prepared in the galley were offered in the restaurant of this ship to asylum seekers and staff members. Typhoid fever was diagnosed and an extensive outbreak investigation was performed. Meanwhile control measures were taken to prevent further spread.

Methods:
Fecal- and blood samples were collected. Asylum seekers and staff members were questioned about their use of meals and tap water at the ship. From April 11th until May 16th the ship was visited six times for microbiological investigation and technical inspection. Cultured isolates were analysed with whole genome sequencing.

Results:
In total, 72 cases of culture confirmed typhoid fever were identified among asylum seekers and staff members of in total 349 exposed people. Cases were diagnosed between April 7th and May 24th. Twenty-five cases were hospitalized and all recovered after treatment. The cultured isolates belonged to the same genetic clade.

Frequent consumption of food and tap water at the ship was associated with infection.

The freshwater and wastewater tanks were found to share a common wall with severe corrosion and small holes, enabling wastewater to leak into freshwater tanks. Salmonella Typhi was cultured from the wastewater tank, matching the outbreak strain. In the freshwater tanks Salmonella spp. DNA was detected by PCR.

Conclusion:
A large outbreak of typhoid fever in the Netherlands was caused by sewage water containing S. Typhi leaking into freshwater tanks of a river cruise ship. Especially in ships, strict supervision on water quality and technical installations is indispensable to guarantee the health and safety of residents.

P141

The selective effect of antimicrobial residues in relation to resistant bacteria

Aram Swinkels[1], dr. ir. E.A.J. Fischer[1], dr. ing. B.J.A. Berendsen[3], prof. dr. J.A. Wagenaar[1,2], dr. A.L. Zomer[1]
[1]Utrecht University, , The Netherlands, [2]Wageningen university and Research Centre, , The Netherlands, [3]Wageningen University and Research Centre, , The Netherlands

Introduction
The global emergence of antimicrobial resistance (AMR) is a cause for concern. Use of antimicrobials contributes to AMR. A particular problem in the use of antimicrobials in livestock is that antimicrobial residues are excreted in the same environment in which the animals are kept. It is believed that after the application of antimicrobials, they are partly excreted through urine and feces and thereafter quickly degrade. However, some antimicrobials proved to be very persistent. In this study, we examined whether persistent antimicrobial compounds remain longer in the animal environment and if the remaining concentrations still have selective properties by determining the minimal selective concentration (MSC).

Methods
The MSC was determined through fecal fermentation studies. An animal trial was conducted to determine the persistence of the three antimicrobials over time. Four groups of broilers were divided in three subgroups (n=12). Groups were left untreated (control) or were treated with amoxicillin (non-persistent), doxycycline or enrofloxacin (persistent). We determined the resistome by shotgun metagenomics using Illumina sequencing and did an phenotypical resistance analysis of E. coli isolates. Antimicrobials were extracted from the fecal samples and analysed by LC-MS/MS.

Results
After treatment, persistent antimicrobials (doxycycline and enrofloxacin) had concentrations equal to or higher than the MSC established in the fermentation study, in contrast to the amoxicillin treatment. The doxycycline treatment showed an increase in phenotypically resistance and an increase in resistance genes in the resistome. In contrast, the amoxicillin treatment only showed an increase in the resistance genes directly after treatment. Enrofloxacin treatment resulted in merely enrofloxacin resistant E. coli and the resistance genes in the resistome increased during the experiment.

Conclusions
Persistent antimicrobials remain longer in the animal environment at selective concentrations. Our findings suggest that persistency of antimicrobials should be taken into consideration in the assessment of priority classification of antimicrobials.

P142

Tracking the development of cable bacteria in marine sediment and the effect on the microbial community.

Jesper Van Dijk[1], Philip Ley[1], Ines Cottignie[1], Jeanine S Geelhoed[1], Filip J R Meysman[1,2]
[1]University of Antwerp, Antwerp, Belgium, [2]Delft University of Technology, Delft, The Netherlands

Cable bacteria are filamentous bacteria that oxidize sulfide in the deeper sediment and reduce oxygen in the top layer of the sediment. This spatial separation of redox reactions creates a flow of electrons, or an 'electric highway'. Moreover, the unique metabolism of cable bacteria creates an electrical ecosystem and may result in interaction with other bacteria, either direct or indirect. However, our knowledge regarding the effect of cable bacteria metabolism on the microbial community is limited.
Therefore, we investigated the spatial and temporal development of cable bacteria and their effect on the microbial community. During the development of cable bacteria the microbial community may be affected due to the strong biogeochemical changes that cable bacteria exert on the sediment (indirect interspecies interactions) and potentially also due to direct interspecies electron transfer. We performed a time series experiment with sediment from Rattekaai salt marsh, with weekly analysis of biogeochemistry of the sediment as well as microbial community analysis using sequencing of 16S rRNA amplicons generated from both DNA and RNA.
The tracking of cable bacteria showed a low abundance in the sediment at the beginning of the incubation. After 35 days, the relative cable bacteria abundance reached a maximum cumulative abundance of 6.3% of 16S rRNA gene amplicon copies before both abundance and biogeochemistry showed a stagnating and lower activity of cable bacteria. Additionally, the composition of 16S rRNA reveals a much larger relative abundance of cable bacteria compared to the DNA-based abundance indicative of high cable bacteria activity. Our results confirm that cable bacteria have different developmental phases and have an effect on the microbial community. Cable bacteria development in the sediment started from low abundance, with growth over time down to 2.5 cm depth, and the development of a highly active electrical microbial community in the sediment.

P143

Towards understanding the synthesis of the key intermediates nitric oxide and hydrazine in anaerobic ammonium oxidation

Femke Vermeir[1], Suzanne Haaijer[1], Lotte Nijman[1], Robert Jansen[1], Laura van Niftrik[1], Wouter Versantvoort[1]
[1]Radboud Universiteit, Nijmegen, Netherlands

Anaerobic ammonium-oxidizing (anammox) bacteria convert substrates ammonium and nitrite to dinitrogen gas via the intermediates nitric oxide and hydrazine in the absence of oxygen. They are of great importance to nature as well as to industry where they contribute significantly to the loss of fixed nitrogen. In the first step of the anammox metabolism an unknown nitrite reductase enzyme reduces nitrite to nitric oxide. This nitric oxide is then combined with ammonium by the enzyme hydrazine synthase to form the 'rocket fuel' hydrazine. It is postulated that within the enzyme, hydrazine synthase first reduces nitric oxide to hydroxylamine. Subsequently, hydroxylamine diffuses through an intra-enzymatic tunnel to the second active site, where it is condensed with ammonium. Thus, hydrazine is formed in a two-step process. Finally, the hydrazine is oxidized to the end product dinitrogen gas. In this project, the enzymes involved in the formation of nitric oxide and hydrazine were examined by 1) enriching physiologically active nitrite reductase(s), and 2) investigating the molecular mechanism by which hydrazine is produced. Therefore, native nitrite reductase was enriched from the anammox species Kuenenia stuttgartiensis, and the production of nitric oxide from nitrite was observed in membrane and soluble protein fractions. To experimentally verify the two-step mechanism in which hydrazine is produced, native hydrazine synthase was anaerobically purified to homogeneity from K. stuttgartiensis and the second half reaction, i.e. the formation of hydrazine from hydroxylamine and ammonium, was measured. This study will shed light on our understanding of the anammox metabolism by characterizing the nitrite reductase(s) that provide nitric oxide for hydrazine production, and by experimentally elucidating the catalytic mechanisms of hydrazine synthase to form biological hydrazine.

P144

Genome evolution of Asgard archaea

Dr. Julian Vosseberg[1], T.J.G. Ettema[1]
[1]Wageningen University & Research, Wageningen, The Netherlands
Asgard archaea are a group of archaea that occupy an interesting position in the tree of life as they represent the closest prokaryotic relatives of eukaryotes. The eukaryotic branch likely sprouted from within the Asgard archaea. Eukaryotes are considered far more complex than prokaryotes and numerous gene duplications, transfers, inventions and fusions shaped the genomes of the emerging eukaryotes. To what extent these genetic innovations have shaped Asgard archaeal genome evolution is unclear. In this study we perform phylogenetic analyses to reconstruct ancestral genomes and illuminate the evolutionary genome dynamics of Asgard archaea. The first results of these reconstructions hint at an increased rate of gene duplications in Asgard archaea. This suggests that the mode of genome evolution of Asgard archaea is to some extent reminiscent of eukaryotes.

P145

ANME-2 archaea mediate metal-dependent anaerobic oxidation of methane in iron-rich coastal sediments

Anna Wallenius[1], A Andziak[1], GHL Nuijten[1], Dr P Dalcin Martins[1], Professor CP Slomp[1], Professor MSM Jetten[1]
[1]Radboud University, Nijmegen, The Netherlands

Anaerobic oxidation of methane (AOM) is an important filter of the greenhouse gas methane in coastal sediments. In brackish and metal oxide-rich sediments, both iron and manganese oxides may be potentially used as alternative electron acceptors in AOM, in addition to sulfate. Here, we studied the occurrence of metal-dependent AOM in oligotrophic, metal-rich and sulfate-poor Bothnian Sea. After a sampling campaign to obtain sediment cores of selected sites, we started batch incubations and long-term bioreactor cultures amended with reactive iron- and manganese oxides and isotopically labelled methane to determine the potential rates and electron acceptors. Furthermore, 16S rRNA amplicon sequencing and metagenomic analysis were used to identify the responsible microorganisms for AOM.
Results: Both manganese and iron oxides stimulated methane oxidation in the deeper sediments where metal oxides were buried. Simultaneous iron reduction and methane oxidation was recorded in the semi-continuous bioreactor four months after inoculation. (Meta)-genomic analysis revealed ANME-2a/b archaea as the most abundant anaerobic methane oxidizer in the selected depths, but methanogens seemed to persist in the bioreactor and might play a role in metal-AOM. Iron-reducing bacteria belonging to Desulfobulbaceae family was enriched over time and is a potential partner for ANME-2a/b in the metal-AOM process.
Conclusion: Together, the results highlighted the importance of metal-dependent AOM in brackish coastal sediments and suggest that ANME-2a/b are capable of mediating this process, either with or without a metal-reducing bacterial partner.

P146

Inhibitors as a tool to differentiate and select for bacterial or archaeal anaerobic methane oxidisers

Ir. Martijn Wissink[1], M. Van der Kolk[2], Prof. dr. ir. M.S.M. Jetten[1], Dr. C.U. Welte[1]
[1]Radboud University, Nijmegen, The Netherlands, [2]Radboud University, Nijmegen, The Netherlands

Introduction. In anoxic environments, methane can be oxidised to CO2 by methanotrophic microorganisms using various electron acceptors thus preventing the emission of this potent greenhouse gas. Methane oxidising bacteria can use either oxygen or nitrite and employ a methane monooxygenase, while methanotrophic archaea are able to use nitrate, metal-oxides, and in syntrophy sulfate as e-acceptor. The archaea rely on methyl-coenzyme M reductase and the reverse methanogenesis pathway for methane conversion. Anaerobic oxidation of methane (AOM) has so far mainly been studied in environmental samples or enrichment cultures. Inhibitors can help to study the contribution of specific methane oxidisers in AOM communities and help to obtain axenic cultures. Here, we investigated the influence of different inhibitors on bacterial and archaeal anaerobic methanotrophy.

Methods. Enrichment cultures of the archaeal nitrate reducing 'Ca. Methanoperedens nitroreducens' and the nitrite reducing bacteria 'Ca. Methylomirabilis oxyfera' were subjected to various (methanogenic) inhibitors and a range of antibiotics. Methane oxidation was measured by GC-FID.

Results. Using puromycin and methanogenic inhibitor 2-bromoethane sulfonate (BES) it was possible to selectively inhibit the archaeal methane oxidiser, while a combination of streptomycin, vancomycin, ampicillin and kanamycin inhibited the bacterial methane oxidiser. Based on the assays it appears that 'Ca. Methanoperedens' is responsible for 70±5% of the apparent methane oxidation rate. Interestingly, the methanogenic inhibitor 3-nitrooxypropanol (3-NOP) did not affect AOM while BES did.

Conclusion. Ultimately, the results of this study will help to understand the microbial interactions of 'Ca. Methanoperedens' and 'Ca. Methylomirabilis' in AOM communities, and can be used to further enrich and to isolate axenic cultures of 'Ca. Methanoperedens' or Ca. Methylomirabilis'.

P148

Predicted lipidomic profile based on genomic analyses of Nitrospina spp. isolated from various marine habitats

Dr. Subhash Yadav[1], Dr. Suzanne C. M. Haaijer[1], Dr. Hanna Koch[1], Ms Linnea Kop[1], Mr. Michel Koenen[2], Dr. Darci Rush[2], Dr. Sebastian Lücker[1]
[1]Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen, The Netherlands, Nijmegen, The Netherlands, [2]NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, P.O. Box 59, 1797AB Den Burg, Texel, The Netherlands, Texel, The Netherlands

Many lipids are synthesized by various microorganisms, but some lipids are unique to specific organisms or to specific biogeochemical processes and hence serve as biomarker lipids. Furthermore, intact polar lipids (IPLs) degrade rapidly upon cell death, indicating recent microbial activity. IPL analysis is a useful tool in microbial ecology, and it has been used successfully in a variety of settings, including marine sediments. Therefore, predicted lipids (core lipids, head groups of IPLs, and bacteriohopanepolyols), complemented with microbiological and molecular methods, can provide valuable information on microbial diversity and activity in marine habitats. The second step of nitrification in these systems is catalyzed by Nitrospina spp., which are chemolithoautotrophic nitrite-oxidizing bacteria with an important role in primary production in the dark ocean. Lipidomic analyses of a few species have been conducted but many lipids remained unidentified and, hence, in-depth analysis is needed. Here, we provide the predicted lipid profile of several Nitrospina species (N. watsonii 347, N. gracilis Nb-3, and N. gracilis Nb-211) based on genomic analyses. We found all the genes necessary for the production of C12:0, C14:0, C16:0, and C18:0, which indicates that they are important core lipids produced in these strains. Genes involved in the biosynthesis of phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylserine were identified, and the presence of genes like ornithine synthase and O-acetyltransferase suggests that under phosphate-limiting conditions, phosphate-reduced cell membranes are produced. 18 distinct genes are present in the genomes of all three strains, which might be responsible for the biosynthesis of diploptene, diplopterol, adenosylhopane, ribosylhopane, aminobacteriohopanetriol, and glycosyl group-containing and unsaturated hopanoids. Collectively, a diverse range of lipid biomolecules are predicted to be produced by Nitrospina spp., which could potentially serve as the biosignature of this taxon and provide information about the present and past nitrogen cycle in marine habitats.

P170

Enhanced and prolonged performance by transcription-mediated amplification in comparison with real-time qPCR for detection of Mycoplasma genitalium in urine

Miss Nikki Adriaens[1], Miss Anne-Marije Pennekamp[1], Dr. Alje van Dam[1,2], Dr. Sylvia Bruisten[1]
[1]GGD Amsterdam, Amsterdam, The Netherlands, [2]Amsterdam UMC, Amsterdam, The Netherlands

Background. Mycoplasma genitalium (MG) is a common causative pathogen of urethritis in males. A recommended method for MG detection in urine includes the use of nucleic acid amplification tests, such as qPCR and transcription-mediated amplification (TMA). A limitation of TMA is the short time span of 24 hours in which the collected urine should be transferred into an Aptima TMA tube, according to the manufacturer's guidelines. In our laboratory, urines received after >24 hours are tested using an in-house qPCR for MG detection, despite its lower sensitivity as compared to TMA. We aimed to compare the MG detection rate of TMA with qPCR after storage of urine samples at different temperatures overtime.

Methods. From June to December 2022, first-void urine samples from STI clinic visitors with a known timepoint of collection, were concomitantly kept at refrigerator (4-8 °C) and room temperature (18-24 °C) for 15 days. They were tested with both an in-house validated qPCR and with MG-TMA after transfer of the original urine samples to Aptima collection tubes at 3, 7, 12 and 15 days from both temperatures.

Results. In total, 47 MG-TMA positive urine samples were collected, stored and tested using qPCR and TMA at 4 follow up times. Molecular detection of MG showed a superior detection positivity rate by TMA compared with qPCR up to 15 days post collection. The temperature at which samples were stored between collection and testing did not have an effect on the molecular MG detection rate, for neither qPCR and TMA methods.

Conclusion. We showed that TMA was superior in detecting MG in urine compared with qPCR at all tested timepoints post sample collection. This indicates that urine samples older than 24 hours should preferably be tested using MG-TMA up to 15 days after sample collection.

P171

Promoting Fc-Fc interactions between anti-capsular antibodies provides strong immune protection against Streptococcus pneumoniae

Phd. Leire Aguinagalde[1], M. A. den Boer[2], S. M. Castenmiller[1], S. A. Zwarthoff[1], C. J. C Gosselaar-de Haas[1], P. C. Aerts[1], F. J. Beurskens[3], J. Schuurman[3], A.J. R. Heck[2], K.P. M. Kessel[1], S. H. M Rooijakkers[1]
[1]University Medical Center Utrecht, Utrecht , The Netherlands, [2]Biomolecular Mass Spectrometry and Proteomics, UTrecht, The Netherlands, [3]Genmab, Utrecht, The Netherladns
Introduction: Monoclonal antibodies (mAbs) that boost the host immune system have emerged as an attractive therapeutic candidates for the treatment of multidrug-resistant pathogens or emerging non-vaccine serotypes. An important effector function of antibody-dependent immunity is the induction of complement activation, a reaction that triggers a variety of immune responses that induce bacterial phagocytosis and intracellular killing by professional phagocytes. Recent studies have shown that specific point mutations in the Fc domain of antibodies can enhance antibody clustering into hexameric structures, required for a more efficient complement activation. This study examines the efficacy of capsule-specific human mAbs as a therapeutic approach against S. pneumoniae, a leading cause of community-acquired pneumonia and an important cause of childhood mortality.

Methods and results: We produced specific mAbs against pneumococcal capsule polysaccharide (CPS) serogroup 6. Using flow cytometry assays, we showed that CPS6-IgG1 harboring the hexamer-enhancing E430G or E345K point mutations potently increase complement activation and phagocytosis of pneumococcal clinical isolates of this serogroup. Bacterial killing assays demonstrated the strong potency of engineered antibodies to induce neutrophil-dependent killing of S. pneumoniae serotype 6B. Moreover, we compared the protective capacity of serogroup-6 specific IgG1 wildtype antibody with its counterpart E345K variant against a mouse model of bacteremic pneumonia. Passive immunization with the complement-enhancing CPS6-IgG1-E345K mutant protected mice from developing sever pneumonia.

Conclusion: This study demonstrates that when human mAbs against pneumococcal capsule have a poor capacity to induce complement activation, their activity can be strongly improved by hexamerization-enhancing mutations. Altogether, the presented work provides an important proof-of-concept for future optimization of antibody therapies against encapsulated bacteria and represents a first systematic approach to design effective therapeutic antibodies against S. pneumoniae with increased potency to activate the human complement system.

P173

High-throughput screening of microbial consortia using microdroplet sorting

Sagarika B Govindaraju[1], Dr. Ir. RJ van Tatenhove-Pel
[1]TU Delft, Delft, The Netherlands

Defined microbial consortia are rarely used in industrial biotechnology. However, in nature, microorganisms co-exist and interact with each other, suggesting that cooperation provides a fitness advantage. By identifying beneficial microbial interactions in nature, we could design novel synthetic microbial consortia for industrial processes. We hypothesize that by using high-throughput screening and selection systems we can decipher interactions between microbes.

Water-in-oil emulsions are a promising high-throughput cultivation tool, as a millilitre of emulsion contains millions of microdroplets. Due to absence of diffusion between microdroplets, each microdroplet acts as an individual cultivation system which can be inoculated by a single cell or combinations of cells. After inoculation, the cells grow and interact with each other. Beneficial interactions result in higher cell concentrations than competitive or inhibitory interactions. Using microdroplet sorting, we could identify consortia with beneficial interactions and selectively enrich for them.

Microdroplet cultivation coupled with sorting could drastically improve the throughput of screening compared to conventional cultivation systems such as shake-flasks and microtiter plates. For a 10 member consortium, to screen all possible combinations of consortium members, we would need 1023 individual cultivation systems. Using a few water-in-oil emulsions, we could in-parallel screen all 1023 combinations of a 10 member consortium and enrich for sub-consortia with beneficial interactions by sorting.

Before we can sort microdroplets with consortia of interest, we first need to develop a workflow to sort microdroplets using FACS. We inoculated microdroplets with a mixture of Lactococcus cremoris MG1363 (92.58%) and L. cremoris MG1363-GFP (7.42%). With 1 hour of sorting, we isolated 59 cells of interest from a starting population of 2·106 cells. Using FACS sorting, we enriched L. cremoris MG1363-GFP from 7.42% to 69.41%. This shows that with our workflow we can enrich for populations of interest that are present in low fractions in the inoculum.

P174

Development of diagnostic and antimicrobial triggered release systems for wound dressings

Ms Gizem Babuccu[1], Ms NV Vavilthota, Dr J.W.D. Drijfhout, Dr R.A.C. Cordfunke, Dr CB Bournez, G.J.P.W. Westen, Dr MR Riool, Assoc. Dr. SAJ Zaat
[1]University of Amsterdam, Amsterdam, the Netherlands

Healthcare-associated infections (HAIs) can be acquired in any healthcare facility. Increasing morbidity, prolonged hospital stay, and increased treatment costs are some of the consequences of HAIs. Thus, there is a critical need for novel antimicrobial strategies and products for emerging antibiotic resistance and the search for cutting-edge infection detection and treatment systems detection systems (theranostics).
This project aims to create rapid screening systems for theranostic antimicrobial devices and to analyze the effectiveness of novel antimicrobial peptides (AMPs)1 that can be used in such devices for wound dressings.

A novel set of peptides was designed using artificial intelligence and synthesized, and their antimicrobial activities under physiological conditions were analyzed against planktonic Staphylococcus aureus JAR060131 and multidrug-resistant Acinetobacter baumannii RUH875. The lethal concentration killing 99.9% of the inoculum (i.e. LC99.9) of these peptides ranged from 0.94-15 µM in the presence of 50% human plasma. The efficacy of selected lead peptides (i.e. AMP-038 and AMP-045) and their retro-inverso (RI) variants were also compared to promising AMPs in the preclinical/clinical phase of development. Moreover, within 2 hours, 60 µM of AMP-038 and 30 µM of AMP-045 showed more than 3-log reduction against biofilm-encased S. aureus. Because resistance development is one of the major concerns, we assessed whether S. aureus and A. baumannii developed resistance to the lead peptides. While the MIC of S. aureus for rifampicin, and of A. baumannii for ciprofloxacin increased ≥4096-fold and >256-fold, respectively, no significant change in MIC was observed when the strains were cultured in the presence of AMP-038 (-RI) and AMP-045 (-RI) peptides for more than 20 passages.

In conclusion, we showed the antimicrobial and antibiofilm efficacy of the candidate peptides, their lack of resistance development, and the rapid action of these AMPs which contribute to the death of the bacteria within minutes

P175

Viral load as a quantification measure instead of the cycle threshold value in hospitalized patients infected with severe acute respiratory syndrome coronavirus 2.

Ines Begovic[1], P van Wunnik[1], Dr. LC Smeets[1]
[1]Reinier Haga Medical Diagnostic Center, Delft, The Netherlands

Introduction: Cycle threshold (Ct) values of the real-time reverse transcription-polymerase chain reaction (RT-PCR) are often used to estimate the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral quantity in a sample. However, the Ct value is not a standardized marker. We aim to establish standardized viral loads in nasopharyngeal swabs in order to understand the range of viral load that can be present. Next, we try to compare the viral loads found in different groups of different patient groups and correlate them with clinical outcome.

Methods: The cobas® SARS-CoV-2 Duo kit is an automated real-time RT-PCR assay and is used for the in vitro qualitative (Ct value) and quantitative detection (International Units per milliliter) of SARS-CoV-2 Ribonucleic acid (RNA) in collected nasal and nasopharyngeal swabs from individuals suspected of Coronavirus Disease 2019 (COVID-19).
Patient samples: we compared admission samples of 52 hospitalized emergency room patients with 277 matched controls from the Community Health Service (GGD), and 14 deceased patients with a control group of 123 living patients.

Results: Hospitalized patients have upon presentation at the emergency room an average viral load of 2.88E+07 IU/ml (range: from 2,92E+02 IU/ml to 1,37E+08 IU/ml), with a standard deviation (SD) of 5.21E+07 IU/ml. The corresponding control group, the Community Health Service (GGD), has an average viral load of 5,01E+07 IU/ml with a SD of 7,15E+07 IU/ml.
Deceased patients have upon presentation an average viral load of 4,91E+06 IU/ml (range: from 1,39E+02 IU/ml to 1,82E+07 IU/ml), with a SD of 8,88E+06 IU/ml. The corresponding control group, patients that survived SARS-CoV-2, have an average viral load of 4,18E+07 IU/ml with a SD of 6,40E+07 IU/ml.

Conclusion: Average viral loads upon presentation differ from 4,91E+06 IU/ml to 2.88E+07 IU/ml. There are no statistically significant differences between patient groups.

P176

Comparison of three real time PCR assays for the detection of adenovirus in clinical samples

Maarten Bongaerts[1], R Alsheghri[1], Dr. ER Heddema[1], Dr. CFM Linssen[1]
[1]Zuyderland Medical Centre, Sittard- Geleen, Nederland

Introduction: adenoviruses are DNA viruses which can cause a range of diseases in humans including pneumonia, gastroenteritis and keratoconjunctivitis.
Methods: two in-house laboratory developed tests (LDTs) based on manuscripts by Wong et al. and Damen et al. were compared to a commercially available real time PCR (AltoStar Adenovirus PCR kit 1.5; Altona Diagnostics GmbH). DNA purification was performed by QiaSymphony (Qiagen) and real time PCR by Rotor-Gene (Qiagen). A lower limit of detection (LLOD) is determined on fecal samples, respiratory samples and eye-swabs. Using Quality Control for Molecular Diagnostics (QCMD) samples a qualitative inter-assay agreement was assessed by pairwise comparison of test results from the three PCRs by calculating the crude percent agreement.
Results: The AltoStar kit showed superior LLOD in feces compared to both LDTs: 100 copies/PCR versus 2000 copies/PCR. In eye-swabs and respiratory samples the LLOD of both the AltoStar kit and the Wong PCR was 100 copies/PCR whilst the PCR described by Damen showed a LLOD of 2000 copies/PCR in eye-swabs. Unfortunately, no results could be generated using the PCR described by Damen in respiratory samples. The highest agreement was found between the AltoStar kit and the Wong PCR with a percentage agreement of 100%. The agreement between the AltoStar kit and the Damen PCR and the Wong PCR and the Damen PCR was 90%.

Conclusion: Both the AltoStar PCR as the PCR described by Wong showed good results in all tested materials. The AltoStar kit showed the lowest LLOD in all tested materials.

P177

The ethnically diverse Dutch gut microbiome from birth until old age: a cross-sectional TRiuMPH-2 study

David Boverhoff[1]
[1]National Institute for Public Health and the Environment, Bilthoven, The Netherlands, [2]University Medical Center Groningen, Groningen, The Netherlands
Introduction: Aging and ethnical background have a major impact on the human gut microbiome. Here, we present a novel attempt investigating associations of gut microbiome diversity, uniqueness and composition from birth until advanced age (0 – 87 years) in an ethnically diverse sample of Dutch individuals (n = 3692). Methods: 16S rRNA gene sequencing was performed on cross-sectionally collected stool samples together with extensive questionnaire data. Gut microbiome properties were compared such as Shannon diversity, uniqueness (Bray-Curtis dissimilarity) and composition. PERMANOVA and multiple linear regression were used to determine associations between gut microbiome features/properties and a collection of variables covering demographic, diet, health, medication, environmental and ethnical background information. Results: Shannon diversity steadily increased during youth which peaked at ~10 years and plateaued at ~25 years old that persisted throughout adulthood. In adults, the highest proportion of variance explained in Shannon diversity and community structure were predominantly positively related with having a Dutch father. Antibiotic usage showed the highest number of significant associations with several genera. In participants of 25 – 64 years old, Akkermansia was negatively associated obesity and Christensenellaceae R−7 group showed a similar negative relationship with obesity, diabetes and fever. Conclusion: Strikingly, the gut microbiome diversity peaks at ~10 years of age as opposed to the widely accepted first years of life in Western populations. We confirmed previously described links of health associated genera with multiple health-related symptoms. To date, this is the most comprehensive characterization of the gut microbiome in an ethnically diverse and representative sample of the Dutch population.

P178

Variation in the capsular structure of serotype 9 Streptococcus suis leads to differences in virulence

Mr. Jaime Brizuela Gabaldón[1,2], Ms. Ruth Bossers-deVries[3], Mr. Thomas J. Roodsant[1,2], Prof. Constance Schultsz[1,2], Dr. Manouk Vrieling[3]
[1]Amserdam UMC, Amsterdam, The Netherlands, [2]Amserdam UMC, Amsterdam, The Netherlands, [3]Wageningen Bioveterinary Research, Lelystad, The Netherlands

Introduction: Streptococcus suis is an emerging zoonotic swine pathogen which causes severe infections in both pigs and humans. S. suis has 31 serotypes based on its capsular polysaccharide (CPS) structure. In Europe, serotype 9 (SS9) S. suis has become a major swine pathogen. Within SS9, there are genetically distinct carriage and invasive S. suis subpopulations. A specific allelic variant of the CPS glycotransferase gene cps9K has been associated with strains that cause invasive disease in Dutch piglets, suggesting that the CPS locus plays a role in virulence. We aimed to characterize the differences in CPS structure between the carriage and invasive SS9 S. suis populations and elucidate its contribution to virulence in pigs.
Methods: We reconstructed a global SS9 S. suis phylogeny based on 188 publicly available genomes and investigated allelic variation in the CPS biosynthesis locus between invasive and carriage populations. In-frame deletion mutants of each glycosyltransferase gene were generated in both invasive and carriage strains. Differences in CPS structure were tested using fluorescently labelled lectins. Isolates were assessed for differences in survival in an opsonophagocytosis and killing assay with porcine neutrophils.
Results: The global SS9 S. suis population was highly diverse, with multiple carriage and clinical subpopulations. Invasive populations shared distinct alleles across all five glycosyltransferase genes (cps9F, cps9G, cps9H, cps9I and cps9K) and displayed a different side chain of the CPS in the lectin binding assay, when compared to carriage isolates. Carriage isolates were readily killed by porcine neutrophils, while invasive isolates were resistant to neutrophil killing and required opsonisation with SS9-specific antibodies to be killed.
Conclusions: Despite the high genomic diversity of SS9 S. suis, distinct alleles of five CPS glycosyltransferase genes correlated with virulence in pigs. Invasive and carriage SS9 S. suis differed in capsule structure, and in survival during phagocytosis by porcine neutrophils.

P179

Effect of anticoccidial approaches on the poultry gut microbiome

Mr. Matteo Buffoni[1,4], J. Kerkvliet[1], H. Enting[2], J.v.d. Stroom[3], M. Rogers[1], J.A.G.M. de Visser[4], F. Paganelli[1], R.J.L. Willems[1], A.C. Schürch[1]
[1]UMC, Utrecht, The Netherlands, [2]Cargill, Schiphol, The Netherlands, [3]Elanco Animal Health Nederland, Utrecht, The Netherlands, [4]Wageningen University & Research, Wageningen, The Netherlands

The gut microbiome refers to all bacteria that colonize the human and animal intestinal tract. It is a reservoir of antimicrobial resistant genes, collectively termed the resistome. In farm animals the taxonomic composition of the gut microbiome is strongly shaped by diet and feed additives. Here, we explore the influence of coccidiosis prevention strategies on the taxonomic composition and resistome of gut microbiome of broilers.

Caeca material was collected from 100 broilers from a single German farm. All broilers were 21 days old, originated from the same hatchery and parental flock, with the same housing and feed and formed two consecutive flocks. Half of the animals were treated with a coccidiostat (Maxiban) and the other half was vaccinated (Paracox-5) against coccidiosis. DNA was extracted and sequenced using a NovaSeq. MetPhlAn 4 was used for taxonomic classification and the MetaMobilePicker (www.metamobilepicker.nl) pipeline was used for resistome determination.

Total bacterial diversity was consistent between the two groups, but significant differences in taxonomic composition were detected. The vaccinated group showed a higher Firmicutes:Bacteroidetes ratio than the coccidiostat group. Also, a higher number of Lactobacillus and Faecalibacterium were found in this group while Alistipes and Bifidobacterium had higher abundance in the coccidiostat-treated samples. Although the comparison of the total resistome composition between the two groups is still pending, an operon encoding for narasin resistance (narAB), was detected significantly more often (15 out of 48 times) in coccidiostat-treated than vaccinated samples (7 out of 47 times) (Fisher's test, p=0.0334).

The two groups show clear differences in microbiome composition. Coccidiostat treatment is associated with a less gram-positive rich microbiome and promote a higher maturation of the gut bacterial composition. Furthermore, higher presence of the narAB operon suggests selection of reduced ionophore susceptibility in the coccidiostat-treated group. Further analysis on resistome composition is currently ongoing.

P180

A comparison of five Illumina, Ion torrent, and nanopore sequencing technology-based approaches for whole genome sequencing of SARS-CoV-2

Ellen Carbo[1,2], K. Mourik[1], SA Boers[1], B. Oude Munnink[3], D. Nieuwenhuijse[3], M. Jonges[2], MRA Welkers[2], S. Matamoros[2], J. van Harinxma thoe Slooten[1], MEM Kraakman[1], E. Karelioti[4], D. van der Meer[4], KE Veldkamp[1], ACM Kroes[1], I. Sidorov[1], JJC de Vries[1]
[1]Leiden Universitair Medisch Centrum, Leiden, , [2]Amsterdam Universitair Medische Centra, Amsterdam, , [3]Erasmus Medisch Centrum, Rotterdam, , [4]Genomescan, Leiden,
Rapid identification of the rise and spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern remains critical for monitoring of the efficacy of diagnostics, therapeutics, vaccines, and control strategies. A wide range of SARS-CoV-2 next-generation sequencing (NGS) methods have been developed over the last years, but cross-sequence technology benchmarking studies are scarce.
In the current study, 26 clinical samples were sequenced using five protocols: AmpliSeq SARS-CoV-2 (Illumina), EasySeq RC-PCR SARS-CoV-2 (Illumina/NimaGen), Ion AmpliSeq SARS-CoV-2 (Thermo Fisher), custom primer sets (Oxford Nanopore Technologies, ONT), and capture probe-based viral metagenomics (Roche/Illumina). Studied parameters included genome coverage, depth of coverage, amplicon distribution, and variant calling.
The median SARS-CoV-2 genome coverage of samples with cycle threshold (Ct) values of 30 and lower ranged from 81.6 to 99.8% for, respectively, the ONT protocol and Illumina Ampliseq protocol. Correlation of coverage with PCR Ct-values varied per protocol. Amplicon distribution signatures differed across the methods, with peak differences of up to 4 log10 at disbalanced positions in samples with high viral loads (Ct-values ≤ 23). Phylogenetic analyses of consensus sequences showed clustering independent of the workflow used. The proportion of SARS-CoV-2 reads in relation to background sequences, as a (cost-)efficiency metric, was highest for the EasySeq protocol. The hands-on time was lowest when using EasySeq and ONT protocols, with the latter additionally having the shortest sequence runtime.
In conclusion, the studied protocols differed on a variety of the studied metrics. This study provides data that assist laboratories when selecting protocols for their specific setting.

P181

The relative role of neutrophils and macrophages in elimination of Staphylococcus aureus

Leonardo Cecotto[1,2], K.P.M van Kessel[1], L de Vor[1], C Passerini[1], C.H. Vogely[2], B.C.H. van der Wal[2], H.H. Weinans[2], S. Amin Yavari[2], J.A.G. van Strijp[1]
[1]UMC Utrecht, Utrecht, The Netherlands, [2]UMC Utrecht, Utrecht, The Netherlands

Background. Staphylococcus aureus is known for its ability to survive antibiotic treatment and immune cells recognition and killing, often by hiding and surviving within host cells. Monoclonal antibody therapy could represent a valid therapeutic alternative to antibiotics, providing host immune cells more effective tools against invading pathogens. Therefore, we aimed to characterize and compare the phagocytic capacity and dynamics of neutrophils versus macrophages, key players involved in the innate immune response, under the same conditions in vitro.

Methods. Neutrophils and macrophages were incubated in suspension together with S. aureus opsonized with different concentrations of serum and monoclonal antibodies (mAb). The phagocytic ability of the two immune cells was determined by flow cytometry and microscopy analysis.

Results. Both neutrophils and macrophages showed a serum-concentration dependent phagocytosis of S. aureus. Bacterial uptake could be further enhanced in both cell types after opsonization with specific mAbs targeting the wall teichoic acid or SDR-proteins expressed on the bacterial membrane. However, expression of protein A by S. aureus jeopardized IgG1 mAbs-induced phagocytosis, which was slightly improved only at concentrations more than 10-times higher than IgG3 class of mAbs. Finally, the highest bacterial uptake in both cell types was recorded after S. aureus opsonization with complement proteins and IgG3-mAbs.

Conclusions. Opsonization with complement proteins and mAb influenced S. aureus recognition and phagocytosis in both immune cells, with neutrophils taking up more pathogens than macrophages. Further studies are needed to characterize and compare the killing ability of each cell type. Accordingly, bispecific mAb might be designed to influence bacterial uptake preferably to one cell type, hence reducing the risk of recalcitrant infections.

P182

Intercontinental travellers' gut microbiota analyses reveal altered gut microbiota preceding the onset of irritable bowel syndrome

Mr Jiyang Chan[1]
[1]Maastricht University, Maastricht, The Netherlands, [2]Gianlucca Galazzo, Maastricht University, The Netherlands, [3]Markia Ward, Maastricht, The Netherlands, [4]Perry van Genderen, Rotterdam, The Netherlands, [5]Niels van Best, Maastricht, The Netherlands, [6]John Penders, Maastricht, The Netherlands

Introduction: Travel related exposures often lead to gastrointestinal complaints. Consequently, several travellers report the onset of irritable bowel symptoms (IBS) after traveller's diarrhoea (TD) which is denoted as post-infectious IBS (PI-IBS). To what extent microbiota alterations precede or are a consequence of the infectious episode remains undescribed. We prospectively characterized the faecal microbiota diversity and community structure in Dutch intercontinental travellers with and without IBS development after TD.
Methods: Our study included 637 travellers and a nested case-control group of 104 travellers within a large longitudinal cohort among travellers. Cases were defined as healthy travellers (without gastrointestinal symptoms at baseline) who experienced diarrhoea during the index travel and met the ROME III criteria for IBS at 6-12 months after travel return. For each case, we matched one control based on age, gender and travel destination. Faecal samples collected pre-travel (T0), immediately post-travel (T1) and 1-month post-travel (T2) were profiled by 16S rRNA gene amplicon sequencing to examine the microbial diversity, composition and community structure.
Results: International travel contributes to the perturbation of the gut microbiota with antibiotics use during travel having the largest influence. 52 Subjects met our case definition for PI-IBS and were matched to 52 controls. Microbial richness prior to the onset of PI-IBS was significantly lower in future cases compared to controls. No difference was observed in the etiological agents for TD between cases and controls. The microbial community structure of cases was significantly different compared controls at T0, T1 and at T2 (PERMANOVA $P< 0.05$ for all time-points). Differential abundance testing indicated increased levels of Eggerthella and decreased level of Lachnospiraceae NK4A136group in cases compared to controls.
Conclusions: We identified an altered microbiota profile preceding the onset of PI-IBS. These results strengthen the evidence for a causal role of the microbiota in the pathophysiology of IBS.

P183

The surface-exposed TypeVII substrate PPE51 facilitates nutrient uptake in Mycobacterium marinum

Vicky Charitou[1], BI Izquierdo Lafuente[2], WB Bitter[1], AS Speer[1]
[1]Amsterdam Umc, Amsterdam, Netherlands, [2]Vrije University, Amsterdam, Netherlands

Mycobacteria have a unique and highly impermeable cell wall that functions as their first line of defense against antibiotics. However, the bacteria must specifically permeabilize the cell wall to allow nutrient uptake. Identifying and characterizing proteins that facilitate nutrient uptake might enable us to understand and improve the uptake of drugs across the cell wall. PPE51, a member of the proline-proline-glutamate PPE family, was shown to be involved in nutrient uptake in Mycobacterium tuberculosis and served as a starting point for this study.

In this work, we focus on Mycobacterium marinum as a model organism for M. tuberculosis, to study the function, secretion, and localization of PPE51.

Deletion of PPE51 and its paralogs hampered M. marinum cells from growing on glucose and glycerol as the sole carbon source in culture. However, zebrafish infection experiments revealed that this growth defect did not impair infectivity in vivo, as there was no difference in virulence between PPE51 knock-out and wild-type. Regarding secretion, we identified PPE51 as an ESX-5 substrate of the type VII secretion system, which is in line with its role in nutrient uptake. Using flow-cytometry, we identified the protein as surface localized and determined PPE51 to be membrane–anchored as we extracted the secreted protein with mild detergents such as Genapol X080. Protein extraction with mild detergents allowed us to study PPE51 protein complex formation, and we observed that the protein might form oligomers but not high-molecular-weight protein complexes.

To conclude, in this study, we further characterized the function and secretion system of PPE51 using the model organism M. marinum. We showed that PPE51 is anchored to the outer membrane and surface exposed. The molecular mechanism of how nutrients are transported across the cell wall remains to be elucidated.

P184

The ability to switch between a walled and cell wall-deficient lifestyle in Escherichia coli: A Blessing or a Curse?

Marjolein Crooijmans[1], Prof Dennis Claessen
[1]Leiden University, Leiden, The Netherlands

The cell wall is a fundamental structure that protects bacterial cells from external stress factors and environmental changes. However after exposure to chemical or enzymatic cell wall targeting agents, certain bacteria are capable of shedding their cell wall and proliferating in osmoprotective conditions. This ability may have important implications for their survival in industrial environments and medical applications, but also for the development of antibiotic resistance. The mechanisms underlying the proliferation of Gram-negative wall-less bacteria remain poorly understood. Recent studies suggest that the accumulation of oxidative damage can inhibit the proliferation of wall-deficient cells. This can be overcome through mutations that reduce reactive oxygen species production and increase membrane blebbing.

We have engineered an E. coli strain that is able to proliferate with or without a cell wall to further investigate this extraordinary growth mechanism. This strain enabled us to study not only the membranes of Gram-negative bacteria, but also the transition between a walled and a wall-less state, and the various types of stressors that are involved in becoming L-forms. Notably, certain pathways related to the wall, such as biofilm formation, were found to be upregulated. Our research has established that lacking a cell wall can have both positive and negative effects on the growth and survival of E. coli and these findings provide novel insight into the mechanism of cell growth without a wall.

P185

Mycobacteria form viable cell wall-deficient cells that upregulate protection against toxic oxidative damage.

Msc. Noortje Dannenberg[1], MSc. T. Weijers[1], MSc. J. Kolk[1], MSc. T. O. Delzenne[1], BSc. C. Santos Gonçalves[1], Dr. V. J. Carrion Bravo[1,2,3], Prof. Dr. H. P. Spaink[1], Prof. Dr. T. H. M. Ottenhoff[4], Prof. Dr. A. Briegel[1,5], Prof. Dr. D. Claessen[1]

[1]Leiden University, Leiden, The Netherlands, [2]Universidad de Málaga, Málaga, Spain, [3]Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands, [4]Leiden University Medical Center (LUMC), Leiden, The Netherlands, [5]Leiden University, Leiden, The Netherlands

The cell wall is a unifying trait in bacteria and provides protection against environmental insults. Therefore, the wall is considered essential for most bacteria. Despite this critical role, many bacteria can transiently shed their cell wall and recent observations suggest a link of such wall-deficient cells to chronic infections. Whether shedding the cell wall also occurs in mycobacteria has not been established unambiguously. In this study we establish several tools and assays to consistently detect and observe mycobacterial cell wall deficiency. Here we provide compelling evidence that a wide range of mycobacterial species, including Mycolicibacterium smegmatis, Mycobacterium marinum, Mycobacterium avium, Mycobacterium bovis BCG and mycobacterial clinical and endophytic isolates, form viable cell wall-deficient cells in response to environmental stressors. Aside to cell wall hydrolyzing enzymes from the host, these stressors include hyperosmotic stress, cell wall-targeting antibiotics and the amino acid glycine, in which supplementation of the magnesium cation has a strong effect on the stability of the wall-deficient cells. Using cryo-transmission electron micrography we show that the complex multi-layered wall is largely lost in such cells. Confocal microscopy and flow cytometry prove the presence of DNA and an intact plasma membrane through propidium iodide staining, and viability is confirmed through detergent treatment quantified with colony counting. Transcriptomic analysis elucidates further insight in the mycobacterial cell wall-deficient life style, such as strong upregulation of catalases/peroxidases (KatG) to protect the cell against toxic reactive oxygen species, which provides potential candidate genes to study this phenomenon. Given that these wall-deficient mycobacteria are undetectable using conventional diagnostic methods, which are typically hypotonic, such cells have likely been overlooked in clinical settings.

P186

Genotyping of Sporothrix brasiliensis reveals high genetic diversity and numerous independent zoonotic introductions in Brazil

Phd Theun De Groot[1,2], BSc B. Spruijtenburg[1,2], PhD A. Bombassaro[1,2], PhD E.F.J. Meijer[1,2], PhD M.E. Grisolia[3,4], PhD V.A. Vicente[3,4], PhD F. de Queiroz-Telles[5], PhD J.F. Meis[1,2,3]
[1]Canisius Wilhelmina Ziekenhuis, Nijmegen, The Netherlands, [2]Radboud University Medical Center/Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands, [3] Federal University of Paraná, Curitiba, Brazil, [4]Federal University of Paraná, Curitiba, Brazil, [5]Federal University of Paraná, Curitiba, Brazil

Sporothrix brasiliensis is the causative agent of human and animal sporotrichosis. Outbreaks, foremost driven by infected cats, were first described in Rio de Janeiro, Brazil and subsequently in adjacent states and countries, possibly involving clonal spread. In order to improve our understanding of the current S. brasiliensis epidemic, we genotyped 173 clinical S. brasiliensis isolates from Brazil, isolated mostly from humans and cats. For this purpose a novel short tandem repeat (STR) genotyping scheme was developed and whole genome sequencing (WGS) analysis was used to determine single nucleotide polymorphism (SNP) differences, mating type loci and genomic population metrics. STR analysis resulted in the identification of 62 different genotypes and ten clusters comprised of more than two isolates. The majority of isolates were highly related according to STR typing and were defined as the Rio de Janeiro (RdJ) clade. STR typing results were compared to WGS SNP analysis of 21 isolates and this correlated highly. SNP differences between isolates ranged from 13 to over 200,000 SNPs, while isolates from the RdJ clade (n=9) differed less than 150 SNPs. Molecular clock analysis estimated the divergence of the sequenced isolates around 51,700 BC while the time to the most recent common ancestor (TMRCA) of the RdJ clade was set to 1995. The majority of isolates outside the RdJ clade exhibited a TMRCA of more than 2000 year. Altogether, we describe the application of a novel and high-resolution STR genotyping scheme, validated by WGS data, to study the hitherto unknown high genetic diversity of S. brasiliensis. While clonal spread across vast distances was observed for isolates allocated to the RdJ clade, various other isolates demonstrated a high genetic diversity and long TMRCA. The latter observation suggests numerous independent introductions in feral populations, as S. brasiliensis infections have only emerged during the last decades.

P187

A large Dutch cluster of tuberculosis analyzed using acquired and mixed single nucleotide polymorphisms comparison with the epidemiological findings, the details matter.

Msc E Ubbelohde[1,2], R De Zwaan[1], A Mulder[1], M Kamst[1], MD PhD G De Vries[3], K Rebel[4], S.N.S Kautz[5], Prof (em). Dr. D Van Soolingen[1], PhD K Kremer[2], Dr R.M Anthony[1]
[1]National Institute For Public Health And The Environment (RIVM), Bilthoven, The Netherlands, [2]KNCV Tuberculosis Foundation, The Hague, The Netherlands, [3]RIVM, Bilthoven, The Netherlands, [4]Municipal Public Health Services, Amsterdam, The Netherlands, [5]Municipal Public Health Services, Rotterdam, The Netherlands

Introduction: Tuberculosis (TB) is caused by Mycobacterium tuberculosis complex species and is still one of the deadliest infectious diseases worldwide. In the Netherlands, TB is a notifiable disease and transmission is investigated by Municipal Public Health Services (MPHSs). Since the early 1990s, source- and contact tracing to investigate TB transmission has been supported by DNA typing. Throughout the years the resolution of DNA typing within the M. tuberculosis complex has improved, it is currently based on whole genome sequencing (WGS). Most TB cases are unique or in small clusters. Occasionally, large clusters spanning many years occur. In this study, the WGS data of a large cluster was examined in detail to infer transmission routes which were subsequently compared to the epidemiological findings.
Methods: All culture positive isolates in the Netherlands were subjected to DNA typing by using Ilumina sequencing and clustered cases reported to MPHSs, who investigate clustered cases by means of interviews. A large cluster, consisting of more than 150 cases spanning over 20 years, was selected for detailed analysis in this study. From this cluster, WGS data was available from 43 cultures isolated between 2003-2022. The “cluster-specific” SNPs, generated automatically by our inhouse sequencing analysis pipeline were manually evaluated and used to predict a transmission scheme (blinded to the epidemiological data). Subsequently, this scheme was compared with the epidemiological data.
Results: Of the 60 cluster-specific SNPs generated by the pipeline, 50 SNPs were considered to be confident after manual screening. Three SNPs were additionally identified as a minority population in the cluster. The links proposed on the basis of WGS data alone were consistent with the epidemiological data.
Conclusion: The analysis of WGS typing data was optimized to better identify transmission routes by detailed analysis of the accumulated SNPs and screening for minority populations of confidently called SNPs.

P188

Investigation of infant microbiome colonization trajectories in LucKi Gut study cohort using whole metagenome sequencing

MSc Evgenia Dikareva[1], Mr NvB Best[1], Ms M Mommers[2], Mr G Le[1], Ms C Driessen[1], Mr J Penders[1]
[1]Maastricht University, Maastricht, Netherlands, [2]Maastricht University, Maastricht, Netherlands

Background and aims: Human gut microbiome (GM) is long known to have an impact on its host health and well-being. Infancy is a crucial time period, when perturbations in the GM establishment can predispose towards diseases later in life. Here we aim to investigate the neonatal GM maturation with the help of frequently collected samples. The large number of time points is an advantage of LucKi cohort in comparison with previous studies.

Methods: Fecal samples from 133 mother (1 week post-partum) and their infant (1, 4, and 8 weeks, 4,5, 6, 9, 11 and 14 months) were collected and profiled using whole-metagenome sequencing. After pre-processing, the samples' (n = 779) taxonomical composition from all nine time points was analyzed on genus and species levels.

Results: Bacterial diversity was stable until 8 weeks, and thereafter increased with age, however was still lower when compared to maternal samples by the time the infants turned 14 months. The infant GM was dominated by Bifidobacteria up to 9 months of age, while Bacteroides were present at all time points. While facultative aerobes were abundant during the first weeks of life, they gradually (Escherichia and Klebsiella) declined towards 14 months or rapidly disappeared (Enterobacter). Other bacterial specialists appeared after the first months (Akkermansia at 4 months, Faecalibacterium at 6 months and Lachnospiraceae at 9 months). An ordination analysis of compositional data showed clear separation by age group. A community typing revealed 6 clusters and transitional modeling showed that majority of the neonatal GM belonged to clusters 1, 3, 5 and 6.

Conclusion: The metagenomic sequencing allowed to investigate microbiome maturation with a resolution on species level. The results of metagenomic sequencing were consistent with previous findings based on 16S rRNA sequencing. The next step will be identifying factors responsible for variation in GM trajectories.

P189

Using the SpyTag/SpyCatcher system to determine periplasmic localization of proteins in mycobacteria

Yuwei Ding[1], Dr. CM Bunduc[1,2,3,4], Prof. Dr. W Bitter[1,5], Dr. ENG Houben[1]
[1]Vrije Universiteit, Amsterdam, Netherlands, [2]Centre for Structural Systems Biology, Hamburg, Germany, [3]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [4]German Electron Synchrotron Centre, Hamburg, Germany, [5]Amsterdam UMC, Amsterdam, Netherlands

Introduction

Mycobacteria have a highly distinct and complex cell envelope, consisting of two membranes with a poorly characterized periplasmic space in between. The high impermeability of this cell structure is crucial for mycobacterial pathogens to resist killing during infection, but it also complicates the identification and localization of cell envelope proteins, in particular proteins that localize to the periplasm. Here, we developed a new tool, based on the SpyTag/SpyCatcher system for irreversible conjugation of recombinant proteins, to determine the periplasmic localization of specific proteins in Mycobacterium marinum.

Methods

We co-expressed a recombinant membrane protein carrying the SpyCatcher facing the periplasm (TM-SpyCatcher) or cytosolic SpyCatcher together with a known periplasmic protein BlaC, bearing the SpyTag and with or without is signal sequence, in M. marinum and analyzed conjugation efficiencies by SDS-PAGE and western-blotting.

Results

We observed highly efficient protein conjugation between BlaC-SpyTag and TM-SpyCatcher, while BlaC-SpyTag without its signal sequence, blocking its export, resulted in no interaction with TM-SpyCatcher. In contrast, cytosolic SpyCatcher reacted with both BlaC-SpyTag variants, as expected.

Conclusion

Our newly developed SpyTag/SpyCatcher-based method is a powerful tool to study if a specific protein has access to the periplasm of mycobacteria. We are currently using the tool to address whether proteins that are secreted via the major secretion pathway in mycobacteria, the type VII secretion system, are exposed to the periplasm during export. Better identification and characterization of periplasmic proteins will help elucidate the role of the periplasm in mycobacterial physiology and virulence.

P190

The added value of whole-genome sequencing to describe an ongoing MRSA outbreak in a nursing home in Friesland.

Dr Marije Doppenberg-oosting[1], Dr M Fliss[2], Dr E Bathoorn[2], Dr D Notermans[3], Dr R Zwittink[3], M Buijsse-van Tilburg[4], A Kramer[4], Dr P Huizinga[1], Dr A Ott[1], Dr A Brandenburg[1]
[1]Certe MMB Friesland Noordoostpolder and Groningen Drenthe, Leeuwarden and Groningen, the Netherlands, [2]UMCG, Groningen, the Netherlands, [3]National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands, [4]Nij Smellinghe, Drachten, the Netherlands
Multiple-locus variable number tandem repeat analysis (MLVA) is used in the Netherlands to type methicillin-resistant Staphylococcus aureus (MRSA) isolates. Starting in 2016 in a nursing home in Friesland, four different MLVA-types were incidentally detected: MT3594, MT2152, MT5330 and MT7004. MT5330 and MT7004 were only found in this nursing home, whereas MT3594 and MT2152 were also found elsewhere, mainly in other nursing homes in the region. All types belong to MLVA complex MC0005 and multi-locus sequence type (MLST) ST5. In September 2021, April 2022 and September 2022 there were three small outbreaks on three different departments in this nursing home. In all outbreaks, more than one of the MLVA-types were detected. It was noticed that the four MLVA types were closely related. The aim of this study was to further unravel the relatedness and origin of these MRSA isolates.
Whole-genome sequencing (WGS) was performed on 20 available strains from this outbreak. The results were also compared with available sequences from seven MT2152 strains detected elsewhere in the region and available ST-5 cluster data from the European database.
Whole-genome MLST revealed high genetic relatedness between isolates from the same nursing home department, independent of MLVA type. Furthermore, two separate, but closely related populations of MRSA could be discriminated. These findings indicate ongoing transmission on a low level in the nursing home possibly originating from two separate introductions.

P191

Human serum inhibits therapeutic phages against Pseudomonas aeruginosa

Julia Egido[1], Simon O. Dekker[1], Suzan H. M. Rooijakkers[1], Bart W. Bardoel[1], Pieter-Jan Haas[1]
[1]Medical Microbiology, University Medical Center Utrecht, , The Netherlands

Introduction

Bacteriophage (phage) therapy uses viruses that specifically attack bacteria to treat infections. Although this is a promising addition to antibiotic-based treatments, its success depends on choosing the optimal phages against each pathogen. We hypothesize that the human immune system may influence the effectiveness of certain phages. To address this, we have studied the binding and killing ability of phages of two different taxa targeting Pseudomonas aeruginosa in presence of human serum.

Methods

We used myophages PB1 and 14-1 and podophages LKD16, LUZ19 and PAXYB1, with P. aeruginosa strain PAO1 as a model. Phage binding was assessed through flow cytometry and confocal microscopy. For these applications, azido-tagged phages were produced in a metZ knockout of the host strain, in medium depleted of methionine and supplemented with L-azidohomoalanine. These were functionalized with a fluorophore by means of click chemistry. Fluorescent bacteria were obtained by transforming PAO1 with a plasmid encoding GFP or sf-Cherry. Phage-mediated killing was assessed using the membrane-impermeant dye Sytox green, which stains damaged bacteria.

Results

Binding of myophage PB1 to P. aeruginosa was compromised by the addition of healthy pooled human serum, while binding of podophage LUZ19 was unaffected. This translated to a reduced killing ability of myophages in presence of serum, compared to podophages. The effect is concentration-dependent, where a higher serum percentage is needed to inhibit a higher multiplicity of infection (MOI). Inactivation of complement system by heat treatment or use of the C3 inhibitor compstatin rescued phage activity, pointing to an involvement of early stages of the complement cascade.

Conclusion

Our results indicate that not all phages perform well in serum. In particular, the complement system may impair myophages, which constitute the biggest group of known Pseudomonas phages. This highlights the importance of using physiologically relevant conditions when screening for phages for therapy.

P192

KPC-85, a novel KPC-3 variant involved in ceftazidime-avibactam resistance in high-risk Klebsiella pneumoniae ST512

Bsc Varisha Ganesh[1], Dr. D.W. Notermans[1], Drs. I. Maat[2], BASc. S. Witteveen[1], BASc. F. Landman[1], Dr. A.P.A. Hendrickx[1]
[1]Centre for Infectious Disease Control, National Institute For Public Health And The Environment, Bilthoven, The Netherlands, [2]Radboud University Medical Center, Nijmegen, The Netherlands

Carbapenemase-producing Enterobacterales (CPE) are of concern for public health. The Klebsiella pneumoniae carbapenemase (KPC) is widely disseminated among CPE and causes carbapenem-resistance. Ceftazidime-avibactam (CAZ-AVI) is used as treatment for CPE infections. Here we describe a case of in-patient CAZ-AVI resistance development in K. pneumoniae harbouring a A175V mutation in KPC-3, resulting in KPC-85.

Four K. pneumoniae isolates originating from one patient, who was treated with CAZ-AVI in Italy and after hospital transfer in the Netherlands, were obtained via the Dutch CPE surveillance. Isolates were characterized by the carbapenem inactivation method, Etest for CAZ-AVI and meropenem, and short-read and long-read sequencing. Sequencing data was used for core-genome multilocus sequence typing (cgMLST), classical MLST, resistance gene analysis by ResFinder v4.0 and plasmid reconstruction.

In one patient, two K. pneumoniae isolates from rectal swabs harboured blaKPC-3 (100% identity), while K. pneumoniae isolates from throat swabs harboured blaKPC-3 with 99.89% identity. The latter two carried an A175V mutation in KPC-3, leading to KPC-85. All isolates were from MLST ST512, produced carbapenemase, and had MICs for meropenem of 32 or higher. cgMLST indicated high genetic relatedness, with an allelic distance of 20 between the KPC-3 and KPC-85 isolates. The KPC-85 isolates lacked plasmid-localized aac(6')-Ib, aac(6')-Ib-cr and blaTEM-1 genes. Both KPC-3 and KPC-85 were located on 99% identical plasmids. The two isolates containing KPC-85 were resistant for CAZ-AVI (MICs 12 and 14 mg/L, R > 8 mg/L), while the KPC-3 isolates were susceptible (MICs 3 and 4 mg/L).

Four K. pneumoniae ST512 isolates were retrieved from one patient of which two contained KPC-85, a KPC-3 variant likely causing resistance to CAZ-AVI. The four isolates may represent one strain, suggesting that CAZ-AVI treatment may lead to selection of the A175V mutation. Since blaKPC-85 is plasmid localized, CAZ-AVI resistance may disseminate in the future.

P193

A cluster of NDM-producing Providencia stuartii in patients from Ukraine in the Netherlands, August-October 2022

Dr. Ing. Antoni Hendrickx[1], Dr. C.H.W. Wielders[1], V.A. Ganesh[1], D.W. Notermans[1], F. Landman[1], S. Witteveen[1], C. Scheeberger-van der Linden[1], R.D. Zwittink[1]
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands
Since March 2022 there is an emergence of carbapenemase-producing Enterobacterales carrying New Delhi-metallo β-lactamase (NDM)-like genes from Ukrainian patients in the Netherlands. Here, we analyzed the genomic epidemiology of carbapenemase-producing Providencia stuartii in the Netherlands.
P. stuartii were characterized by the carbapenem inactivation method, Etest for meropenem, carbapenemase PCR on OXA, VIM, IMP, KPC and NDM-genes, and short-read and long-read sequencing. Sequencing data was used for core genome (cg)SNP analysis, resistance gene detection and plasmid reconstruction.
From July to October 2022, nine P. stuartii isolates from nine patients were submitted to the CPE surveillance by seven medical microbiology laboratories, while between 2014-2021 only seven P. stuartii isolates were received. Eight of the nine patients were male, the median age was 38 years, and five were from patients from Ukraine. Recent hospitalization in Ukraine was reported for 3/4 Ukrainian patients with information available. Sampling materials were swabs (n=4), wound/pus (n=4) and catheter-related urine (n=1). All P. stuartii isolates produced carbapenemase, and none were meropenem-resistant (MICs 0.38-4 mg/ml). PCR revealed that eight out of nine isolates carried an NDM-gene and one a VIM-gene. Six P. stuartii isolates were sequenced, including four from Ukrainian patients. Based on cgSNP analysis, four isolates from Ukrainian patients were genetically related (1-4 SNPs difference) and carried blaNDM-1. A P. stuartii isolate from a Dutch female patient carried blaNDM-1 and differed 11-13 SNPs from the four Ukrainian isolates. These five highly related isolates (1-13 SNPs) had identical resistomes conferring resistance towards multiple classes of antibiotics. One Dutch P. stuartii isolate carrying blaVIM-1 obtained in July was not related (2277-2282 SNPs different). Lastly, blaNDM-1 was localized on a 157-kb IncC resistance plasmid.
In conclusion, we observed a genetic cluster of multidrug-resistant NDM-producing P. stuartii from Ukrainian patients in the Netherlands in 2022 with potential transmission to a Dutch patient.

P194

Development of a split-luciferase assay to monitor protein secretion in Bacillus subtilis

Msc Mariah Kes[1], BSc D. van Es[1], dr B. Wang[2], dr T. van Rij[3], prof dr L.H. Hamoen[2], dr S. Luirink[1]
[1]Vrije Universiteit Amsterdam, , The Netherlands, [2]Universiteit van Amsterdam, , The Netherlands, [3]Koninklijke DSM N.V., , The Netherlands

Introduction: Bacillus subtilis is a Gram-positive bacterium that is widely studied, partly because of its ability to secrete proteins of biotechnological interest. To study the efficiency and kinetics of expression and secretion, protein levels in cell and medium fractions have to be determined. Standard assays for protein detection such as SDS-PAGE, western blotting and enzyme activity are used most often but are laborious, hampering analysis of different conditions and mutants. The goal of this study was to establish a luminescence-based assay to investigate protein secretion in B. subtilis with short handling times, high sensitivity and a quick read-out.
Methods: The split luciferase system NanoBiT, which is based on the luciferase NanoLuc, consists of a small peptide, HiBiT, and a large unit, LgBiT, that assemble with high affinity. We tagged proteins secreted by B. subtilis with HiBiT, sampled cultures grown in a microplate reader during protein secretion and measured luminescence upon addition of LgBiT and the substrate furimazine.
Results: We show that this system can be used to detect low amounts of proteins quantitatively, quickly, and in a medium-throughput manner, facilitating analysis of protein secretion in B. subtilis. In addition, initial secretion analyses using this approach are presented, in which various cargo proteins are compared.
Conclusion: This assay appears a useful addition to the toolbox for monitoring protein secretion by B. subtilis.

P195

QUANTITATIVE AND FUNCTIONAL DIFFERENCES IN SARS-COV-2 SPECIFIC MUCOSAL ANTIBODY RESPONSES FOLLOWING INFECTION AND VACCINATION

Mrs Vera Koomen[1,2], Janeri Fröberg[1], Fred Van Opzeeland[1], Joyce Rust[1], Christa Gaast-deJongh[1], Elles Simonetti[1], Ria Philipsen[1,2], dr. Renate van der Molen[1], prof.dr. Martijn Huijnen[3], dr. Marien de Jonge[1], prof.dr. Luuk Hilbrands[2], dr.ir. Dimitri Diavatopoulos[1]
[1]Radboudumc, Nijmegen, The Netherlands, [2]Radboudumc, Nijmegen, The Netherlands, [3]Radboudumc, Nijmegen, The Netherlands

Introduction

Neutralizing antibodies play a critical role in the prevention of SARS-CoV-2 infection by blocking the interaction of the receptor binding domain (RBD) with the angiotensin converting enzyme 2 (ACE2) receptor on cells. Little is known about the differences in quantitative and functional aspects of antibodies induced by infection vs vaccination, particularly with regards to mucosal antibodies. In this study, we compared the nasal antibody response to primary infection with SARS-CoV-2 and primary vaccination with two doses of mRNA-1273.

Methods

For this study we used two convenience cohorts including COVID-19 cases (n=88) who tested positive for SARS-CoV-2 during the first wave and healthy controls who were vaccinated with mRNA-1273 (n=50). Mucosal lining fluid (MLF) was obtained via nasosorption at study start (d0), 28 days after infection or the 2nd dose of mRNA-1273 (d28), and at 6 months or 9 months, respectively. IgG and IgA concentrations in MLF against Spike (Wuhan), RBD (Wuhan, Delta, Omicron) and N were quantified by multiplex immune assay. To assess the neutralizing capacity of MLF samples, we developed a Luminex-based pseudoneutralization ACE2 competition assay, which we validated against the gold standard, i.e. the plaque reduction neutralization test (PRNT) (Erasmus MC, Rotterdam).

Results

Although vaccination induced significantly higher mucosal IgG concentrations than infection, neutralizing antibody levels were similar at d28. Whilst IgG concentrations correlated very strongly with neutralizing capacity for the vaccination cohort, significantly lower correlation was observed post-infection. Conversely, neutralization capacity post-infection was more strongly correlated with IgA. For both cohorts, neutralizing antibody levels at follow-up timepoints had declined to baseline level.

Conclusion

Our results suggests that infection and vaccination can both achieve viral neutralization, but are dependent on distinct antibody responses.

P196

Development of robust primary human respiratory epithelial infection models for respiratory syncytial virus and influenza A virus to support vaccine research

Rosanne Koutstaal[1]
[1]Rijksinstituut voor Volksgezondheid en Mileu, Bilthoven, The Netherlands
This research project is part of the Inno4Vac consortium, which aims to develop primary in vitro models for early evaluation of vaccine efficacy and prediction of immune protection, in the context of respiratory syncytial virus (RSV) and influenza A virus (IAV). We hypothesize that these models can be used as a robust and semi-high-throughput platform to study immune responses against respiratory viruses.

Primary nasal and bronchial epithelial cells from different donors were differentiated at air-liquid interface (ALI) for 6 weeks. The epithelium was characterized using immunofluorescence and trans-epithelial electrical resistance(TEER). Cultures were infected with RSV-A/0594, RSV-B/9761, or influenza A/Wisconsin/588/2019. Virus production was measured using TCID50 assays and qPCR, cytokine production was analyzed using Legendplex. Virus neutralization experiments were done with either sheep serum against IAV (NIBSC code 21/120) or Palivizumab against RSV-A/B.

After differentiation, cells formed a stable barrier as measured by TEER and all relevant cell types were present. Viral replication kinetics were reproducible in 3 independent donors. Upon infection, cells consistently produced antiviral and pro-inflammatory cytokines. Virus production could be inhibited by adding either antibodies or serum against the virus. When RSV and IAV were compared, IAV replicated to higher titers, induced higher cytokine levels and caused a (temporary) drop in TEER, whereas RSV did not seem to have cytopathic effects.

The developed ALI model was able to demonstrate differences between IAV and RSV infection, which might indicate that these viruses have different ways of activating the immune system. This model is thus a robust and stable candidate to study infection mechanisms and their associated immune responses. In the next phase of this project, we aim to set up co-cultures of epithelial cells and different immune cell types to study their response to infection and their role in the protection or enhancement of respiratory viral infections.

ThisWorkHasReceivedSupportFromTheIMI2/EU/EFPIAJointUndertakingInno4VacGrant n°101007799.

P197

The respiratory syncytial virus attachment protein affects dendritic cell maturation

Anke Lakerveld[1,2], S. M. A. Mijnhardt[1], P. B. van Kasteren[1]
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands,
[2]Leiden University Medical Center (LUMC), Leiden, The Netherlands

Respiratory syncytial virus (RSV) infection is a major cause of severe lower respiratory tract infections in infants and elderly. RSV reinfections occur frequently throughout life, suggesting a defective memory response. Dendritic cells (DCs) play an important role in the generation of memory T cell responses. RSV is known to influence DC maturation, but the underlying mechanisms remain unclear.

Here, we studied the effect of the RSV attachment protein (G) on DC maturation using monocyte-derived DCs from healthy adult donors. To study the effect of the G protein, we stimulated immature DCs with RSV, RSV lacking the G protein (RSVΔG), and RSVΔG in combination with recombinant G soluble protein. Using flow cytometry, we measured the percentage infected DCs and the expression of several maturation markers on DCs. Additionally, a multiplex immunoassay was used to measure the concentration of cytokines secreted by DCs.

We show that RSVΔG infects an increased percentage of DCs compared to RSV, which is decreased in the presence of recombinant G. In contrast, the expression of several maturation markers, e.g. CD38 and CD86, is reduced upon stimulation with RSVΔG compared to RSV and remains low in the presence of recombinant G. Additionally, secretion of certain cytokines (e.g. IFN-β, CXCL10) by DCs is also decreased upon stimulation with RSVΔG compared to RSV.

These results suggest that the RSV G protein plays a role in DC maturation which might be of importance for the design of novel vaccines. Future research will focus on further elucidating the role of specific regions within the G protein using our in-house RSV reverse genetics platform.

P198

A mutation in codY of Staphylococcus aureus USA300 results in small colony variants formation and a persistent phenotype in human lung epithelial cells

Xiaofang Li[1], Dr Sjouke Piersma[1], Dr Min Wang[1], Dr Girbe Buist[1], Prof. Dr Jan Maarten van Dijl[1]
[1]University Of Groningen, Groningen, The Netherlands

Introduction: S. aureus is a leading cause of pneumonia. This is a result of its ability to adapt to the host's intracellular environments. The emergence of metabolically adapted S. aureus subpopulation pose a threat to human health as these metabolically adapted strains present decreased virulence and survive longer in the host cells, leading to chronic infection and relapse of infection. In order to find the effective treatments, a better understanding of the metabolic adaptation strategies is urgently needed. In a previous study we dissected the dynamic interplay between epithelial cells and the internalized S. aureus over four days. The abundance changes of SigB, CodY and SaHPF dependent proteins were identified in reaching the dormancy state. SigB and CodY are global regulators of S. aureus while SaHPF is known to be involved in the survival of S. aureus during nutrient deprivation. In this project, we investigated and compared the roles of SigB, CodY and SaHPF in a human lung epithelial cell infection model. Methods: The growth behavior, cell morphology, antibiotic resistance and invasion/replication inside human lung epithelial cells of the strains were analyzed by flow cytometry, fluorescence confocal microscopy and transmission electron microscopy.
Results: The results showed that the sigB, codY, saHPF mutant strains presented differential growth behavior, antibiotic resistance and invasion/replication ability in lung epithelial cells compared with S. aureus USA300 wildtype strain. In particular, the codY mutant displayed small colony variants formation and persister cell phenotype which survive longer in human lung epithelial cells.
Conclusions: Altogether, this study indicates that SigB, CodY and SaHPF play important roles in the interactions between S. aureus and human lung epithelial cells and gives a better understanding of the S. aureus metabolic adaptation mechanisms.

P199

Production of γ-aminobutyric acid by Akkermansia muciniphila and characterization of its glutamate decarboxylase

Kate Ligthart[1], Prokopis Konstanti[1], Dr. C. Fryganas[2], Dr. C. Patinios[1], Prof. V. Fogliano[2], Prof. dr. W. M. de Vos[1], Prof. dr. H. Smidt[1], Dr. C. Belzer[1]
[1]Wageningen University & Research, Wageningen, The Netherlands, [2]Wageningen University & Research, Wageningen, The Netherlands

Introduction:
Gut bacteria hold the potential to produce a broad range of metabolites that can modulate human functions. Besides the well-studied production of compounds such as short chain fatty acids (SCFAs), in recent years the neuroactive potential of human gut bacteria is increasingly being acknowledged. Here, we explore Akkermansia muciniphila's potential to produce γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the central nervous system. A. muciniphila is predicted to encode the glutamate decarboxylase, the enzyme that catalyzes the conversion of glutamate to GABA, but has not yet been experimentally verified to produce GABA.

Methods:
A. muciniphila was grown in basal medium supplemented with either monosodium glutamate or glutamine. In batch cultures, samples were taken every 24 hours and SCFA and amino acid production and consumption was measured. To verify these findings, similar experiments were performed in bioreactor where, as opposed to the bath cultures, pH was controlled. Samples started at pH 5.8 and after stationary phase was reached, the pH was decreased every 24 hours until pH 4. Amino acid and SCFA production were measured using HPLC-UV(?) and GABA production was confirmed with HPLC-ESI-MSMS. Next to that, A. muciniphila's GAD protein was cloned and expressed in E. coli. After purification, protein assays were performed to determine the effect of pH on the enzymatic activity.

Results:
GABA production was observed only when A. muciniphila was grown in low pH and in the presence of either glutamate or glutamine in the growth medium. We also demonstrate that A. muciniphila's GAD protein was active in pH levels between 6 and 4, with optimal activity at pH 5.

Conclusion:
A. muciniphila produces GABA as a response to acid stress (pH < 5.5) in the presence of either glutamate or glutamine in the growth medium.

P200

Investigating the role of lipoproteins in pathogenic Mycobacteria

Robin Lissner[1], Coen Kuijl[2], Wilbert Bitter[1,2]
[1]Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, [2]Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam Institute of Infection and Immunity, Amsterdam, The Netherlands

Introduction:
By being the most deadly bacterial pathogen, causing over 1.5 million deaths per year, Mycobacterium tuberculosis (Mtb) is a constant global threat. Understanding its biology and pathogenesis is essential for the development of new and better treatments, which are urgently needed. A crucial role for the virulence of Mtb plays its unusual cell wall, which serves as a protective layer and directly interacts with the host. One important, but so far understudied group of proteins in this cell wall are the lipoproteins. A small fraction has been reported to be involved in lipid transport, nutrient acquisition and virulence, but the function of the majority of lipoproteins remains unknown. The objective of this study was to investigate the function of all lipoproteins by characterizing a collection of knockout strains.

Methods:
Using CRISPR/Cas9 technology, we created knockout strains for each of the 75 highly conserved lipoproteins in the closely related Mycobacterium marinum. These strains were subsequently tested for their ability to grow on different media, for their susceptibility to several antibiotics and the permeability of the cell wall to Ethidium bromide.

Results:
Multiple mutant strains had a decreased growth rate and an abnormal colony morphology. Certain strains showed a greater susceptibility to various antibiotics and an increased cell wall permeability.

Conclusion:
These findings point towards crucial roles of lipoproteins in the organization of the cell envelope. Further characterization of these key lipoproteins will help to understand the complex architecture and biogenesis of the cell envelope and may offer interesting candidates for new drug targets.

P201

Reproducible switching between a walled and cell wall-deficient lifestyle of actinomycetes using gradient agar plates

M.sc. Maarten Lubbers[1], Prof. Dr. D. Claessen[1], Prof. Dr. G.P. van Wezel[1]
[1]Institute Of Biology Leiden, Leiden University, Leiden, Netherlands

The cell wall is a shape-defining structure that envelopes almost all bacteria, protecting them from biotic and abiotic stresses. Paradoxically, some filamentous actinomycetes have a natural ability to shed their cell wall under influence of hyperosmotic stress. These wall-deficient cells can revert to their walled state when transferred to a medium without osmoprotection but often lyse due to their fragile nature. Here, we designed plates with an osmolyte gradient to reduce cell lysis and thereby facilitating the transition between a walled and wall-deficient state. Furthermore, our method allows precise determination of the osmolyte concentration where reversion takes place, allowing careful and reproducible comparison between mutants. For instance, we show that Kitasatospora viridifaciens alpha, an unstable L-form strain obtained after induction with penicillin and lysozyme, can either grow filamentous or wall-deficient depending on the osmolarity of the gradient. Most interestingly, heteromorphic colonies were formed on the gradient that consisted of a mixture of filaments and wall-deficient cells. Besides, we show that gradient agar plates can also be applied for another purpose, namely to study chemical differentiation in streptomycetes as a response to osmotic stress. Streptomyces coelicolor produced aerial hyphae and spores on non-osmoprotective medium, but readily lost the ability to develop when medium osmolarity increased. Interestingly, this developmental arrest coincided with the formation of a green-blue metallic pigment. Furthermore, the production of the blue-pigmented antibiotic actinorhodin increased. Exploring these transitions could give valuable insights into the ecology of actinomycetes and their biotechnological applications.

P202

C-type lectin receptor sequencing as a platform to study the selective immune induction of bacterial antibody responses at host barrier sites

Msc Jasper Mol[1], PhD Astrid Hendriks[1], Professor Christoph Rademacher[2], PhD Hilde Herrema[3], Mark Davids[3], Professor Nina Van Sorge[1,4]
[1]Amsterdam Umc, Amsterdam, The Netherlands, [2]University of Vienna, Vienna, Austria, [3]Amsterdam UMC, Amsterdam, The Netherlands, [4]Amsterdam UMC, Amsterdam, The Netherlands
Bacterial communities located at host barrier sites harbor species essential for human health, but also species with pathogenic potential. Therefore, the human immune system has the task to distinguish 'friend' from 'foe'. Infection induces antibody immunity, contributing to durable protection against the same pathogen, but microbiota species also contribute to natural antibody immunity. However, in this context of homeostasis, the underlying molecular mechanisms for selection and induction of immunity are unknown. Antigen presenting cells (APCs) are local immune cells that constantly 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, even in the absence of an infection. We aimed to identify microbiota species that can be recognized by APC-expressed CLRs in a comprehensive manner. To this end, we have developed CLR-sequencing, which applies soluble CLR receptors as probes to identify interacting microbiota species through bacterial cell sorting and subsequent 16S rRNA gene sequencing. We established and optimized our experimental pipeline using the fluorescently-labeled CLRs langerin and MGL, and fecal microbiota samples from healthy donors. Langerin and MGL bound on average 9% and 4% of the fecal microbiota, respectively, with substantial interindividual variation. Analysis revealed donor-specific enrichment of taxa in the CLR-sorted samples, but also shared enrichment of genera such as Ruminoclostridium_9, Coprococcus_1 and Butyricicoccus. Current work focuses on validating the CLR-mediated interaction with enriched species common to studied donors, and expanding the CLR repertoire in the experimental pipeline. With this experimental approach we can gain insight into immunologically-relevant glycans in healthy microbiota communities. Using this as a blueprint, future research on clinically-relevant patient samples or CLRs with naturally occurring mutations could reveal crucial disparities in glycan-based immune signaling.

P203

Targeting 16S rRNA by reverse complement PCR-next-generation-sequencing: specific and sensitive detection and identification of microbes directly in clinical samples

Simone Moorlag[1], Jordy Coolen[1], Bart van den Bosch[1], Elisabeth Hui-Mei Jin[1], Jochem Buil[1], Heiman Wertheim[1], Willem Melchers[1]
[1]Radboudumc, Nijmegen, The Netherlands

Introduction: Detection and accurate identification of bacterial species in clinical samples is crucial for diagnosis and appropriate antibiotic treatment. To date, sequencing of the 16S rRNA gene is widely used as a complementary molecular approach when identification by culture fails. The accuracy and sensitivity of this method is highly affected by the selection of the 16S rRNA gene region targeted. Currently, many routine clinical microbiology laboratories use a single primer set targeting two variable regions. As a result of this approach, discriminatory power in clinical samples for certain genera may be limited and resolution at the species level is often unfeasible.
Methods: In this study, we assessed the clinical utility of 16S rRNA reverse complement polymerase chain reaction (16S RC-PCR) for the identification of bacterial species, a novel method based on targeted next-generation sequencing (NGS) using multiplex target amplification of short amplicons by RC-PCR, covering about 80% of the 16S rRNA gene. We investigated the performance of 16S RC-PCR on bacterial isolates, polymicrobial community samples and on clinical samples from patients suspected of a bacterial infection. Results were compared to culture results if available and compared to 16S Sanger sequencing.
Results: By 16S RC-PCR, all bacterial isolates were accurately identified to the species level. Moreover, we show that this method can detect and identify different bacterial species present within polymicrobial samples. Furthermore, in culture-negative clinical samples the identification increased from 17.1% (7/41) to 46.3% (19/41) when comparing 16S Sanger to 16S RC-PCR.
Conclusions: We conclude that the use of 16S RC-PCR in the clinical setting leads to an increased sensitivity of detection of bacterial pathogens resulting in an higher number of diagnosed bacterial infections and thereby can improve patient care.

P204

Septicaemia cases in pigs due to the emergence of hypervirulent ST25 Klebsiella pneumoniae in the Netherlands

Mr Soe Yu Naing[1], Aldert Zomer[1], Manon Houben[2], Karin Junker[2], Otto Schreurs[3], Annet Heuvelink[2], Jaap Wagenaar[1], Jobke van Hout[2]
[1]Utrecht University, Utrecht, Netherlands, [2]Royal GD, Deventer, Netherlands, [3]Advee Dierenartsen, Ysselsteyn, Netherlands

Introduction: Hypervirulent K. pneumoniae (hvKP) is a concerning pathotype that differs from classic K. pneumoniae (cKP) because it has acquired additional virulence factors, causing community-acquired infections in humans and severe infections in animals, including pigs. Seasonal outbreaks of septicaemia in neonatal pigs due to hvKP have been reported in Australia and the United Kingdom. In the Netherlands, septicaemia cases in 15 farms have been reported to Royal GD from 2013 to 2020. In the current study, we aimed to perform a genomic investigation of septicaemia cases due to (presumably) hvKP in pigs.

Methods: 41 isolates were collected from outbreaks on 15 pig farms in the Netherlands between 2013 and 2020. Whole genome sequencing was performed, and the results were analyzed using Kleborate to type virulence and resistance-associated loci. Additional genomes of ST25 of human origin (n=230) and all ST types of pig origin (n=87) from public databases were included in the analysis to examine the emergence and spread of these infections.

Results: Molecular characterisation showed that 87% of isolates were identified as ST25; the rest were ST5, ST37, ST219, ST292, and ST1480. All ST25 isolates were hypervirulent with K2 hyper capsule type and carried plasmids associated with virulence genes encoding aerobactin, yersiniabactin, and rmpA, conferring hypermucoviscous phenotype. The cgMLST analysis revealed two different clonal groups within ST25 - CG25 and CG3804. Nine CG25 isolates obtained from two farms were multidrug-resistant (MDR), conferring resistance to streptomycin/spectinomycin, sulphonamide, tetracycline, and trimethoprim. A global phylogenetic tree showed the divergence of the Netherlands hvKP isolates (CG25) from global isolates. CG3804 is a novel clone observed in pigs for the first time.

Conclusion: Based on the genomic findings and epidemiological observations, we identified the emergence of hypervirulent ST25 K. pneumoniae in pigs and showed the presence of two clonal groups in the Netherlands.

P205

Rise in Neisseria meningitidis Genogroup E Carriage Prevalence Three Years after menACWY Vaccine Implementation in the National Immunization Programme in the Netherlands

Gerlinde Pluister[1], Marcia Tappel[1], Meike Sikking[1], Willem Miellet[2], Linda Visser[1], Janieke van Veldhuizen[1], Tessa Nieuwenhuijsen[2], Krzysztof Trzcinski[2], Rob Mariman[1]
[1]RIVM, Bilthoven, The Netherlands, [2]Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

Introduction:
Carriage of Neisseria meningitidis is an accepted endpoint in monitoring meningococcal vaccines effects. We have assessed the impact of menACWY implementation in 2019, as well as non-pharmaceutical interventions (NPIs) during the COVID-19 pandemic on meningococcal carriage by comparing N. meningitidis and vaccine-type genogroup carriage prevalence before and after the introduction of the tetravalent vaccine.

Methods:
In the Fall of 2018, prior to the introduction menACWY vaccine, we established a baseline for N. meningitidis carriage and genogroup-specific prevalence rates among college students. We conducted a follow-up study in the Fall of 2022 in the same demographic setting. For this, saliva samples collected from 601 students were cultured for meningococcus on selective plates. The DNA extracted from all bacterial growth was subjected to qPCRs to detect meningococcal and genogroup-specific genes.

Results:
Carriage of genogroupable meningococci, defined as saliva positivity for metA and ctrA, was detected in 125 students. The difference in carriage prevalence between both studies was not significant (17.4.% vs 20.8%, p=0.25). We conducted genogroup-specific qPCR assays for all vaccine-types plus menB, menE, menX and menZ, covering 107/109 (98.2%) of genogroupable samples. The 2022 prevalence of vaccine-type genogroups A, C, W, and Y was none, 0.7%, 0.5% and 0.7%, respectively. There was a substantial decline in vaccine-type carriage compared with the pre-vaccination cohort in 2018 (1.8% vs 6,4%, p<0.001, O.R. 0.27, 95%CI 0.13-0.60). Importantly, we observed a 9,0 fold increase in non-vaccine type menE carriage. Changes in prevalence of other non-vaccine-type genogroups, including menB, were not significant.

Conclusion:
The introduction of the menACWY vaccine and NPIs had limited impact on the overall carriage of N. meningitidis. However, we observed a decline in vaccine-type genogroups and a rise in genogroup E carriage prevalence. Ongoing genomic-based analyses examines functionality of the capsular loci and assesses clonality of genogroups for vaccine-induced shifts.

P206

Rapid identification of respiratory tract microbiota by Molecular Culture

Lauren Remijas[1]
[1]inbiome, Amsterdam, The Netherlands

Introduction

The respiratory tract microbiome (RTM) is a key contributor to respiratory health and may play a role in triggering exacerbations in disease. Routine detection of RTM changes could serve to predict exacerbation and reduce healthcare burden. Molecular assays, such as the Molecular Culture Microbiota test, have the potential to rapidly and comprehensively analyze the RTM. This PCR-based assay utilizes differences in intergenic lengths (IS) between 16S and 23S rDNA to identify bacterial species. However, to best interpret this data, libraries are necessary to translate DNA fingerprints to species. Here, we build a translational library using Nanopore sequencing and evaluate it on respiratory samples.

Methods

3197 respiratory samples, including bronchoalveolar lavage (BAL), throat swabs, sputum, nasopharyngeal and nasal swabs were subjected to DNA isolation followed by Molecular Culture (inbiome, the Netherlands). Common DNA fingerprints were identified and 71 samples containing these species were sequenced. Species containing at least 20 consensus reads and present in at least 8 sequenced samples were included in the translational database, then incorporated into the MLE-based bacterial classifier for identification of Molecular Culture profiles.

Results

278 species were identified by Nanopore sequencing, of which 45 were added to the database, accounting for 70.4% of the total Molecular Culture profile intensity of the sequenced samples. Following integration of the translational database with the classifier, 81.2% of the total dataset was identified. Dominant species in nasal and nasopharynx swabs included Staphylococcus epidermidis, Dolosigranulum pigrum, and Granulicatella adiacens. Conversely, the lower respiratory tract and throat swabs were defined by Streptococcus species, Prevotella melaninogenica, Bacillus smithii, and Haemophilus parainfluenzae.

Conclusions

We generated a translational database to rapidly identify RTM. Applying this database, we observed species-level distinct niches within the RTM. This study demonstrates the value of Molecular Culture in identifying the RTM composition, with potential use for exacerbation-related risk assessment.

P207

The streptococcal phase-variable Type I Restriction-Modification system SsuCC20p dictates Streptococcus suis methylome and impacts virulence

Thomas Roodsant[1,2], B. van der Putten[1,2], JPM Coolen[3], T.J.H. Baltussen[3], K. Schipper[2], Y. Pannekoek[2], K. van der Ark[1,2], C. Schultsz[1,2]
[1]Amsterdam UMC, , Amsterdam, The Netherlands, [2]Amsterdam UMC, , Amsterdam, The Netherlands, [3]Radboud UMC, , Nijmegen, The Netherlands

Introduction
Phase-variable Type I Restriction Modification (RM) systems are epigenetic regulation systems that can impact gene expression and virulence of bacterial pathogens. Type I RM systems are encoded by three hsd genes: a specificity subunit (HsdS), a modification subunit (HsdM) and a restriction subunit (HsdR). A trimeric subunit complex (2HsdM,1HsdS) can methylate specific sequences of the bacterial genome. Phase-variable Type I RM systems can recombine the hsdS gene to form different hsdS alleles. The emerging zoonotic lineage of Streptococcus suis clonal complex (CC) 20 has acquired a Type I RM system named SsuCC20p, which is hypothesized to be phase variable and impacting virulence.

Methods
S. suis genomes and the NCBI Refseq Genomes Bacterial Database were searched for SsuCC20p using (t)blastn. Phase variability of SsuCC20p was assessed using a FAM-labelled PCR with subsequent endonuclease digestion and fragment analysis (FAM assay). The methylome of S. suis wildtype, locked mutants (LM) expressing only a single hsdS allele and hsdS KO mutant were characterized by PacBio SMRT and HiFi sequencing. LM virulence was compared in a zebrafish larvae infection model.

Results
SsuCC20p was identified in 22 S. suis, 17 S. agalactiae and 5 other streptococcal isolates. Three SsuCC20p alleles were identified within a single isolate using the FAM assay. Knocking out the site-specific recombinase encoded in SsuCC20p (xerD) halted phase variation. Multiple methylation profiles were detected in a WT strain, while the hsdS KO lacked genome methylation. The methylation profile of three hsdS alleles were characterized in the LMs. The LMs differed in virulence in a zebrafish larvae infection model.

Conclusions
The Type I RM system SsuCC20p is phase-variable, present in multiple streptococcal species and methylates the S. suis genome. hsdS phase variability depends on xerD. Different hsdS alleles result in unique genome methylation profiles, which impacts the virulence of zoonotic CC20 S. suis.

P208

Translocation across a human enteroid monolayer by zoonotic Streptococcus suis is mediated by Gb3+ cells

Thomas Roodsant[1,2], K. van der Ark[1,2], C. Schultsz[1,2]
[1]Amsterdam UMC, , Amsterdam, The Netherlands, [2]Amsterdam UMC, , Amsterdam, The Netherlands

Introduction
Zoonotic infections are mostly caused by S. suis serotype 2 (SS2) isolates from clonal complex 1 (CC1). In the Netherlands, the zoonotic SS2CC20 lineage has emerged from the non-zoonotic but virulent pig lineage SS9CC16. The consumption of undercooked pig products is an important risk factor for human S. suis infection, identifying the gastrointestinal tract as potential entry site. We studied the adhesion, invasion and translocation of zoonotic and non-zoonotic S. suis in a human enteroid model.

Methods
Human 3D enteroid cultures were generated from proximal and distal small intestine fetal tissue. Differentiated enteroid monolayers grown on transwells were infected with S. suis (MOI 50) and translocation was monitored for 6 hours. Monolayer permeability during infection was monitored with 4kDa FITC-dextran permeation. Enteroid monolayers were grown in 48-wells plates to quantify S. suis adhesion and invasion. Monolayers were analyzed by confocal imaging and flow cytometry after immunofluorescence staining.

Results
Zoonotic S. suis strains from SS2CC1 and SS2CC20 showed increased translocation across the proximal compared to distal enteroid monolayers. Zoonotic SS2CC1 translocated more than the non-zoonotic SS9CC16, without affecting the monolayer permeability as measured by FITC-dextran permeation. Translocation occurred without damaging the tight junctions or adherens junctions that seal off the paracellular route. The tested CCs did not differ in adhesion and invasion. Translocation of SS2CC1 depended on the presence of Gb3+ cells within the monolayer.

Discussion
Zoonotic S. suis from SS2CC1 is better in translocating across the human small intestine than non-zoonotic SS9CC16. Similar to observations made in the Caco-2 model. In contrast to the Caco-2 model, the adhesion and invasion capabilities did not differ between the tested CCs and translocation did not affect the adherens or tight junctions. Translocation of SS2CC1 depended on the presence of Gb3+ cells within the monolayer, a receptor also used by other human pathogens.

P209

SEASEEDS project: towards understanding the role of the microbiome in development of sugar kelp

Federica R. Schanz[1], Otto van der Linden[2], Dr. Tijs Ketelaar[2], Renier Nauta[3], Dr. ir. Detmer Sipkema[1]
[1]Wageningen University & Research, Wageningen,, Netherlands, [2]Wageningen University & Research, Wageningen,, Netherlands, [3]Wageningen Research, Yerseke,, Netherlands

While seaweed cultivation for food, feed and chemicals is well established in the Eastern part of the world, the European sector is still developing. A major challenge is the up to 90 % loss of seeded seaweed in cultivations. The current work is part of the SEASEEDS project that aims to lift related constraints limiting the progression of large-scale seaweed cultivation in the Dutch North Sea by improving the sugar kelp attachment. To do so, we aimed to improve our understanding of the development of early life-stages of sugar kelp and the role of microbes therein. To identify key developmental stages during which attachment is a limiting factor and to explore microbiota-based methods to improve attachment, we studied gametophyte development and embryogenesis in the presence of the associated microbiota. We have microscopically characterized the distinct life-stages of the sugar kelp lifecycle as a reference for key developmental stages including meiospores, gametophytes and the fertilization as the turning point whereafter embryogenesis takes place. We developed a method to generate axenic cultures that we will use to investigate the consequences of microbiota on attachment and development. This provides fundamental knowledge of developing sugar kelp individuals, where we can specifically focus on the microbial impact onto next. This allows us further to identify new stages to optimize the system regarding attachment.

P210

Genetic variation of the Group A Carbohydrate biosynthesis genes in the Streptococcus pyogenes population reveals rare structural diversity

Ing. Kim Schipper[1], Dr. Y Pannekoek[1], Prof. Dr. N.M. van Sorge[1,2]
[1]Amsterdamumc, Amsterdam, The Netherlands, [2]AmsterdamUMC, Amsterdam, The Netherlands

Streptococcus pyogenes, also known as Group A Streptococcus (GAS), causes millions of relative mild infections such as pharyngitis, but also severe life-threatening infections, resulting in an estimated global disease burden of 700 million infections and 500,000 deaths annually. The World Health Organization (WHO) has prioritized vaccine development to reduce the public health burden of GAS infections. The surface-exposed Group A Carbohydrate (GAC) is an attractive vaccine antigen due to its assumed universal and conserved expression among the S. pyogenes population. Moreover, we have previously shown that presence of specific GAC epitopes, such as the N-acetylglucosamine side chain and glycerolphosphate (GroP) moieties, strongly impacts host-pathogen interaction. We investigated potential structural diversity of GAC by studying the genetic variation of the genes implicated in GAC biosynthesis across the S. pyogenes population.

Whole genome sequences of 2,044 S. pyogenes were uploaded to the PubMLST database. The genes implicated in GAC biosynthesis (gacA-gacL) were annotated and screened for genetic variation, where allele 1 was assigned to reference strain MGAS5005.

All 2,044 S. pyogenes isolates contained the gacA-gacL gene cluster. Nucleotide sequence variation was most prominent in gacH (286 alleles) with three most prominent alleles of gacH representing only 11% of all gacH sequences. In contrast, gacJ represented the most conserved gene of the cluster with only 34 alleles, with the three most frequently found gacJ alleles covering 80% of the population's variability. The 286 alleles of gacH represent 181 different protein sequences and 12 isolates contained internal stop codons. We are currently investigating whether these truncated proteins and amino acid substitutions in active domains affect the function of GacH, which is responsible for adding the GAC-GroP modification.

GAC biosynthesis genes showed a high level of sequence conservation although some interesting natural mutants were identified that may be hampered in virulence and vaccine coverage.

P212

Transmembrane mucin MUC1 modulates the NFkB/p65 response during Salmonella infection of intestinal epithelial cells

Ms. Jinyi Su[1], Dr X Li, Ms. E Floor, Dr K Strijbis
[1]Utrecht University, Utrecht, The Netherlands

The transmembrane mucin MUC1 is expressed on the apical surface of the intestinal epithelium. MUC1 consists of an extracellular domain (ED) that is highly glycosylated and a cytoplasmic tail (CT) that has signaling capacity. Previously, we have reported that Salmonella Enteritidis (S.Enteritidis) can employ MUC1 as a receptor for apical invasion of enterocytes, HT29-MTX cells. The bacterium utilizes the giant adhesin SiiE to interact with sialic acids on the MUC1-ED. Here, we investigate the exact roles of MUC1-ED and MUC1-CT during invasion. Using CRISPR/Cas9, we created HT29-MTX MUC1 knockout cells (ΔMUC1) and cells that lacked the MUC1-CT (ΔMUC1-CT). With the EHEC-derived mucinase StcE, we can remove the MUC1-ED while leaving the transmembrane domain and CT intact. Using these cell lines and conditions, a significant reduction in bacterial infection was observed in ΔMUC1 and StcE-treated cells that lacked the MUC1-ED, pointing at the importance of the ED. No difference in bacterial infection was observed for MUC1-ΔCT cells, showing that the CT is not essential for bacterial invasion through MUC1. We next investigated if the MUC1-CT played a role in downstream NFκB signaling. Mucins were previously linked to regulate of this central epithelial pathway. In MUC1-ΔCT cells, the NFκB p65 subunit is more abundant compared to wild-type cells even without S.Enteritidis invasion. At the transcriptional level, a significant upregulation in the NFKBIA gene was observed in MUC1-WT than in MUC1-ΔCT cells after S.Enteritidis infection. ELISA experiments showed equal IL-8 production in wild-type and MUC1-ΔCT cells after S.Enteritidis invasion, while ΔMUC1 cells showed a more moderate IL-8 production. RNAseq identified NFκB genes to be differentially regulated after S.Enteritidis invasion in wild-type cells compared to MUC1-ΔCT and ΔMUC1 cells. In conclusion, while MUC1 serves as a receptor for S.Enteritidis invasion, the MUC1 cytoplasmic tail might have an anti-inflammatory function by suppressing the NFκB pathway.

P213

Frontline defense against Streptococcus pyogenes by human Langerhans cells

Sara Tamminga[1], L. Gonizzi[1], Dr. A. Hendriks[1], Dr. Y. Pannekoek[1], Prof. N.M. van Sorge[1,2]
[1]Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands, [2]Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam, The Netherlands

Introduction

Streptococcus pyogenes or Group A Streptococcus (GAS) is a major cause of non-invasive and invasive infections resulting in an estimated 500,000 deaths worldwide annually. The streptococcal M protein, encoded by emm, is a major virulence factor through a wide range of interactions with host molecules. Consequently, immunity against M protein provides protection from infection, but protection is M-restricted. The skin and pharynx are prime entry sites for GAS. Subsequent local infections are on the obligatory causal pathway to more severe disease manifestations. Langerhans cells (LCs), which are characterized by the expression of the C-type lectin receptor langerin, are the only innate sentinel cells in the skin epidermis and are present in oral mucosa. LCs can take up, process and present antigens to local memory T cells as well as naïve T cells in the draining lymph nodes. Our study aimed to investigate whether and how LCs recognize a diverse range of GAS M-types to locally detect and eliminate invading bacteria.

Methods

In this study, we stimulated in vitro-generated LCs (muLCs) with wild-type GAS and determined cytokine production and muLC maturation by ELISA and flow cytometry, respectively. Furthermore, we used soluble human recombinant langerin to probe interaction with a diverse range of wild-type GAS M-types as well as genetically-modified strains.

Results

In vitro-generated muLCs interacted with GAS, leading to increased cytokine production and muLC maturation. Binding of recombinant human langerin differed across GAS isolates and M-types. In addition, binding to GAS M3 was abrogated in the presence of mannan and by deletion of emm3.

Conclusion

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

P214

Universal targeting of Staphylococcus aureus through antibodies binding wall teichoic acid

Dr. Robin Temming[1], Alessia Di Maggio[2], Paul M. P. van Bergen en Henegouwen[2], Jeroen D. C. Codée[3], Thijs Voskuilen[3], Sabrina Oliveira[2,4], Nina M. van Sorge[1,5]
[1]Amsterdam UMC, location AMC, Amsterdam, The Netherlands, [2]Utrecht University, Utrecht, The Netherlands, [3]Leiden UMC, Leiden, The Netherlands, [4]Utrecht University, Utrecht, The Netherlands, [5]Amsterdam UMC, location AMC, Amsterdam, The Netherlands

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

P215

A Core-Genome Multilocus Sequence Typing Scheme for Detection of Genetically Related Streptococcus pyogenes Clusters

Dr. Myrthe Toorop[1], MEM Kraakman[1], Drs. IV Hoogendijk[1], Dr. J. van Prehn[1], Dr. ECJ Claas[1], Dr. E. Wessels[1], Dr. SA Boers[1]
[1]Leiden University Medical Center, , Leiden

In the Netherlands, a post-COVID19 increase in invasive Group A streptococcal disease was noted by the national surveillance program. However, conventional emm-typing and multilocus sequence typing (MLST) have limited resolution for detecting local genetically related clusters and transmission, indicating a need for higher-resolution molecular typing methods to assess genetic relatedness between S. pyogenes strains. Here, we aimed to develop a novel core-genome MLST (cgMLST) scheme and compared its discriminatory ability to conventional emm-typing and MLST on a suspected local cluster and publicly available datasets.

A cgMLST scheme comprising 1095 target genes was designed in the commercially available Ridom™ SeqSphere+ software package using whole-genome sequence (WGS) data of publicly available S. pyogenes isolates. The scheme was then evaluated using three different data-sets: (1) eight possible outbreak related clinical S. pyogenes isolates collected from December 2021-October 2022 at Leiden University Medical Center (the Netherlands), (2) WGS data of 101 S. pyogenes isolates provided by the Netherlands Reference Laboratory for Bacterial Meningitis and 3) WGS data of 30 S. pyogenes isolates from recent outbreaks in England [1]. The cgMLST results were compared to the standard MLST-scheme and emm-typing.

In the first dataset, five isolates belonged to ST 36 and emm-type 12.0. cgMLST analysis revealed that among those isolates, the maximum distance between target genes of four isolates was two target genes, possibly identifying a cluster. Minimum distance between the cluster and isolates from dataset 2 was twelve. For data from confirmed outbreaks, maximum distance within outbreaks between target genes was five.

The proposed cgMLST scheme shows a higher discriminatory ability when compared to conventional typing methods, and identified potential clusters that were not related to other community isolates. The robustness of this cgMLST scheme, in combination with easy-to-use software enables widespread improvement of genomic surveillance of S. pyogenes, allowing increased detection of transmission.

P216

The effect of microbial exposure on vaccination responses of laboratory mice

Rienke Uijen[1,2], Fred van Opzeeland[1,2], Diane ten Have[3], Martijn H Salomons[3], Elles Simonetti[1,2], Martin F Toft[4], Axel K Hansen[5], Jeroen D Langereis[1,2], Marien I de Jonge[1,2]
[1]Radboudumc, Nijmegen, The Netherlands, [2]Radboudumc, Nijmegen, The Netherlands, [3]University of Groningen, Groningen, The Netherlands, [4]QM Diagostics, Nijmegen, The Netherlands, [5]University of Copenhagen, Copenhagen, Denmark

Introduction
Vaccination studies are usually performed with laboratory mice that are kept under specific pathogen free (SPF) conditions. However, in recent studies, it was found that the microbiome and immune system of these SPF mice is underdeveloped compared to mice that have been exposed to microbial stimuli. Studies have shown that the immune system of mice with increased microbial exposure resemble the human immune system to a larger extend and, therefore, might be better in predicting the outcome of clinical studies. Here, we determined whether increased microbial exposure has an impact on vaccination responses of laboratory mice.

Methods
To investigate the effects of microbial exposure, groups of 24 mice were either kept under regular SPF conditions, with or without pre-treatment with a mix of inactivated murine pathogens, or kept outdoors in open cages resulting in environmental exposure. Within each condition, eight mice received the PCV13 vaccine, the Boostrix vaccine or salt solution as negative control. Vaccines were administered intramuscularly three times. Serum samples were collected pre- and post-vaccination. Eleven days after the last vaccination, mice were euthanized after which blood and bronchoalveolar lavage were collected. Antibody levels against vaccine-included antigens were measured in blood and mucosal samples using a fluorescent bead-based multiplex immunoassay.

Results
The effects of microbial exposure on the antibody response were generally minor for the measured antigens. However, a clear inhibitory effect of pre-immunization with the pathogen mixture was seen on the antibody response against the 18C pneumococcal polysaccharide. Interestingly, naïve mice that were housed outdoors had higher polyreactive IgM antibodies, binding to 11 out of 13 pneumococcal polysaccharides but not the Bordetella pertussis protein antigens.

Conclusions
The microbial exposure as tested in our experiment did not lead to major changes in the vaccine-induced antibody responses. However, a few interesting phenomena were observed which require further research.

P217

Secretory IgA impacts the microbiota density in the human nose

Dr. Rob van Dalen[1], A.M.A. Elsherbini[1], M. Harms[1], S. Alber[1], R. Stemmler[1], A. Peschel[1]
[1]University of Tübingen, Tübingen, Germany

Respiratory mucosal host defense relies on production of secretory IgA (sIgA) antibodies, but we currently lack fundamental understanding of how sIgA is induced by contact with microbes and how such immune responses may vary between humans. Defense of the nasal mucosal barrier through sIgA is critical to protect from infection and to maintain homeostasis of the microbiome, which influences respiratory disorders and hosts opportunistic pathogens. Through IgA-seq analysis of nasal microbiota samples and sIgA deposition experiments using nasal sIgA from male and female healthy volunteers, we identified which bacterial genera and species are targeted by sIgA on the level of the individual host. We observed that the amount of sIgA secreted into the nasal mucosa by the host varied substantially and was negatively correlated with the bacterial density, suggesting that nasal sIgA limits the overall bacterial capacity to colonize. The interaction between mucosal sIgA antibodies and the nasal microbiota was highly individual with no obvious differences between potentially invasive and non-invasive bacterial species. Importantly, we show that for the clinically relevant opportunistic pathogen and frequent nasal resident Staphylococcus aureus, sIgA reactivity was in part the result of epitope-independent interaction of sIgA with the antibody-binding protein SpA through binding of sIgA Fab regions. This study thereby offers a first comprehensive insight into the targeting of the nasal microbiota by sIgA antibodies. It thereby helps to better understand the shaping and homeostasis of the nasal microbiome by the host and may guide the development of effective mucosal vaccines against bacterial pathogens.

P218

WGS-based pathogen surveillance of Yersinia spp. shows circulation of pathogenic strains in the Netherlands with limited clustering

Dr. Maaike Van Den Beld[1], Dr. Roan Pijnacker[2], Ing. Kim van der Zwaluw[1], Ing. Ilse Zutt[1], Ing. Sjoerd Kuiling[1], Dr. Eelco Franz[2], Dr. Thijs Bosch[1], On behalf of participating Medical Microbiological Laboratories
[1]RIVM, Bilthoven, The Netherlands, [2]RIVM, Bilthoven, The Netherlands

Introduction: Yersiniosis is a zoonotic foodborne gastro intestinal infection in humans. Because there is currently no surveillance system for Yersinia infections in the Netherlands, there is no insight in long-term trends or clustering of human cases. Therefore, a pathogen surveillance of Yersinia spp. was implemented using existing laboratory infrastructure of other foodborne species based on whole genome sequencing (WGS).
Methods: In April 2022, Medical Microbiological Laboratories (MMLs) were requested to share Yersinia isolates with the National Institute for Public Health and the Environment (RIVM) on a voluntary basis. All isolates were typed using MALDI-TOF and WGS with Illumina techniques. Paired end reads were produced, quality checked, de novo assembled and species identified, using ISO 15189 validated procedures. In Ridom SeqSphere, McNally 7-locus MLST typing and cgMLST using the Enterobase Yersinia V1 scheme were performed. Cluster cut-off was defined as maximum 5 alleles difference.
Results: Twenty-two MMLs shared 112 Yersinia isolates of 110 unique patients. Forty-six cases (42%) were male and 63 (57%) were female with a median age of 29 years (range: 0-92). Forty-four isolates (40%) were assigned to known virulent sequence types; ST12 (n=10), ST14 (n=1) and ST18 (n=33). Thirteen (12%) isolates clustered with at least one other isolate. Five clusters were identified, of which one cluster contained five isolates, four clusters contained two isolates each. Cases in three clusters were geographically dispersed throughout the Netherlands, the other two clusters contained cases that reside within 25 and 35 km ranges.
Conclusion: WGS-based pathogen surveillance gave more insight in clustering and age-gender distribution of yersiniosis cases in the Netherlands. Preliminary results showed that pathogenic strains were encountered throughout the Netherlands, with limited clustering. In order to monitor circulation and clustering of Yersinia spp. in the Netherlands, we propose that this surveillance should be continued with our partners in the field.

P219

Staphylococcus aureus α-Toxin pH-Dependent Cytotoxicity

Tristan Van Der Linden[1], A.N. Spaan[1]
[1]UMC Utrecht, , The Netherlands

Introduction: Staphylococcus aureus is a commensal colonizer of the skin. Colonization is a risk factor for invasive perioperative infections. To facilitate its survival in the host, S. aureus strains produce an arsenal of virulence factors. Most strains secrete α-toxin (AT), a β-barrel pore-forming toxin that is secreted as a water-soluble monomer. Upon binding to the host target cell surface, AT heptamerizes into cytolytic pores. The main AT-receptor, ADAM10, is expressed by many cell types. Hence, AT targets a large array of hematopoietic and non-hematopoietic cells. Interestingly, despite the broad cellular specificity of AT, S. aureus infections display a tropism to the skin and lungs. One explanation for the tropism of S. aureus infections may be found in the non-physiological pH present in these organ systems, of which the epithelial barriers are naturally more acidic. This study aims to elucidate the mechanisms and effects of the environmental acidity on AT-induced cellular viability and organ injury.
Methods: We used various non-hematopoietic cell lines (A549, HaCaT and HEK293T) and hematopoietic cell lines (U937 and THP-1). AT cytotoxicity was evaluated with cell viability and cell death assays. Medium pH was altered and measured under live-cell conditions. ADAM10 expression and activity were measured at varying pHs in A549 cells.
Results: In non-hematopoietic cell lines, we show a potentiation of AT cytotoxicity in acidic environments. The capacity of AT to activate ADAM10 in these cells appears to be a function of the pH, but the expression of ADAM10 is not. Interestingly, we do not observe such pH-dependent potentiation of AT in hematopoietic cell lines.
Conclusion: Our findings show a cell-type dependent relation between the environmental pH and AT cytotoxicity. This suggests that environmental acidity plays a role in the organ tropism of S. aureus infections.

P220

Viral load dynamics in healthcare workers with COVID-19 during Delta and Omicron era

Dr. Brian van der Veer[1], Dr. J Dingemans[1], MD LEA Bank[1], Dr. CJH von Wintersdorff[1], Y Nijsen[1], Dr. IHM van Loo[1], Prof PHM Savelkoul[1]
[1]Department of Medical Microbiology, infectious diseases & Infection prevention, Maastricht University Medical Center+, Maastricht, Netherlands., Maastricht, The Netherlands

Background
During the SARS-CoV-2 pandemic, several variants of concern (VOC) have emerged and became the dominant strain. These are considered VOC because of traits like increased transmissibility, increased severity, and immune-evasion. Understanding their viral load dynamics, with the use of longitudinal follow-up data, can help to understand transmission and could inform policy makers on for example infection prevention guidelines. However, longitudinal follow-up studies are scarce. In this study, we used an unique longitudinal dataset to monitor the viral load dynamics of the Delta, Omicron BA.1 and BA.2 variants.

Methods
Retrospective longitudinal follow-up viral load data of healthcare workers at our institution that was obtained as a consequence of the implemented infection prevention guidelines.

Results
We found that the dynamics are different between Delta, Omicron BA.1, and Omicron BA.2 variants, the latter having the highest viral load on day five (5·1 log10 copies/mL compared to 4·4 log10 copies/mL for Delta and 4·8 log10 copies/mL for BA.1) and day seven (4·1 log10 copies/mL compared to 3·2 log10 copies/mL for Delta and 3·7 log10 copies/mL for BA.1). However, the infection duration does not appear to be different between these variants. Still, considerable viral loads even after the suggested quarantine period were observed, in particular for the Omicron BA.2 subvariant.

Conclusion
The considerable differences highlight the need for a tailored approach per variant as results of previously circulating variants do not always match. This is important for healthcare workers in particular as they can transmit SARS-CoV-2 to vulnerable patients

P221

Despite no ceftriaxone resistance in the Netherlands many Neisseria gonorrhoeae isolates have a mosaic penA gene

Dr. Brian van der Veer[1], Prof CJPA Hoebe[1,2,3], Dr. LB van Alphen[1], Dr. PFG Wolffs[1]
[1]Department of Medical Microbiology, Infectious diseases and Infection prevention, Maastricht University Medical Center (MUMC+), PO Box 5800, 6202 AZ Maastricht, the Netherlands, Maastricht, The Netherlands, [2]Department of Sexual Health, Infectious Diseases and Environmental Health, South Limburg Public Health Service, PO Box 33, 6400 AA Heerlen, the Netherlands, Heerlen, The Netherlands, [3]Department of Social Medicine, Care and Public Health Research Institute (CAPHRI), Maastricht University, the Netherlands, Maastricht, The Netherlands

Background: Neisseria gonorrhoeae (NG) is increasingly resistant towards all classes of antibiotics, including ceftriaxone. Ceftriaxone resistance can occur by recombination with DNA of commensal Neisseria species forming mosaic penA genes. To study the presence of commensal Neisseria species and possible penA recombination during an oropharyngeal NG infection, penA gene and flanking regions were long read sequenced.

Methods: Men who have sex with men (MSM, n=57) who were tested more than once and had at least one NG positive oropharyngeal sample between January 2017 and June 2018 were selected retrospectively. NG positivity was based on the cobas 4800 CT/NG assay and all NG positive oropharyngeal swab samples (64/176) were included. The penA gene and adjacent regions (3.3kb), used for species identification, were PCR amplified and sequenced (Nanopore). Amino acid sequences of penA were compared between Neisseria species when a mosaic gene was observed in NG.

Results: A total of 61/64 NG positive samples were penA PCR positive. In all samples N. subflava was observed, N. cinerea was present in 35/61 samples, N. elongata 31/61, N. meningitidis 29/61, N. mucosa 26/61, N. lactamica 8/61, and N. oralis 9/61. A NG consensus sequence could be constructed in 28/61 samples of which 8/28 were mosaic. This part was similar to one of the commensal Neisseria species within the same sample for all of these samples.

Conclusion: Despite no ceftriaxone resistance in the Netherlands, in 29% of the NG consensus sequences, a mosaic penA gene was observed. Our study provides further evidence that commensal Neisseria species are frequently present during a NG infection. This suggests that recombination of DNA from commensal Neisseria species could occur frequently during an oropharyngeal NG infection. Therefore, future studies should also focus on the commensal Neisseria species as they could have a pivotal role in AMR development in NG.

P222

The antimicrobial activity of Graphene Quantum Dots

Laure Van Hofwegen[1], Muhammad Hassnain[2], Dr. Payal Balraadjsing[1], Associate Professor Sedat Nizamoglu[2], Assistant Professor Sebastian Zaat[1]
[1]Amsterdam UMC, Amsterdam, The Netherlands, [2]KOC University, Istanbul, Turkey

One of the most common complications related to implantation of a biomaterial is biomaterial-associated infection (BAI). BAI is predominantly caused by the commensal bacteria Staphylococcus aureus and Staphylococcus epidermidis, which can become pathogenic in the presence of a biomaterial. These infections may lead to chronic inflammation and in severe cases, loss of implant function. Biomaterial surfaces coated with antimicrobials are a promising strategy for prevention of BAI, but the use of antibiotics is discouraged due to resistance development. Graphene quantum dots (GQD) have good antimicrobial properties with low cytotoxicity and might provide an alternative for antibiotics to protect against infection. GQD consist of a single layer of carbon atoms in a honeycomb-like structure with photoactivation properties. Upon photoactivation, GQD can produce reactive oxygen species (ROS) which can kill bacteria. The aim of our study was to test a novel GQD coating for its antimicrobial activity against S. aureus. The coating consisted of several alternating layers of GQD and polymer applied on glass slides. To test the antimicrobial activitiy, we used the Japanese Industrial Standard (JIS) assay where we photoactivated the GQD coating with 450nm blue light. Surprisingly, the GQD coating showed promising antimicrobial activity with, as well as without photoactivation. The properties of the polymer are likely responsible for the antimicrobial activity without photoactivation. With photoactivation, the GQD further enhanced the antimicrobial activity, which resulted in complete killing of the bacteria. Taken together, these data show that the GQD coating has promising (dual) antimicrobial activity against S. aureus. Therefore, GQD could make a suitable candidate for application on wound dressings or central vein catheters.

P223

In-host adaptation of Aspergillus fumigatus in chronic fungal airway infections in cystic fibrosis patients.

Msc Jacq Van Neer[1]
[1]Utrecht University, Utrecht, The Netherlands

Aspergillosis due to Aspergillus fumigatus is a lung-associated infection which can develop into an invasive form in especially immunocompromised patients, or a noninvasive form for example in patients with cystic fibrosis. A recent survey revealed that aspergillus fumigatus is the most frequently isolated fungus in patients with cystic fibrosis with a mean prevalence of 31.7%. Fungal infections with Aspergillus fumigatus in cystic fibrosis patients are often chronic and patients have been shown to carry the same fungal genotype for years. As bronchial airway mucosa is not the natural habitat of Aspergillus fumigatus, it can be expected that chronic infections require in-host adaptation to allow growth and survival in the lungs.

Chronic Aspergillus fumigatus isolates from several cystic fibrosis patients from two cystic fibrosis centres in the Netherlands were selected and genotyped with a short tandem repeat analysis. For each isolate it was determined whether or not an identical genotype could be found multiple times over the span of three months or more in the same patient. This resulted in the discovery of multiple chronic isolates. The genomes of 57 isolates have been sequenced and the genetic changes between the first found isolate and the last, with the same genotype, have been determined to uncover possible mutations responsible for the successful in-host adaptation. To check our findings we have done multiple phenotypical analyses on the chronic isolates as well. Their sensitivity to reactive oxygen species and cell wall damaging compounds have been investigated as well as their morphology on different media.

We found the presence of chronic infections mostly in older patients and phenotypic changes do occur during chronic infections. The sensitivity to reactive oyxgen species and cell wall damaging compounds is changed in around 50% of the chronic isolates. We expect the genomic data to reflect this pattern as well.

P224

A genomic description of methicillin-susceptible Staphylococcus aureus in patients on home parenteral nutrition and their caregivers (CARRIER-TRIAL)

Msc Ingrid Van Weerdenburg[1], M Gompelman[3], G.T.J. Wezendonk[3], Msc. J.P.M. Coolen[1], Prof. dr. C.P. Rovers[2], Dr. G.J.A. Wanten[3], Prof. dr. H.F.L. Wertheim[1]
[1]Radboudumc, Nijmegen, The Netherlands, [2]Radboudumc, Nijmegen, The Netherlands, [3]Radboudumc, Nijmegen, The Netherlands

Background: Patients on home parenteral nutrition (HPN) are, due to their dependence of a central venous access device, susceptible for catheter-related infections. S. aureus is a frequent cause of severe catheter-related infections. To avoid S. aureus infections, decolonization treatment can be an effective strategy. Importantly, the HPN patients' caregiver assists in catheter care and may serve as an important reservoir for S. aureus carriage. We aimed to investigate S. aureus genetic relatedness in HPN patients during eradication and between HPN patients with their caregiver.
Methods: This was a prospective, observational study among 241 HPN patients, of whom 127 (53%) had a caregiver to culture (2018-2021). Samples from multiple body sites were collected to screen for S. aureus. Sixty-three HPN patients received decolonization treatment and follow-up swabs were collected every three months during one year. Whole genome sequencing (WGS) was performed on cultured S. aureus strains. Bioinformatic and statistical analysis was done to describe genetic relatedness, which was defined as ≤ 30 single-nucleotide polymorphism (SNP) difference.
Results: In almost 60% of the HPN patients who received eradication treatment, one or more genetic related S. aureus strains were found over the 1 year follow-up period. Forty-one S. aureus positive HPN patients (32%) had a S. aureus positive caregiver. HPN patients were more likely to be a carrier if their caregiver was also a carrier, OR 2.32 (95% CI 1.13, 4.76, p = 0.02). Genetic relatedness was present in 68% of these cases.
Conclusion: Although patients were subjected to decolonization treatment, repeated cultures of genetically related S. aureus strains were found. We also demonstrated that S. aureus carriage among HPN patients is more likely if their caregiver is also a carrier. This suggests transmission between HPN patients and their caregivers. Therefore, screening and decolonization of caregivers should be considered as well.

P225

Effect of antibiotic treatment on commensal Neisseria species load and presence in the oropharynx.

Mr. Sem Vellema[1], Dr. BMJA van der Veer[1], M Lucchesi[1], Drs. JM van Niekerk[1], Prof. Dr. CJPA Hoebe[1,2,3], Prof. Dr. PHM Savelkoul[1], Dr. PFG Wolffs[1]
[1]Department of Medical Microbiology, Care and Public Health Research Institute (CAPHRI), Maastricht University Medical Center+, Maastricht, Netherlands, , , [2]Department of Sexual Health, Infectious Diseases and Environmental Health, Living Lab Public Health, South Limburg Public Health Service, Heerlen, the Netherlands, , , [3]Department of Social Medicine, Care and Public Health Research Institute (CAPHRI), Maastricht University, the Netherlands, ,

INTRODUCTION Ceftriaxone-resistant Neisseria gonorrhoeae (NG) poses a threat to successful first-line empirical treatment of gonorrhoeae. Ceftriaxone resistance is partially conferred through mosaic penA alleles, which are mainly obtained through transformation of DNA from commensal Neisseria species colonizing the oropharynx. These commensals themselves may exhibit high-level ceftriaxone resistance. Little is known about the effect of antibiotic treatment on their load and species-specific effect on presence within the oropharynx. Therefore, this study aimed to evaluate patterns in Neisseria species load and presence before and after antibiotic treatment.

METHODS A total of 96 oropharyngeal swabs were selected from follow-up consultations of 10 patients who visited our STI clinic between 2019 and 2021. Selection was based on frequency of positive PCR tests for NG. Samples were tested for bacterial load of commensal Neisseria and total bacterial load through PCR. Commensal Neisseria species identification was performed through targeted metagenomic sequencing of penA and flanking regions using Nanopore sequencing. Patients were treated with ceftriaxone 500mg IM according to national guidelines when testing positive for NG.

RESULTS PCR results showed that proportions of commensal Neisseria varied from 0.003% to 33%, with no clear observable difference between samples taken before and after antibiotic treatment. Targeted metagenomic sequencing showed a high degree of stable presence of N. subflava and stable absence of N. lactamica and N. oralis after antibiotic treatment. Other Neisseria species showed varying degrees of losses and gains after antibiotic treatment.

CONCLUSION Commensal Neisseria form a reservoir of AMR for NG. Our results indicate that commensal Neisseria are not evidently affected in load after antibiotic treatment compared to other commensal bacterial species inhabiting the oropharynx. Furthermore, results indicate that some Neisseria species are more stable than others in response to antibiotic treatment, indicating that some Neisseria may provide a more suitable reservoir of AMR than others.

P226

Spectroscopic insights into the mechanism of anammox hydrazine synthase

Dr. Wouter Versantvoort[1], Prof R. Hienerwadel[2], Dr. C. Ferousi[3], P. van der Velden[1], Dr. C. Berthomieu[4], Dr. F. Baymann[5]
[1]Radboud University, Nijmegen,, The Netherlands, [2]Aix-Marseille University, Marseille,, France, [3]National Technical University of Athens, Athens,, Greece, [4]CEA-Caderache, Saint-Paul-Lez-Durance,, France, [5]CNRS/AMU, Marseille,, France

Anaerobic ammonium oxidizing (anammox) bacteria are chemolithoautotrophic microorganisms that make a living by converting nitrite and ammonium to dinitrogen gas, with the rocket fuel hydrazine as a unique free intermediate [1]. The c-heme containing protein complex hydrazine synthase catalyzes the formation of hydrazine in anammox metabolism and has been purified directly from native biomass as an αβγ dimer. Analysis of the crystal structure [2] allowed for the hypothesis of a two step reaction mechanism where nitric oxide is first reduced to hydroxylamine at active site heme γI. Hydroxylamine then diffuses through an intra-protein tunnel to a second active site heme in the α subunit, where it is proposed to condense with ammonium to form hydrazine. Anammox bacteria thus seem to utilize the oxidative power of hydroxylamine to anaerobically activate inert ammonium, resulting in the formation of hydrazine. To assess this proposition and to assign individual redox properties to each heme at both active sites, the redox properties of the hemes were determined by spectroelectrochemistry and EPR spectroscopy and this data was combined with structural information and redox-induced FTIR difference spectra. Spectroscopic analysis in the presence of substrates and reaction intermediates was performed, giving first insights into the reaction mechanism of hydrazine synthase.

[1] Kartal, B., de Almeida, N.M., Maalcke, W.J., Op den Camp, H.J., Jetten, M.S. and Keltjens, J.T., How to make a living from anaerobic ammonium oxidation. FEMS Microbiol Rev, 37 (2013): 428-461.
[2] Dietl, A., Ferousi, C., Maalcke, W., Menzel, A. de Vries, S. Keltjens, J.T., Jetten, M.S.M., Kartal, B., Barends, T,R.M., The inner workings of the hydrazine synthase multiprotein complex. Nature 527 (2015), 394–397.

P227

Development of a rapid viability RT-PCR assay for detection of intact SARS-CoV-2

Msc Judith Veugen[1,2,3,4], MSc T Schoenmakers[5], Dr. IHM van Loo[3,4], Dr. BL Haagmans[6], Dr. MPG Leers[5], Prof. Dr. RMMA Nuijts[1,2,7], Prof. Dr. PHM Savelkoul[3], Dr. MM Dickman[1,2], Dr. PFG Wolffs[3,4]
[1]Maastricht University Medical Center, Maastricht, The Netherlands, [2]Maastricht University, Maastricht, The Netherlands, [3]Maastricht University Medical Center, Maastricht, The Netherlands, [4]Maastricht University, Maastricht, The Netherlands, [5]Zuyderland Medical Center, Heerlen, The Netherlands, [6]Erasmus Medical Center, Rotterdam, The Netherlands, [7]Zuyderland Medical Center, Heerlen, The Netherlands

Introduction: Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) is an enveloped virus that causes COronaVIrus Disease 2019 (COVID-19). The most commonly used methods to detect SARS-CoV-2 RNA cannot conclude whether samples that tested positive for SARS-CoV-2 contain infectious virus particles. In viability PCR (v-PCR), samples are pre-treated with a photoreactive dye that binds to RNA of compromised virions and prevents RNA amplification. The aim of this study was to develop a rapid and sensitive v-PCR assay that can distinguish between intact and compromised SARS-CoV-2.
Methods: PMAXX was used as a photoreactive dye. Mixtures were prepared with concentrations ranging from 100% to 0% viable SARS-CoV-2 and each concentration was divided in a PMAXX-treated sample and a non-PMAXX-treated sample. After PMAXX-treatment, Reverse transcription-PCR (RT-PCR) using an in-house developed SARS-CoV-2 viability assay was applied to both PMAXX-treated and non-PMAXX-treated samples. Delta Cycle threshold (Ct) value was determined by subtracting the Ct value of the non-PMAXX-treated sample from the Ct value of the PMAXX-treated sample to determine the difference in intact SARS-CoV-2.
Results: Each experiment was performed for both high and low viral loads. Mixtures containing 100%, 50%, 10%, 1%, 0.1%, and 0% viable SARS-CoV-2 showed mean delta Ct values of 2.1, 3.5, 6.2, 9.4, 12.3, and 14.2 respectively in high viral load samples. In low viral load samples, mean delta Ct values were 2.4, 3.8, 5.8, 8.5, ≥11.6, and ≥11.5 respectively. The smaller the delta Ct value, the higher the percentage of intact SARS-CoV-2.
Conclusion: High and low viral load mixtures containing different percentages of viable SARS-CoV-2 showed similar results, making this assay suitable for both high and low viral loads. In the current study, a rapid v-PCR assay has been developed that can distinguish between intact and compromised SARS-CoV-2. The presence of intact virions may be a measure of SARS-CoV-2 infectiousness.

P228

Sequencing-based surveillance of circulating Bordetella pertussis strains in the Netherlands from 2015-2020

Dr. Linda Visser[1], Valentijn Tromp[1], Ramon Noomen[1], Gerlinde Pluister[1], James Groot[1], Rob Mariman[1]
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Introduction
Whooping cough is a notifiable disease in the Netherlands. Since the introduction of the acellular pertussis vaccine there is active surveillance on the circulating strains, with an emphasis on presence of vaccine antigens in these strains and early detection of possible vaccine-escape mutants.

Methods
Diagnostic laboratories participate voluntarily to the pertussis surveillance by sending in pertussis isolates. These isolates were cultured and tested biochemically before being subject to next-generation sequencing. Here we perform a analysis of all strains submitted in the 5 years prior to the COVID-19 pandemic. A population structure is determined using cgMLST and we provide a detailed analysis of the trends in vaccine antigen expression.

Results
276 pertussis isolates were sequenced between 2015 and 2020. We compared the cgMLST schemes available at the BIGSDB instance of Institut Pasteur, and the recently published wgMLST scheme. While the wgMLST scheme had the highest discriminatory power, we found that roughly 10% of alleles of this scheme were missing from our isolates. Meanwhile, neither cgMLST scheme provided sufficient resolution to differentiate all our isolates. Pertactin-deficiency is the most important vaccine escape mechanism. We subjected all isolates to Luminex analysis of pertactin expression, and predicted pertactin expression based on the WGS-data. In earlier years (2015-2017) 10% of strains were deficient in pertactin, while this increased to 24% in later years (2018-2020).

Conclusions
The pre-COVID-19 pandemic circulating B. pertussis strains are genetically homogenous. This is highlighted by the lacking resolution of cgMLST to separate isolates without an epidemiological link. We see a trend towards more pertactin deficient strains in later years. Since the pandemic, all epidemiologic evidence suggest little to no circulation of pertussis. When pertussis re-emerges and which genetic lineages will resurface is unknown. The data presented here provide a baseline of pre-pandemic pertussis, to which future strains may be compared.

P229

The respiratory microbiome of piglets displays temporal development and harbors strains with putative probiotic use against common respiratory pathogens.

MSc. Abel Vlasblom[1], Ph.D. S. Patel[2], Ph.D. C. Huseyin[2], Ph.D. M. Claesson[2], Ph.D. D. Crespo-Piazuelo[4], Ph.D. L. Wadja[4], Ph.D. P. G. Lawlor[4], Ph.D. R. E. C. Luiken[1], Prof. J. A. Wagenaar[1,3], Ph.D. B. Duim[1], Ph.D. A. L. Zomer[1]

[1]Utrecht University, Utrecht, The Netherlands, [2]University College Cork, Cork, Ireland, [3]Wageningen University and Research, Lelystad, The Netherlands, [4]Animal & Grassland Research and Innovation Centre Teagasc, Moorepark, Ireland

Introduction

Bacterial pathogens inhabiting the porcine respiratory tract undermine animal health and welfare and some are a zoonotic risk. This study aimed at investigating the development of the porcine nasal microbiome and the identification and isolation of bacteria suitable for interventions against respiratory pathogens in pigs.

Methods

Nasal swabs were obtained from 252 piglets, 7 per litter from 36 sows, from 9 farms in 3 countries (Germany, Ireland, the Netherlands), from birth till week 10 (n=4032). DNA was isolated and duplicate samples were stored at -80C for bacterial isolation. Using V3-V4 16S rRNA and tuf gene sequencing data, network analysis was performed to identify microbial associations (SparCC). Sequencing data was supplemented with S. aureus specific qPCR data. Species negatively associated with S. aureus (mixed models, rmcorr) were isolated and identified (MALDI-TOF, 16S sequencing), followed by in vitro (phenotypical antimicrobial resistance testing) and in silico (whole genome sequencing, taxonomy, antimicrobial resistance genes, virulence factors) safety screening, following EFSA guidance. These species were subsequently utilized in an in vivo colonization experiment.

Results

From the developing nasal microbiome data, network analysis identified 26 unique taxa negatively associated with pathogens as Bordetella sp., Glaesserella parasuis, Mycoplasma hyorhinis, Pasteurella multocida, Staphylococcus aureus, and Streptococcus suis presence in all three countries. qPCR supplementation to amplicon sequencing data identified 54 species negatively associated with S. aureus. Screening resulted in 15 probiotic candidates against S. aureus, consisting of lactic acid bacteria (LAB) and species closely related to the Staphylococcus genus. A culturomics effort, followed by in vitro and in silico safety and efficacy studies, yielded three LAB strains, meeting the Qualified Presumption of Safety (QPS) status from EFSA.

Conclusion

Investigation of the developing porcine nasal microbiome resulted in probiotic candidates aimed at pathogen reduction strategies. Probiotic LAB strains against S. aureus are currently being tested in vivo.

P230

Induction of CtsR-regulated chaperones improves Xylanase production in Bacillus subtilis

Dr. Biwen Wang[1], Dr. F. van der Kloet[1], Prof. Dr. L. W. Hamoen[1]
[1]University of Amsterdam, Amsterdam, The Netherlands

Introduction: Bacillus subtilis is extensively used for the commercial production of enzymes due to its efficient secretion system. However, the efficiency of secretion varies greatly between enzymes, and despite many years of research, optimization of enzyme production is still largely a matter of trial-and-error. To identify relevant secretion bottlenecks, genome-wide transcriptome analysis seems a useful tool, yet to this day, only a limited number of transcriptome studies have been published that focus on enzyme secretion in B. subtilis.

Methods: RNA-seq, molecular cloning, enzyme activity measurement, In-depth transcriptome analysis using operon, regulon and functional category information with in-house developed software GINtool.

Results: We examined the effect of high-level expression of the commercially important endo-1,4-β-xylanase XynA on the B. subtilis transcriptome using RNA-seq. We developed a new software tool, GINtool, to interpret the transcriptome data at the regulon level and functional category level. Rather unexpectedly, we found a reduced expression of several protein chaperones, including ClpC, ClpE and ClpX, when XynA was overproduced. Expression of these chaperones is controlled by the transcriptional repressor CtsR. CtsR levels are, in turn, directly controlled by regulated proteolysis, involving ClpC and its cognate protease ClpP. Preventing this downregulation by knocking out the involved transcriptional repressor CtsR resulted in increased XynA production by more than 25 %.

Conclusion: These data show that transcriptome is a useful tool to identify bottlenecks that hamper the optimal production of enzymes. In addition, transcriptome evaluation making use of functional category and regulon information could avoid discrete and arbitrary gene selection by subjective threshold values.

P231

Enabling genomic surveillance of mpox virus by development of a novel whole genome sequencing approach

Dr. Matthijs Welkers[1]
[1]AmsterdamUMC, Amsterdam, Netherlands, [2]Public Health Services Amsterdam, Amsterdam, The Netherlands

Introduction: Mpox is the clinical syndrome caused by infection with the human monkeypox virus (hMPXV). After the initial description of accumulating cases internationally, the first mpox cases in The Netherlands were identified in May 2022 in Amsterdam. While rapid and accurate diagnostics were immediately implemented to aid outbreak control, a relatively low-cost and fast whole genome sequencing approach was not available. Therefore, we developed a novel 2,5 kB amplicon-based Nanopore sequencing protocol and investigated its usability to characterize the initial introduction and subsequent spread of hMPXV within the Amsterdam region.

Methods: Our sequencing approach was developed with two PCR platforms in mind: 1) the Nextgen PCR platform, which allows for ultrafast PCR amplification using custom reagents allowing one-day NGS analysis and 2) the widely used Q5 polymerase-based approach which is globally applied for genomic surveillance of SARS-CoV-2. To verify the usability of the approaches for genomic surveillance, we initially used dilutions of a single hMPXV positive specimen to provide information on robustness and sensitivity. Next, we analyzed samples from multiple epidemiologically confirmed transmission pairs as well as samples that were epidemiologically unlinked in place and/or time.

Results: Using a straightforward Q5 polymerase amplification and Nanopore sequencing protocol we were able to successfully obtain full hMPXV genome sequences from hMPXV-positive specimens. Within the generated hMPXV outbreak sequences we observed evidence of ongoing viral evolution. Sequences from known transmission pairs were highly similar and were in agreement with the transmission route. We also observed previously unknown local clusters as well as identified import cases related to international travel.

Conclusion: Genomic surveillance of hMPVX using our fast and relatively cheap sequencing approach generates data on hMPXV transmission and potential vaccine escape mutations which can help guide outbreak management and public health measures.

P232

The stomatin-like protein StlP confines apical growth in filamentous actinobacteria under hyperosmotic stress

Xiaobo Zhong[1], Dr S Shitut, Dr E Vijgenboom, Dr D Claessen
[1]Leiden University, Leiden , The Netherlands

Filamentous actinobacteria are ubiquitous in almost all soil environments, where they are frequently exposed to environmental insults. Recent work indicates that some actinobacteria have a natural ability to shed their cell wall under influence of hyperosmotic stress, while others, such as the model organism Streptomyces coelicolor, appear unaffected. We here identify the stomatin-like protein StlP as a crucial factor for growth under hyperosmotic stress conditions. StlP localizes at hyphal tips, where it is important to spatially confine growth. In the absence of StlP, filaments start to branch frequently coinciding with a delocalized pattern of cell wall synthesis and an increased membrane fluidity at hyphal tips. Surprisingly, filaments of the S. coelicolor ΔstlP mutant extrude cell-wall-deficient cells, while the constitutive expression of StlP in actinobacteria that naturally form such cells blocks their extrusion. Consistent with other stomatin proteins, we show that StlP oligomerizes and interacts with several proteins involved in tip growth. Altogether, these data indicate that StlP plays a central role in coordinating tip growth by organizing cell wall synthesis in localized microdomains and imply that stomatin-like proteins provide competitive advantage to actinobacteria that are frequently exposed to hyperosmotic stress.

P233

A genetic cluster of methicillin-resistant Staphylococcus aureus potentially associated with intravenous drug use and men who have sex with men in the Netherlands, 2020-2022

Dr. Romy Zwittink[1], Annelot Schoffelen[1], Sandra Witteveen[1], Angela de Haan[1], Jeroen Bos[1], Lam Trang[2], Laura Kasteel[3], Mariette Lokate[4], Erik Bathoorn[4], Alewijn Ott[5], Daan Notermans[1], Antoni Hendrickx[1], . on behalf of the Dutch MRSA Surveillance Study Group
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, [2]GGD Fryslân, Leeuwarden, The Netherlands, [3]GGD Groningen, Groningen, The Netherlands, [4]University Medical Center Groningen, Groningen, The Netherlands, [5]Certe, Groningen and Drenthe, The Netherlands

Surveillance of methicillin-resistant Staphylococcus aureus (MRSA) provides insights in molecular characteristics and relatedness of MRSA in the Netherlands. Two cases of MRSA with multiple-locus variable number of tandem repeat analysis (MLVA) type MT6494 suggested an association with men who have sex with men (MSM) and intravenous drug use. To further explore this, patient and isolate characteristics of all MRSA MT6494 collected until June 2022 were analysed.

Submitted isolates were characterised by MALDI-ToF, MLVA typing, mecA and PVL gene detection, and next-generation sequencing (NGS). Antimicrobial susceptibility testing results and pseudonymised patient data were provided by medical microbiological laboratories and information regarding MSM and drug use were requested.

Forty-one MRSA MT6494 isolates from 38 persons (median age: 51 years, 82% male) from eight provinces were submitted from January 2020 until June 2022. In 23 persons (56%), the culture was taken in context of infection. Isolates were mecA positive and PVL negative. From 29/41 isolates, antimicrobial susceptibility results were available, indicating that all were sensitive to rifampicin and mupirocin and resistant to tetracycline and fusidic acid. Five isolates (17%) were resistant to co-trimoxazole. NGS data of isolates from 11/38 persons were available. The isolates belonged to multi-locus sequence typing (MLST) ST5 and whole-genome MLST indicated high genetic relatedness between ten isolates (2-9 allelic differences). All isolates harboured genes associated with resistance to fusidic acid (fusC), penicillin (blaZ), florfenicol-chloramphenicol (fexA) and tetracyclin (tet(M)) and seven isolates carried the erm(C) gene, associated with macrolide resistance. Additional information of 19 persons was obtained. Intravenous drug use was reported in five cases (26%), MSM in three (16%) and in two cases both was reported (11%).

In conclusion, a genetic cluster of MRSA MT6494 ST5 potentially associated with intravenous drug use and MSM was identified in the Netherlands. Additional isolates and patient data are currently being investigated.

P234

Multidrug-resistant organisms in patients from Ukraine in the Netherlands, March to August 2022

Dr. Romy Zwittink[1], Cornelia Wielders[1], Daan Notermans[1], Nelianne Verkaik[2], Annelot Schoffelen[1], Sandra Witteveen[1], Varisha Ganesh[1], Angela de Haan[1], Jeroen Bos[1], Jacinta Bakker[1], Caroline Schneeberger-van der Linden[1], Ed Kuijper[1], Sabine de Greeff[1], Antoni Hendrickx[1], . on behalf of the Dutch CPE and MRSA Surveillance Study Groups
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]Erasmus University Medical Center, Rotterdam, Netherlands

Since March 2022, an emergence of multi-drug resistant microorganisms (MDRO) from patients originating from Ukraine was observed in the Netherlands through the mandatory notification of carbapenemase-producing Enterobacterales (CPE), and the national CPE, carbapenemase-producing Pseudomonas aeruginosa (CPPA), carbapenem-resistant Acinetobacter baumannii complex (CRAB) and methicillin-resistant Staphylococcus aureus (MRSA) surveillance. We determined the epidemiology and genetic characteristics of MDRO from these Ukrainian patients (n=58).

Submitted MDRO were characterised by the carbapenem inactivation method, Etest for meropenem and next-generation sequencing for multi-locus sequence typing (MLST) and antimicrobial resistance gene detection.

From March-August 2022, 39 patients with CPE originating from Ukraine were notified. In addition, 47 CPE, 12 CPPA, 3 CRAB and 13 MRSA isolates from 56 patients originating from Ukraine were submitted, representing 21% (CPE), 52% (CPPA) and 1.2% (MRSA) of the total number of submitted isolates during this period. Recent hospitalisation abroad (<1 year ago) was reported for 54%, 86% and 17% of patients with CPE, CPPA and MRSA, respectively. Twenty percent of the patients carried multiple MDRO. All CPE, CPPA and CRAB isolates were carbapenemase positive and 63% were meropenem-resistant. The majority of isolates was K. pneumoniae (60%) followed by P. aeruginosa (20%) and E. coli (10%) and these belonged to globally spread MLST types. Isolates from Ukrainian patients were mainly endowed with blaNDM-like carbapenemase alleles (60%) and 26% harboured isolates with double carbapenemase allele combinations, whereas this was respectively 31% and 9% in other Dutch surveillance isolates. MRSA isolates from Ukrainian patients were of varying MLST types, they all carried mecA and 15% harboured Panton-Valentine leucocidin.

In conclusion, we observed an increase of MDRO from globally epidemic high-risk sequence types retrieved from Ukrainian patients in the Netherlands. Professionals working with MDRO should be aware of the emergence of these microorganisms for patient treatment and public health.