P001 Four patients with severe infections of hypervirulent Klebsiella pneumoniae ST 23, diagnosed in the Netherlands.

Drs. Fardau Anema[1,2], E.J. Kuijper[1], E Bathoorn[3], M. A. Fliss[3], L. van Dommelen[4], D. F. Stoop[5], B.C.A.M. van Ginneken[5], M.P. den Reijer[6], J.W. Raymakers[7], A.P.A. Hendrickx[1], D. W. Notermans[1,7]
[1]National Institute for Public Health and the Environment (RIVM), [2]Leiden University Medical Center, [3]University Medical Center Groningen, [4]Stichting PAMM, [5]Maxima Medical Centre (MMC), [6]Gelre hospitals, [7]Amsterdam University Medical Centers

Introduction
Klebsiella pneumoniae is mainly known as a nosocomial and opportunistic pathogen. However, severe community-acquired hypervirulent K. pneumoniae (hvKp) infections are emerging.
While hvKp strains are rarely resistant, in 2021 the European Centre for Disease Control published a rapid risk assessment notifying that sequence type (ST) 23 hvKp isolates could carry carbapenemase genes. Subsequently, the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) announced a pilot surveillance study and was informed on four previous patients with presumed hvKp infections before the start of the pilot period. Patients records were analysed and isolates were subjected to whole genome sequencing.

Case descriptions
Four patients (aged 41 to 61, two female) were reported with presumed hvKp infections diagnosed in 2020 and 2021. One patient was immunocompromised, one had an unknown medical history and two others were relatively healthy. Three patients originated from Asia and one patient was born in The Netherlands. None of the patients had travelled abroad in the preceding six months. All infections were community acquired, presenting with bacteraemia and abscesses in liver, lungs, brains, prostate and/or eye. One patient died as a consequence of advanced cerebral infection and one patient developed persisting hearing loss and mild residual cognitive dysfunction. All strains tested sensitive to cephalosporins, carbapenems, fluoroquinolones and aminoglycosides. Next-generation sequencing confirmed that all isolates belonged to Klebsiella pneumoniae ST 23 and did not contain carbapenemase producing genes. All isolates contained a virulence plasmid KpVP-1, colibactin, aerobactin, yersiniabactin, salmochelin, wzi-1 and truncated rmpA-2, resulting in a Kleborate virulence score of 5 out of 5.

Discussion
While hvKp reports initially originated mainly from Asia, cases are now reported widely distributed. Infections with hvKp are life-threatening and can develop suddenly in previously healthy individuals. To date, no carbapenem resistant strains have been reported in the Netherlands.

P002 Empiric use of ceftriaxone, cefuroxime or co-amoxiclav and the prevalence of ESBL-producing gram-negative bacteria in Dutch hospitals

Ali Auzin[1], I Maat[1], J ten Oever[2], A.F. Schoffelen[3], W Altorf-van der Kuil[3], HFL Wertheim[1], on behalf of the ISIS-AR Study Group

[1]Radboudumc, [2]Radboudumc, [3]National Institute for Public Health and the Environment (RIVM)

Background:
Increased (suspected) infections with ESBL-producing gram-negative bacteria are causing an increase in carbapenem use. The rise in ESBL may be a result of cephalosporin use in empiric antibiotic treatments (EAT). Focusing on sepsis EAT, hospitals in the Netherlands can be roughly divided into cefuroxime, ceftriaxone or co-amoxiclav hospitals. In this ecological study, we aim to focus on the difference in prevalence of ESBL-producing Escherichia coli and Klebsiella pneumoniae between these hospitals.

Methods:
An anonymous dataset containing data from Dutch medical microbiological laboratories (MMLs) that consistently reported to ISIS-AR between 2014 and 2018 was obtained. Each hospital was labelled according to local guidelines for sepsis without focus, urosepsis and abdominal sepsis. The first ESBL-positive E. coli and K. pneumoniae isolate per person per year was selected, or, if no ESBL-positive isolate was cultured, the first isolate per person per year. ESBL prevalence in diagnostic samples was calculated separately for hospitals using different EATs and were compared using Chi-square tests.

Results:
The dataset comprised data from 35 MMLs and 75 hospital locations. Overall, 5 years data on a total of 185,581 isolates were included. A higher ESBL prevalence was seen in E. coli and K. pneumoniae in the ceftriaxone hospitals (8.3% of the samples, 95% confidence interval 8.0%-8.5%) when compared to the co-amoxiclav (5.5%, 5.2%-5.7%) and the cefuroxime hospitals(7.7%, 7.5%-7.9%). In general, the ESBL prevalence was lower for E. coli (6.7%, 6.6%-6.9%) in comparison with K. pneumoniae (11.1%, 10.7 %-11.5%).

Conclusions:
In this ecological study, a higher prevalence of ESBL-producing E. coli and K. pneumoniae was consistently seen in hospitals that use ceftriaxone when compared to hospitals that use cefuroxime or co-amoxiclav for EAT. To definitively confirm any conclusions on the association between increased pressure by third-generation cephalosporins and ESBL prevalence, further research is needed.

P003 Virulence genes and autopsy findings in fatal community-acquired infection with hypervirulent Klebsiella pneumoniae ST23 in the Netherlands

Monika Fliss[1], CHSB van den Berg[2], E Kuijper[3], A Hendrickx[3], M Schoots[4], Erik Bathoorn[1]
[1]University of Groningen, University Medical Center Groningen, [2]University of Groningen, University Medical Center Groningen, [3]National Institute for Public Health and the Environment , [4]University of Groningen, University Medical Center Groningen

Introduction
Infections with hypervirulent Klebsiella pneumoniae (hvKp) have frequently been reported in South-East Asia and are associated with sepsis, liver abscesses and metastatic spread to eye, brain, and/or lungs. In recent years, several lineages of hvKp have spread globally. Here, we characterize a devastating community-acquired infection with hvKp infection in the Netherlands.
Methods
Typing and virulence gene detection in Illumina short-read sequencing data were performed using Pathogenwatch and the Kleborate genotyping tools. Antibiotic susceptibility was tested by Vitek2. At autopsy, standard samples were taken and H&E slides were histopathological examined.
Results
A 74-year old female patient with a history of steroid use for suspected polymyalgia rheumatica, and recurrent urinary tract infections after previous bladder surgery, was admitted to the intensive care with a septic shock. She had not recently travelled abroad. Pulmonary imaging showed diffuse consolidations. Blood, urine and sputum cultures grew K. pneumoniae (wild-type susceptible). Molecular characterization revealed MLST ST23, K-locus 1, O-locus O1V2, presence of a KpVP-1 virulence plasmid, and virulence genes encoding for yersiniabactin, colibactin, aerobactin and salmochelin, rmpADC rmp 1, and rmpA2 (truncated). The Kleborate virulence score was 5/5.
Despite adequate antibiotic therapy and optimal supportive care, the patient died after 15 hours of admission of overwhelming septic shock with multi-organ failure. Autopsy and histomorphology showed acute bronchopneumonia, vasculitis, pyelonephritis, septic thromboemboli, and abscesses in lungs, kidney and perirenal fat. Lung-tissue showed mixed infiltrates in the bronchi and alveoli with multifocal alveolar damage, focal capillary fibrin thrombi, and vasculitis with neutrophils.
Conclusion
We report a case of fatal infection with hvKp strain belonging to ST23, acquired in the Netherlands. Autopsy results showed bronchopneumonia, extensive vascular involvement and abscesses in kidney and lung. Based on this observation and other similar reported patients, a pilot national surveillance has been started to assess the incidence rate of hvKp infection.

P004 A national survey on antimicrobial resistance of clinical Bacteroides and Prevotella isolates in the Netherlands

Kathleen Boiten[1], DW Notermans[2,3], RJ Rentenaar[4], J van Prehn[5], LGM Bode[6], I Maat[7], W van der Zwet[8], NLA Arents[9], TJH Siebers[1,10], SC de Greeff[2], APA Hendrickx[2], JWA Rossen[1,11,12], EJ Kuijper[2,5], ACM Veloo[1]
[1]University of Groningen, University Medical Center Groningen (UMCG), [2]Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), [3]Amsterdam University Medical Center, Amsterdam, the Netherlands, [4]University Medical Centre Utrecht, [5]Leiden University Medical Center (LUMC), [6]Erasmus University Medical Center, [7]Radboud University Medical Center, [8]Maastricht University Medical Center+, [9]PAMM Laboratories, [10] Certe, [11]University of Utah School of Medicine, [12]Isala Hospital

Introduction
Antimicrobial resistance among anaerobic bacteria is increasing, especially in the Bacteroidales order. However, multi-drug resistance (MDR, defined as resistance to 3 or more classes of antibiotics) was assumed to be rare in the Netherlands until 2019, when various MDR clinical isolates were found and confirmed at the reference center in Groningen. This prompted the development of a nationwide survey to determine the antimicrobial susceptibility of anaerobic bacteria. From March till June 2021, 8 hospitals throughout the Netherlands collected Bacteroides and Prevotella isolates. The antimicrobial susceptibility profile for 10 different antibiotics was assessed.

Methods
In each laboratory, a well-defined selection of clinical punctures and drainages of abscesses and material obtained from sinusitis was cultured on selective media for the isolation of 20-25 Bacteroides and 10-15 Prevotella isolates per laboratory.
Isolates were sent to the reference laboratory at the UMCG and identified using MALDI-TOF MS. Antimicrobial susceptibility was determined, using agar dilution, for amoxicillin, meropenem, and metronidazole, and will be determined for amoxicillin-clavulanic acid, piperacillin-tazobactam, imipenem, clindamycin, erythromycin, tetracycline, and moxifloxacin. MIC values were interpreted using EUCAST breakpoints (V11.0, 2021).

Results
The participating laboratories collected a total of 184 Bacteroides and 99 Prevotella isolates. Resistance to amoxicillin was found in 174/183 (95%) Bacteroides and 47/85 (55%) Prevotella isolates. Meropenem resistance was observed in 7/159 (4%) Bacteroides (2 Bacteroides fragilis and 5 Bacteroides non-fragilis) isolates. No meropenem resistance was detected among Prevotella isolates. Metronidazole resistance was detected in 1/182 (0.5%) Bacteroides and 3/96 (3%) Prevotella isolates.

Discussion
These data indicate that resistance for metronidazole and meropenem is not rare in the Netherlands and show the importance of national surveillance. Future studies should focus on revealing resistance mechanisms and risk factors for infection with these micro-organisms, in order to control the further increase and spread.

P005 No differences in 90-day mortality following hospitalisation with influenza, respiratory syncytial virus, rhinovirus or human metapneumovirus infection

PhD H Boon[1], MD AJ Meinders[2,3], MD, PhD M Tersmette[1], MD, PhD E Schaftenaar[1]
[1]St Antonius Hospital, [2]St Antonius Hospital, [3]St Antonius Hospital

Introduction
In the pre-COVID-19 era, influenza virus was the only viral agent of respiratory infection for which hospital-wide infection control measures were in effect. However, a comparison of mortality rates of patients admitted with other well-known respiratory viruses, such as respiratory syncytial virus (RSV), rhinovirus or human metapneumovirus (hMPV), has not yet been made. Therefore, we decided to study whether, in patients admitted to the hospital with these viral respiratory infections, hazard of mortality was dependent on the pathogen.

Methods
This retrospective cohort study included all adults presenting to our hospital in whom an infection with influenza A, influenza B, RSV, rhinovirus or hMPV, as determined by positive RT-PCR, was detected. This analysis contains data between July 1st 2017 and March 1st 2020. Primary outcome was 90-day mortality for the subset of patients that was admitted for an overnight stay or longer. A Cox proportional hazards (PH) regression model was fitted to assess the association between mortality and respiratory virus.

Results
Between July 2017 and March 2020, the following number of patients had a positive RT-PCR for a respiratory virus: 875 for influenza A, 510 for influenza B, 796 for rhinovirus, 346 for hMPV and 356 for RSV. Of these, 625 patients (71 %) were admitted with influenza A, 373 with influenza B (73 %), 565 (71 %) with a rhinovirus infection, 263 (76 %) with an hMPV infection and 271 (76 %) with a RSV infection. Covariate-adjusted hazard rates for mortality of respiratory viruses were comparable ($p > 0.05$). Overall 90-day mortality rate for the viruses combined was 11.5 % (95% CI: 10-13 %).

Conclusion
Hazards for 90-day mortality for patients admitted with different respiratory viruses were comparable.

P006 Contact investigations of contact patients and screening of the environment after identifying carbapenem-resistant Pseudomonas aeruginosa: a systematic review of the literature

Md Andrea Büchler[1], S.N. Shahab[1,2], Professor J.A. Severin[1], Professor M.C. Vos[1], Dr. A.F. Voor in 't holt[1]
[1]Erasmus MC , [2]Universitas Indonesia

Introduction
Carbapenem-resistant Pseudomonas aeruginosa (CRPA) are a global threat due to the limited treatment options. However, little is known about how to perform a contact investigation after identifying a CRPA in the healthcare setting. Therefore, this systematic review aims to summarize contact investigations performed after detection of CRPA in the hospital setting.
Methods
The guidelines presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (Prospero registration number CRD42020194165) were followed. Articles related to our research question were identified through a literature search in seven databases (i.a. Embase, Medline Ovid, Cochrane) without time limitation until 30st of June 2020. An updated search until January 12, 2022 is currently performed. First, studies were reviewed independently by two researchers for eligibility based on the title and abstract, second, studies were reviewed based on the full text. Seven components of contact investigations after identification of CRPA were collected: identification of contact patients, screening of contact patients, universal screening on admission, screening during hospitalization, healthcare worker screening, environmental screening of the wet and/or dry environment, and others.
Results
Out of 3300 potentially relevant articles, we included 124 studies in the systematic review. The included studies were published between 1989 and 2020, and described either an epidemic (n=79) or endemic setting (n=45). In the preliminary analysis, a median of 2.1 components of contact investigations were performed, environmental screening being the most common one (79.8%). In the epidemic setting, most outbreaks could be terminated by identifying and combatting an environmental source (59.5%). Overall, only seven studies (5.6%) reported the method used to identify contact patients.
Conclusion
This systematic review shows that environmental source screening is important after detection of CRPA in the epidemic setting. However, detailed description of contact investigations concerning identification and screening of contact patients are scarce.

P008 Standardized methods to rear high-quality Galleria mellonella larvae for the study of bacterial and fungal infections

MSc A.W. Jong[1,2], D. van Veldhuizen[3], Prof. dr. A.T. Groot[3], dr. F. Hagen[1,2,4]
[1]Westerdijk Fungal Biodiversity Institute, [2]University Medical Center Utrecht, [3]University of Amsterdam, [4]Jining No.1 People's Hospital

Background: The greater wax moth, Galleria mellonella, has gathered widespread interest as an alternative non-mammalian model to study microbial infections in humans. The immune response of G. mellonella shows many similarities with the innate immunity of mammals. However, most laboratories rely on commercial breeding companies that grow these larvae in bulk. Variation in rearing conditions and shipping of the larvae affects the response of G. mellonella to infection, both decreasing the reproducibility and comparison of infection experiments. We present a standardized rearing protocol, resulting in high-quality larvae to study microbial infections.
Material/methods: For the propagation of G. mellonella, we used an optimized artificial diet and created cost-effective housing solutions. To verify that our standardized rearing methods result in higher quality larvae, we compared the inoculum densities needed to kill at least 50% of larvae obtained from a commercial supplier and our own reared larvae, using two reference strains of Candida albicans. Larvae were infected by intra-hemocoel injection. The survival was monitored for 10 days and analyzed by Log-Rank (Mantel-Cox) test.
Results: With our protocol, we were able to rear G. mellonella larvae in high quantities at low costs. The survival analysis showed significantly higher survival of standardized reared larvae after infection with C. albicans compared to the commercially obtained larvae. The higher quality of standardized larvae is shown by the fact that double the inoculum was needed for standardized larvae to reach 50% mortality compared to commercial larvae.
Conclusions: 1) Our protocol results in high quality larvae with better resistance to fungal infections compared to commercially bred larvae. 2) The low costs and easy to obtain materials make it convenient to implement this setup in other laboratories. 3) Using a standardized rearing protocol and the inclusion of standard reference strains will greatly improve inter-laboratory comparisons of virulence studies.

P009 Detecting inappropriate total duration of antimicrobial therapy using semi-automated surveillance

Drs. A.K. van den Broek[1], Drs. J.R. de la Court[1,2], T. de Groot[2], Dr. R.M. van Hest[3], Dr. C. Visser[2], Dr. R.P. Schade[2], Prof. Dr. J.M. Prins[1]
[1]Amsterdam UMC, [2]Amsterdam UMC, [3]Amsterdam UMC

Background
Evaluation of the appropriateness and duration of antimicrobial use is a cornerstone of antibiotic stewardship programs (ASP), but this is time-consuming. Assessment of the antibiotic treatment duration is therefore often restricted to antibiotics prescribed during hospital admission only, while a considerable part is prescribed post-discharge. This study aimed to determine whether mandatory prescription-indication registration enables reliable assessment of both the appropriateness and the duration of antibiotic therapy, including post-discharge duration, broken down per indication.
Method
Antibiotic prescriptions and admission data, from 1-6-2020 to 31-12-2021, were extracted from the Electronic Medical Record (EMR) of two hospital locations (AMC and VUmc) using mandatory indication registration. Systemic antibiotic prescriptions (J01) linked to an indication registered in the first 24h of admission, were selected and merged with consecutive antibiotics prescribed during hospital admission and post-discharge. The accuracy of the data was evaluated by comparing 400 clinical notes with the data extraction results. Length of therapy (LOT) per indication was provided and compared to the local antibiotic guideline recommendations.
Results
A total of 3,466 antibiotic courses met the inclusion criteria. LOT was retrieved accurately in 95% of evaluated antibiotic courses. In 17% of these courses the registered indication of therapy did not match the clinical notes, resulting in an incorrect assessment of guideline adherence in 9%. Post-discharge prescriptions accounted for 27% of the treatment duration, this proportion was highest for E.N.T. infections (52%). Guideline adherence regarding treatment duration ranged from 26-75% and was lowest in respiratory tract infections.
Conclusion
Mandatory prescription-indication registration data can be used to reliably assess total treatment course duration, including post-discharge antibiotic therapy. This enables to target ASPs by semi-automated surveillance of the extent and appropriateness of antibiotic use and the effect of ASP interventions. Repeated data validation, however, is necessary to assure accuracy of the extracted data over time.

P010 Simultaneous profiling of SARS-CoV-2 and host transcriptome in airway samples: a pilot study

Ellen C. Carbo[1], Hailiang Mei[1], Simon P. Jochems[1], Jutte J.C. de Vries[1]
[1]*Leiden University Medical Center*

Introduction: SARS-CoV-2 infection can result in target organ damage from direct viral replication, immunopathology, and a combination of both. Simultaneous assessment of both viral and the host immune transcriptome in airways can increase our understanding of viral persistence, adaptation, and its clearance from the host by the immune system.

Methods: A dual transcriptomic, untargeted RNA sequencing approach was designed and validated for bronchial aspirates and tissues of SARS-CoV-2 positive patients. For sequencing, clinical samples were processed with the NEBNext RNA Ultra Directional kit in combination with unique molecular identifiers (UMI) followed by Illumina NovaSeq6000 sequencing. Data analysis was performed for both viral and host transcriptome of immune-related genes using standard filtering followed by mapping via STAR in combination with corresponding transcriptomic references. Differential expression (DE) analysis was carried out using HTseq.

Results: In samples with high concentrations of SARS-CoV-2 and human cells, both the viral and the human transcriptome was sequenced successfully. Viral transcriptome data enabled differentiation of + and - genome strands, and the UMI tags enabled highly accurate quantitative measurements of virus and host gene RNA. Analysis of transcription of patient immune-related genes resulted in differences in expression of up to 3 log10 in different samples.

Conclusion: Simultaneous profiling of virus and host signatures in airways of patients with respiratory infections increases insights into host-pathogen interaction.

P011 Chlamydia trachomatis coinfection does not influence Mycoplasma genitalium bacterial load in urogenital samples

Dr. A Dirks[1], dr. IHM van Loo[1], dr. NHTM Dukers-Muijrers[2,3], dr. PFG Wolffs[1,3], Prof. CJPA Hoebe[1,2,4]
[1]Department of Medical Microbiology, MUMC+, [2]Department of Sexual Health, Infectious Diseases and Environmental Health, [3]Department Health Promotion, Maastricht University, [4]Department Social Medicine, Maastricht University

Introduction
Mycoplasma genitalium (MG) is associated with non-gonococcal urethritis in men and weakly associated with pelvic inflammatory disease and infertility in women. Coinfections of MG and Chlamydia trachomatis (CT) are commonly reported (~10%). MG coinfections increase the bacterial load of Neisseria gonorrhoeae and therefore the transmission potential. Little is known of the impact of CT on MG transmission, and vice versa. Therefore, we investigated if patients with CT/MG coinfections have a higher bacterial load than patients with either one infection.

Methods
1673 patients were included from a population-based chlamydia trial and the STI-clinic in South Limburg. Clinical data included age, gender, ethnicity and symptoms. The CT load was quantified targeting the OmpA gene and for the MG load the MgPa adhesin gene was used.
Non-parametric tests compared the CT and MG load between groups. Linear regression analyses compared the CT and MG load within a patient.

Results
Of 60 MG positive patients, MG load ranged from 1.7 to 6.0 log10 copies/ml (median 3.6 log10 copies/ml). The CT load ranged from 0.75 to 7.1 log10 CT copies/ml (median 4.5 log10 copies/ml).
In the MG-negative group, the CT load was similar (0.75 to 7.93 log10 copies/ml, n.s.). Only 6 patients were MG-positive and CT-negative, but the MG load distribution was similar to that of CT-positive patients (2.9 – 5.1 log10 copies/ml vs 1.71 – 6.0 log10 copies/ml respectively, n.s.). The MG and CT load was unrelated within one person (p=0.19).

Conclusion
In short, no correlation exists between the CT and MG load in urogenital samples, and the MG load distribution is similar in CT-positive and CT-negative patients. Although coinfections are seen in up to 10% of the population, the bacterial load by itself does not appear to increase the transmission risk of either pathogen during coinfections.

P012 Human complement system inhibits binding of phages of the Myoviridae family to Pseudomonas aeruginosa

Julia Egido[1], SO Dekker[1], CJ Toner-Bartelds[1], BW Bardoel[1], SHM Rooijakkers[1], PJ Haas[1]
[1]*UMC Utrecht*

Bacteriophage (phage) therapy is gaining momentum as an alternative to antibiotics. However, the interactions of phages with our immune system might limit their success as therapeutic agents. Here, we have studied the activity of phages targeting Pseudomonas aeruginosa in presence of human serum.

We developed a method based on a fluorescent dye that stains the DNA of both phages and damaged bacteria, emitting a signal as bacteria lyse and new phage progeny is produced. Using P. aeruginosa strain PAO1 as a host, we screened a panel of phages in presence of serum. Our results reveal that human serum reduces the ability of phages from the Myoviridae family to infect bacteria. The effect was observed, among others, with phage 14-1, known to be used in therapeutic cocktails. Phage activity was not compromised when using heat-inactivated serum, which suggests an involvement of the complement system. Compstatin, a C3 cleavage inhibitor, can also counteract the negative effect of serum on phage activity.

Next, we fluorescently labelled Pseudomonas myovirus PB1 to monitor its binding to PAO1 by means of flow cytometry and confocal microscopy. In this way, we were able to detect that human serum prevents phage PB1 from binding to its host.

We conclude from our data that human serum has an inhibitory effect on phages of the Myoviridae family. This effect is likely mediated by the early stages of the complement system, and takes place at the stage of host recognition and binding. Phages currently used in the clinic to treat P. aeruginosa infections could be rendered ineffective as a result of this phenomenon. Our findings highlight the importance of considering the human immune system when applying phage therapy, and may impact the design and content of future phage cocktails.

P013 FMT resulted in clinical improvement and microbiota changes in two patients with immune checkpoint inhibitor-induced colitis

Msc. Bas Groenewegen[1,5], MD PhD E.M. Terveer[1], MD PhD A. Joosse[2], MD PhD M.C. Barnhoorn[3,4,5], PhD R.D. Zwittink[1]

[1]Leiden University Medical Center, [2]Erasmus Medical Center, [3]Alrijne Hospital, [4]Haga Hospital, [5]Leiden University Medical Center

Introduction

Immune checkpoint inhibitors (ICIs) have improved the prognosis in multiple cancer types. However, ICIs can induce immune-related adverse events such as immune-mediated enterocolitis (IMC). The gut microbiota may be implicated in IMC development. Therefore, we investigated faecal microbiota transplantation (FMT) as treatment option for two IMC patients.

Methods

Two patients with metastatic cancer suffering from refractory IMC (resistant to prednisone, infliximab, tacrolimus and vedolizumab) were treated with respectively one and three FMTs after vancomycin pre-treatment. We monitored defaecation frequency, faecal calprotectin, and microbiota composition (16S rRNA gene amplicon sequencing).

Results

Both patients improved in defaecation frequency after FMT. Patient one developed an invasive pulmonary aspergillosis deemed to be related to prolonged steroid exposure. Patient two suffered of a Campylobacter jejuni infection after the first FMT and was treated with meropenem, resulting in a low-diversity microbiota profile and increased calprotectin levels and defaecation frequency. After a second and third FMT, bacterial diversity increased and defaecation frequency and calprotectin levels decreased. Pre-FMT, both patients showed low bacterial richness, but varying bacterial diversity. After FMT, diversity and richness were similar to donor levels. Both patients demonstrated decreased relative abundance of Escherichia/Shigella, and increased relative abundance of Collinsella, Faecalibacterium, Dialister and Bilophila within one month after FMT. Patient one, but not patient two demonstrated a microbiota profile more similar to his respective donor in the first month after FMT. Patient faeces samples taken respectively 47 and 50 days after the first FMT were dissimilar to respective donor samples and revealed dominance of Enterococcus in both patients, accompanied by Escherichia/Shigella dominance in patient two.

Conclusion

In two cancer patients with refractory IMC, improvement of IMC symptoms and microbiota changes were observed after FMT. While more research is warranted, microbiome-modulation could be a promising new therapeutic option for IMC.

P014 Study protocol on the role of intestinal microbiota on breast cancer treatment with hormone therapy: are bacteria the key to new therapeutic options?

Msc, MD Lars Hillege[1,2], dr. R Aarnoutse[1,2], BSc J.M.A. Waelen[2], drs. J Ziemons[1,2], dr. J de Vos-Geelen[1,3], dr. M de Boer[1,3], drs. E.A.Y. van Riet[4], drs. J Vincent[5], Prof. dr. K Venema[6,7], dr. S.S. Rensen[8], drs. J.B. Simpson[9], Prof. dr. R.M. Redinbo[9], dr. J Penders[7,8,10], Prof. dr. M.L. Smidt[1,2]

[1]Maastricht University, [2]Maastricht University Medical Centre, [3]Maastricht University Medical Centre, [4]Catharina Hospital, [5]Elkerliek Hospital, [6]Maastricht University, [7]Euregional Microbiome Center, [8]Maastricht University, [9]University of North Carolina, [10]Maastricht University Medical Centre

Purpose

The aim of this observational study is to explore the relationship between the intestinal microbiota composition, bacterial ß-glucuronidase activity and systemic hormone therapy in postmenopausal estrogen receptor positive breast cancer patients before and during hormone therapy.

Introduction

In recent years, there has been a growing interest in the role of the human intestinal microbiota in different types of cancer. One possible link between breast cancer and the intestinal microbiota is the estrobolome, which is defined as the aggregate of intestinal bacterial reactions involved in metabolizing estrogens. The enzyme β-glucuronidase, produced of the estrobolome, has been shown to increase intestinal estrogen reabsorption from the intestine into the circulation. Specifically, in breast cancer, high levels of circulating estrogens are related to the development of estrogen receptor-positive breast cancer. However, there is currently no knowledge on the role of the intestinal microbiota before and during systemic hormone therapy.

Methods

In total sixty-six postmenopausal histologically proven estrogen receptor positive and Human Epidermal growth factor Receptor 2 negative (ER+/HER2-) breast cancer patients scheduled for adjuvant endocrine therapy (tamoxifen) will prospectively be enrolled in a multicenter cohort study in the Netherlands. After informed consent, patients collect a fecal sample and complete a questionnaire before and during hormone therapy. Microbiota composition will be analyzed by amplicon sequencing of the 16S rRNA V4 gene region. Bacterial functional activity of ß-glucuronidase will be measured by means of an enzyme activity assay using fecal lysate. Blood estrogens and endoxifen metabolites will be quantified by ultra-high performance-liquid-chromatography-mass-spectrometry.

Conclusions

We aim to investigate the influence of the intestinal microbiota and its related ß-glucuronidase activity on estrogen metabolism as well as the metabolism of tamoxifen and its related metabolite endoxifen before and during hormone therapy. These insights will be important to improve efficiency of systemic hormone therapy in the future.

P015 SARS-CoV-2 antigen self-testing is inferior to professional testing

T Lodder[1], F Dusseldorp[1], C Stals[1], M Jilissen[2], J van den Brand[2], Dr. Vishal Hira[1]
[1]Groene Hart Ziekenhuis, [2]GGD Hollands Midden

Introduction
Rapid antigen tests (RATs) are a valuable addition in the diagnostic test arsenal against COVID-19. Compared to RT-PCR, SARS-CoV-2 rapid antigen tests (RATs) are faster and easier to perform. RATs have a sensitivity of approximately 70 to 80% and a specificity near to 100% when performed professionally, but self-testing with RATs shows lower sensitivity. Studies comparing self-testing and professional testing using the same test in the same population are scarce. Therefore, we performed the Gouda SARS-CoV-2 Rapid Antigen Test evaluation (GORAT) study to evaluate the JOYSBIO SARS-CoV-2 RAT against RT-PCR and to determine how self-testing compares to professional testing.

Methods
For this study, we included adults who visited the Gouda municipal health SARS-CoV-2 test location in April 2021 and suffered from mild COVID-19 like symptoms for no longer than 7 days. We performed the RAT with a mid-turbinate swab, according to manufacturer's instructions. For the RT-PCR one sided oro-/nasopharyngeal sampling was performed. Participants received an antigen test kit for self-testing at home. Results of different testing methods were compared.

Results
We included 1265 and 1183 participants for the professional test and the self-test, respectively. The median of days after symptom onset was two. For the professional test and self-test, the sensitivity was 75% and 66%, respectively. Among samples with Ct-value <25, sensitivity was 94% and 84% respectively. The specificity was 97% and 99%, respectively. Among the 84 true positive professional tests, 13 corresponding self-tests were negative. Among 72 true positive self-tests just one corresponding professional test was negative. Age, self-testing experience and education level were not correlated with discrepancies between self-testing and professional testing.

Conclusions
1. the JOYSBIO SARS-CoV-2 RAT has a sensitivity and specificity that matches the characteristics of other rapid antigen tests.
2. Self-testing was inferior to professional testing. Indications for self-testing should therefore be limited.

P016 Can SARS-CoV-2 rapid antigen tests be used on virus transport medium instead of buffer?

T Lodder[1], F Dusseldorp[1], C Stals[1], M Jilissen[2], J van den Brand[2], Dr. Vishal Hira[1]
[1]Groene Hart Ziekenhuis, [2]GGD Hollands Midden

Introduction

During the COVID-19 pandemic, rapid testing of healthcare workers is essential. Rapid RT-PCR is preferable, but capacity is often scarce. Rapid antigen tests (RATs) could be used as pre-screening for RT-PCR. However, RATs need to be sampled in the included buffer, whereas PCR tests are usually sampled in (virus) transport medium (VTM), making double sampling necessary. To determine if just one sample is sufficient, we evaluated the JOYSBIO SARS-CoV-2 RAT using VTM instead of the included buffer.

Methods

For this evaluation we used 119 RT-PCR positive and 140 RT-PCR negative samples from the Gouda SARS-CoV-2 Rapid Antigen Test evaluation (GORAT) study. In this study, the JOYSBIO SARS-CoV-2 RAT was validated in a cohort of mildly symptomatic patients, who visited the municipal health service for SARS-CoV-2 RT-PCR testing in April 2021. The samples in VTM were diluted (1:1) with RAT buffer and the RAT was performed according to manufacturer's instructions. The performing researcher was blinded for RT-PCR results. When the testline in the antigen cassette was clearly visible, the test was positive. When the testline could only be seen with help of external illumination, the test was weak positive. If the testline was not visible, the test was negative. RAT results were compared with RT-PCR results.

Results

The sensitivity of the test when considering weak positives as positive was 81%. The sensitivity using samples with a Ct-value under 30 and 25 was 91% and 98% respectively. Specificity was 87%. When weak positives were considered as negative, sensitivity was 66%, 74% and 92%, respectively. Specificity was 99%.

Conclusions

The JOYSBIO SARS-CoV-2 RAT performed on VTM shows high sensitivity in persons with high viral load and could be an import addition to rapid RT-PCR. However, due to the large number of weak positives, specific indications need to be determined in advance.

P017 Susceptibility results within 6 hours after direct inoculation of urine samples

BSc. I.J. Janssen[1], dr. N.L.A. Arents[1], Bsc. B.M.C.S. Sweegers[1]
[1]*Stichting PAMM*

Introduction

The urinalysis of General practitioners takes at least two workingdays. During this period, the pathogen is obtained in pure culture and a susceptibility test is preformed. Pending the results, the patient is prescribed broad-spectrum empiric antibiotic therapy. This study investigates a urinalysis method that can be performed within a few hours.

Methods

Direct inoculation of urine samples for disc diffusion AST (antimicrobial susceptibility testing) encounters several challenges which have been adressed in this study: the growth rate of Gram negative rods (GNr), the ability of WASP/WASPLab® [Copan](WASP) to detect growth zones within 6 hours of incubation, the influence of commensal flora and an unknown inoculum on growth zones. A Gram stain selection was used to identify urine sample with a higher likelihood of a positive culture (>100.000 GNr cfu/ml). By this method 300 urine samples were selected and directly plated on Mueller Hinton agar (MHa) by WASP. Antibiotic disk for amoxicillin/clavulanic acid, ceftazidime, ciprofloxacin, cefotaxime, nitrofurantoin, fosfomycin, trimethoprim/sulfamethoxazole and trimethoprim were dispersed on the plate and incubated for 16 hours. Diameters were measured automatically by WASP and interpreted by EUCAST breakpoints at 4, 5, 6, 7, 8 and 16 hours of incubation. Results were compared to the standard method (overnight AST of identified pathogens with disc diffusion using EUCAST breakpoints).

Results

Of the 300 urine samples in this study the AST performed by the test method showed an overall Categorial agreement (CA) of 84% after 6 hours of incubation (86% for the 197 Escherichia coli). The percentage very major errors stayed under 0,5% and major error under 3%. 5% of the positive urines showed no growth in 6 hours.

Conclusion

- The study method showed that an accurate AST can be available after 6 hours of direct inoculation of urine samples in 84% of the urine samples with GNr.

P018 Detection and consequences of Streptococcus dysgalactiae subsp. equisimilis (SDSE) as a causative pathogen in group A streptococcal infections

Dr. Koos Korsten[1,2], Dr. W. Freudenburg[1,2], Ing W. Bril-Keijzers[1], MSc B. van der Putten[1,2], Prof. N.M. van Sorge[1,2]

[1]Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam University Medical Center, [2]Amsterdam University Medical Center, University of Amsterdam

Introduction Invasive group A streptococcal (iGAS) infections, often caused by Streptococcus pyogenes, cause a large disease burden worldwide. Invasive infections caused by Streptococcus dysgalactiae subsp. equisimilis (SDSE) are clinically indistinguishable from S. pyogenes. SDSE is generally classified as Lancefield group C or G (GCG-SDSE), but can also present as Lancefield group A (GA-SDSE), which complicates GAS species discrimination. Dutch guidelines recommend specific treatment and prophylaxis for iGAS-infections without the need to specify for the causative pathogen. In contrast, to fulfill the case definition for GAS as a notifiable disease, specification for S. pyogenes is required. The contradiction between these guidelines questions the clinical relevance of specification for GA-SDSE within GAS infections.

Methods In this study, we compared the behavior of (GA-)SDSE in different diagnostic procedures using 39 clinical SDSE isolates from the Netherlands Reference Laboratory for Bacterial Meningitis. Additionally, we sent out a questionnaire to the Dutch microbiological laboratories to gain insight into their diagnostic procedures for hemolytic streptococci.

Results The proportion of GA-SDSE (n=14) among 591 submitted GAS isolates between 2019-2021 was 2.4%. Biochemical tests showed that GA-SDSE behaved more similar to S. pyogenes than to GCG-SDSE, suggesting these are not suitable for species discrimination. Although 95% of Dutch laboratories could discriminate hemolytic streptococci at the species level using MALDI-ToF, only 55% reported on species level to the clinic. Moreover, 47% of the Dutch laboratories are theoretically able to distinguish GA-SDSE, by combining MALDI-ToF with Lancefield typing.

Conclusion We conclude that 2.4% of iGAS-infections are caused by GA-SDSE. Only half of the Dutch laboratories are able to discriminate for GA-SDSE using their routine diagnostic workflow. Current literature is inconclusive about the requirements for specific treatment and prophylaxis of GA-SDSE infections. Routine determination at species level with Lancefield typing is required to address this current gap in knowledge.

P019 High speed COVID screening in the emergency department using the Abbott ID NOW: helpful or a nuisance?

R Vrijhoeven[1], M Knops[1], Dr E Heddema[1], Dr S Postma[2], Dr R Mostard[3], Dr C.F.M. Linssen[1]
[1]Zuyderland Mc, [2]Zuyderland Mc, [3]Zuyderland Mc

Introduction: The ID NOW COVID-19 system seems a promising tool for rapid screening of patients. However, the performance of this system outside the controlled laboratory environment has been debated. We evaluate the ID NOW system as a screening method to determine the need of admission under isolation precautions.
Methods: In a two month period all patients clinically suspected of COVID-19 were included. A dry nasal swab and a nasopharyngeal swab were taken from each patient. At the emergency department the dry nasal swab was used to perform the test on the ID NOW (according to the manufacturer's instructions). The nasopharyngeal swab was collected in GLY medium, combined with the nasal swab, and transported to the laboratory for subsequent RT-PCR using the Abbott Alinity-M system. The result of the ID NOW, combined with the degree of clinical suspicion of COVID-19, determined whether or not a patient was admitted under isolation precautions .
Results: 1202 ID NOW/RT-PCR results were available, including 295 PCR positive samples (prevalence 24.5%). 106 tests resulted in an invalid result, in 61% of cases repeating the test did result in a valid result. The ID NOW results demonstrated a total of 0.25% false positive results and 4.16% false negative results compared to the RT-PCR. CT values of the false negatives varied between CT 20.70 and 39.96 with an average of CT 35.96. This resulted in a sensitivity of 83.1%, a specificity of 99.6%, a positive predictive value of 0.99 and a negative predictive value of 0.95.
Conclusion: 1) the ID NOW COVID-19 test is a fast and reliable test to diagnose COVID-19 in the emergency department. However the clinical context and prevalence of the disease should be taken into account. 2) Furthermore, a relatively high number of invalid results were found, compared to what was initially expected.

P020 MycetOS 2.0: using an open source drug discovery approach to identifying novel compounds able to inhibit Madurella mycetomatis, the main causative agent of the mycetoma

Jingyi Ma[1], Hung Phat Duong[2], Dmitrij Melechov[3], Mickey Konings[1], Dr Kymberly Scroggie[2], Prof Ahmed Fahal[4], Dr Ben Perry[5], Prof Matthew Todd[2], Dr Wendy van de Sande[1]
[1]ErasmusMC, [2]University of Sydney, [3]University College London, [4]University of Khartoum, [5]DNDi

Background :
Mycetoma is a Neglected Tropical Disease characterized by large subcutaneous swellings and the formation of grains. Madurella mycetomatis is the most common causative agent. Currently mycetoma is treated with a combination of itraconazole therapy and surgery with low success rates, resulting often in amputation and social sigma. To improve the current therapeutic success rates a novel drug is needed. Due to the lack of interest of pharmaceutical industry, an open source drug discovery program for mycetoma was established called MycetOS. In this program out of 1360 compounds screened, the fenarimols appeared to be promising. Preliminary data showed that a logD<2.5 was associated with in vivo activity. To investigate this hypothesis further a large collection of fenarimols was screened.
Methods :
In total 185 fenarimol analogues were evaluated for their potential to inhibit M. mycetomatis growth in vitro. 18 compounds were further evaluated in vivo in our G. mellonella larvae model.
Results :
76 analogues were able to inhibit M. mycetomatis growth at 100 uM and 41 at 25 uM. Compounds with a logD>2.5 were associated with a lower growth percentage at 100 uM. Five compounds significantly improved larval survival and one enhanced larval death. For compounds with a logD <2.5 a trend towards enhanced larval survival was noted.
Conclusions :
The fenarimols is a novel class of antifungal agents which seemed to be able to penetrate mycetoma grains in vivo. Although a higher logD value seems to be linked to a higher activity against M. mycetomatis in vitro, only the compounds with a low logD value seemed to be able to penetrate the mycetoma grain. This indicates that in vitro screenings might not always be predictive for therapeutic success in mycetoma.

P021 Genomic detective work on recurrent infections with MDR Staphylococcus epidermidis in a subcutaneous ureteral bypass device in a feline patient treated with bacteriophages

Mr Soe Yu Naing[1], Dr Birgitta Duim[1], Dr Els M Broens[1], Dr Marloes AM van Dijk[1], Ms Marian Broekhuizen-Stins[1], Dr Linda van der Graaf-van Bloois[1], Dr Emma van Soest[2], Dr Nicolien van Klaveren[3], Dr Aldert Zomer[1], Prof Jaap A Wagenaar[1]

[1]Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, [2]AniCura Dierenkliniek de Waterpoort, [3]Medisch Centrum voor Dieren

Introduction: Staphylococcus epidermidis is a leading cause of infections associated with indwelling medical devices due to its ability to form biofilms over foreign bodies. This biofilm formation and its antimicrobial resistance (AMR) often result in recurrent infections and treatment failures. Bacteriophage therapy is being revived and considered as a last resort treatment. This study aimed to describe a feline patient that received two different phage therapies for recurrent infections with multidrug-resistant (MDR) S. epidermidis in a subcutaneous ureteral bypass (SUB) device.

Methods: After trimethoprim/sulfamethoxazole and nitrofurantoin treatment failures, a standard bacteriophage and a tailor-made bacteriophage therapy from Eliava Institute, Georgia, were administered through the SUB system six months apart between January 2021 and July 2021. A total of ten urine samples were collected at different time points before, during, and after phage therapy. We also performed antimicrobial susceptibility (MIC), mecA PCR, and a spot test. Bacterial culture yielded 45 S. epidermidis isolates in which all methicillin-resistant (MRSE, n=10) and a selection of methicillin-susceptible (MSSE, n=8) were included for whole-genome sequencing.

Results: All isolates were MDR displaying no susceptibility for ≥3 antimicrobial classes. Spot tests showed that two MRSE isolates were not sensitive to the tailor-made phage. Genomic results revealed that MRSE isolates displayed multilocus sequence type 2 (ST2), and MSSE isolates differed in one allele. MRSE isolates shared almost identical antimicrobial resistance phenotypes and genotypes. They carried virulence factors, including the arginine catabolic mobile element (ACME) and polysaccharide intercellular adhesin (PIA). The ACME and PIA were absent in MSSE isolates.

Conclusion: This study described recurrent infections with MSSE and MRSE before and after phage therapy. We identified virulence genes in MRSE isolates that may have favored the colonization of the SUB system by S. epidermidis. Furthermore, this case underscores the challenge in treating recurrent infections of MRSE effectively with bacteriophages.

P022 Reducing ambiguous weakly positive SARS-CoV-2 PCR results caused by remnant RNA using RNAse

Dr. Roel Nijhuis[1], Annick Groot[1], Anne Russcher[1]
[1]*Meandermc*

Introduction
Detection of SARS-CoV-2 by real-time PCR enables generating Ct-values, providing information on viral load. Using such a highly sensitive method, RNA can be detected up to several months after infection, resulting in weakly positive results and difficulties in interpretation for clinicians. It is unclear whether detected RNA is part of replicating viral particles or whether these are mainly RNA remnants of disintegrated viruses. In this study, we evaluated a simple pre-analytical procedure to deplete remnant RNA in clinical specimens, allowing discrimination between the presence of intact viral particles and viral remnants.
Methods
All oro-/nasopharyngeal specimens that tested positive in our in-house real-time PCR (Ct-value <45) in the period of March 8 – June 28 2021 were re-tested the next day using exactly the same procedure, but now including a RNAse (6U) pre-treatment prior to extraction.
Results
A total of 158 specimens (111 patients) were evaluated. In routine diagnostics, 61 specimens tested positive with Ct-value <30 (range 17.26–29.95, median: 25.84), 48 with Ct-value 30-35 (range 30.10–34.89, median: 32.86) and 49 with Ct-value >35 (range 35.27-44.81, median: 40.79). In the corresponding RNAse pre-treated specimens, all specimens of Ct-value <30 tested positive as well (range 17.39-35.48, median: 27.53). In the Ct 30-35 group, 36 specimens (75%) tested positive with a mean increase in Ct-value of 4.56 (range 29.88– 44.95, median: 36.79). Finally, in the Ct >35 group, 37 (75.5%) tested negative. All specimens testing negative after RNAse pre-treatment were obtained ≥8 days after onset of disease (range: 8-127, median 22).
Conclusion
This study suggests that RNAse pre-treatment of SARS-CoV-2 positive specimens can possibly differentiate between active infection, when intact viral particles are present, and past infection, when only non-infectious RNA remnants might be present. This assay could be a new valuable tool in assessment of infectivity in individuals with SARS-CoV-2 infection.

P023 Resistance of hyperinvasive clinical meningococcal isolates to complement-mediated killing in vaccinated adolescents and adults

Milou Ohm[1], J. Wolf[1], D.M. van Rooijen[1], N.M. van Sorge[2], A. van der Ende[2], M.J. Knol[1], G.A.M. Berbers[1], G. den Hartog[1]

[1]National Institute for Public Health and the Environment (RIVM), [2]Amsterdam UMC, location Amsterdam Medical Center

Introduction: During a recent outbreak of invasive meningococcal serogroup W disease (IMD-W) in the Netherlands, the majority of cases was caused by the genetic clonal complex (cc)11 lineage. This indicates a role for cc11-associated traits in the invasiveness of this lineage, also because a similar genetic lineage was responsible for a previous IMD-C outbreak. Antibody-induced, complement-mediated bacterial killing is an important protection mechanism but it is unknown whether vaccine-induced antibodies are equally capable of inducing killing of invasive lineages of meningococci.

Methods: Resistance to killing was assessed in the serum bactericidal antibody (SBA) assay, which is well established as the 'gold standard', with a titer of ≥8 as the internationally-accepted correlate of protection. With pooled sera from vaccinated individuals, we tested IMD strains (n=58) from different hyperinvasive lineages in the SBA assay. Furthermore, we determined geometric mean titers (GMTs) and protection levels for the default laboratory SBA non-cc11 strains (MenC C11, MenW MP01240070) and a hyperinvasive cc11 MenC and MenW strain in individual sera from adolescents (n=55; 15-20 years of age) and adults (n=56; 55-70 years of age) who received a MenACWY vaccination 5 years prior to serum collection.

Results: The hyperinvasive isolates differed in their resistance to killing (SBA titers ranged from 128-3072, median 512), but no clear trend was observed for the serogroup or type of clonal complex and the corresponding SBA titer. While a significantly lower GMT was observed against cc11 MenW in adults but not in adolescents, we found no significant differences in individual protection levels between the non-cc11 and a hyperinvasive cc11 meningococcal serogroup C or W strain.

Conclusion: These data show that hyperinvasive cc11 lineage meningococci are not resistant to complement-mediated killing in the SBA assay and vaccine-induced antibodies are effective against these invasive isolates.

P024 ClonalTracker: A new pipeline to survey vancomycin-resistant Enterococcus faecium outbreaks

Victoria Pascal[1], Anita Schürch[1]
[1]*Umc Utrecht*

Introduction
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-resistant genes and provide insight into transmission networks. We have built ClonalTracker to easily compare any two given VRE genomes.

Methods
The pipeline uses blastN to detect the van type, TETyper for transposon typing, MASH for whole genome comparison and PopPUNK to contextualize the genomes within a larger VRE collection. The results and default thresholds chosen were validated by using two previously analysed VRE datasets: 333 vanA VRE genomes (Arredondo-Alonso et al, 2021) and 39 vanB VRE isolates (Zhou et al, 2018).

Results
The pairwise comparison between the vanA genomes grouped the genomes into 40 clusters and 28 singletons while MLST classified them into a total of 38 groups. Representatives within the same group belonged to the same MLST group and also agreed with BAPS1-3 clustering, suggesting that the ClonalTracker results are highly comparable to those reported by more sophisticated and computationally intensive tools. Comparing the transposon types (TPs) found with the ones reported by Zhou et al highlighted our ability to find more differences within TP2 transposons but failed to detect a small inserted sequence in TP4. Regarding clonal relatedness, a MASH score of 95% similarity linked a total of 265 vanA genomes to be clonally related to at least another isolate. Similarly, within the vanB dataset, 4 genomes seem to be clonally related.

Conclusions
Overall, this approach provides a more fine-grained clustering than using MLST/BAPS and useful insights regarding the transposon and can provide a timely assessment of potential outbreak isolates for hospital surveillance. Nonetheless, these results also highlight areas of improvements for future versions of this pipeline.

P025 Dried Chicory Root: A New Intrinsic Fiber Product That Boosts Bowel Function, Gut Microbiota and Its Products in Subjects At Risk for Type 2 Diabetes

MSc Marie-Luise Puhlmann[1,2], MSc R. Jokela[3], MSc KCW van Dongen[4], PhD TPN Bui[1,5], PhD RWJ Hangelbroek[6], Prof. dr. H Smidt[1], Prof. dr. WM de Vos[1,3], Prof. dr. EJ Feskens[2]

[1]Wageningen University, [2]Wageningen University & Research, [3]University of Helsinki, [4]Wageningen University & Research, [5]Caelus Health, [6]Euretos B.V

Introduction: Dietary fiber intake negatively correlates with various diseases, yet minimally processed, intrinsic fibers are rarely studied.

Methods: We investigated the impact of dried chicory roots, an intrinsic multifiber product, on bowel function, fecal microbiota and its products, and glucose homeostasis in a three-week randomized trial with 55 subjects at risk for type 2 diabetes that consumed dried chicory roots (30 gram/day) or placebo (iso-caloric maltodextrin).

Results: A strong dose-dependent and reversible treatment effect was observed on bowel function and microbiota. In the treatment group, stool softness (+1.1 ± 0.3 units; P = 0.034) and frequency (+0.6 ± 0.2 defecations/day; p < 0.001) increased. The treatment strongly modulated gut microbiota composition (7% variation; P = 0.001) and dramatically increased relative levels (3-4-fold) of Anaerostipes and Bifidobacterium spp. Representative members of these genera formed a butyrogenic trophic chain from dried chicory roots in a synthetic in vitro community. In contrast, Blautia spp, Ruminococcus spp and other Lachnospiraceae levels decreased. The treatment increased fecal levels of acetate, propionate and butyrate by 25.8% (+13.02 ± 0.2 mmol/kg; P = 0.023) and their fasting circulating levels by 15.7% (+7.7 ± 3.9 µM; P = 0.057). In the treatment group the coefficient of variation in continuous glucose monitoring decreased from 21.3 ± 0.94% to 18.3 ± 0.84% (P = 0.004). Treatment responders (> 10% HOMA-ir decrease) differentiated from non-responders in baseline abundance of Blautia spp, which revealed to impact metabolic outcomes with distinct decreases in fasting glucose and insulin resistance markers in subjects with low baseline Blautia levels (-0.3 ± 0.1 mmol/L fasting glucose; P = 0.0187; - 0.14 ± 0.1 HOMA-ir; P = 0.045).

Conclusion: Dried chicory root is a prebiotic that rapidly and reversibly affects bowel function, gut microbiota and its products, benefits butyrogenic trophic chains and offers potential to improve glycemic control.

P026 Minocycline boosts the activity of recommended treatment regimens for Mycobacterium avium complex pulmonary disease in a dynamic hollow-fibre system

Jelmer Raaijmakers[1], Dr. M.M. Ruth[1], E. van den Hombergh[2], Prof. Dr. R. Aarnoutse[2], Dr. E Svensson[2,3], Prof. Dr. H Wertheim[1], Dr. W Hoefsloot[4], Dr. J van Ingen[1]
[1]Radboudumc, [2]Radboudumc, [3]Uppsala University, [4]Radboudumc

Introduction
Mycobacterium avium complex bacteria cause chronic pulmonary disease (MAC-PD) in susceptible patient populations. The recommended treatment regimen of rifampicin, ethambutol and azithromycin yields cure rates around 65%, but with considerable toxicity and drug-drug interactions. Minocycline proved active in a monotherapy experiment using the hollow-fibre model. We compared the efficacy of the recommended treatment regimen versus a minocycline, ethambutol and azithromycin regimen in the hollow-fibre model.

Methods
In an in vitro hollow-fibre experiment, epithelial lining fluid pharmacokinetic profiles of either the recommended rifampicin, ethambutol, azithromycin regimen or a minocycline, ethambutol, azithromycin regimen was simulated. THP-1 cells infected with M. avium ATCC 700898 were exposed to these regimens for 21 days. Pharmacokinetic profiles were determined at day 0 and at steady-state. The pharmacodynamic effect was measured by determining bacterial densities at day 0, 3, 7, 14 and 21 for both intracellular and extracellular fractions. The emergence of macrolide-resistance was monitored by inoculating azithromycin-containing agar (3x MIC), MIC measurements and resistance mutation analysis.

Results
The minocycline-containing regimen showed a higher initial kill rate than the recommended rifampicin-containing regimen. Both regimens failed to sustain their activity over time. Treatment failure was not linked to macrolide-resistance as measured by bacterial densities on azithromycin-containing agar, azithromycin MICs and resistance mutation analysis. The pharmacokinetic profiles simulated in the model matched with those described for patients with MAC-PD.

Conclusions
Replacing rifampicin with minocycline increased the antimycobacterial activity of the MAC-PD treatment regimen in the hollow-fibre model, without jeopardizing the prevention of macrolide-resistance. This promising minocycline-containing regimen may be of interest for inclusion in future clinical trials.

P027 The effect of resuscitation protein factor to the physiological state of clarithromycin exposed mycobacterium avium.

Jelmer Raaijmakers[1], H. de Man[1], Dr. J. van Ingen[1]
[1]*Radboudumc*

Introduction
Mycobacterium avium complex (MAC) bacteria cause pulmonary disease in susceptible populations; relapse rates after successful antibiotic treatment are as high as 30-40%. Antibiotic-induced stress causes MAC to change to a nonculturable - dormant state. After treatment termination, dormant MAC might be resuscitated by bacterial resuscitation promotion factors (RPF), causing a relapse. Here, we explore the effect of RPF on clarithromycin-induced dormant MAC bacteria.

Methods
Growth medium of unexposed and clarithromycin exposed (2x MIC; 4 µgml-1) M. avium ATCC 700898 was enriched with RPF (obtained from M. tuberculosis H37Rv liquid culture supernatants) on day 0, day 7, both, or not. To determine bacterial densities, samples were drawn and inoculated on 7H10 agar after a serial 10-fold dilutions on each day for 2 weeks, except for days 6 and 11 to 13.

Results
An increased growth rate and maximum bacterial carrying capacity is seen in growth controls after enriching growth medium with RPF, independent on the moment of enrichment. Initial RPF supplementation in clarithromycin-exposed strains caused mycobacteria to regrow, after an initial killing phase. The addition of RPF solely at day 7 did not cause a regrowth of the bacterial culture.

Conclusions
RPF promotes bacterial growth in both exposed and clarithromycin-exposed M. avium. The incapability of clarithromycin to suppress bacterial growth can possibly be explained by the prevention of clarithromycin induced dormancy M. avium and the activation of alternative mechanism of drug tolerance. Addition of RPFs solely on day 7 did not cause M. avium regrowth, possibly by increased clarithromycin susceptibility after RPF resuscitation.

P028 Self-reported symptoms associated with the development of SARS-CoV-2 antibodies among healthcare workers in Dutch hospitals

Claudia Recanatini[1], C GeurtsvanKessel[2], S Pas[3], M Maas[4], R van Mansfeld[5], AJG van Oudheusden[6], M van Rijen[7], E Schippers[8], A Tami[9], KE Veldkamp[10], H Visser[11], A Voss[12], M Wegdam-Blans[13], H Wertheim[14], P Wever[15], MPG Koopmans[2], JAJW Kluytmans[1,7], MFQ Kluytmans-van den Bergh[1,7], COCON study group

[1]Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, The Netherlands, [2]Viroscience department, Erasmus University Medical Center, Rotterdam, The Netherlands, [3]Microvida Laboratory for Medical Microbiology, Roosendaal, The Netherlands, [4]Hospital Bernhoven, Uden, The Netherlands, [5]Amsterdam University Medical Centers, University of Amsterdam, The Netherlands, [6]Elisabeth-TweeSteden Hospital, Tilburg, The Netherlands, [7]Amphia Hospital, Breda, The Netherlands, [8]Haga Hospital, The Hague, The Netherlands, [9]University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, [10]Leiden University Medical Center, Leiden, The Netherlands, [11]Beatrix Hospital, Gorinchem, The Netherlands, [12]Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, [13]Catharina Hospital, Eindhoven, The Netherlands, Hospital St. Jans Gasthuis, Weert, The Netherlands, and stichting PAMM Veldhoven, [14]Radboud University Medical Center, Nijmegen, The Netherlands, [15]Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands

Introduction: We aimed to estimate the rate of asymptomatic SARS-CoV-2 infections among healthcare workers (HCWs) employed in Dutch hospitals during the first wave of the COVID-19 pandemic in 2020 and describe the self-reported symptoms most associated with the development of SARS-CoV-2 antibodies.
Method: COCON (Control of COVID-19 in hospitals) was a cross-sectional study enrolling 2328 HCWs across 13 Dutch hospitals in June-July 2020. The serostatus was determined at enrolment by testing for total antibodies against the SARS-CoV-2 spike protein (ELISA, Wantai). Participants (aged 20-68 years, 81.5% women) completed a questionnaire on symptoms suggestive of COVID-19 experienced since January 2020, their date of onset and duration.
Results: 343/2328 (14.7%) HCWs were seropositive at enrolment:13% (n=45) of them were asymptomatic, 20% (n=70) had symptoms at enrolment and 67% (n=228) reported previous symptoms. The self-reported duration of symptoms was longer for seropositive than for seronegative participants (median, interquartile range:2,1-3 vs. 1,0-3 weeks; p<.001). Fatigue (61%) and fever (53%) were the most common symptoms among seropositive HCWs. In a multivariable model containing all symptoms, loss of smell or taste, fever, fatigue and muscle aches were independently associated with the development of antibodies, while sore throat, shortness of breath, chills and interscapular pain were negative predictors.
Conclusions: Most HCWs with SARS-CoV-2 antibodies suffered from mild disease, with 13% being asymptomatic. The voluntary participation in the study might have led to selection bias: HCWs believing they had COVID-19 might have attended more likely, and those with severe disease participated less likely. Loss of smell and taste were confirmed to be specific COVID-19 symptoms. Fever, muscle aches and fatigue were also independent predictors of SARS-CoV-2 antibodies in a cohort of non-vaccinated HCWs with mild COVID-19 infection, while sore throat and shortness of breath seemed predictive of other conditions.

P029 SARS-CoV-2 seroprevalence in relation to occupational and personal risk factors among healthcare workers in Dutch hospitals after the 2020 first epidemic wave

Claudia Recanatini[1], C GeurtsvanKessel[2], S Pas[3], M Maas[4], R van Mansfeld[5], AJG van Oudheusden[6], M van Rijen[7], E Schippers[8], A Tami[9], KE Veldkamp[10], H Visser[11], A Voss[12], M Wegdam-Blans[13], H Wertheim[14], P Wever[15], MPG Koopmans[2], JAJW Kluytmans[1,7], MFQ Kluytmans-van den Bergh[1,7], COCON study group

[1]University Medical Center Utrecht, [2]Viroscience department, Erasmus University Medical Center, Rotterdam, The Netherlands, [3]Microvida Laboratory for Medical Microbiology, Roosendaal, The Netherlands, [4]Hospital Bernhoven, Uden, The Netherlands, [5]Amsterdam University Medical Centers, University of Amsterdam, The Netherlands, [6]Elisabeth-TweeSteden Hospital, Tilburg, The Netherlands, [7]Amphia Hospital, Breda, The Netherlands, [8]Haga Hospital, The Hague, The Netherlands, [9]University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, [10]Leiden University Medical Center, Leiden, The Netherlands, [11]Beatrix Hospital, Gorinchem, The Netherlands, [12]Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, [13]Catharina Hospital, Eindhoven, The Netherlands, Hospital St. Jans Gasthuis, Weert, The Netherlands, and stichting PAMM Veldhoven, The Netherlands, [14]Radboud University Medical Center, Nijmegen, The Netherlands, [15]Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands

Introduction: We aimed to estimate SARS-CoV-2 seroprevalence and describe its determinants among healthcare workers (HCWs) in Dutch hospitals after the first wave of the COVID-19 pandemic.
Method: COCON (Control of COVID-19 in hospitals) was a cross-sectional study with prospective follow-up enrolling 2328 HCWs across 13 Dutch hospitals in June-July 2020. The serostatus was determined at enrolment and after three months by testing for total antibodies against the SARS-CoV-2 spike protein (ELISA, Wantai). Participants (aged 20-68 years, 81.5% women) completed a questionnaire on risk factors for occupational and community exposure to SARS-CoV-2 since January 2020. Adjusted odds ratios (aOR) for seropositivity were determined using logistic regression.
Results: The seroprevalence at enrolment was 14.7% (343/2328), with differences across hospitals (min:3.8%, max:30.0%) and remained stable after follow-up (16.1%, 357/2220). Older and younger HCWs had higher seroprevalence (16.2% ≤35 and ≥50 years vs. 11.6% 36-49). Nurses (aOR 1.99, 95%CI 1.24-3.19) had a higher infection risk than physicians. The emergency department (ED) (aOR 1.78, 95%CI 1.11-2.87) was the ward with the highest infection risk, whereas HCWs working in ICU (aOR 0.46, 95%CI 0.31-0.69) were relatively protected. Flu vaccination, chronic respiratory disease, smoking and having a pet were associated with lower seroprevalence in our cohort.
Conclusions: In 2020, SARS-CoV-2 seroprevalence in HCWs reflected regional differences in the Netherlands and the viral circulation during the summer was minimal. We identified occupational risk and protective factors (high risk in ED and nurses, low risk in ICU) that could inform risk assessment and infection control policies. We confirmed the cross-protection between influenza vaccination and COVID-19 suggested by other studies. A prudent behaviour of vaccinated HCWs could also explain the lower seroprevalence, same as for HCWs with a chronic respiratory disease. The lower seroprevalence in smokers could be the effect of a reduced immune response to infection.

P030 A longitudinal study using an enzyme linked immunosorbent spot assay reveals a delayed SARS-CoV-2 specific T-cell response in patients with severe COVID-19

L.W. Rümke[1], A. Bossink[2], G. Limonard[2], L. van Lelyveld-Haas[2], S. van der Wal[2], B. Thio[2], J. Reimerink[3], C. Reusken[3], M. Nijhuis[1], A.M.J. Wensing[1], M. Heron[2], S.F.T. Thijsen[2]
[1]UMCU, [2]Diakonessenhuis, [3]Rijksinstituut voor Volksgezondheid en Milieu (RIVM)

INTRODUCTION:
Progression to severe COVID-19 is associated with maladapted immune responses with ongoing cytokine production and lymphopenia. To what extent SARS-CoV-2 specific T-cell responses relate to disease progression remains to be elucidated.

METHODS:
Cellular and humoral responses were longitudinally characterized in hospitalized patients with moderate (n=86) and severe (n=75) COVID-19. T-cell immunity was measured by enzyme-linked immunosorbent spot (ELISpot) interferon-γ release assay using four peptide pools covering the immunodominant regions of spike (S and S1), membrane and nucleocapsid. Concomitant antibody responses were assessed with a commercial ELISA targeting the receptor binding domain (Wantai). Samples were collected at enrolment (<3 days after admission), 14 days after enrolment or at discharge, 1 and 3 months after discharge.

RESULTS:
At hospital admission, median T-cell responses measured by ELISpot in moderate and severe patients were 35 vs 10 spot forming cells (SFC; $P<0.001$). In both groups, the median symptom duration at hospital admission was 11 (IQR 9-15) days. The majority of SFCs was induced by the S1 peptide pool (mean percentage of total SFCs resp. 44% and 30%) and the membrane peptide pool (resp. 28% and 30%). The proportion of SARS-CoV-2 specific T-cells of the total T-lymphocyte repertoire were 0.042% vs 0.013% ($P<0.05$). These reduced SARS-CoV-2 specific T-cell responses in severe cases persisted up to 40 days after symptom onset. In all patients antibodies were detected 10 days after symptom onset. No differences in antibody responses were observed.

CONCLUSION:
- Our data demonstrate a delayed SARS-CoV-2 specific T-cell response in severe COVID-19 patients compared to patients with moderate disease.
- This might play a role in progression to severe disease possibly due to suboptimal viral clearance with ongoing infection and inflammation.

Samples of mild COVID-19 patients (n=65) are currently tested and will be added to the data.

P031 The role and perception of clinical microbiology and infectious diseases trainees during the COVID-19 crisis

Valentijn Schweitzer
[1]*UMC Utrecht*

Introduction:
The COVID-19 pandemic has had a major impact on healthcare workers around the globe. During the pandemic, trainees in infectious diseases (ID) and clinical microbiology (CM) have often been at the forefront. It is unknown to which extend the pandemic has influenced the working conditions and mental health of ID and CM trainees. Therefore, we performed an European survey to evaluate the role and perceptions of ID and CM trainees during the COVID-19 pandemic.

Methods:
An online survey was designed with questions regarding working conditions and mental health perception. The survey consisted of 19 multiple-choice questions and 13 questions with a 5-point Likert scale (5=strongly agree, 1=strongly disagree), divided into 4 sections: (a) Demographic characteristics, (b) Role of ID and CM trainees, (c) Impact on training, (d) Perception and feelings during the pandemic. The survey was distributed online by the Trainee Association of ESCMID (TAE) using their contact persons, Twitter, and through newsletters.

Results:
The survey was completed by 235 participants from 26 different countries, representing both ID (56.2%) and CM (37.4%). A total of 91% (215/235) experienced a disruption of training activities, and 33.6% (79/235) was relocated to another department. Working over hours was common (70.6%, 166/235), with 23.5% (39/235) working an additional >20 hours/week. A total of 72% (169/235) experienced more stress than usual, and 19% (45/235) experienced worsening or new health-related issues. In addition, 76% (179/235). Despite this, only 35% (26/235) reported the possibility of psychological support.

Conclusion:
The COVID-19 pandemic has had a substantial impact on European ID and CM trainees. These results show it is important to safeguard the continuity of training and to come up with solutions for possible delays. In addition, as the pandemic might have important mental health consequences for trainees, sufficient psychological support should be ensured.

P032 Regulation of the intestinal epithelial tight junctions by the transmembrane mucin MUC13

MSc C Segui-Perez[1], Dr. D. A. C Stapels[1], MSc Z Ma[2], Dr. W Wu[2], MSc J Su[1], Dr. B Westendorp[1], Prof. dr. Dr J Van Putten[1], Dr. K Strijbis[1]
[1]Utrecht University, [2]Utrecht University

Mucins are known as barrier proteins that prevent inflammation and infection, but it is starting to emerge that they are also important regulators of epithelial responses. The transmembrane mucin MUC13 is highly expressed on the apical surface of enterocytes but is also overexpressed in the inflamed colonic mucosa of inflammatory bowel disease (IBD) patients and colorectal cancer (CRC). Cell junctions, including tight junctions (TJs) and adherens junctions (AJs), play a crucial role in maintaining epithelial barrier integrity and sealing the paracellular space between cells. The loss of cohesion between these structures can lead to the translocation of particles and bacterial compounds, contributing to systemic inflammation. Using CRISPR/Cas9 technology, we knocked out the MUC13 gene in the intestinal cell line HRT18 (ΔMUC13). We observed that MUC13 localized near the TJ region on the lateral membrane in HRT18 cells. ΔMUC13 cells built up a tighter barrier as measured by the transepithelial resistance (TEER) assay, and this process was dependent on protein kinase C (PKC). Removal of MUC13 also led to a slight reduction in translocation of the Lucifer Yellow (LY) tracer and 4kDa FITC-Dextran particles across the epithelial barrier. Mass spectrometry performed on membrane fractions indicated an increased accumulation of TJ proteins such as claudins, occludin, and ZOs in ΔMUC13 cells. Furthermore, by targeted deletion of only the cytoplasmic tail of MUC13 (ΔCT-MUC13), we showed that the MUC13 tail was not responsible for the increased TEER. ΔCT-MUC13 cells had increased membranal levels of ZO-1 and some, but not all of the claudins. As a negative regulator of epithelial barrier function, MUC13 could be involved in the opening of intestinal cellular junctions, consequently contributing to chronic intestinal inflammation.

P034 Lessons-learned: preventive SARS-CoV-2 PCR-screening of newly admitted patients without COVID-19-related symptoms or recent COVID-19 exposure, Radboudumc January 2022.

EB Bowles[1], Dr Alma Tostmann[1], MS Marije Stoffer[2], LG Gommers[2], CM Meijer[1], Dr MM McCall[1], Prof. Dr. HW Wertheim[1], Prof. Dr. CB Bleeker-Rovers[3]
[1]Radboudumc, [2]Radboudumc, [3]Radboudumc

Background
In the Netherlands hospitalised patients are tested for SARS-CoV-2 when they have symptoms suggestive of COVID-19 or in case of a recent high-risk contact. Additionally, pre-operative screening is performed in patients over 65 years who are not fully vaccinated and/or immunocompromised.
Given the high transmissibility of the omicron variant and reports of high levels of nosocomial transmission from the UK, we extended testing to all admitted patients.

Methods
The screening started on 6 January 2022 and is envisaged to continue until at least the end of February. The electronic patient record system (EPIC) automatically generates a PCR test order for all newly admitted patients. Their test results are coupled to infection prevention analyses and presented in a real-live dashboard. After implementation, screening was evaluated daily using the plan-do-study-act cycle. Because not all positive tests indicated infectiousness, we will describe for how many patients isolation precautions were required. Evaluation will be completed at the end of February.

Provisional Results
Between 6 and 27 January 2022 1,008 patients were tested, of whom 31 patients (3.1%) had a positive SARS-CoV-2 PCR. Of these, 23 patients (74%) were considered infectious and contact investigations were initiated among patients residing in the same patient room. So far, no transmission has been identified among these contacts.

Discussion
At the conference we will share the results of the evaluation of this preventive screening and the lessons learned. This is relevant in the context of the possible emergence of a new Variant of Concern during the COVID-19 pandemic, and in the context of preparedness for future pandemics. We would also like to share the outcomes of the (scientific) methods we used to implement the PCR screening process and the impact it had on our patients, health care personnel and the organisation.

P035 The need for culture for surveillance of Shiga toxin-producing Escherichia coli (STEC): how best to treat your fecal samples

Mr Kim van der Zwaluw[1], Dr. I Friesema[1], Dr. T Bosch[1], Dr. E Franz[1], Dr M van den Beld[1]
[1]*National Institute for Public Health and the Environment (RIVM)*

Introduction: In the Netherlands, Medical Microbiological Laboratories are obliged to report STEC-infections to the municipal health services and voluntarily submit isolates to the National Institute for Public Health and the Environment for confirmation and typing. These isolates are crucial to monitor circulating types and detect clusters. Currently, only 26% of reported STEC-infections are submitted. This percentage decreases annually, resulting in an incomplete picture of circulating STEC and hampering our preparedness and response in case of outbreaks. To improve our surveillance, we will accept STEC-PCR positive fecal samples in addition to isolates. Here we describe the optimization of storage and transport conditions for fecal samples.
Methods: Fecal samples were spiked with two STEC isolates (O26:H11 and O157:H7) and diluted from 10^7 to 10^1 cfu/ml. These dilutions were stored with and without Cary-Blair medium, at room temperature or refrigerated for up to nine days. Samples were cultured on CHROMagar at different time-points. After establishing the optimal conditions, the STEC isolation protocol will include culturing on CHROM and MacConkey agar followed by PCR on three suspected colonies from CHROMagar or up to ten colonies from MacConkey.
Results: The addition of Cary-Blair medium improves the recovery of STEC isolates by up to 1000-fold. When only added for the last 48 hours, there was a 10-fold beneficial effect. Furthermore, refrigerated storage seems to result in a 10-fold reduction of STEC-recovery. Future testing will include the use of commercially available swabs in Cary-Blair and other media.
Conclusion: For the recovery of STEC isolates, fecal samples can best be stored and transported in Cary-Blair medium at room temperature. It is anticipated that the option to submit fecal samples in addition to isolates will result in a more effective national surveillance of STEC. Meanwhile, STEC PCR-positive fecal samples are needed from MMLs to validate our STEC isolation protocol.

P036 Torque teno virus loads as marker of functional immunity, indicating infection and rejection after solid organ transplantation; A systematic review

Aline van Rijn[1], R Roos[2], Dr. JI Rotmans[3], Dr. MCW Feltkamp[1]
[1]Leiden University Medical Center, [2]HagaZiekenhuis, [3]Leiden University Medical Center

Background: Balancing immunosuppression to prevent infection and rejection in solid organ transplant recipients (SOTx) remains a challenge. Torque teno virus (TTV), a commensal non-pathogenic virus, has been proposed as marker of functional immunity: higher loads correspond to over-immunosuppression, and lower loads to under-immunosuppression. This review offers an overview of the current literature and evidence on the association between TTV load, and infection and rejection in SOTx recipients.

Methods: A systematic literature search strategy, explained in PROSPERO, resulted in 548 records. After screening, 23 original and peer-reviewed articles were included in qualitative assessment, investigating the association between TTV load, and either infection or rejection in SOTx. The Quality in Prognosis Studies tool was used to assess the risk of bias.

Results: Of 23 included studies, most involved retrospective cohorts in which the TTV load was measured within 1-2 years post-transplantation, once or longitudinally. Defined outcomes differed between studies: infection outcomes included viral (CMV, BK, respiratory), bacterial, and fungal infections of varying severity. Rejection outcomes included start of rejection treatment or confirmation by biopsy. Twelve out of 17 studies reported an association between high TTV loads and infection (71%), and 13 out of 15 an association between low TTV loads and rejection (87%). The qualitative assessment showed varying risks of bias between studies.

Conclusion: Systematic review of the current literature indicates uncertainty in the proposed association between TTV loads and infection and rejection. Inconsistent results between studies may reflect the heterogeneity of the endpoints and/or might signify a limited predictive value of TTV for anticipating these outcomes. While the presented literature explores the association, externally validated prediction models remain to be built, as these will offer individualized diagnostic or prognostic value to predict infection and rejection in SOTx recipients.

P037 Culture methods to detect carriage of carbapenem-resistant Pseudomonas aeruginosa (CR-PA): a systematic review.

MSc A van Veen[1], MD SN Shahab[1,2], MD AC Büchler[1], Dr. YR Saharman[2], Prof.dr. A Karuniawati[2], Prof.dr. MC Vos[1], Dr. AF Voor in 't Holt[1], Dr. JA Severin[1]

[1]Erasmus MC University Medical Centre, [2]Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo General Hospital

Background

Detection of carbapenem-resistant Pseudomonas aeruginosa (CR-PA) carriage in humans is important for controlling its spread. However, routine culture methods may fail to detect CR-PA. This systematic review aims to determine the most accurate method for detecting CR-PA in humans and to identify which detection methods are used in outbreak management and surveillance activities.

Methods

We systematically searched the electronic databases Embase, Medline Ovid, Cochrane, Scopus, Cinahl, and Web of Science until September 7, 2020 (updated search ongoing). All studies comparing two or more culture methods for detecting CR-PA (i.e. diagnostic accuracy studies) and outbreak or surveillance reports of CR-PA in humans which describe culture methods and their results, were eligible for inclusion.

Results

Eight diagnostic and thirty-nine outbreak/surveillance studies were included. The diagnostic studies showed a large diversity in study design and included a variety of (selective) media for the detection of Gram-negative bacteria (including P. aeruginosa and/or CR-PA). Selective culture media used were modified CHROMagar-Pseudomonas supplemented with meropenem, chromID® CARBA SMART, Brilliance™ CRE/ESBL, Chromart CRE, BBL™ CHROMagar™ CPE, Pseudomonas chromogenic medium, HardyCHROM CRE agar, Cetrimide Agar (CN), and IsoSensitest agar supplemented with meropenem. One study found that CR-PA detection significantly increased when inoculation on a selective medium was preceded by a (non-)selective enrichment broth. For outbreak/surveillance studies, CR-PA prevalence ranged from 0.5-25.2% (n=12) in studies with a variety of microorganisms and from 5-64% (n=8) in studies with P. aeruginosa only. A cotton-tipped swab was most frequently used (n=13, 33.3%). Seven studies (17.9%) described using enrichment broths prior to identification. Selective media were used in fifteen studies (38.5%), of which nine (60%) used CN.

Conclusions

A variety of methods are used for detecting CR-PA in humans. Our preliminary results show the potential value of using an enrichment step and selective media, although more research is needed.

P038 Long term linezolid use (>28 days) in bone and joint infections is safe and well-tolerated

Drs Karin Veerman[1], Drs. Denise Telgt[1], Dr. Jon Goosen[1], Dr. Petra Heesterbeek[1]
[1]*Sint Maartenskliniek*

Introduction
Linezolid is frequently prescribed in bone and joint infections. Its use is restricted because of the potential serious side effects in prolonged treatment (>28 days). The aim of this study is to evaluate the tolerability and safety of linezolid when used for >28 days.

Methods
This single center retrospective cohort study was conducted in the Sint Maartenskliniek, The Netherlands. Patients were included if linezolid was prescribed between December 2013 and January 2020 for the intended use of >28 days. The unintended discontinuation rate, the reason for unintended discontinuation and the occurrence of side effects related to the linezolid use were analyzed.

Results
A total of 78 patients were included in the analysis. Sixy-seven patients used linezolid therapy for >28 days, mean: 47 (SD: 15). Side effects were reported in 68% (53/78) of the cases: gastrointestinal symptoms (n=43), malaise (n=31) and neurological symptoms (n=21). Possible optic neuritis occurred in 1 case. In 26% (20/78) of the cases, side effects resulted in unintended linezolid discontinuation, most frequently due to gastrointestinal symptoms (n=10) and malaise (n=8). Nine of the 20 patients stopped after >28 days of treatment, due to gastro-intestinal symptoms (n=5), malaise (n=5), cytopenia (n=3), renal failure (n=1) and rash (n=1).

After >28 days of treatment hemoglobin level became <6.0 mg/l in 8 cases, leucocytes count <4.0x10^9/l in 3 cases and thrombocytes count <150x10^9/l in 3 cases. This resulted in unintended discontinuation of linezolid in 3 cases. Recovery of the hematopoiesis within 4 weeks after stopping the linezolid was seen in 100% of the cases in which follow-up measurements were available (n=7).

Conclusion
Prolonged use of linezolid of >28 days is safe provided that side effects are accurately monitored. The main reasons for discontinuation after 28 days of treatment were gastrointestinal symptoms and malaise. Bone marrow suppression was reversible after discontinuation.

P039 Cluster of symptomatic graft-to-host transmission of herpes simplex virus type 1 in an endothelial keratoplasty setting

Dr J.J.A. van Kampen[1], Dr J.A. Guerra-Assunção[2], Dr S. Roy[2], Dr L. Remeijer[3], Prof J Breuer[2], Dr Georges Verjans[1]

[1]Erasmus MC, [2]University College London, [3]Rotterdam Eye Hospital

Introduction. Descemet's membrane endothelial keratoplasty (DMEK) is becoming the gold standard to treat corneal endothelial dysfunctions worldwide. Compared with conventional penetrating keratoplasty, infectious complications after DMEK are ill-defined. We describe the clinical picture of 2 DMEK recipients, operated on the same day and in the same clinic, who developed atypical herpes simplex virus type 1 (HSV-1) infection in the transplant recipient eye within days post-DMEK. Because recipients received cornea tissue from 2 different donors prepared by the same eye bank, the likelihood of a common HSV-1 source was determined.

Methods. Surplus cornea donor (pre-DMEK cornea remnants and conditioned cornea storage and transport media) and recipient samples (post-DMEK aqueous humor) were assayed for HSV-1 DNA and infectious virus by real-time polymerase chain reaction (RT-PCR) and cell culture, respectively. Target-enriched whole viral genome sequencing was performed on HSV-1 DNA–positive ocular specimens.

Results. HSV-1 DNA was detected in aqueous humor and donor cornea specimens of both DMEK cases, but not in the cornea remnants of 6 randomly selected donors processed by the same eye bank. Infectious HSV-1 was isolated from the cornea remnant and corresponding culture medium of 1 cornea donor. Notably, whole-genome sequencing of virus DNA-positive specimens demonstrated exceptionally high genetic similarity between HSV-1 strains in recipient and donor specimens of both DMEK cases.

Conclusions. Data indicate cross-contamination of cornea grafts during DMEK preparation with subsequent graft-to-host HSV-1 transmission that caused atypical sight-threatening herpetic eye disease shortly after DMEK. Ophthalmologists should be aware that HSV-1 transmission by DMEK is possible and can lead to atypical ocular disease, a condition that can easily be prevented by taking appropriate technical and clinical measures at both eye bank and surgical levels.

P040 Infections caused by the Delta variant of SARS-CoV-2 are associated with 4-fold increased viral loads compared to infections with the Alpha or non-Variants of Concern

Dr Christian Von Wintersdorff[1], Dr. J Dingemans[1], Dr. LB van Alphen[1], Dr. PFG Wolffs[1], Dr. BMJW van der Veer[1], Prof. Dr. CJPA Hoebe[2], Prof. Dr. PHM Savelkoul[1]
[1]Maastricht University Medical Center+, [2]Public Health Service South Limburg

Introduction:
There has been a growing body of evidence that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant (B.1.617.2) shows enhanced transmissibility and increased viral loads compared to other variants. A recent study has even suggested that respiratory samples from people infected with the Delta variant can harbor up to 1000 times higher viral loads compared to samples with variants that are more closely related to the original Wuhan strain.
Methods:
In this study, we have compared the viral load in 16,185 samples that were obtained mainly from public health testing in periods during which non-VOC, the Alpha (B.1.1.7) or Delta variant (B.1.617.2) were dominant in the corresponding region as evidenced by genomic surveillance. Furthermore we compared the viral loads of a subset of 1,958 samples that were whole genome sequenced.
Results:
We found that the Delta variant contained about 4-fold higher viral loads compared to the non-VOC or Alpha variants. Furthermore we found that this difference in viral load was present only in age categories 0-20 and 21-40 years, but not in age categories 41-60 or 61+ years. This trend in age categories was inversely related to the amount of fully vaccinated individuals in these categories.
Conclusion:
We found that the Delta variant contained about 4-fold higher viral loads compared to those of the non-VOC or Alpha variants, which is significantly lower than what was reported earlier.

P041 Automated detection and classification of SARS-CoV-2 rapid diagnostic tests using machine learning

E Mintjes[1], F Khan[1], J de Swart[1], K Bouaissa[1], R Nolet[1], M Loois[1], Dr. B Herpers[2], F Metz[2], Dr. Alex Wagemakers[2]
[1]Amsterdam University of Applied Science, [2]Streeklab Haarlem

Introduction
Rapid Diagnostic Tests (RDTs) are widely used antigen tests for detection of SARS-CoV-2 infections. While literature is scarce, lower than expected sensitivities have been described in a self-test setting, possibly due to inadequate test performance or interpretation. Moreover, it is currently impossible to track the incidence of SARS-CoV-2 infections when tests are performed in a self-test setting. The objective of this study was to develop an algorithm to detect and classify RDT results based on an digital image of the test.

Method
SARS-CoV-2 (diluted) positive and negative samples were used for 6 different brands of SARS-CoV-2 RDTs, and were photographed under different angles and light conditions. Images were divided into a set for training (n=1188) and validation (n=302), and data augmentation was performed on the training set to obtain 1200 images with varying photography settings. A detection model (YoloV3 Convolutional Neural Network(CNN)) was trained to crop and save the RDT test window containing the test- and control bands. Cropped window images were used to train a CNN classification model (Custom Vision, Microsoft Azure). Finally, the detection- and classification algorithms were combined in Python and a separate set of photographs (181 positive tests, 121 negative tests) was used for validation.

Results
Across the 302 validation images, the algorithm yielded an accuracy of 99.0 percent and a sensitivity and specificity of 98,9 and 99,2 percent respectively.

Conclusion
This study confirms that it is possible to detect and classify images of RDTs using machine learning techniques. In our laboratory-generated dataset, accuracy was high. Further studies should validate the clinical sensitivity and specificity of the algorithm on a real-life dataset. If successful, the algorithm could be used to aid individuals with the interpretation of their RDT and provide a tool for epidemiological tracking of SARS-CoV-2 incidence in the self-test setting.

P042 Fluorescence in situ hybridization for detecting Coxiella burnetii in tissue samples

Mr. Jesper Weehuizen[1], Sheila B Buijs[1], prof. dr. Tim K Jensen[2], dr. Mirjam MA Hermans[3], dr. Peet TGA Nooijen[4], prof. dr. Andy IM Hoepelman[1], prof. dr. Chantal P Bleeker-Rovers[5], dr. Jan Jelrik Oosterheert[1], dr. Peter C Wever[3]

[1]University Medical Center Utrecht, [2]Technical University of Denmark, [3]Jeroen Bosch Hospital, [4]Jeroen Bosch Hospital, [5]Radboud University Medical Center and Radboud Expertise Center for Q fever

Introduction: Diagnosing chronic Q fever, caused by the intracellular bacterium Coxiella burnetii, is notoriously difficult and is based on a combination of clinical, microbiological, and radiologic characteristics. Fluorescence in situ hybridization (FISH) might be a promising technique, but its value in comparison with PCR is uncertain. In this study we evaluated the diagnostic value of FISH for detecting C. burnetii in tissue samples.

Methods: FISH and PCR were performed on tissue samples from Dutch chronic Q fever patients collected during surgery or autopsy. Sensitivity, specificity, and overall diagnostic accuracy was calculated. Additionally, data on patient and disease characteristics were collected from electronic medical records.

Results: Of 39 chronic Q fever patients, 49 tissue samples from mainly vascular walls, heart valves, or placentas were examined by FISH and PCR. The sensitivity and specificity of FISH compared to PCR for detecting C. burnetii in tissue samples from chronic Q fever patients was respectively 45.1% and 85.7%. The overall diagnostic accuracy was 57.8% (95% CI, 42.2% - 72.3%). Two C. burnetii PCR negative placentas were FISH positive. Four FISH results (8.2%) were deemed inconclusive because of autofluorescence.

Conclusion: With an overall diagnostic accuracy of 57.8%, we cannot recommend the replacement of PCR by FISH in the routine diagnostics of chronic Q fever. Given the FISH positive results in PCR negative placentas, FISH does seem to have an additional value next to PCR and remains an interesting technique to further examine in chronic Q fever patients.

P043 Methicillin-resistant Staphylococcus aureus (MRSA) prevalence among samples of healthcare workers (HCW) in contact tracings in a Dutch teaching hospital, 2010-2021

Mrs Veronica Weterings[1], W. van den Bijllaardt[1,2], Y. Hendriks[1], A. Joziasse[1], H. Kievits[1], L. Kilsdonk[1], A. Melsen[1], G. Moen[1], I. Vincenten[2], S. van de werken[1], E. Weterings[1], Dr M. van Rijen[1]
[1]Amphia, [2]Microvida Laboratory for Microbiology

Introduction
The Dutch guideline on MRSA prevention and control advocate screening of HCW after unprotected exposure to MRSA carriers. Although this strategy at large is successful, contact tracing of staff is a time consuming and costly component. We evaluated our contact tracing policy for HCW over the years 2010 – 2021.

Methods
A retrospective, observational study was performed in a Dutch teaching hospital. All samples of HCWs who were tested for MRSA carriage as part of contact tracing from 2010 until 2021 were included. A pooled nose, throat and perineum swab was collected using the eSwab medium (Copan) and inoculated on chromID MRSA agar plates (bioMérieux) after enrichment in a broth. Molecular typing was performed using MLVA.

Results
In total, we included 10,007 samples from a total of 368 contact tracings. Thirty-four HCWs were colonized with MRSA (0.36%; 95%CI 0.24 – 0.47). Eight HCWs (0.08%; 95%CI 0.05% – 0.16%) were colonized with the same MLVA type as the index case, and were detected in 6/368 contact tracings (1.6%). In 3/8 cases it was clear that the HCW who was identified in the contact tracing was the source of the outbreak and was the cause of invasive MRSA infections in patients. Notably, a different MLVA type as the index case was found in 26 HCWs (0.26%; 95%CI 0.18 – 0.38) of which 7/26 HCW (26,9%) were intermittent carriers.

Conclusion
This study revealed a sustained low MRSA prevalence among samples in contact tracing of HCW, over twelve years. Furthermore, it shows that when MRSA contact tracing is performed according to the national guideline only one out 1250 samples results in a secondary case. More frequently, an unrelated strain is found. These findings raise question marks regarding the efficacy of the current strategy to perform contact tracing after unprotected exposure.

P044 Detection of Actinotignum from urine samples in the Netherlands: large variation between medical microbiological laboratories

C.C.H. Wielders[1], A.F. Schoffelen[1], D.W. Notermans[1,2], E.J. Kuijper[1,3], W. Altorf-van der Kuil[1], M.A. Leversteijn-van Hall[4], on behalf of the Infectious Diseases Surveillance Information System-Antimicrobial Resistance (ISIS-AR) Study Group

[1]Rijksinstituut voor Volksgezondheid en Milieu (RIVM), [2]Amsterdam University Medical Center, [3]Leiden University Medical Center, [4]Alrijne Hospital

Introduction

The genus Actinotignum (reclassified Actinobaculum) contains uropathogenic species. These bacteria are small, gram-positive coccoid rods, facultative anaerobic and CO2-requiring, and may be underdiagnosed in aerobic routine culture methods. We aimed to assess differences in the detection rate of Actinotignum spp. between Dutch medical microbiological laboratories (MMLs) in urine samples.

Methods

From the Dutch national surveillance system for antimicrobial resistance (ISIS-AR) database, we selected the first urinary Actinotignum spp. isolate per patient per year for 2010–2019. The first urinary Escherichia coli isolate per patient per year was used as reference. Per MML (n=49) we calculated the number of detected Actinotignum spp. per 1,000 E. coli isolates (A/E ratio) as well as the overall median and interquartile range (IQR). Furthermore, A/E ratios were calculated by sex, age and type of MML using Poisson regression.

Results

The median A/E ratio was 1.60 per 1,000 E. coli (IQR 0.16–3.22). Nine MMLs (18%) never identified Actinotignum spp., while twelve (24%) detected Actinotignum spp. statistically significantly more often than the mean of all MMLs. Statistically significant higher A/E ratios were found for men (7.64 vs 0.97 for women), for increased age (0.68, 2.35, and 3.90 for <49, 50-70, and >70 years old, respectively), and for MMLs in academic centres (0.42, 2.38, and 11.34 for serving mainly general practitioners, general hospital, and academic centre, respectively).

Conclusion

1. Large, statistically significant differences were found between MMLs in the detection rate of Actinotignum spp. in urine cultures, suggesting that urinary tract infections caused by Actinotignum spp. may be missed by some MMLs.
2. Differences in the culture methods used by MMLs and/or used for different patient populations may be the underlying reason. A questionnaire has been sent out to the MMLs to obtain insight in culture methods of urine samples and potential explanations for the differences found.

P045 Dose-Response Relationship Between Proton Pump Inhibitor Use and Acquisition of Carbapenemase and Extended-Spectrum Beta-Lactamase-Producing Enterobacterales in Tertiary-Care Patients: A Nested Case-Control Study

MD RPJ Willems[1], PhD MC Schut[2], AM Kaiser[1], TH Groot[1], PhD A Abu-Hanna[2], PhD JWR Twisk[3], MD, PhD K van Dijk[1], MD, PhD CMJE Vandenbroucke-Grauls[1,4]
[1]Amsterdam UMC, [2]Amsterdam UMC, [3]Amsterdam UMC, [4]Aarhus University

Introduction:
Importance: Proton pump inhibitor (PPI) use appears to be associated with risk of colonization with multidrug-resistant Enterobacterales; whether this risk is associated with dose or increased risk of infection is unknown.
Objective: To assess the association between PPI use and the risk of ESBL-E/CPE colonization or infection, and to evaluate a dose-response association.
Methods:
Design: A retrospective nested case-control study of 2,244 adult patients (≥18 years) identified from the microbiology laboratory database in two Dutch tertiary-care hospitals between December 31, 2018 and January 6, 2021. Cases yielding newly-detected ESBL-E/CPE (positive clinical cultures) were matched (1:5) by age and culture date to control patients with cultures negative for ESBL-E/CPE using risk-set sampling.
Exposure: Use of PPIs within 0-30-days and 0-90-days prior to the date of culture; PPI use was extracted from the (hospital) electronic data repository.
Main Outcome Measure: Incidence rate ratios (IRRs) and 95% confidence intervals (CIs) of ESBL-E/CPE acquisition by PPI dose and time-risk windows using conditional logistic regression, adjusting for sex, body mass index, coexisting diseases, and total intensive care duration. Newly-detected ESBL-E/CPE were ascertained by routine laboratory confirmation in clinical specimens (e.g. feces, urine, blood, ascites, or sputum)
Results: Overall, 374 cases and 1,865 matched controls were included (mean age [SD] 60.9 [17] years; 48.9% female; 26.1% PPI use). After adjustment for important confounders, the IRR for PPI use in this patient population was 1.48 (95% CI, 1.15-1.91). A dose-response effect was observed: once-daily PPI use: IRR, 1.44 [95% CI, 1.10-1.89] versus twice-daily PPI use: IRR, 1.75 [95% CI, 1.03-2.98].
Conclusion: PPI use was associated with an increased risk of ESBL-E/CPE acquisition in hospitalized patients; this risk is lower than shown previously. Severity of illness appears as the major confounder in the association between PPI use and risk of colonization with ESBL-E/CPE.

P046 The additive value of BACTEC Mycosis IC/F blood culture bottles in a tertiary referral hospital in the Netherlands.

Dr. Rose Willemze[1], H.J. Schutte[1], N.J. Verkaik[1]
[1]*Erasmus MC*

Introduction:
The BD BACTEC™ Mycosis IC/F blood culture bottles (MycF) are designed for selective culture and recovery of fungi and yeasts from blood (mycBSI). However, their additive value has been debated and not all referral hospitals make use of MycF. We evaluated the additive value of MycF in our setting.

Methods:
We retrieved all positive blood cultures that yielded either yeasts or fungi from 2016 to April 2021. We selected cultures where both a MycF and a BD BACTEC™ PLUS - Aerobic/F Mediumc or BD Peds Plus™ M were performed within 24 hours. Then, we analysed whether the microorganism was found in MycF, aerobic/Peds bottle or in both bottles in children aged 0-18 and adults ≥ 18 years.

Results:
In adults, there were 481 bottles positive with a yeast or fungus (97% Candida spp.) (296 aerobic bottles and 185 MycF (of in total 11679 obtained MycF)). This concerned 128 different episodes of mycBSI of 115 patients. 75 episodes were detected in both MycF and an aerobic bottle, 30 episodes only in an aerobic bottle and 23 episodes were only detected in MycF. These patients were haematology patients, had positive SDD cultures for yeasts or had a recent history after complex abdominal surgery including liver transplantation.
In children, no episode of mycBSI was missed by using Peds culture bottles.

Conclusion:
In adults, 23 of 128 (18%) episodes of mycBSI over a period of 5 years would have been missed, without including a MycF. This is a low amount of the total number of MycF. We suggest that a MycF should be considered in patients at high risk for mycBSI only, since the cost of error of missing mycBSI is high.
We found no additive value for the use of MycF in children under the age of 18 years.

P047 Levels of gut bacteria-derived short chain fatty acids in metastatic colorectal cancer patients during chemotherapy with capecitabine

Mrs Janine Ziemons[1,2], Dr. R Aarnoutse[1,2], A. Heuft[3], L. Hillege[1,2], Dr. J. de Vos-Geelen[1,3], Dr. L. Valkenburg-van Iersel[1,3], Dr. G.J.M. Creemers[4], Dr. A. Baars[5], Dr. H.J.H.M.J. Vestjens[6], Dr. J. Penders[7,8,9], Prof. Dr. K. Venema[9,10], Prof. Dr. M.L. Smidt[1,2]

[1]Maastricht University, [2]Maastricht University Medical Centre, [3]Maastricht University Medical Centre, [4]Catharina Hospital, [5]Hospital Gelderse Vallei, [6]VieCuri Medical Centre, [7]Maastricht University, [8]Maastricht University Medical Centre, [9]Euregional Microbiome Center, [10]Maastricht University – Campus Venlo

Introduction

In recent years, it became increasingly evident that the gut microbiota plays a crucial role in the development, manifestation and treatment of different types of cancer. Short chain fatty acids (SCFA) are produced by microbial fermentation of non-digestible carbohydrates and were shown to modulate human metabolism and gut barrier function, as well as to exert potent anti-inflammatory and anti-carcinogenic effects. In addition, in-vitro studies indicated that the administration of chemotherapeutic drugs influenced the growth of SCFA-producing bacteria. Consequently, it was hypothesized that fecal SCFA would decrease during treatment with the chemotherapeutic drug capecitabine and would be associated with chemotherapy-induced toxicity, nutritional status and physical performance.

Methods

Forty-four patients with metastatic colorectal cancer, who were scheduled for treatment with capecitabine (±bevacizumab), were prospectively enrolled in a multicentre cohort study in the Netherlands. Patients collected a fecal sample and completed a questionnaire before, during and after three cycles capecitabine. Several clinical characteristics, including chemotherapy-induced toxicity, tumour response, physical performance (Karnofsky Performance Score), nutritional status (MUST score) and systemic inflammation were recorded. Fecal SCFA concentrations were determined by means of gas chromatography-mass spectrometry (GC-MS) and corrected for dry weight.

Results

During the course of three cycles of capecitabine, fecal levels of valerate and caproate decreased significantly, while the concentrations of acetate, propionate and butyrate remained unchanged. Nutritional status and physical performance did not change significantly during capecitabine treatment. At the three timepoints under investigation, different associations between SCFA, chemotherapy-induced toxicity, clinical parameters as well as blood inflammatory markers were detected.

Conclusions

(1) The present research indicated that some SCFA might play a role during treatment with capecitabine in patients with metastatic colorectal cancer (2) However, in order to gain insight into cause-effect relationships, a more detailed investigation of molecular interactions and underlying mechanisms is required.

P051 A novel Streptomyces strain isolated from a maple leaf encodes an alcohol dehydrogenase with a putative lanthanide-binding site

Dr. Tom Berben[1], A Grozza[1], J Wesseling[1], S Usón Salinas[1], TA van Alen[1], G Cremers[1], Prof. Dr. HJM op den Camp[1]
[1]*Radboud University*

Streptomyces are a genus of Gram-positive filamentous bacteria within the phylum Actinobacteria, that mainly live as saprophytes. They have been primarily studied as (rhizo)soil organisms, but in recent years it has been shown that they can effectively colonize plant tissue as well. During a third-year practical course centred on the cultivation and sequencing of plant leaf-associated methylotrophs, we noticed a filamentous bacterium growing on agar plates containing a mineral salt medium supplemented with methanol and trace minerals including lanthanide salts. Lanthanides are a group of transition metals that are of great importance to e.g. modern technology and diagnostic medicine. They were long thought to have little biological relevance, until the discovery of a lanthanide-dependent methanol dehydrogenase (XoxF, a PQQ-dependent beta propeller enzyme). Since then, XoxF-type dehydrogenases have been shown to be ubiquitous among the known methylotrophs within the proteobacteria and verrucomicrobia. We isolated the filamentous Streptomyces strain and used a combination of Nanopore and Illumina Miseq sequencing to fully resolve its genome. Annotation revealed five alcohol dehydrogenases, including a polyvinyl alcohol dehydrogenase (PVAdh). This PVAdh contains a PQQ-binding motif as well as a metal-binding site that contains the aspartate residue that in XoxF is essential for binding a lanthanide ion. A BlastP search against the non-redundant protein database revealed close to 100 highly similar sequences with these features within the Actinobacteria, Acidobacteria, Deltaproteobacteria and Cyanobacteria. This hints at a much richer diversity of lanthanide biochemistry than thus far considered.
Current research focuses on the expression and purification of the Streptomyces PVAdh, to study its biochemical properties, including the ability to bind lanthanides.

P052 Culturing novel nitrifiers through targeted cell sorting

Pieter Blom[1], prof. dr. ir. Mike Jetten[1], dr. Maartje van Kessel[1], dr. Sebastian Lücker[1]
[1]*Radboud University Nijmegen*

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 shift of this paradigm. The, to date, only available comammox Nitrospira pure culture yielded interesting physiological insights, but more isolates are needed to test whether the observed traits are common to all comammox bacteria and to understand their contribution to nitrogen cycling in natural and engineered ecosystems. 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 (complete) ammonia oxidizing microorganisms 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 ammonia. All wells were regularly screened for the production of nitrite and nitrate in order to detect activity of canonical ammonia oxidizers and complete ammonia oxidizers, respectively. Wells containing active nitrifiers were selected for further cultivation and physiological characterization. Applying this workflow to complex enrichment cultures derived from activated sludge and recirculating aquaculture system biofilters, we managed to obtain four highly enriched cultures containing comammox Nitrospira and three apparently pure Nitrosomonas-related ammonia oxidizing bacteria. In conclusion, we demonstrate that our approach is well-suited to isolate novel ammonia oxidizers from complex environmental samples, circumvents challenges involved in classical cultivation techniques that hindered the isolation of many relevant nitrifying microorganisms, and thus will greatly advance our understanding of the environmental role and biotechnological potential of these intriguing microorganisms.

P053 The influence of backwashing and filter age on the performance and microbial community of rapid sand filters used in the production of drinking water.

Alje Boersma[1], Signe Haukelidsaeter[2], Dr. Maartje A.H.J. van Kessel[1], Dr. Paul W.J.J. van der Wielen[3], Prof. dr. ir. Mike S.M. Jetten[1], Prof. dr. Caroline P. Slomp[2], Dr. Sebastian Lücker[1]
[1]Radboud University, [2]Utrecht University, [3]KWR Water Research Institute

The production of safe drinking water from groundwater can be achieved through biofiltration using rapid sand filters (RSFs). RSFs contain complex microbial communities shaped by the chemical parameters of the inlet water. In conjunction with a variety of geochemical processes, these microbial communities are responsible for the removal of contaminants. In most RSFs, methane is removed by a preceding aeration step, with subsequent removal of iron, ammonium and manganese in the filter bed. Backwashing and the periodical replacement of filter medium are ubiquitously used to maintain filter performance. However, the effect of backwashing and filter age on RSFs is poorly understood.

We use a complementary set of geochemical and molecular methods to study the effect of backwashing and filter age on filter performance. Both water and filter medium samples were taken along the total height of the filter and used to follow to removal of iron, ammonium and manganese. Additionally, changes in microbial community structure were studied using 16S rRNA amplicon sequencing, qPCR and metagenomics.

Chemical analyses of water samples show that the removal of iron, ammonium and manganese is negatively influenced by flocculent iron accumulation. Backwashing is successful in restoring filter performance but does not influence the microbial community composition in the filter bed. Our data also indicate that filter age has a large effect on performance and microbial community. Young filters show incomplete removal of manganese and ammonium, and contain distinct microbial communities compared to older filters, with especially much lower abundances of known nitrifiers.

Backwashing successfully restores filter performance by removing flocculent iron but does not influence microbial community structure. Additionally, we show how filter age influences contaminant removal efficiency as well as microbial community composition, indicating that periodical complete filter medium exchange is not a good strategy to for RSF operation.

P054 Gut microbiota perturbations precede the onset of Post-Infectious IBS in intercontinental travelers

Mr. Jiyang Chan[1], PhD Gianluca Galazzo[1], MSc Markia Ward[1], MD and PhD Maris Arcilla[2], MD and PhD Perry van Genderen[3], PhD John Penders[1]
[1]Maastricht University, [2]Erasmus University Medical Centre, [3]Erasmus University Medical Centre

Introduction
A proportion of Irritable Bowel Syndrome (IBS) patients report the onset of symptoms after infectious gastroenteritis (IG), 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, community structure and dynamics in Dutch intercontinental travellers with and without PI-IBS development after travellers' diarrhoea.

Methods
We conducted a nested case-control study within a 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, immediately post-travel and 1-month post-travel were profiled by 16S rRNA gene amplicon sequencing to examine the microbial diversity, composition and community structure.

Results
52 subjects met our case definition for PI-IBS and were matched to 52 control subjects. Microbial richness prior to the onset of PI-IBS was significantly lower in future cases compared to controls ($p=0.0028$). The microbial diversity (Shannon-index) was also lower in PI-IBS cases before and immediately after travel ($p=0.0338$ and $p=0.0105$ respectively). In addition, the microbial community structure of PI-IBS cases was significantly different as compared to healthy controls pre-travel, immediately after travel and one-month post-travel (PERMANOVA $p< 0.05$ for all time-points). Longitudinal differential abundance testing indicated increased levels of Bacteroides and Streptococcus in PI-IBS cases compared to a higher abundance of Ruminococcus and Butyricoccus among healthy controls.

Conclusions
In a longitudinal study of faecal microbiota of intercontinental travellers from pre-travel through post-travel, 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.

P056 Ammonium removal in oxygen-limited coastal ecosystems

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

The microbial nitrogen (N) cycle is one of the major biogeochemical cycles on earth. Due to anthropogenic activities, the N cycle in many marine coastal ecosystems is out of balance, leading to severe environmental problems. Especially nitrate, but also to some extent nitrite, is an important electron acceptor for microorganisms in oxygen-limited environments, and their conversion to N_2O and N_2 leads to loss of bioavailable nitrogen. However, the processes controlling N loss from oxygen-limited coastal ecosystems are poorly understood.

Since several decades, marine Lake Grevelingen in the Netherlands increasingly suffers from oxygen depletion in the lower part of the water column. Over the summer months, the oxycline (the transition zone from oxic to anoxic water), shifts upwards in the water column. This transition to increased anoxia causes a shift in metabolically active microorganisms, due to the different electron acceptor availabilities and their respective concentrations. However, it is not well understood how this influences N cycling in the lake and especially how the microorganisms adapt to these changing redox conditions. Besides proliferation of denitrification rates, it is hypothesized that the activity of anaerobic ammonium oxidizing bacteria will increase during anoxic periods. To investigate the nitrogen cycling in Lake Grevelingen, we determined aerobic and anaerobic ammonium- and nitrite-oxidation rates at several depths at the Scharendijke basin. Furthermore, high resolution biogeochemical data of the water column showed that ammonium was released from the sediment in late summer, and that a secondary nitrite peak occurred at the oxycline. The analysis of 16S rRNA gene-based microbial community surprisingly revealed that the diversity and abundance of known N cycle microorganisms was relatively low. This study will yield valuable insight into the microbial N cycle network and its spatiotemporal dynamics and thereby contribute to a better understanding of the impact of human activity on oxygen-limited coastal ecosystems.

P057 Substrate loading affects the community composition of a complex anaerobic chemolithotrophic microbial community

Ms Maider Junkal Echeveste Medrano[1], Dr Cornelia Welte[1], Prof Dr Mike Jetten[1]
[1]*Radboud University*

Microbial communities are key drivers of carbon, sulfur and nitrogen cycling in coastal ecosystems, where they experience dynamic shifts in substrate availability and exposure to toxic compounds. However, how these shifts affect microbial interactions and function is poorly understood. Our research aims to better comprehend how such microbial community changes occur in a controlled laboratory reactor mimicking coastal sediment conditions. This will allow to shed light on the environmental distribution and resilience of microbial communities under current and future disturbances.

Two methane saturated anoxic bioreactors were inoculated with Stockholm Archipelago's coastal anoxic sediments. These two systems were subjected to varying N (ammonium and nitrate) and S (sulfide) concentrations, high vs low. We used physicochemical measurements, 16S rRNA gene sequencing and metagenomics to investigate: (1) distinct nutrient concentration effect on microbial community structure and interactions; (2) compare microbial communities enriched to those observed in the original coastal anoxic sediment inoculum and, (3) aim for novel microbial metabolic reactions associated to the anaerobic oxidation of methane.

Our results indicated that distinct microbial communities were enriched compared to the initial inoculum as well as in between conditions. From inoculum to both enrichments, microbial community richness and diversity decreased, while microbial community structure and growth-type varied between the two conditions. Microbial functional potential for both systems remained comparable, with sulfide oxidation as the dominant metabolism. Bacterial taxonomical analysis showed enrichment of well-characterized microorganisms. On the contrary, we enriched for novel and uncharacterized archaea, distinct to better-studied anaerobic methanotrophs.

To the best of our knowledge, our study is the first one that studies complex anaerobic chemolithotrophic microbial communities that both: mimic low environmental substrate conditions and compare high/low substrate loading effects. These insights will help to understand and predict coastal ecosystem responses to anthropogenically enhanced naturally occurring metabolites such as sulfide and nitrate.

P058 Dissimilatory nitrate reduction to ammonium by Acididesulfobacillus acetoxydans

Msc Reinier Egas[1], Dr. JM Kurth[2], Dr. CU Welte[2], Dr. I Sánchez-Andrea[1], Prof. DZ Sousa[1]
[1]*Wageningen University & Research,* [2]*Radboud University*

Acididesulfobacillus acetoxydans is a recently characterized acidophilic sulfate reducing bacterium (aSRB), capable of dissimilatory nitrate reduction to ammonium. While the nitrate reductase is of the cytoplasmic oriented Nar-type, A. acetoxydans does not encode a known nitrite reductase.
This study aims to identify and describe the enzymes and reactions responsible for nitrite reduction in A. acetoxydans. Knowledge gained would increase understanding of the nitrogen cycle and the ecophysiology of aSRB. This could potentially identify a missing link between the nitrogen and sulfur cycle in acid rock and acid mine drainage environments.
To identify nitrite reductase candidates, comparative transcriptomics and proteomics was performed in quadruplicate on A. acetoxydans using either sulfate (20 mM) or nitrate (20 mM) as terminal electron acceptor with glycerol (5 mM) as electron donor and carbon source. To test the utilization of potential intermediates, resting cell assays were performed in triplicate with A. acetoxydans using either nitrate, nitrite or hydroxylamine as sole electron acceptor at different concentrations (1, 0.5 and 0.25 mM) with glycerol (2 mM) as electron donor.
The comparison of nitrate to sulfate reducing conditions showed the same three candidates highly upregulated (>16-fold) in the transcriptome and more abundant (>29-fold) in the proteome. Two candidates (DEACI_4025-4027 and DEACI_1836) were annotated as the anaerobic sulfite reductase AsrABC and an NAD(P)H dependent oxidoreductase, respectively. The third (DEACI_0275) was annotated as the hydroxylamine reductase Hcp. In resting cell assays no extracellular hydroxylamine was observed (detection limit of 5 µM) and all acceptors could be reduced to ammonium. This study showed: (1) A. acetoxydans can reduce nitrate, nitrite and hydroxylamine to ammonium. (2) Hydroxylamine in A. acetoxydans is a potential intermediate in nitrite reduction.

P059 The promiscuous alcohol dehydrogenases of Desulfofundulus kuznetsovii strains 17T and TPOSR

Lukas Friedeheim[1], prof. dr. DZ Sousa[1], dr. I Sánchez-Andrea[1], prof. dr. ir. AJM Stams[1,2]
[1]*Wageningen University & Research,* [2]*University of Minho*

Methanol acts as energy and carbon source for anaerobic microorganisms. Methanogens and acetogens convert methanol using a pathway involving a vitamin B12-dependent methyltransferase (MT). The MT-system was thought to be prevalent in anoxic environments for methanol conversion, whereas in oxic environments methanol is oxidized by alcohol dehydrogenases (ADH). The simultaneous occurrence of both pathways was recently described in the sulfate reducer Desulfofundulus kuznetsovii strain 17T.

D. kuznetsovii strain TPOSR was isolated at our laboratory. In contrast to D. kuznetsovii strain 17T, strain TPOSR's genome lacks essential genes of the MT pathway and therefore relies on a vitamin B12-independent ADH for methanol metabolism. The characterization of the ADHs of both strains helps to understand the advantage of either pathway, for example in competition with other microbial groups.

Cells were grown on selected alcohols, in presence and absence of vitamin B12 and cobalt. Total protein was extracted, and protein composition was analyzed using protein aggregation capture. ADH genes associated with growth on methanol were cloned into plasmids and transformed to E. coli BL21. Cellular protein was extracted and heterologous ADH was purified using HIS-tag purification. Activity of purified ADHs in several substrates was assessed colorimetrically based on NAD+ reduction rate.

Strain TPOSR utilizes a wide range of alcohols. Proteomics analysis revealed highly upregulated expression of an ADH with high similarity to the ADH expressed in strain 17T. The same ADH is expressed during growth on other alcohols despite presence of several other ADHs in the genomes. Heterologous expressed ADH genes from strain TPOSR showed activity with various C1 to C6 alcohols.

In summary, we were able to identify single ADH genes with potential activity with a broad range of alcohols. Using heterologous expression, we were able to purify these and confirm their activity with a wide range of alcohols.

P060 Metal(loid) dependent anaerobic methane oxidation

Dr. Martyna Glodowska[1], Professor Mike Jetten[1], Associate Professor Cornelia Welte[1]
[1]*Radboud University*

Methane (CH_4) is a potent greenhouse gas significantly contributing to the climate warming we are currently facing. Research in recent decades advanced our understanding of CH_4 oxidation, which until 1976 was believed to be a strictly aerobic process. Anaerobic oxidation of methane (AOM) coupled to sulphate and nitrate reduction are now known to occur in many marine and freshwater ecosystems. Furthermore, recent studies suggest that AOM is possibly linked to other electron acceptors including some metal(loid)s such as Fe(III), Mn(IV), As(V), Cr(VI), Se(V), Sb(V), V(V), and Br(V). Several of these proposed electron acceptors are highly toxic water contaminants. If indeed efficient metal(loid) dependent AOM can be achieved, it could provide an attractive and cheap bioremediation strategy. Anaerobic methanotrophic archaea (ANME), are key players in the AOM process, yet we are still lacking a deeper understanding of their metabolism, electron acceptor preferences and their interaction with other microbial community members.

In this study, we explored the metabolic potential of two ANME-2 enrichment cultures to use various metals and metalloids as electron acceptors and CH_4 as electron donor and sole C source. Our preliminary results based on batch incubations suggest that indeed most of the proposed electron acceptors can be reduced by both ANME-2 cultures. Higher valence toxic metal(loid)s are transformed into insoluble nanoparticles and efficiently removed from the water. The role of CH_4 in this process is however elusive as no clear pattern of CH_4 oxidation and CO_2 formation was observed. With a dedicated bioreactor approach, we intend to investigate whether intermediate C compounds are being produced that potentially fuel metal(loid)s reduction. Even though we still need to unravel the exact mechanisms of metal(loid) reduction, both ANME-2 enrichment cultures show high potential for the bioremediation of contaminated wastewater.

P061 Haloalkaline biodesulfurization reactors share a core microbiome

Suyash Gupta[1,4], Dr. CM Plugge[1,2], Dr. ir. JBM Klok[1,3], Prof.dr G Muyzer[4]
[1]Wetsus, [2]Wageningen University and Research, [3]Paqell B.V., [4]University of Amsterdam

Biodesulfurization (BD) is a biological process that can efficiently remove malodorous and toxic sulfide (H_2S) from gaseous streams. The process uses haloalkaliphilic sulfur-oxidizing bacteria (SOB) that oxidize sulfide to elemental sulfur. Even though BD technology is employed in over 350 plants, information about the microbial populations in the full-scale reactor is still limited. This study fills this knowledge gap by characterizing the microbial communities of eight full-scale and two pilot-scale BD reactors. In this work, we studied the effect of physicochemical parameters on the microbial community composition, such as alkalinity, pH, Salinity and gas stream source. For this purpose, 16S rRNA gene-based amplicon sequencing using the Illumina MiSeq platform and QIIME2 analysis was performed. The analyses of sequencing data revealed a dominance of SOBs such as Thioalkalivibrio sulfidiphilus, Guyparkeria, Thiomicrospira, Alkalilimnicola, and Halomonas. The Bray Curtis distance-based beta diversity measurements showed that the microbial population differs by microbial community composition and the contaminants present in the feed gas. Although populations differed vastly amongst the different BD bioreactors, we found that all these reactors shared a core community of 30 bacteria comprising ~70 – 90% of the total population. The phylogeny based analyses illustrated that most of the core community members are SOB. Furthermore, functional-based analysis confirmed it by depicting potential high expression of sulfide oxidizing genes. The redundancy analyses showed that Guyparkeria and Halomonas are potential indicators of sulfate and thiosulfate in the system.

From the above results, it can be concluded that irrespective of variations in operational parameters or source of sour gas, all BD reactors have common SOB. The operational conditions play important role in change of microbial composition in SOB. On the other hand SOB can influence the ultimate percentage sulfur conversions. The results from this study forms basis for optimizing the performance of full-scale reactors.

P062 The Indigestibles: resource sharing within the infant gut microbiota.

Ms. Athanasia Ioannou[1], Jan Knol[1,2], Clara Belzer[1]
[1]*Wageningen University & Research,* [2]*Danone Nutricia Research*

The infant gut microbiota starts developing from birth, and breastfeeding is a crucial constituent. The indigestible fraction of human milk, the human milk oligosaccharides (HMOs), creates carbon source pressure which is an advantage for bacteria capable of degrading HMOs. Research so far suggests that HMO-degraders share by-products that sustain the bacteria surrounding them. However, it is not fully clear how resource sharing happens at a community level and how it is affected by varying compositions of HMOs. To elucidate this, we created a community model with species commonly found in the gut of pre-weaning infants. To test our model, we grew the strains in minimal medium with different HMO mixes as sole carbon sources and without an added carbon source. The gut microbes selected for our model have different abilities to grow on HMOs. Our data suggest niche segregation of microbes able to use a broad range or a selected range of HMOs as a carbon source. The complete model will combine a selection of species, and it will show how HMOs are collaboratively degraded by the gut microbiota, as well as which metabolites are exchanged, in the cross-feeding between bacteria that can stimulate infant health.

P063 MetaMobilePicker: a novel tool for the computational identification of mobile genetic elements in metagenomes

Mr. Jesse Kerkvliet[1], Prof. Dr. R. Willems[1], Dr. A. Schürch[1]
[1]*UMC Utrecht*

Introduction
Mobile genetic elements (MGEs) play a key role in the dissemination of genetic traits between different hosts and environments. Comparing the presence of MGEs between different environments can give insights on the flow of genes like antimicrobial resistance genes (ARGs) in and between bacterial communities. We selected tools suitable for identification of MGEs from metagenomes with a focus on MGEs contributing most to the spread of ARGs and developed an open-source, modular computational pipeline called MetaMobilePicker.
Methods
MetaMobilePicker combines tools in a snakemake framework to identify plasmids, insertion sequences (IS) and bacteriophages in metagenomes. For these, PlasClass, ISEScan and DeepVirFinder are used, respectively. Additionally, ATLAS is used for read quality control, MetaSPAdes for metagenomics assembly and the ResFinder database to annotate ARGs. MetaMobilePicker outputs annotated contigs containing one or more MGEs.
To validate the pipeline's performance, we identified MGEs and ARGs in a simulated community based on representative genomes of seven clinically relevant bacterial species and five species-associated bacteriophages. Using InSilicoSeq, a metagenomic dataset of 40 million reads was simulated. Performance of MetaMobilePicker was assessed by calculating classification metrics (recall, precision and F1) for plasmid and virus classification, and percentage retrieved hits for annotation of IS and ARGs.
Results
In the simulated dataset, plasmids were identified with an F-score of 0.66. The performance suffered from a large number of false positives due to a potential algorithmic bias on contigs not specific to plasmids or chromosomes, like transposons. Per species, 86.5% of IS families were recovered on average. A per species average of 67% of ARGs was retrieved by the pipeline, suffering from assembly collapse of similar genes, resulting in underestimation of the true copy-number of ARGs.
Conclusions
MetaMobilePicker combines multiple MGE prediction tools into one convenient pipeline and is available at https://metamobilepicker.readthedocs.io/en/latest/

P064 Revisiting the metabolic and phylogenetic diversity of the genus Nitrospira

Hanna Koch[1], L. F. M. Kop[1], S. C. M. Haaijer[1], M. S. M. Jetten[1], E. Spieck[2], S. Lücker[1]
[1]Radboud University, [2]University of Hamburg

Autotrophic nitrification, the stepwise oxidation of ammonia to nitrate via nitrite, is performed either in a mutualistic interaction of ammonia oxidizers and nitrite-oxidizing bacteria (NOB), or by complete nitrifiers that catalyze both nitrification steps on their own. The genus Nitrospira represents one of the most physiologically diverse nitrifying clades and comprises complete nitrifiers and canonical NOB. This globally distributed genus can be phylogenetically divided into at least six lineages and its members drive ammonia/nitrite oxidation in various engineered and natural ecosystems.
Although distribution studies identified Nitrospira-like bacteria in a wide range of natural ecosystems, the majority of genomes analyzed to date was obtained from isolates or metagenomes derived from engineered systems. Moreover, until now genome-sequenced Nitrospira belong just to three of the six known phylogenetic lineages.

In this study, we sequenced the genomes of several Nitrospira species obtained mainly from different natural habitats to analyze the genomic potential of yet understudied phylogenetic lineages.

While the core genome of Nitrospira includes key pathways like the reductive tricarboxylic acid cycle for CO2 fixation, all five respiratory chain complexes and the nitrite oxidoreductase for nitrite oxidation, the accessory genome enables the use of alternative substrates such as hydrogen and formate as well as alternative nitrogen sources for assimilation, like nitrite, cyanate and urea. The genomic repertoire enabling uptake and degradation of these alternative nitrogen source differs remarkably in Nitrospira, especially in representatives of the marine Nitrospira. In addition, comparative analysis of marine host-associated vs. free living Nitrospira revealed a clear trend toward genome reduction in host-associated species.

Taken together, this metabolic versatility opens Nitrospira-like bacteria the possibility to occupy different niches and to respond to fluctuating nutrient availability, which might be one reason for their global distribution and the large diversity.

P066 Genomic insights into the biology of the planctomycetal Phycisphaerae

Wouter Lenferink[1], Dr. G.J. Smith[1], Prof. Dr. Ir. M.S.M. Jetten[1], Dr. M.A.H.J. Van Kessel[1], Dr. S. Lücker[1]
[1]*Radboud University*

Members of the class Phycisphaerae within the Planctomycetes phylum are ubiquitously found in diverse environments. Cultured representatives of this class are affiliated with the orders Phycisphaerales, Sedimentisphaerales, and Tepidisphaerales. However, the vast majority of Phycisphaerae are uncultured and known only from their putative genome sequence. The Planctomycetes gained special interest over the past decades with its members showing traits such as anaerobic ammonium oxidation, budding cell division, and secondary metabolite production. Nonetheless, the Phycisphaerae remain largely unstudied despite their presence in copious metagenomic datasets.
To resolve the biology of Phycisphaerae, we analysed a dereplicated set of metagenome-assembled genomes including all Phycisphaerae in the Genome Taxonomy Database and in-house sequenced genomes with >70% completeness and <10% contamination, and their phylogeny was resolved using the up-to-date bacterial core gene pipeline. The genomes were annotated using Anvi'o and dbCAN2 and MEROPS for carbohydrate-active enzymes (CAZymes) and peptidases, respectively.
Together, 187 genomes were recovered from orders Phycisphaerales, Sedimentisphaerales, and Tepidisphaerales and the unclassified FEN-1346, JAAYCJ01, SM23-33, and UBA1845 groups. All genomes suggest an obligatory heterotrophic lifestyle. The majority belonged to the Phycisphaerales and originated from oxic marine, fresh-, or wastewater environments. An aerobic lifestyle was supported by the apparent presence of a respiratory chain harbouring the alternative complex III instead of the bc1 complex. Other groups showed obligately anaerobic or facultatively anaerobic lifestyles. Genomes of the Tepidisphaerales, Sedimentisphaerales, and the family Phycisphaeracaea were enriched in CAZyme gene clusters. The Sedimentisphaerales, JAAYCJ01, and SM23-33 furthermore lacked most respiratory and TCA cycle genes, suggesting an obligately fermentative lifestyle.
In conclusion, the Phycisphaerae comprise a diverse group of heterotrophic bacteria from both oxic and anoxic environments. The elevated presence of CAZYme gene clusters in the Phycisphaerae suggests that these bacteria are degraders of complex carbon products. This could potentially explain their frequent detection in autotrophic enrichment cultures.

P067 In vitro screening of butyrate boosting dietary fibres to stimulate bacterial butyrate production in the intestine of ulcerative colitis patients

Z Liu[1], E Tsompanidou[2], D Kusuma[2], J Gerritsen[2], B Witteman[3,4], H Smidt[1], EG Zoetendal[1]
[1]Wageningen University & Research, [2]Winclove Probiotics B.V., [3]Wageningen University & Research, [4]Hospital Gelderse Vallei

Introduction: Short chain fatty acids (SCFA) such as butyrate are considered beneficial for human health and are produced by intestinal bacteria during the fermentation of dietary fibres (DF). Intestinal butyrate production capacity is determined by microbiota composition and DF intake and is found to be compromised in active ulcerative colitis (UC) patients. Recently, sustained remission induced by faecal microbiota transplantation was associated with increased butyrate production capacity in UC patients with relatively low abundance of Bacteroidetes during active disease. We hypothesized that in UC patients butyrate production could be boosted by specific DF intake, and that intervention efficacy would be dependent on faecal Bacteroidetes levels.
Methods: In vitro incubations were done in batch cultures with microbiota from UC patients as inoculum. UC microbiota were classified as either low Bacteroidetes (LB, n=6) or high Bacteroidetes (HB, n=5). Cultures were supplemented with four butyrate boosting DF prototypes (A: kiwi powder + resistant starch, B: kiwi power + resistant starch (different proportion with A), C: acacia gum + guar gum + resistant starch, D: acacia gum + kiwi powder + guar gum). Gas production, SCFA concentrations, and microbial composition were determined at 0, 24, and 48 h.
Results: Prototype C induced the highest stimulation of SCFA production, including butyrate, with the lowest gas production in the LB group, whereas no differences among the four prototypes were observed in the HB group, supporting a Bacteroidetes level-dependent butyrate production boosting capacity in UC patients. Although all four prototypes induced different microbiota composition dynamics, the increase in relative abundance of potential butyrate producers such as Eubacterium, Blautia, and Coprococcus_3 was similar.
Conclusion: 1) Butyrate production can be stimulated by butyrate boosting DF in UC patients. 2) Successful increase in butyrate production due to DF is dependent on the level of Bacteroidetes at start of intervention.

P068 A longitudinal study of the gut microbiota during the first three years of life: links with problem behavior at age three

Mrs. Yangwenshan Ou[1], Phd candidate Y. Willemsen[2], Dr. Associate Professor C. Belzer[1], Dr. Professor H. Smidt[1], Dr. Associate Professor R. Beijers[2], Dr. Professor C. de Weerth[2]
[1]Laboratory of Microbiology, Wageningen University & Research, [2]Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center

Background: Research into the associations of the gut microbiota with psychopathological disorders has rapidly increased over the past decade. However, evidence from longitudinal studies in low-risk populations is lacking. Therefore, we investigated the potential associations of the developing gut microbiota in the first three years of life with problem behavior in low-risk children at age three.

Methods: Fecal samples were collected in the first months of life, and at one and three years of age in 79 participants of the BINGO cohort. Microbial composition was analyzed by 16S ribosomal RNA gene sequencing. Mothers reported problem behavior (i.e., internalizing and externalizing behavior) at child age three by means of the Child Behavior Checklist (Achenbach & Ruffle, 2000) and the Strengths and Difficulties questionnaire (Goodman, 1997). Random forest models were first used to select microbial taxa contributing most strongly to predicting behavioral problems. Bayesian linear regression models were subsequently used to validate the association of these taxa with behavioral problems.

Results: Among the selected taxa, Bayesian models showed that increased internalizing behavior at age three was significantly related to lower levels of Parabacteroides (a GABA producer), Ruminococcus 2 and Butyricicoccus (a butyrate producer) at two weeks, one and three years of age, respectively. Significant positive associations were observed between Barnesiella at age three and both internalizing and externalizing behavior.

Conclusions: (1) Our findings suggest potential associations between gut microbiota composition and problem behavior in the first three years of life in a low-risk sample of children. (2) Interestingly, some of the bacterial groups associated with problem behavior have the potential to produce the neurostimulatory GABA and the short-chain fatty acid butyrate. Further validations in other cohorts are required to confirm our findings.

P069 Constraint based modelling and thermodynamic analysis of the metabolism of Ca. Accumulibacter explains the need for glycogen under dynamic conditions.

Ir Timmy Paez[1], Beatriz Diniz[1], Mark. C. M. van Loosdrecht[1], S. Aljoscha Wahl[2]
[1]TU Delft, [2]Friedrich-Alexander University Erlangen-Nürnberg

Introduction

In wastewater treatment plants, Ca. Accumulibacter thrives when oxygen is periodically unavailable by relying on the use of polymers. Anaerobically, these organisms rapidly uptake organic carbon (mainly acetate) and store it as poly-hydroxyalkanoates (PHAs). PHAs are more reduced per carbon than acetate, thus this anaerobic conversion requires the input of electrons. The source of reducing power has been hypothesised over the years, amongst which feature the conversion of internal storage of glycogen into PHA and/or the anaerobic operation of the tricarboxylic acid (TCA) cycle. In this study, we modelled the metabolism of Ca. Accumulibacter in an anaerobic/aerobic cycle with the combination of constraint based models and thermodynamics to further elucidate the source of electrons for PHA storage.

Methods

The metabolism of Ca. Accumulibacter in cyclic conditions was modelled using conditional Flux Balance Analysis (cFBA). The feasibility of different solutions from the model (i.e. at different initial glycogen levels) was tested by applying a thermodynamic analysis assuming NADH / NAD+ ratios typically described for bacteria under anaerobic and aerobic conditions.

Results

cFBA results indicated that the anaerobic source of NADH for PHA synthesis differs depending on the amount of glycogen degraded. Without degradation of glycogen, the imported acetate could be funnelled into the TCA cycle to generate the required NADH. Closer analysis of the resulting fluxes from this solution indicated that the reaction malate dehydrogenase was not feasible under anaerobic conditions ($\Delta G_r > 0$ kJ/mol). Implementation of thermodynamic limits on reactions' feasibility led to a reduced range of solutions in which glycogen was always required for NADH generation.

Conclusion

Glycolysis is required for the generation of NADH to enable the uptake of acetate and subsequent incorporation into PHAs in Ca. Accumulibacter. The operation of the TCA cycle alone cannot generate the required NADH for this process due to thermodynamic bottlenecks.

P070 Hydrogen oxidation by the methanotroph Methylocystis bryophila

Ida Peterse[1,2], Dr. A. Pol[1], Dr. A.J. Veraart[2], Dr. S. Lücker[1], Prof. dr. H.J.M. Op den Camp[1]
[1]Radboud University, [2]Radboud University

Hydrogen gas (H_2) is a powerful electron donor used by many microorganisms in a wide array of environments. Recently it was demonstrated that the verrucomicrobial methanotroph Methylacidiphilum fumariolicum SolV could grow as a "Knallgas" bacterium. Additionally, this strain could oxidize H_2 at subatmospheric concentrations employing a high-affinity, membrane-associated Group 1h [NiFe] hydrogenase. H_2 oxidation can serve as an additional energy source particularly for methanotrophs growing with atmospheric concentrations of CH_4 (1.86 ppmv), as such low CH_4 concentrations alone cannot provide enough energy for cell maintenance. The ability to conserve energy from H_2 might increase fitness and enhance growth of methanotrophs in many different ecosystems especially when CH_4 concentrations are low. As a consequence, methanotrophs possessing such high-affinity [NiFe] hydrogenases could be important contributors to the mitigation of the potent greenhouse gas CH_4. Several methanotrophs from the class Alphaproteobacteria including Methylocystis bryophila contain various hydrogenases genes including the high-affinity Group 1h [NiFe] hydrogenases in their genomes. Here we show that M. bryophila pregrown on limiting CH_4 concentrations immediately oxidized H_2, suggesting a constitutive expression of hydrogenase genes during growth under CH_4-limited conditions. With the use of membrane inlet mass spectrometry (MIMS) a high apparent affinity for H_2 was measured (Km(app) = 59 nM). O_2 concentrations above 2% (v/v) showed an inhibitory effect on H_2 consumption, indicating O_2 sensitivity. Moreover, preliminary results indicated that additions of H_2 stimulated CH_4 oxidation below the atmospheric CH_4 level of 1.86 ppmv. These findings are a first step towards a revision of the role of mesophilic methanotrophs and the importance of H_2 in ecosystems where CH_4 concentrations are naturally low.

P071 Metagenomic and transcriptomic analysis of paracetamol biodegradation in a microbial community of a hospital wastewater treatment plant

MSc Ana B Rios-miguel[1], GJ Smith[1], G Cremers[1], T van Alen[1], P Blom[1], S Lücker[1], MSM Jetten[1], HJM Op den Camp[1], CU Welte[1]
[1]*Radboud University*

Pharmaceuticals are relatively new to nature and often not completely degraded in wastewater treatment plants (WWTPs). Consequently, they end up in water bodies all around the world posing a great environmental risk. One exception is paracetamol, whose full degradation has been linked to numerous microorganisms. However, the genes involved in its biodegradation are still unknown. Therefore, we aimed at sequencing the genome and transcriptome of a paracetamol-degrading microbial community in order to identify these genes. A bioreactor was inoculated with the sludge of a hospital WWTP in Delft (Pharmafilter) and fed with paracetamol as the sole carbon source. Paracetamol was fully degraded without any lag phase throughout the operation period. The enriched microbial community in the bioreactor was very diverse. In addition, plating the biomass on agar medium and using untargeted cell sorting yielded eight different isolates growing on paracetamol. In a fast-growing Pseudomonas isolate, an operon containing an amide transporter and an amidase were upregulated. However, this amidase was not detected in the bioreactor metagenome suggesting that other as-yet uncharacterized amidases may be responsible for the first biodegradation step at low concentrations of paracetamol. Furthermore, cross-feeding might be occurring to efficiently degrade paracetamol and its intermediates. These results suggest that rapid evolution towards paracetamol biodegradation might be the result of mutations in different amidases leading to a broad substrate spectrum.

P072 Life on a protein diet in wastewater treatment plants: Metabolism of Ca. Accumulibacter utilizing aspartic acid as sole carbon source

Dr. Samarpita Roy[1], Mr. T. P. Watson[1], Asst. Prof. M. Pabst[1], Prof. M. C. M. van Loosdrecht[1]
[1]*TU Delft*

Introduction
Microorganisms inhabiting natural environments (e.g., estuarine or coastal sediments) and wastewater treatment plants (WWTPs) adapt to fluctuations in nutrient and dissolved oxygen concentrations. In WWTPs, Ca. Accumulibacter plays an important role in removing phosphorus (P) by enhanced biological phosphorus removal (EBPR). These organisms thrive in periodic alterations between anaerobic conditions with organic carbon uptake and storage, and aerobic conditions where growth occurs. The metabolism of Ca. Accumulibacter utilising volatile fatty acids (VFA) anaerobically as carbon source is well studied but, their ability to utilise complex carbon such as proteins is obscure. In this study, we evaluated the potential of Ca. Accumulibacter enrichment to anaerobically accumulate an α-amino acid commonly found in wastewaters – L-aspartic acid.

Methods
Two reactors of working volume 750 mL were operated in duplicates. The reactor was operated sequentially in anaerobic (with substrate present) and aerobic conditions (no external substrate). The conversion kinetics of the culture was characterized. Community was characterized by metaproteomics. 29 high-quality Ca. Accumulibacter genomes were downloaded from NCBI assembly database for pangenome analysis.

Results
The ratio of anaerobic phosphorus release to acetate uptake was around 0.45 molP/molC with complete P uptake in aerobic phase. Pangenome analysis indicated aspartate conversion to intracellular polyhydroxyalkanoates could be achieved through two key pathways- (i) conversion to poly-hydroxybutyrate (PHB) via oxaloacetate, and (ii) conversion to poly-hydroxyvalerate (PHV) from fumarate via either the urea cycle or purine nucleotide cycle. Subsequent proteomic analysis revealed proteins from both key pathways, e.g., glutamate/aspartate periplasmic-binding, aspartate ammonia-lyase, and aminotransferase proteins.

Conclusions
Aspartate utilization via split pathways through oxaloacetate or fumarate indicates a mechanism of redox balancing where conversion of aspartate to PHV via fumarate helps meet redox cofactor demand of aspartate conversion via oxaloacetate to PHB. These results show an increased versatility of anaerobic organic carbon sequestration in environments with aerobic/anaerobic dynamics.

P073 Host specificity in the root microbiome of Lotus, Arabidopsis and barley down to the strain level

dhr Gijs Selten[1], dr. R. de Jonge[1], Dr. F. Lamouche[2], Dr. S. Kelly[2], Dr. S. Radutoiu[2]
[1]Utrecht Universiteit, [2]Aarhus University

Despite its complexity, the root microbiome is recruited by the plant in a consistent and reproducible manner. This indicates the presence of core microbes; microbes that are consistently recruited by the plant roots and core microbial genes or functions; genes or traits that these core microbes possess that make them efficient root colonizers. We aim to find these core microbes for three model plant species; Lotus japonicus, Arabidopsis thaliana and barley (Hordeum vulgare) with which to answer the following research questions: which bacteria are the most efficient root colonizers and which bacterial genes/functions play a key role in root colonization of these plant species? To investigate this, we cultivated the bacterial culture collections that were isolated prior from Lotus, Arabidopsis and barley roots and assembled them in highly complex synthetic communities (SynComs). The largest SynCom that we used consisted of 1250 strains. These SynComs were inoculated with each plant species and the plant microbiomes were harvested and sequenced after three weeks of growth. The results show that each plant host does not recruit all strains from the same genus/family/order but recruits specific strains, indicating a host specificity to the strain level (1). Recruited bacteria consist of mainly nodulating Mesorhizobium for Lotus roots and, interestingly, similar recruitment for Arabidopsis and barley roots which consist of Acidovorax, Caulobacter, Flavobacterium, Pseudomonas and Pseudoxanthomonas species (2). This suggests that these recruited strains might have important bacterial genes and functions required for host-specific root colonization in comparison to other strains of the same genus/family/order. The first functional analyses of these microbiomes indicate an enrichment of xyloglucan catabolic processes in the root microbiomes of all plant species, while different plant species also seem to recruit host-specific bacterial functions such as leucine biosynthesis in Arabidopsis or enterobactin biosynthesis in barley (3).

P074 Using meta-omics to obtain insights into the ecology and physiology of Thorarchaeota in anoxic Aarhus Bay sediments

Ir. Jolanda ter Horst[1], Dr. P Geesink[1], Prof. Dr. KU Kjeldsen[2], Prof. Dr. Ir. TJG Ettema[1]
[1]*Wageningen University and Research,* [2]*Aarhus University*

In the past decades, metagenomic studies have revealed the existence of novel taxa and entire branches in the tree of life. Many of these microbial lineages still lack cultivated representatives. Among these microorganisms are members of the Asgard superphylum, a group of archaea that received great attention after the discovery of their role in bridging the evolutionary gap between pro- and eukaryotes. Although some complete metagenome assembled genomes (MAGs) have been reconstructed, little is known about their activity and ecological role within microbial communities. Especially, limited research has been performed on the Asgard phylum Thorarchaeota. Marine sediments of Aarhus Bay (Denmark) have been found to contain high but varying relative abundances of Thorarchaeota in different depths. Here, we combine genome-resolved metagenomics with metatranscriptomics across a depth gradient within Aarhus Bay sediments from 5 to 35 centimeters below the sea floor. A detailed analysis of nine recovered MAGs showed that Thorarchaeota have the potential to fix CO2 via the Wood Ljungdahl pathway but can also utilize complex organic substrates, pointing towards a mixotrophic lifestyle. For some Thorarchaeota MAGs, transcriptomic profiles across depths show that genes involved in translation are highly expressed in shallow sediment layers while for other MAGs genes related to replication were mainly expressed in deeper layers. This could indicate that different Thorarchaeota thrive in different layers of the sediment. Furthermore, a large fraction of the expressed genes are to date of unknown function, highlighting the importance of elucidating the role of these genes and their relevance for Thorarchaeota in marine sediments. Our results demonstrate the importance of combining meta-omic techniques to gain insights into the lifestyle of Asgard archaea to guide novel enrichment, isolation and cultivation strategies and ultimately enable a wider understanding of the physiology and role of Thorarchaeota in marine sediments.

P075 Sialic acids: special sugars overlooked in environmental microbiology

Mr. Sergio Tomas Martinez[1], Dr. M. Pabst[1], Dr. D.G. Weissbrodt[1], Prof. M.C.M van Loosdrecht[1], Dr. Y. Lin[1]
[1]*Delft University of Technology*

Sialic acids are a family of nine-carbon negatively charged sugars normally found as terminal residues on cell-surface glycans of animal cells. They play important roles in regulation, interaction and protection processes. Sialic acids have been mainly studied in animal tissues and pathogenic bacteria due to their importance in the host immune response. Pathogens can synthesize sialic acids or acquire them from the environment to delay the host immune response by mimicking the host's glycosylation pattern. However, sialic acids have been overlooked in other type of organisms, such as environmental microbial biofilms, where a host-pathogen interaction is not present.
Recently, a genome level study and a large-scale mass spectrometry survey showed a wide occurrence of different forms of sialic acids and their biosynthetic genes in the microbial world, including non-pathogenic species. Specifically, sialic acids and other related molecules were detected in several environmentally relevant biofilms such as aerobic granular sludge (AGS), anammox granular sludge and biofilms growing in cooling towers. In the case of AGS dominated with "Candidatus Accumulibacter", fluorescence lectin staining revealed the presence of sialic acids as sialoglycoproteins in the extracellular polymeric substances (EPS). Moreover, sialic acids were measured through enzymatic quantification, resulting in 1.1% of volatile solids, highlighting the importance of these sugars. Further genomic analysis of "Ca. Accumulibacter" revealed its potential to produce different type of sialic acids as part of its EPS. Due to their known roles as recognition molecules in other organisms, sialic acids might be involved in processes such as biofilm formation in environmental bacteria. Moreover, enzymatic removal experiments, showed that they may have a protective role avoiding the hydrolysis of underlying glycan chains. The widespread presence of sialic acids in the microbial world urges their investigation to fully understand their role in environmental biofilms, where their production is not driven by host-pathogen interaction.

P076 MICROBIAL METHANE OXIDATION IN THE WATER COLUMN OF MARINE LAKE GREVELINGEN

Msc Jessica Venetz[1], Msc O.M. Zygadlowska[2], PhD W. K. Lenstra[2], PhD N. A. G. M. Van Helmond[2], Msc A. J. Wallenius[1], PhD P. Dalcin Martins[1], Msc G. H. L. Nuijten[1], Prof. Dr. Ir. C. P. Slomp[2], Prof. Dr. Ir. M. S. M. Jetten[1], Dr. A. J. Veraart[3]
[1]Radboud University, [2]Utrecht University, [3]Radboud University

Coastal ecosystems are hotspots for marine methane emissions. Particularly during summer hypoxia, methane production can exceed methane consumption in the sediment, resulting in a net methane efflux to the overlying water and atmosphere. Therefore, the microbial aerobic removal of methane in the water column is of critical importance to mitigate CH4 emissions. However, the geochemical drivers of methane oxidation and the methanotrophic community structure in the water column of coastal ecosystems are not well understood.
To shed light on the fate of water column methane in marine Lake Grevelingen during summer stratification, we combined 16S rRNA gene sequencing and biogeochemical analysis of water column samples at a high spatial resolution. Furthermore, we tested potential methane oxidation rates at four different oxygen concentrations (1-2-5-10% headspace v/v).
Methane concentrations peaked at 45 µM in anoxic bottom water, and rapidly decreased in the oxycline. High potential methane oxidation rates were measured at all sampling depths, even below and above the methane-oxygen interface. Microbiome analysis using16S rRNA gene as marker also showed the presence of aerobic methanotrophic bacteria (Methylomonadacaea) in the entire water column. However, their relative abundance was highest at the methane oxygen counter-gradient. In our incubation experiments, supplied oxygen concentrations did not affect methane oxidation rates. Therefore, oxygen availability does not seem to be the only control of methane oxidation in this system and limitation by other nutrients or competition with other microorganisms are more important.
Even at low oxygen concentrations, methane oxidation is possible in the entire water column, making this an important process to mitigate methane emissions from Lake Grevelingen. Following seasonal dynamics of methane cycling in the water column will give further insight into how well the methanotrophic community can adapt to rapid changes in chemistry and the main drivers for sustaining an effective water column methane filter.

P077 Methane cycle out of balance: methanogenic microorganisms triumph over anaerobic methanotrophs in the anoxic sediments of Lake Grevelingen

Anna Wallenius[1], MSc O.M. Zygadlowska[2], Dr. W.K Lenstra[2], Dr. N.A.G.M van Helmond[2], Dr. P Dalcin Martins[1], Prof C.P Slomp[2], Prof M.S.M Jetten[1]
[1]Radboud University, [2]Utrecht University

Coastal ecosystems contribute to the vast majority of marine methane (CH4) emissions. CH4, a potent greenhouse gas, is produced in deep sediments by methanogenic archaea, and up to 90% of it is typically removed in upper layers by anaerobic CH4-oxidizing archaea (ANME) in the sulfate-methane transition zone (SMTZ). In coastal sediments, CH4 is not removed as efficiently due to eutrophication, hypoxia and other fluctuating conditions that often favour methanogens over ANMEs. This imbalance in CH4 cycling may lead to CH4 release into the water column and ultimately to the atmosphere, but factors affecting these processes are not fully understood. To better understand mechanisms for increased CH4 emissions, we investigated sediment microbial communities with a focus on CH4-cycling archaea, together with seasonal CH4 production and oxidation rates in the anoxic sediments of marine Lake Grevelingen (NL).
Sediment cores collected in 2020 and 2021 were processed anoxically for in-situ and potential rate incubations. Core sub-samples were used for DNA isolation and 16S rRNA gene sequencing. The sediment depth profile of targeted gene sequences revealed high relative abundances of methanogenic archaea (15-80 %), with members of Methanomicrobiaceae and Methanosarcinaceae dominating at every depth. ANMEs from clade 2 were present in lower abundance and mainly in the SMTZ, indicating potential for sulfate-dependent CH4 oxidation. Methane production and oxidation rates were stable across seasons but did not directly correspond to microbial community composition at depth.
In conclusion, we found 1. a vast methanogenic potential in Lake Grevelingen sediments, but 2. a potentially smaller role for (anaerobic) CH4 oxidation and 3. stable in-situ rates throughout the year. Further investigation of other factors affecting the metabolic pathways together with metagenomic analysis will provide insights on the imbalance of the CH4 cycle in these sediments.

P080 Community science: online microbiome course proves usefull for research purposes.

Msc Maaike Alkema[1], PhD Clara Belzer[1]
[1]*Wageningen University*

Introduction. The Massive Open Online Course (MOOC) “Nutrition and Health: Human Microbiome” was developed on edX by the laboratory of microbiology from Wageningen University. Almost 50.000 people have enrolled since 2017 globally. As such large and diverse numbers of people were reached, we wanted to investigate if MOOCs can be used for research purposes.

Methods. Learners were asked to complete assignments throughout the MOOC. One of the assignments (completed by 858 learners) contained questions about early life events and their impact on gut microbiota development and non-communicable diseases later in life (if any). Another assignment (completed by 328 learners) consisted of questions about pre-and probiotic products that learners could find in local stores. To see if MOOCs could be effective tools to recruit people for participating and/or engaging in research projects, all learners (n = 46.936) were invited via e-mail to register to our recently developed “WUR microbiome research panel”: a panel for people who would like to volunteer and/or engage in microbiology focused research projects.

Results. Self-reported preterm birth rates (9%) were comparable to the >1 in 10 reported by the WHO. The c-section rate of 14% lies within the “ideal” rate (10-15%). The percentage of people that received breastmilk in their first year of life (74%) is comparable to the global breastfeeding rate. A large variety of pre-and probiotic products was reported to be seen in local shops, where Lactobacillus acidophilus and Bifidobacterium lactis were counted most often. In response to our e-mail invite to the panel, within four weeks 760 learners (1.6%) subscribed.

Conclusion. Due to the high numbers and global diversity of learners, MOOCs can be powerful tools to reach out to individuals for research purposes. Our analyses show that apart from educational purposes MOOCs can also be used for questionnaire based microbiology focused research.

P081 The mysterious inserts in mitochondrial carrier proteins of Plasmodium falciparum.

Msc Cas Boshoven[1], A. K. King[2], Dr. E. R. S. Kunji[2], Dr. T. W. A Kooij[1]
[1]*Radboudumc,* [2]*University of Cambridge*

Plasmodium spp., the causative agent of malaria, harbour only a single mitochondrion, which is essential in all life-stages, highly divergent from its hosts and a proven drug target. Metabolite transfer is an important process in this metabolically active organelle. This process is performed by a well conserved transporter family, called mitochondrial carriers (MCs). Plasmodium spp. have 13 MCs encoded in their genome, however only few of their transported substrates are predicted, let alone confirmed. In this study, we computationally compared the P. falciparum MC protein sequences to their well characterised human and yeast orthologues. A phylogenetic analysis aligning the substrate binding transmembrane domains identified 2 potential substrates for previously unknown PfMCs and substrate-classes for 2 others. We additionally identified specific inserts linking the well conserved transmembrane domains, a feature which has not been described before. We analysed these inserts further and revealed that they are conserved within Plasmodium species. The length and amino acid composition of these inserts are different between species, however their charge is conserved. Mapping these charges over the predicted protein structure revealed a pattern of positively charged inserts pointing towards the mitochondrial matrix, while negatively charged inserts are directed to the inter membrane space. With the canonical characteristic of the positively charged intermembrane membrane space in mind, we hypothesize that these inserts are required for correct insertion of MCs into the mitochondrial inner membrane.

P082 Visualization of SpoVAEa protein dynamics in dormant spores of Bacillus cereus and dynamic changes in their germinosomes and SpoVAEa during germination

MSc Y. Wang[1], MSc N.O.E. Vischer[1], BSc D. Wekking[1], MSc A. Boggian[1], Prof. dr. P. Setlow[2], Prof. Stanley Brul[1]
[1]University Of Amsterdam, [2]UConn Health

Bacillus cereus spores, like most Bacillus spores, can survive for years depending on their specific structure, and germinate when their surroundings become suitable. Spore germination proteins play an important role in the initiation of germination of this toxigenic microorganism. Because germinated spores lose the extreme resistance of the dormant state, more information related to the function of germination proteins could be useful to develop new strategies to control B. cereus spores. Prior work has shown that: i) the channel protein SpoVAEa exhibits high frequency movement in the outer leaflet of the inner membrane (IM) in dormant spores of B. subtilis; (ii) the dynamics of germinosome formation in developing spores of B. cereus indicate that the formation of germinosome foci is slower than foci formation of germinant receptors GerR and scaffold protein GerD. However, the dynamics of movement of SpoVAEa in B. cereus spores, and the complete behavior of the germinosome in germinated spores of B. cereus is still unclear. In this study, we found that the SpoVAEa fluorescent foci in dormant spores of B. cereus redistribute at a lower frequency than in B. subtilis, and likely colocalize with GerD in dormant spores. Our results further indicate that: i) overexpression of GerR(A-C-B)-SGFP2 and SpoVAEa-SGFP2 with GerD-mScarlet-I from a plasmid leads to more heterogeneity and lower efficiency of spore germination in B. cereus; ii) germinosome foci composed of GerR(A-C-B)-SGFP2 and GerD-mScarlet-I were lost prior to the phase transition in germination; and iii) GerD-mScarlet-I foci spread out but continued to exist beyond the phase transition of B. cereus spores from phase bright to phase dark, typical for water uptake in germinating spores.

P083 Influence of coccidiosis prevention strategies on the chicken gut microbiome

Mr. Matteo Buffoni[1,4], Ph.D. H Enting[2], J.v.d. Stroom[3], M. Rogers[1], M. Viveen[1], Prof. J.A.G.M de Visser[4], Ph.D. R.J.L. Willems[1], Ph.D. A.C. Schürch[1], Ph.D. F.L. Paganelli[1]
[1]UMC Utrecht, [2]Cargill, [3]Elanco, [4]Wageningen University & Research

Introduction: Humans' and animals' intestinal tract is densely populated by bacteria, collectively called gut microbiome. This is an important reservoir of antibiotic resistance genes, called the resistome. Diet and feed additives play an important role in shaping the microbiome composition and consequently the resistome in farm animals. In this study, we aim to assess the effect of two commonly used control strategies to prevent coccidiosis in broilers.
Material and Methods: We collected caeca material from 100 broilers, 21 days of age, from a single German farm. The samples were taken from two consecutive flocks (same hatchery and parentflock), kept in the sample conditions (house and feeding). These samples were equally distributed (50 animals per group) by sex and in two groups (coccidiostat or Paracox 5 vaccine). DNA was extracted from all samples and the 16S rRNA V3-V4 region was amplified and sequenced with Illumina MiSeq technology.
Results: Total bacterial diversity (alpha diversity), did not show significant differences between the groups, while beta-diversity analysis showed significant differences in the taxonomic composition. Using supervised analysis, 21 genera were found to be statistically different between the groups. Overall, the coccidiostat group showed a lower Firmicutes:Bacteroidetes ratio than the vaccinated group. In addition, in this group, lower proportions of beneficial bacteria, especially Lactobacillus, Butyricicoccus and Oscillospiraceae, were observed, with exception of Bifidobacterium, which was more abundance. Furthermore, some other genera were more abundant as Alistipes, Parabacteroides and Parasutterella.
Conclusion: The gut microbiome changes differently in the two groups. Overall, a shift toward some genera including potential pathobionts, but at the same time a more mature microbiome is observed in the coccidiostat group. The mature microbiome leads to healthier birds, less foot pad lesions, better growth and feed conversion rate. Whether this shift in microbiome composition results in changes in resistome will be investigated next.

P084 Host defense peptides and their immunomodulatory and antibacterial functions against Staphylococcus aureus

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

Introduction: Staphyloccous aureus represent one the most challenging cause of infections to treat by traditional antibacterial therapies. Thanks to their lack of microbial resistance described so far, host defense peptides (HDPs) represent an attractive therapeutic alternative to antibiotics. Furthermore, HDPs have been showed to control infections via a dual function: direct antimicrobial activity and regulation of immune response. However, HDPs functions characterization and comparison is controversial, as changing test conditions or cell type used might yield different effects from the same peptide. In the current study, we aim to characterize and compare the immunomodulatory and antibacterial functions under the same conditions in vitro of 3 well-known HDPs: IDR-1018, CATH-2, and LL-37.

Methods: S. aureus, strain SH1000, was incubated with different concentrations of each HDP and bacterial growth was monitored overnight. Primary human monocytes were isolated from peripheral blood using Ficoll-Paque density and CD14 microbeads, and differentiated for 7 days to macrophages. After 24h incubation in presence of LPS and HDPs, macrophages cytokines production was measured by ELISA. Macrophages cultured for 24h in presence of HDPs were infected with serum-opsonized S. aureus. 30 min and 24h after infection, bacterial phagocytosis and intracellular killing by macrophages were measured by flow cytometry and colony forming units (CFU) count respectively.

Results: All HDPs efficiently inhibit macrophages LPS-mediated activation, as observed by a reduced production of TNF-α and IL-10. Despite a comparable anti-inflammatory action, only CATH-2 shows direct antibacterial properties at concentrations 10-times lower than those needed to stimulate immune cells. Finally, stimulation with HDPs fails to improve macrophages ability to kill intracellular S. aureus, while only IDR-1018 decrease the proportion of cells phagocytosing bacteria.

Conclusions:
1. All HDPs show anti-inflammatory effects
2. Only CATH-2 has direct antibacterial activity
3. HDPs do not improve macrophages antibacterial functions

P085 Effect of silver ions and nanoparticles on immune cells antibacterial functions

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

Introduction: Staphyloccous aureus represent one the most challenging cause of implant infections to treat by traditional antibacterial therapies. As an alternative therapeutic approach, implants releasing metallic silver ions (AgNO3) or nanoparticles (AgNP) are currently employed in clinical applications. However, the strong broad-spectrum antibacterial properties of silver correlates with toxic effects on host cells, particularly against immune cells.
In the current study, we aim to identify a therapeutic window where AgNO3 and AgNP maintain their antimicrobial properties while not affecting immune cells viability and functions.

Methods: S. aureus, strain SH1000, was incubated with different concentrations of AgNO3 or AgNP 20 nm, and bacterial growth was monitored overnight. The following day, bacteria were plated for counting. Primary human monocytes were isolated from peripheral blood using Ficoll-Paque density and CD14 microbeads. Monocytes were differentiated to macrophages for 7 days and then incubated with AgNO3 or AgNP for 24h. Then, cells viability was measured by alamar blue assay. Macrophages stimulated with silver were infected with serum-opsonized S. aureus. 30 min and 6h after infection, bacterial phagocytosis and intracellular killing by macrophages were measured by flow cytometry and colony forming units (CFU) count respectively.

Results: Both AgNP and AgNO3 inhibit S. aureus growth, however only high AgNO3 concentrations result in complete killing of the bacteria. Although macrophages viability is not affected by incubation with silver, their antibacterial functions are negatively affected. Despite a decreased uptake of bacteria after 30 min, both AgNO3 and AgNP interfere with the reduction of macrophages bacterial load after 6h. Additionally, silver treatment does not kill S. aureus surviving intracellularly.

Conclusions:
1. At non-cytotoxic concentrations, AgNP have a direct antibacterial effect, while high AgNO3 concentrations completely kill S. aureus.
2. Silver treatment negatively affects macrophages antibacterial functions while not reducing S. aureus intracellular survival

P086 Isolation, characterization and localization of bacterioferritin nanoparticles in the compartmentalized anammox bacterium Kuenenia stuttgartiensis

Mrs. Laura Claret Fernandez[1], Dr Simon Lindhoud[2], Dr Rob Mesman[1], Dr Wouter Versantvoort[1], MSc S H Peeters[1], Prof. Dr. M.S.M. Jetten[1], Prof. Dr. Laura van Niftrik[1]
[1]Radboud University, [2]Wageningen University and Research

Anaerobic ammonium-oxidizing (anammox) bacteria convert ammonium and nitrite to dinitrogen gas with nitric oxide and hydrazine as intermediates. The energy metabolism of anammox bacteria is heavily dependent on heme-containing proteins. Anammox bacteria are important players in the global nitrogen cycle and are applied in wastewater treatment for the removal of nitrogen compounds. Anammox bacteria have an extraordinary cell plan with a major "prokaryotic organelle" called the anammoxosome. The anammox reaction takes place in the anammoxosome and is proposed to be coupled to energy conservation over the anammoxosome membrane via proton motive force and subsequent ATP synthesis. In addition, the anammoxosome contains iron-loaded nanoparticles of unknown function. The genome of the anammox bacterium Kuenenia stuttgartiensis encodes two bacterioferritins (kuste3640 and kuste4480). Bacterioferritins are iron storage multimeric proteins that can compartmentalize up to ~2000 iron atoms. Typically, bacterioferritin functions in regulating the cellular levels of free iron to ensure availability while at the same time preventing iron-induced toxicity. However, there is evidence that they could be involved in oxygen stress response in anaerobic bacteria.
Here we isolated, characterized and localized K. stuttgartiensis bacterioferritin ksute3640, one of the two bacterioferritins encoded in its genome. The bacterioferritin was purified from K. stuttgartiensis cells using a novel protocol. The bacterioferritin was characterized after purification, confirming the presence of heme groups, absorption bands and size. Also, the ability to load the bacterioferritin with iron was investigated, and a 3D model was computed using other known bacterioferritins as templates. Immunogold localization was used to determine the location of bacterioferritin in the K. stuttgartiensis cell.

P087 Elucidating the role of two secretion systems in conjugative plasmids in Mycobacteria

Sita Coenraads[1], R Ummels[2], Prof. Dr. W Bitter[1,2]
[1]*Vrije Universiteit Amsterdam,* [2]*Amsterdam UMC*

Introduction
Conjugation is one of the most efficient ways of horizontal gene transfer and plays an important role in the genetic diversity of bacteria. The mechanism of most widespread plasmid conjugation systems depends on a type IV secretion system. Recently, a conjugative plasmid (pRAW) was discovered in Mycobacterium marinum. An unusual characteristic of this plasmid is the presence of both a type IV-like and a type VII secretion system for conjugation. These unusual conjugative plasmids seem to be widespread among the mycobacteria.

Results
We are interested in the conjugation process and studied the essentiality of the different genes for conjugation using a CRISPR-Cas9 directed mutagenesis approach. Using this knockout approach we now show that the majority of conserved genes located in the type IV-like locus are essential for plasmid conjugation with only two exceptions. This suggests that these genes might take over the functions of the absent type IV secretion system components.
Furthermore, not only the type VII secretion system itself is essential for conjugation, but also five genes that are predicted to encode type VII substrates or chaperones and are located on a different part of the plasmid. As both secretion systems are essential for conjugation, these secreted substrates might play a role in the connection with the type IV secretion system. A hypothesis that is currently tested.

P088 ADP-heptose-mediated NF-kB activation is inhibited by a secreted protein from Limosilactobacillus

Mr Jiannan Cui[1]
[1]*UMCU*

Jiannan Cui, Natascha Wolf, Jos P.M. van Putten & Marcel R. de Zoete. Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands

The nucleotide sugar ADP-heptose is a newly identified pathogen-associated molecular pattern (PAMP) that binds and activates the cytosolic receptor alpha-kinase 1 (ALPK1), resulting in TRAF-interacting protein with forkhead-associated domain (TIFA)-mediated nuclear factor kappa-B (NF-κB) activation. As ADP-heptose is a crucial metabolic intermediate in the synthesis of lipopolysaccharide from Gram-negative bacteria and therefore presumably abundantly present in the intestinal tract, we screened the fecal microbiotas of 10 healthy donors for ADP-heptose-mediated NF-κB activation. Surprisingly, we found that the microbiotas of 8 out of 10 donors exhibited potent ADP-heptose neutralizing activity. By testing the culture supernatants of a wide panel of isolated intestinal bacteria, a Limosilactobacillus sp. was shown to strongly and specifically inhibit ADP-heptose-mediated NF-κB activation but not TNF-α and Toll-like Receptor 5 signaling. Heat and proteinase K treatment abolished the inhibitory activity of the Limosilactobacillus supernatant, suggesting that the active compound is of proteinous nature. Fractionation of the supernatant by size and charge revealed that the inhibitory compound has a size of 50-100 kDa and a slightly negative charge at pH 8. This research describes the first natural inhibitor for ADP-heptose and identifies a previously unknown anti-inflammatory mechanism in Limosilactobacillus in the intestinal tract.

P091 Mobile colistin-resistance gene mcr-10.1 in Enterobacter spp. in the Netherlands

BSc A de Haan[1], BSc F Landman[1], BSc S Witteveen[1], BSc M.G. van Santen-Verheuvel[1], Dr. L.M. Schouls[1], Dr. D.W. Notermans[1], Dr. Ing. A.P.A. Hendrickx[1]
[1]*RIVM*

Introduction
Plasmid-based dissemination of mobile colistin resistance (mcr) genes among Enterobacterales are of global concern. Colistin is considered a last-line treatment option for infections caused by multi-drug resistant and carbapenemase-producing Enterobacterales (CPE). The objective was to analyze the presence of the mcr-10.1 gene in isolates retrieved from the Dutch CPE surveillance.
Methods
All isolates were sequenced by short-read sequencing (Illumina) and a subset by long-read sequencing (Nanopore). Illumina data were used for multi-locus sequence typing (MLST), pan-genome MLST (pgMLST), AMRFinder and PlasmidFinder. Combined Illumina and nanopore data were used to reconstruct plasmids. Colistin resistance was determined by broth microdilution (BMD). The carbapenem inhibition method (CIM) was used to assess carbapenemase activity. Mcr-10.1-plasmid was cured from one isolate by repeated subculturing.
Results
The mcr-10.1 gene was identified in eight of the 268 Enterobacter spp. isolates (3%) from the Dutch CPE surveillance from 2012-2020 and not among other CPE. Seven of the eight isolates produced carbapenemase according to the CIM, had ST125, and differed 45-105 alleles from each other based on pgMLST, while the eighth isolate differed in 1731 alleles. BMD showed that three of the eight Enterobacter spp. isolates were colistin resistant (one with an MIC of 16 mg/L and two with ≥64 mg/L). Hybrid assembly enabled reconstructions of four of the eight mcr-10.1-plasmids. The mcr-10.1-plasmids differed in size, ranging from 81 to 127 kb and all harbored the IncFII replicon. Mcr-10.1 was located in the chromosome in one isolate. The mcr-10.1-plasmid was cured from one isolate leading to a reduction of the MIC for colistin of ≥64 mg/L to 16 mg/L.

Conclusion
The presence of the plasmid-localized mcr-10.1 gene in Enterobacter spp. isolates is low in the Netherlands and confers resistance to colistin. Circulation of mcr genes among CPE is worrying and future monitoring colistin resistance may be needed.

P092 Standardizing the assessment of amoebicidal efficacy of contact lens solutions against Acanthamoeba species

Lieke De Kroon[1], H Otten[2], B Schimmer[3], M Tehupeiory-Kooreman[1], C Arias Claro-Handgraaf[1,4], Dr. F.F. Stelma[1]

[1]Radboudumc, [2]Visser Contactlenzen, [3]National Institute for Public Health and the Environment (RIVM), [4]CWZ

ABSTRACT

Introduction

To date no standardized methods are used in order to assess the amoebicidal efficacy of commercial contact lens solutions for both trophozoites and cysts of Acanthamoeba species. Here we present two methods that are suitable for this purpose; the Spearman Karber log reduction method and XTT colorimetric assay.

Methods

Acanthamoeba castellanii (ATCC 50370) and A. polyphaga (ATCC 30461) trophozoites were cultured in peptone-yeast extract-glucose medium. Cysts were developed in Neff's encystment medium during 1 week. Spearman Karber and XTT colorimetric assay were used to evaluate trophozoite and cysticidal efficacy of multi-purpose contact lens solutions (MPS).

Results

The Spearman Karber and XTT colorimetric assay both yielded quantitative results and can be used to evaluate the amoebicidal efficacy of contact lens solutions. The Spearman-Karber method yields an estimate of kill, whereas XTT colorimetric assay provides an estimate of decreases amoebic metabolic activity.

Conclusion

Both methods give reproducible estimates of amoebicidal efficacy of contact lens solutions, however, XTT colorimetric assay should be followed by a compete kill assay to test for viable cysts.

Keywords: Acanthamoeba; MPS; contact lens fluids; XTT colorimetric assay; Spearman Karber log reduction method

P093 Implementation of Multi Locus Sequence Typing based on Nanopore Single Molecular Real-Time sequencing

BSc Q. D. Dijkstra[1], BSc A. K. Kant[1], Dr T. J. B. G. Gonzalez[1], BSc F. H. Harders[1], Dr K. V. Veldman[1], Dr M. B. Brouwer[1]
[1]*Wageningen Bioveterinary Research*

Multi-locus sequence typing (MLST) is the most commonly used methods for studying microbial lineage. However, the traditional MLST protocol using Sanger sequencing is time-consuming and expensive. To tackle these issues a comprehensive multiplex sequencing and analysis method was set up using Nanopore Single Molecule Real-Time (SMRT) sequencing with Escherichia coli as model organism.

A total of 50 E.coli samples obtained from fecal rectal swabs of veal calves were analyzed using Illumina whole-genome sequencing for reference, and the Nanopore amplicon sequencing method. Amplicons of the 7 MLST genes were pooled per isolate, prepared using the SQK-LSK109 kit, barcoded using the EXP-NBD196 kit and subsequently loaded onto a flowcell (R9.4.1)(Oxford Nanopore Technologies). The read depth of the MLST genes were analyzed in real time using RAMPART v1.1.0 and the run was halted when a read depth of at least 150x was reached in order to reuse the flowcell for the next run. The data was mapped using Artic v1.1.3 and then compared to the MLST database from pubmlst.org.

A read depth of at least 150x was shown to be sufficient for all isolates and reached within 2 hours of sequencing. Because of the real time analysis of RAMPART, the flowcell could safely be stopped and reused. The data was then compared to the sequences obtained using Illumina technology and showed a 100% match.

This is the first description of a workflow for real-time sequencing and analysis of MLST amplicons on a collection of bacterial isolates. Because the results between Nanopore SMRT sequencing and Illumina technology showed a 100% match it can be said that no compromise had to be made regarding reliability and quality. This method could prove to be useful for quick and cost-effective screening of isolate collections. Furthermore, the method can easily be adopted to include further sets of amplicons.

P094 Fusobacterium nucleatum secretes small molecules that induce intestinal DNA damage and an ALPK1-dependent inflammatory response

Mr. Coco Duizer[1], M.J. Stok[1], M.A. Sijbranda[1], Prof. Dr. R.J.L Willems[1], Dr. M.R. de Zoete[1]
[1]*Umc Utrecht*

Fusobacterium nucleatum is an oral commensal anaerobe that can migrate to different organs in the human bodies upon disease. Outside the oral cavity, the pathogenic potential of F. nucleatum is especially apparent in the colon, where it is thought to contribute to colorectal carcinogenesis. Research aimed at elucidating the molecular mechanism underlying the effect of F. nucleatum on colorectal cancer (CRC) has mainly focused on bacterial proteins responsible for adhesion to and proliferation of tumor cells. We hypothesized that, similar to other carcinogenic bacteria, F. nucleatum induces a potent inflammatory response and DNA damage in intestinal epithelial cells. By examining sterile F. nucleatum culture supernatants, we have identified a released molecule, smaller than 3 kDa and not of proteinaceous nature, that induces a Nuclear Factor κB (NF-κB) response and subsequent Interleukin (IL)-8 release in HT29 and HeLa-57a cells. These responses are lost upon genetic inactivation of ALPK1 in both HT-29 and HeLa-57a cells. Testing multiple species and strains of Fusobacterium showed that all were able to induce the inflammatory response. Additionally, an F. nucleatum culture supernatant factor smaller than 3 kDa and not of proteinaceous nature drove the formation of DNA double stranded breaks in HT-29 cells, as shown by γH2AX western blot staining and 53BP1 confocal microscopy staining. Combined, a small, unknown factor within the culture supernatant of Fusobacterium spp. induces DNA damage and drives NF-κB response in an ALPK1-dependent manner. These findings could prove pivotal in understanding the pathogenesis of F. nucleatum and pave the road for new therapeutics that inhibit F. nucleatum-mediated development of colorectal cancer.

P095 Glycosylation-dependent binding of Akkermansia muciniphila to mucin

Msc Janneke Elzinga[1], Yoshiki Narimatsu[2], Henrik Clausen[2], Hanne L.P. Tytgat[1], Willem M. de Vos[1,3]
[1]Laboratory of Microbiology, Wageningen University & Research, The Netherlands, [2]Copenhagen Center for Glycomics, Department of Cellular and Molecular Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark., [3]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Introduction
The intestinal tract is lined by mucus, which serves as a protective barrier, acts as a lubricant for food passage and provides attachment sites and nutrients for intestinal bacteria. Mucus is mainly composed of mucin, a highly O-glycosylated protein. The commensal bacterium Akkermansia muciniphila is a mucin-degrading expert: it is able to cleave and degrade the glycans decorating the mucin protein backbone as well as the protein core itself. A. muciniphila plays a key role in the intestinal host-microbe ecosystem at the mucosal interface and has been shown to reinforce the mucosal barrier in mice and humans. A. muciniphila has potential for next-generation therapies in the context of obesity and associated metabolic disorders. Although the mucin-degrading properties of this bacterium have been extensively studied, its mucin-binding properties have not been fully elucidated yet.
Methods
In this study, a previously developed a glycoengineered HEK293 cell-based platform for the display and production of human tandem repeat (TR) mucin domains with tunable structures and patterns of O-glycans, was used. We investigated ELISA-based binding of A. muciniphila to different glycoforms of purified mucin reporter proteins encoding the TR domains of transmembrane mucin MUC1 and secreted mucins MUC2, MUC5AC and MUC7.
Results
Our experiments demonstrated that A. muciniphila prefers binding to mucin TRs expressed in HEK293 wild-type carrying core2 and core3 and that binding was reduced or lost for core1 and truncated glycoforms of mucin reporters. Moreover, desialylation of wild-type mucin TRs led to increased binding of A. muciniphila.
Conclusion
Additional experiments are needed to investigate the recognition of clustered patches or multiple O-glycosylation patterns by A. muciniphila and identify the bacterial carbohydrate-binding proteins responsible for binding. Further investigation of microbe-mucin binding will provide novel molecular insights into mucus colonization, recognition and degradation by mucus-adapted bacteria sustaining the intestinal host-microbe ecosystem.

P096 Interactions of bacterial pathogens with specific glycan structures on the transmembrane mucin MUC1

Msc Koen Giesbers[1], Dr. Karin Strijbis[1]
[1]*Utrecht University*

The intestine has the highest density of bacteria anywhere in the body. To prevent constant infection and/or penetration of bacteria, the intestine is lined with a layer of glycosylated proteins, called mucins. Mucins are mostly comprised of O-linked glycans. The differences in glycosylation of mucins in relation to carcinogenesis and inflammation, in particular for the transmembrane mucin MUC1, has been extensively studied. Intra- and extra-cellular processes resulting in aberrant hypo-glycosylation of MUC1, mediated by high expression of sialyltransferases, have been implicated in chronic inflammation and cancer metastasis. Under healthy conditions, the glycosylated MUC1 has an essential barrier function for pathogenic bacteria such as Helicobacter pylori and Campylobacter jejuni. However, our recent work demonstrated that Salmonella Enteritidis expresses a giant adhesin SiiE that specifically interacts with sialic acids on MUC1 and facilitates apical entry into enterocytes. Currently, we are investigating which MUC1 glycan structures are responsible for Salmonella binding and which glycan structures pose a barrier that prevents bacterial invasion. Using lectin blocking in combination with a Salmonella invasion assay, we found that blocking α2-3 linked sialic acids with MALII, significantly decreased the invasion of Salmonella into HT29-MTX cells. Blocking α2-6 linked sialic acids with SNA on the other hand had no effect on Salmonella invasion. These results indicate that the bacterium utilizes α2-3 linked sialic acids on MUC1 for SiiE adhesion. The specificity of this interaction will be confirmed using specific neuraminidases and mass spectrometry analysis. In addition, we will generate CRISPR/Cas9 knockout and lentiviral overexpression cell lines for sialyltransferases ST3Gal1-6. We hypothesize that Salmonella invasion will be blocked in the absence of the specific α2-3 linked sialic acid. In future experiments, we will identify novel MUC1-binding bacteria using different screening methods. With this work, we will gain insight into bacteria-mucin interactions in the healthy and inflamed intestine.

P097 Novel mobile elements and plasmid plasticity of Staphylococcus pseudintermedius are involved in the acquisition of multiple antimicrobial resistance genes

Dr Linda Graaf - van Bloois[1], A. Wegener[1], Dr. E.M. Broens[1], A.L. Zomer[1], A. Timmerman[1], J.A. Wagenaar[1,2], B. Duim[1]

[1]Utrecht University, [2]Wageningen Bioveterinary Research

Introduction

The population of Staphylococcus pseudintermedius is characterized by clonal methicillin-resistant (MRSP) isolates which are often multidrug resistant isolates. In this study, we performed an extensive genomic analysis on mobile genetic elements and plasmids, to identify all mobile elements in the closed genomes of S. pseudintermedius and investigate their association with the dissemination of antimicrobial resistance genes.

Methods

In total 223 Dutch S. pseudintermedius isolates were included, consisting of 98 MRSP isolates and 125 methicillin-susceptible (MSSP) isolates from canine (n=190) and human (n=33). This set was sequenced with short-read Illumina sequencing and 25 isolates containing different antimicrobial resistance (AMR) genes were selected, to close their chromosomes and plasmids using additional long-read Nanopore sequencing.

Results

The isolates carried multiple AMR genes, and almost all MRSP isolates were multi-drug resistant, compared to 40% of the MSSP isolates.

A highly diverse novel mobile element was identified in the closed chromosomes, carrying aminoglycoside-streptothricin resistances, but also variations of this element additionally carrying macrolide resistance ermB gene and/or the pleuromutilin-lincosamide-streptogramin A resistance genes. Two novel SCCmec elements were identified. The conjugative pRE25-like element carrying cat, erm(B), aphA3, aadK, and sat resistance genes showed to be highly conserved. Furthermore, transposons containing resistance genes tet(M), tet(L) and the bifunctional aminoglycoside aac(6')-Ie-aph(2'')-Ia gene were identified, as well as multiple insertion sites of gene blaZ.

Plasmid were present in 19/25 closed genomes, and carried often AMR genes. Two plasmids carried tetracycline resistance gene tet(K) that was also integrated in the chromosome. Different plasmids carrying lincosamide lnu(A) resistance and chloramphenicol acetyltransferase (cat) genes were detected.

Conclusion

The novel mobile elements with multiple resistance genes, novel SCCmec elements, and multiple plasmids carrying AMR genes, indicated the rapidly ongoing acquisition of antimicrobial resistances in S. pseudintermedius, but also provides an ecological source for horizontal transfer of resistance genes to other pathogens.

P098 COCHLEA Study: Proof of principle of a fetal human inner ear model

Ms. Lucia Grijpink[1], Dr. H Locher[2], Dr. J.C.M.J. de Groot[2], Dr. A.C.T.M. Vossen[1]
[1]Leiden University Medical Center, [2]Leiden University Medical Center

Congenital cytomegalovirus (CMV) infection is the most common non-genetic cause of sensorineural hearing loss. In addition, vestibular impairment is frequently observed in children infected with CMV. However, the pathogenesis of CMV-induced hearing loss and vestibular impairment remains unclear, and there is a need for a human disease model. We describe a fetal inner ear model, which can be used to perform ex vivo viral infection and to study the expression of CMV entry proteins during fetal development of the human inner ear.

Ex vivo infection
A fetal human inner ear of 13 weeks fetal age (W13) was obtained by means of elective abortion. To investigate if ex vivo infection of the fetal inner ear is possible, a GFP-labeled adenovector was injected through the oval window. After culturing for 4 days, the inner ear was fixed, decalcified and embedded in paraffin. Sections were immunostained to show hair cells (anti-myosin VIIa). The GFP signal was detected in the epithelial lining of the cochlea. In the vestibular labyrinth, GFP was expressed in the dark cells and transitional epithelium of the ampulla.

Entry proteins
Paraffin sections of inner ears from both the first (W7-12; N=6) and second trimester (W13-14, N=3) were used and immunostained for CMV entry proteins EGFR and PDGFRα. EGFR was expressed in the mesenchyme, epithelial cells, the spiral ganglion and stereocilia in the vestibular organs. As early as W10, it could be detected in the organ of Corti. PDGFRα was also expressed in the mesenchyme, but was not always co-expressed with EGFR.

Conclusion
This study shows that:
1. Ex vivo viral infection of a fetal human inner ear is feasible.
2. CMV entry proteins in the fetal inner ear are already expressed during the first trimester, both in the cochlea and vestibular organs. These cells are a potential target for CMV.

P099 Heterogeneity of New Delhi Metallo-β-lactamase (NDM) plasmids among carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in the Netherlands from 2014-2019

Dr. Ing. Antoni Hendrickx[1], S. Lipworth[2], F. Landman[1], W. Matlock[2], S. Witteveen[1], E. Kuijper[1], D. W. Notermans[1], N. Stoesser[2], L. M. Schouls[1], Dutch CPE surveillance study group[1]
[1]RIVM, [2]University of Oxford

Introduction
Genes encoding NDM carbapenemases are the second most abundant carbapenem resistance genes among Enterobacterales submitted to the Dutch CPE surveillance. Therefore, we investigated the characteristics of NDM-plasmids in Escherichia coli (Eco) and Klebsiella pneumoniae (Kpn) isolates collected in 2014-2019 and compared these with NDM-plasmids from all continents.

Methods
Hybrid assemblies (Illumina/Nanopore) enabled reconstruction of NDM-plasmids from Eco and Kpn isolates. Mash, MLST, wgMLST, ResFinder and PlasmidFinder analyses were performed. Comparisons were made with 1,227/34,513 NDM-plasmids from PLSDB and with blaNDM sequences from the AMRFinder database. The Etest for meropenem was used to determine carbapenem susceptibility.

Results
Fifty-three and 96 NDM-plasmids were reconstructed from 152 Eco and 148 Kpn isolates, respectively. NDM-plasmids dominated among Eco ST167 and ST405 and Kpn ST11 and ST147. Eight Dutch NDM-plasmid groups clustered with NDM-plasmids (n=1,227) reported from all continents. NDM-plasmid groups varied in size, %G+C, resistance genes, and replicons. One group of IncX3 blaNDM-5/blaNDM-7 plasmids was conserved (84-99%) among Eco and Kpn. Another group of blaNDM-1 plasmids shared 64-99% similarity and was associated with the blaCMY-6 and sul1 resistance genes and the IncA/C2 replicon in Kpn. Two groups of blaNDM-1 plasmids were restricted to Kpn, containing the aac(6')-Ib-cr resistance gene and various replicons. One group of Kpn blaNDM-1/blaNDM-5 plasmids harbor the aph3-VI resistance gene and the IncHIB replicon. The blaNDM-1 gene conferred resistance to meropenem in Kpn (42/84; 50% with MICs for meropenem >8mg/L), and to a lesser extent in Eco (3/32; 9%). In contrast, blaNDM-5 caused resistance in Eco (63/98; 64%), but less so in Kpn (7/9; 78%). The blaNDM-7 gene was rare, and caused resistance to meropenem in Eco (6/11; 55%) and Kpn (6/7; 86%) isolates.

Conclusion
The composition of the NDM-plasmids of Eco and Kpn is highly diverse in the Netherlands and comparable to NDM-plasmids reported globally, confirming international dissemination.

P100 Positive carbapenem inhibition method of Enterobacter spp. due to minor class C type carbapenemases in the Netherlands 2014-2019

Dr. Ing. Antoni Hendrickx[1], Angela de Haan[1], Daan W. Notermans[1], Wil van der Zwet, Gert-Jan Godeke[1], Sandra Witteveen[1], Marga van Santen-Verheuvel[1], Ed Kuijper[1], Leo M. Schouls[1]
[1]*RIVM*

Background: Carbapenemase-producing Enterobacterales (CPE) represent a health care problem. For the Dutch surveillance, laboratories submit suspected CPE to the RIVM for characterization. Between 2014-2019, 28.7% (54/188) of the Enterobacter cloacae complex produced carbapenemase according to the carbapenem inhibition method (CIM) without having a detectable carbapenemase gene (OXA, KPC, NDM, VIM, IMP), a phenotype termed CIM+Carba-. The majority 81% (44/54) of these isolates were susceptible for meropenem (MIC ≤2 mg/mL). The objective was to characterize the mechanism responsible for the CIM+Carba- phenotype.

Methods: Next-generation sequencing (NGS) was performed on 188 Enterobacter spp. to determine the carbapenemase allele and was used for MLST, pgMLST, resistome and replicome analyses by using SeqSphere, CARD and PlasmidFinder, respectively. The CIM and CarbaNP test were used to assess carbapenemase production or imipenem hydrolysis. CIM supernatants were analyzed by nanoLC-MS/MS.

Results: pgMLST of Enterobacter revealed eight genogroups differing ≤750 alleles from each other, and were characterized by varying resistomes, replicomes, MLST-types and minor class C-type carbapenemase genes. Enterobacter spp. with blaACT-20, blaACT-24, blaACT-25 produced a known carbapenemase and associated with E. hormaechei ST114 and ST78 and E. xiangfangensis ST121, respectively. blaACT-28 (11/54; 20%) and blaACT-28-like (97-99% identity, 17/54; 31%) alleles were enriched (28/54; 52%) in CIM+Carba- isolates which all yielded a positive CarbaNP test and 10/54 (19%) and 6/54 (11%) were E. kobei ST125 and ST32, respectively. The remainder CIM+Carba- isolates had various STs, contained blaCMH-1 (5/54; 9%), blaMIR-like (8/54; 7%) or other blaACT-variants (13/54; 20%), and were devoid of other resistance genes and replicons. CIM supernatants from E. kobei CIM+Carba- with blaACT-28 revealed a dominant protein on SDS-PAGE and nanoLC-MS/MS confirmed ACT-28 identity.

Conclusion: A proportion of the Enterobacter isolates submitted to the Dutch CPE surveillance carry class C-type carbapenemases, including ACT-28, and are associated with a positive CIM in the absence of major carbapenemase genes.

P101 Mapping the antibody repertoire to Staphylococcus aureus wall teichoic acid in invasive infection; a protective role for IgM?

Dr. Astrid Hendriks[1], S Ali[2], M.R.J. Varkila[3], J Haitsma-Mulier[3], C.J.C de Haas[4], P.C. Aerts[4], O.L. Cremer[5], M.J.M. Bonten[3,4], J.A.G. van Strijp[4], J.D.C. Codée[2], N.M. van Sorge[1,6]

[1]Department of Medical Microbiology and Infection Prevention, Amsterdam Umc, location AMC, University of Amsterdam, [2]Leiden Institute of Chemistry, Leiden University, [3]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, [4]Department of Medical Microbiology, University Medical Center Utrecht, [5]Department of Intensive Care, University Medical Center Utrecht, [6]Netherlands Reference Center for Bacterial Meningitis, Amsterdam Umc, location AMC, University of Amsterdam

Staphylococcus aureus is one of the leading causes of hospital-acquired infections with high overall mortality. Pre-existing immunity to S. aureus is common among healthy individuals due to natural exposure, although this is not always sufficient to protect from (re-)infection. Antibodies are believed to play a key role in bacterial killing through antibody opsonization and complement activation, which enhances bacterial uptake and neutrophil recruitment. A large proportion of the anti-S. aureus antibody pool is directed against Wall Teichoic Acid (WTA), an abundant cell wall glycopolymer, which shows structural variation through glycosylation with N-acetylglucosamine (GlcNAc). Overall, three WTA glycotypes are currently distinguished and IgG antibody responses to these distinct glycotypes are detected in healthy individuals. How these antibody profiles are affected during invasive S. aureus infection is not known but may help to identify protective responses. We analyzed the antibody repertoire to S. aureus WTA in plasma from healthy individuals (n=31) and patients with culture-confirmed S. aureus bacteremia (n=38) on the intensive-care unit (ICU) using in vitro-glycosylated synthetic WTA fragments that resemble the three S. aureus WTA glycotypes. Robust IgM responses to all three WTA modifications were detected in nearly all healthy individuals, whereas WTA-specific IgM responses were significantly decreased (three-fold lower) in patients with S. aureus bacteremia. No differences in IgG2 responses were observed. Moreover, patients that died on the ICU had even lower levels of WTA-specific IgM. For 13 patients, we correlated the WTA glycoprofile of the infecting S. aureus isolate to the detected WTA-specific antibody responses and observed that the absence of glycotype-specific IgM, but also decreased glycotype-specific IgG2 responses, was associated with patient mortality. This study supports the existence of a broad antibody repertoire to S. aureus WTA glycotypes, and hints towards a protective role of WTA-specific IgM against invasive S. aureus infections.

P102 Translocation of water during the cultivation of Agaricus bisporus

MSc. Koen Herman[1], Dr. RJ Bleichrodt[1]
[1]*Utrecht University*

Agaricus bisporus forms its mushrooms after colonised compost is topped by a layer of casing soil. Mushrooms contain ~90 % water. It is unclear to what degree the different parts of the culture supply water to the developing mushrooms. Therefore, we measured the water content, water potential and osmotic potential of distinct compost layers, casing soil, and mushrooms during the cropping process. A total of 1.1 kg of water was lost from the culture. 590 g of water was lost from the casing layer, compared to 210 g, 179 and 100 g from the top, middle and bottom compost layers respectively. Water can translocate from areas with high- to areas having lower water potentials. After harvesting two flushes, the water potential of the casing layer decreased from 0 to -1.7 MPa, whilst the water potential of the compost (-2 MPa) decreased to -2.9, -3 and -2.4 for the top, middle and bottom layer, respectively. Our results show (i) the importance of the casing and the top compost layer in supplying the mushrooms. During the 1st flush the osmotic potential of the fruiting bodies (-0.9 MPa) already equaled the water potential of the casing (-0.9 MPa). Paradoxically, this suggests that (ii) fruiting bodies would not be able to extract water from the casing after the 1st flush, and (iii) could not extract water from the compost. In reality, mushrooms do extract water from both layers. We point to the turgor of the mycelium during fructification as a missing link in understanding the flow of water to developing mushrooms.

P103 Investigation of a potential compound modulating Esx-5 secretion system in Mycobacteria

Mr Quang Tri Vien Ho[1]

[1]*Amsterdam University Medical Center-location VU*

Tuberculosis caused by Mycobacterium tuberculosis is still one of the most dangerous infectious disease nowadays. Mycobacterium tuberculosis and its closed genetic relative Mycobacterium marinum possess Esx-5 secretion system which is responsible for importing nutrients or virulence. Previously, we conducted a high-throughput screening of compounds that can target Esx-5 secretion system in Mycobacterium marinum. We identified compound 36 as one of the hit compounds from this screening whose antimicrobial activity has not been reported. Strikingly, we observed through immunoblot analysis that compound 36 was able to induce hypersecretion of Esx-5 substrate PE-PGRS proteins in Mycobacterium marinum. Proteomic analysis of secreted fraction confirmed this finding as bacteria treated with compound 36 showed highly abundant amount of one of the PE-PGRS protein. Moreover, this compound also increased susceptibility of Mycobacterium marinum towards detergent SDS. More importantly, compound 36 was able to reduce bacterial burden in zebrafish model infected with Mycobacterium marinum. In conclusion, we have identified a potential compound which can modulate Esx-5 secretion system in mycobacteria and possess in vivo activity.

P104 Mobile colistin resistance mcr-4-plasmids in livestock- and human-retrieved Enterobacterales isolates in the Netherlands

Casper Jamin[1], Dr. M. Brouwer[2], Dr. K. Veldman[2], S. Witteveen[3], F. Landman[3], Dr. E. Heddema[4], Prof. Dr. C. Hoebe[1,5], Prof. Dr. P. Savelkoul[1,6], L. van Alphen[1], A. P. A Hendrickx[3]
[1]Mumc+, [2]Wageningen Bioveterinary Research, [3]National Institute for Public Health and the Environment (RIVM), [4]Zuyderland Medical Center, [5]South Limburg Public Health Service, [6]Amsterdam UMC, location VUmc

Introduction
Since the first report of the mobile colistin resistance gene mcr-1, ten mcr variants were identified among antibiotic-resistant Enterobacterales and were mostly located on plasmids. Until now, the presence of mcr-4 has not been described before in The Netherlands. The major objective of this study was to analyse mcr-4 encoding plasmids from Enterobacterales spp. obtained from humans and livestock in The Netherlands.
Methods
Mcr-4-containing Enterobacterales isolates were collected for either National surveillance or typing purposes by the RIVM, WUR and Zuyderland MC. Isolates were subjected to short-read and long-read sequencing. Hybrid assembly of short-read and long-read sequencing data was performed using Unicycler to reconstruct mcr-4 plasmids, which were compared to mcr-4 plasmid sequences from NCBI using BioNumerics. Colistin resistance was determined by broth microdilution.
Results
Seven highly similar (76-99%) and small (12.8 kb) mcr-4 plasmids with a ColE10 replicon were identified with either a mcr-4.3 allele in four human Enterobacter cloacae complex isolates (MIC colistin <1 mg/L), or a mcr-4.6 allele in three Escherichia coli livestock isolates (MIC colistin 4-8 mg/L) collected between 2012-2020. The mcr-4 plasmids identified in the Netherlands were distinct from the mcr-4 plasmids in the NCBI database. The Dutch mcr-4 plasmid architecture was comparable and comprised the phosphoethanolamine lipid A transferase gene mcr-4, followed by a type II toxin/antitoxin system, TraD conjugal transfer protein, MobA/X mobilization proteins and transposases. Dutch livestock-retrieved E. coli isolates with mcr-4.6 plasmids differed from the human-derived plasmids by a single SNP in the mcr-4.3 gene, leading to a mcr-4.6 allelic variant.

Conclusion
The occurrence of mcr-4 plasmids among Enterobacterales is low in the Netherlands. The mcr-4.6 plasmid may cause decreased colistin susceptibility in livestock E. coli, while mcr-4.3 in E. cloacae complex not. The mcr-4 plasmids from human and livestock were highly similar, indicating the zoonotic potential of colistin resistance.

P105 Serology reveals LIPyV as a feline polyomavirus rather than a human polyomavirus

Md Sergio Kamminga[1], PhD PZ van der Meijden[1], PhD CB Buck[2], PhD P Pesavento[3], PhD MCW Feltkamp[1]
[1]Leiden University Medical Center, [2]National Cancer Institute, [3]UC Davis

Introduction
In recent years, the number of known human polyomaviruses (HPyVs) has increased rapidly. HPyVs cause disease in long-term immunocompromised patients, such as nephropathy (BKPyV) in kidney transplant patients, and PML (JCPyV) in Natalizumab-treated MS patients, while healthy individuals remain asymptomatically infected. The Lyon-IARC polyomavirus (LIPyV) was discovered in 2017 in a human skin swab sample and grouped in species Human polyomavirus 14. However, LIPyV seroprevalence determined in blood donors was much lower (6%) compared to most HPyVs. Furthermore, LIPyV has been detected in faeces samples from a small number of cats suffering from diarrhea. Here, we investigate seroresponses against LIPyV and other HPyVs, among cats and dogs, in comparison to healthy humans and present evidence that LIPyV should be considered a feline rather than a human polyomavirus.
Methods
Serologic analyses based on a customized Luminex immunoassay were preformed to detect LIPyV and other HPyV VP1 IgG antibodies in 38 dog and 40 cat serum samples, and in 87 sera from healthy humans. An arbitrary cut-off for seropositivity was set at 1500 MFI.
Results
The seroresponse against LIPyV was significantly higher in cat serum samples compared to both dog and human serum samples. On average, cats serum samples had an Median Fluorescence intensity (MFI) value of 13100 MFI, which higher than the dog serum samples (1296 MFI, Mann-Whitney U-test $P<0.001$) and human serum samples (69 MFI, $P<0.001$). Moreover, the LIPyV seroprevalence was extremely high (92.5%) among cats compared to humans (2.3%) and dogs (31.6%). Crossreactivity against other HPyVs was not observed in the cats.
Conclusions
The high seroprevalence and the high intensity of serologic responses against LIPyV in the cat serum samples compared to human sera suggests LIPyV to be a feline virus, rather than a human virus, Whether LIPyV infection can trigger diarrhea in cats remains to be established.

P106 Unravelling the viremia of enterovirus-D68

Dr Brigitta Laksono[1], Syriam Sooksawasdi Na Ayudhya[1], Muriel Aguilar Bretones[1], Carmen Embregts[1], Laurine Rijsbergen[1], Lisa Bauer[1], Gijs van Nierop[1], Rik de Swart[1], Debby van Riel[1]
[1]*Erasmus Mc*

Introduction
Enterovirus-D68 (EV-D68) is an emerging respiratory virus that often causes mild respiratory infections. However, it can also lead to severe respiratory infections, systemic spread and central nervous system (CNS) complications, especially acute flaccid myelitis (AFM). EV-D68 can also be detected outside the respiratory tract, but the mechanism of systemic spread is not understood. In this study, we aim to unravel the viremia of EV-D68.

Methods
We inoculated human peripheral blood mononuclear cells (PBMC), activated B cells, and monocyte-derived dendritic cells (DCs) with EV-D68. Cells and supernatants were collected at several time points for detection of viral capsid protein VP1, viral RNA replication and production of new virus particles.

Results
We found that PBMC B cells were infected by the virus, without evidence for infection in T cells and monocytes. However, we did not detect any virus production, which might be related to the resting state of these cells. In order to get more insight into the role of B cells, we included activated B cell models and found that these cells were not only susceptible to infection, but also able to facilitate replication and production of new infectious virus particles. To understand how EV-D68 can reach activated B cells, which are mostly located in lymphoid tissues, we inoculated DCs and found that these cells were susceptible and permissive to EV-D68 infection and could spread the virus to B cells.

Conclusion
We conclude that immune cells play an important role in the systemic spread and viremia during an EV-D68 infection, which seems to be the essential step towards development of extra-respiratory tract complications, including AFM. As the incidence of EV-D68-associated CNS complications increased in the last decade, combined with the emerging potential of the virus, it is important to understand the pathogenesis of EV-D68 infection to counteract the disease.

P107 Elucidating Haemophilus influenzae type b epidemiology in the Netherlands using whole genome sequencing

Dr. Rob Mariman[1], Dr. L. Visser[1], Dr. A. Steens[1], C. Schot[1], Dr. A. Van Der Ende[2], B. Van Der Putten[2], K. Chung[4], Dr. K. Trzcinski[3], W. Miellet[3], Dr. N. Van Sorge[2], Dr. T. Bosch[1]
[1]RIVM, [2]Amsterdam UMC, University of Amsterdam, [3]UMC Utrecht, [4]Leiden University Medical Center (LUMC)

Introduction
Haemophilus influenzae serotype b (Hib) is the cause of severe invasive diseases like meningitis and sepsis. In the Netherlands, childhood vaccination was implemented in 1993, resulting in a low but fluctuating incidence. From 2000 onwards, the number of Hib cases is gradually increasing. In 2020 and 2021, we noted an unexpected faster increase in vaccinated and non-vaccinated individuals.
In this study, we aimed to elucidate the changes in epidemiology of invasive Hib by genotypic characterization of clinical isolates.

Methods
Hib isolates from 276 children diagnosed with invasive Hib infection were obtained from the -------collection of the NRLBM. Of these, 20 were randomly selected from the pre-vaccine era and 256 strains, from both vaccinated and unvaccinated children, represented the vaccine era. All Hib isolates were subjected to whole-genome-sequencing. Cluster analyses using core genomes, comprising 1599 genes, were performed by minimum spanning tree (MST) algorithm in Grapethree.

Results
Of 276 isolates, 254 (92%) belonged to either Sequence Type (ST)-6 or ST-190. Clinical isolates in the MST did not cluster based on year of isolation, age, vaccination status, or invasiveness. However, principal component analysis (PCA) on the binary transformed core genome data revealed three distinct clusters of isolates within the dominant ST-6. One cluster that appeared after the introduction of the vaccine is gradually increasing and now comprises 81% of all clinical isolates. Statistical analysis identified 71 genes that were significantly different in any of the comparisons between the three clusters. Among these, genes encoding Immunoglobulin A1 protease autotransporter and Outer membrane protein P1 might be of interest in the context of disease.

Conclusions
The preliminary data suggests that the recent increment in Hib cases may be caused by expansion of a more successful genotypical Hib cluster. Ongoing research will be presented that focuses on the genes that drive these clusters.

P108 Staphylococcus aureus langerin binding is regulated by cell wall glycan presence and glycosyltransferase modifications

Jasper Mol[1], Dr. Astrid Hendriks[1,2], Dr. Rob van Dalen[1], Sacha Vrendenbarg[1], Prof. Dr. N.M. van Sorge[1,3]
[1]Amsterdam UMC, [2]UMC Utrecht, [3]Amsterdam UMC

The Gram-positive bacterium Staphylococcus aureus (S. aureus) is a major cause of skin and soft tissue infections, yet the local immune response upon bacterial invasion remains largely unknown. Langerhans cells (LCs) are the primary antigen-presenting cells in the upper skin layer, allowing early detection of incoming pathogens. We have previously identified that S. aureus is sensed by LCs through the C-type lectin receptor langerin. Langerin recognizes specific glycan modifications on wall teichoic acid (WTA), which are attached by the glycosyltransferases TarS and TarP. However, residual binding to S. aureus is observed in the absence of WTA glycosylation, suggesting the presence of a secondary langerin ligand. Here, we aimed to unravel the full repertoire of langerin-binding ligands on the S. aureus surface and determine the impact for LC activation. Using synthetic WTA fragments, we demonstrated that the WTA glycosylation by either TarS or TarP is sufficient for human langerin binding. Treatment of WTA glycan-deficient S. aureus with trypsin reduced interaction with recombinant langerin, suggesting that the putative secondary langerin ligand is a surface-exposed protein. Using S. aureus cell wall extracts for lectin blot analysis, revealed that the langerin protein target has a high molecular weight around 200 KD. We are currently testing the hypothesis that the serine-rich repeat protein SraP, which is one of several glycosylated cell wall proteins, is the target for langerin in addition to WTA. Understanding the pathways and mechanisms involving S. aureus recognition by LCs and subsequent skin immune responses could assist in developing strategies to prevent or treat infections.

P109 Experimental evidence of the large-scale flexibility in Pseudomonas fluorescens genome

Dr. Pabitra Nandy[1], Dr. Jenna Gallie[1]
[1]*Max Planck Institute For Evolutionary Biology*

Microorganisms in natural niches often have to survive conditions that are not well-suited for growth. In order to survive in diverse niches, bacterial genomes often have redundancies built into them that shield beneficial genes from mutational hazards. Pseudomonads are remarkably diverse, featuring soil dwellers, plant dwellers, as well as important human pathogens. Naturally, their genomes are diverse, with synteny between species being maximum near the origin of replication and minimal near the terminus of the genome.

We evolved a population of Pseudomonas fluorescens SBW25 under prolonged starvation conditions in the laboratory. After 6-months, we isolated a starvation-adapted mutant (termed SP2), and identified two mutations. The first is a massive deletion of 213,799 bp around the terminus region (Δbig), and the second is a shorter, 99 bp deletion in pyrB (ΔpyrB). Genomic analysis of Δbig revealed 178 genes, many of which lack annotation. Further functional classification of the deleted region uncovered an abundance of amino acid transporters. We constructed the Δbig deletion in the wild-type background (SBW25-Δbig), and observed that this massive deletion has no noticeable effect on growth dynamics. Furthermore, across diverse stress factors like antibiotics, and incubation temperatures, the SBW25-Δbig behaves similarly to the wild-type, indicating no effect on stress responses. The molecular mechanism by how the deletion increases survival under nutrient-depleted conditions is currently being studied.

Conclusion
1. These results indicate a high degree of flexibility near the terminus of P. fluorescens SBW25 genome. The genomic redundancy is so much that we have not yet detected a way in which the absence of almost 3.1% of the genome would significantly affect the microorganism's growth dynamics or stress response.

P110 No evidence of aberrant amyloid β and phosphorylated tau expression in herpes simplex virus-infected neurons of the trigeminal ganglia and brain

MSc DN Tran[1], MSc ATCM Bakx[1], Dr V van Dis[2], Dr. E Aronica[3], Dr RM Verdijk[2], Dr Werner Ouwendijk[1]
[1]Erasmus MC , [2]Erasmus MC, [3]Amsterdam University Medical Centers

Increasing evidence supports the role of neurotropic herpes simplex virus 1 (HSV-1) in the pathogenesis of Alzheimer's Disease (AD). However, it is unclear whether previously reported findings in HSV-1 cell culture and animal models can be translated to humans. Here, we analyzed clinical specimens from latently HSV-1 infected individuals and individuals with lytic HSV infection of the brain (herpes simplex encephalitis; HSE). Latent HSV-1 DNA load and latency-associated transcript (LAT) expression was identical between trigeminal ganglia (TG) of AD patients and controls. Amyloid β (Aβ) and hyperphosphorylated tau (pTau) were not detected in latently HSV-infected TG neurons. Ageing-related intraneuronal Aβ accumulations, neurofibrillary tangles (NFT) and/or extracellular Aβ plaques were observed in brain of some HSE patient, but these were neither restricted to HSV-infected neurons nor brain regions containing virus-infected cells. Analysis of unique brain material from an AD patient with concurrent HSE showed that HSV-infected cells frequently localized close to Aβ plaques and NFT, but were not associated with exacerbated AD-related pathology. HSE-associated neuroinflammation was not associated with specific Aβ or pTau phenotypes. Collectively, we observed that neither latent nor lytic HSV infection of human neurons is directly associated with aberrant Aβ or pTau protein expression in ganglia and brain.

P111 Bordetella pertussis in the Netherlands, 2015-2021: a sharp increase in pertactin (PRN)-deficient isolates

Gerlinde Pluister[1], Rob Mariman[1], Corrie Schot[1], Linda Visser[1], James Groot[1], Frans Reubsaet[1], Ramón Noomen[1], Thijs Bosch[1]
[1]*National Institute for Public Health and the Environment (RIVM)*

Background
In the Netherlands, pertussis notifications resurged since 1996, likely due to strain adaptation. We applied molecular methods to characterize B. pertussis clinical isolates with emphasis to vaccine antigens. Here, we report on the surveillance data from 2015 to 2021.

Method
All B. pertussis samples were cultured and suspected colonies were subjected to MALDI-TOF MS for species confirmation, followed by whole genome sequencing (WGS). The resulting data were used for identifying the pertussis toxin (ptxA, ptxP), fimbriae (Fim3) and pertactin (Prn) genotypes. Furthermore, core-genome MLST, using an in-house scheme consisting of 3,180 genes, was used to infer genetic relationships between the isolates.

Results
WGS revealed that 99% of all our 223 B. pertussis isolates that have been collected the past 6 years had a ptxA1 and ptxP3 genotype. A minimum spanning tree (MST) based on cgMLST, showed limited variation within the Dutch B. pertussis population with an average distance of 5 genes between two neighboring isolates. There was no clustering in the MST based on year of isolation or age, but there was a clear distinction between fim3-1 (39%) and fim3-2 (60%) isolates with a higher genetic diversity among fim3-1 isolates. The majority of isolates were prn-2, but no prn allele could be determined in 12% of the isolates isolated between 2015-2017. In 2018 and 2019, a sharp increase of Prn-deficient isolates was observed (24% of all), caused by multiple changes including inversion of ~22 kb in the promotor, IS481 element in the prn-gene, and the insertion of a stop codon.

Conclusions
The current Dutch B. pertussis population represents a homogenous group dominated by isolates that harbor ptxP3 and prn-2 as genotypes. In 2018-2019, a sharp increase in prn-deficiency strains was observed. In 2020-2021, COVID-19 related restrictions in society resulted in a sudden and dramatic drop of pertussis notifications.

P112 Waning Severe Acute Respiratory Syndrome Corona Virus 2 antibodies, a comparison of serological assays in Dutch blood donors.

FA Quee[1], PhD BM Hogema[1,2], M Molier[1], K Van den Hurk[1], HL Zaaijer[1,3]
[1]Sanquin Research, [2]Sanquin Diagnostic Services, [3]Amsterdam University Medical Centre

Introduction
To advise policy makers on preventive measures to combat the Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) pandemic, insight in the course of SARS-CoV-2 antibodies over time is important. To assess how long potentially protective anti-nucleocapsid (NC), anti-receptor binding domain (RBD), and anti-Spike (S) antibodies remain detectable in blood of infected or vaccinated individuals, longitudinal sero-surveillance studies are necessary. We studied the performance of various SARS-CoV-2 antibody assays in serial samples of infected donors.

Methods
7361 plasma donations collected April 1-15th 2020, were tested for presence of SARS-CoV-2 antibodies using the Wantai SARS-CoV-2 total Ab assay. When positive, earlier archived samples of these donors were tested to check for true seroconversion. Positive donors were followed over time by testing subsequent donations, using one or more of the following assays for detection of RBD or S antibodies: Wantai tAb assay, Euroimmun IgG ELISA, Euroimmun IgA ELISA, and Roche Elecsys assay; and two NC antibody assays: Abbott Architect, and Roche Elecsys.

Results
For prolonged detection of RBD and S antibodies, the Wantai and Roche RBD assay are superior to both Euroimmun ELISA's, probably because of their double antigen sandwich format. In the Wantai and Roche RBD assay more than 95% of donors still tested positive after 12 months, but only 49% respectively 36% in Euroimmun IgA respectively IgG. After 12 months, in the Roche NC assay 86% of donors still tested positive, in Abbott Architect NC only 3%.

Conclusion
The Wantai and Roche RBD assays detect antibodies long after initial infection, making them useful for studying whether persons ever experienced SARS-CoV-2 infection or vaccination. Architect NC shows rapid waning. However, this assay can be used when studying recent, re- and breakthrough infections. This study helps researchers to choose the right assay for specific purposes.

P113 Characterization of Streptococcus suis phase-variable type I restriction modification system SsuCC20p and its contribution to zoonotic potential

Thomas Roodsant[1,2], K van der Ark[1,2], B van der Putten[1,2], J Brizuela Gabaldon[1], C Schultsz[1,2]
[1]Amsterdam UMC, [2]AIGHD

Introduction
Streptococcus suis is an opportunistic pathogen in pigs and an emerging zoonotic pathogen. In the Netherlands, a unique zoonotic serotype 2 clonal complex (CC) 20 clade has diverged from a non-zoonotic serotype 9 CC16 clade. The zoonotic CC20 clade has acquired three genomic regions, of which one encodes a phase-variable type I restriction-modification system, named SsuCC20p. In Streptococcus pneumoniae it was shown that a phase-variable type I restriction-modification system regulates virulence. Here we characterize SsuCC20p and assess its potential role in virulence.

Methods
Quantification of phase-variable hsdS alleles was performed by fragment length analysis of endonuclease digested (SmlI) fluorescently labelled PCR products amplified from genomic DNA of strain 861160. Mutants containing single hsdS alleles ('locked') were generated and long read sequencing of isolated genomic DNA was used to quantify hsdS allele distribution in the wild type (WT) and to analyze genome methylation patterns in WT and locked mutant strains. Virulence of locked hsdS mutants was assessed in a zebrafish larvae infection model.

Results
Within sequence type 20, three unique and functional hsdS alleles were identified, which could all be detected within strain 861160. SsuCC20p expression was confirmed with RT-qPCR. Knocking out the recombinase xerD that is present within the SsuCC20p locus halted phase variation. Methylome analysis of the WT and hsdS knockout genomes showed that SsuCC20p actively methylates the genome and identified two different methylation patterns (GCAN^{5}GTC/GACN^{5}TGC and GCAN^{5}CTC/GAGN^{5}TGC). Pilot data of the zebrafish larvae infection show different zebrafish mortality after locked hsdS mutant infection.

Conclusion
SsuCC20p phase variability is xerD dependent and three different hsdS alleles can be detected within a single isolate. SsuCC20p is expressed and actively methylates the S. suis genome. Phase variation of SsuCC20p results in differential genome methylation and potential changes in virulence in a zebrafish larvae infection model.

P114 Transformation of Staphylococcus aureus is enhanced by inhibition of TagO

Ing. Kim Schipper[1], Msc. M.M. Kuijk[1], Msc. S.M. Tamminga[1], Dr. N.M. van Sorge
[1]*Amsterdamumc*

Background
Staphylococcus aureus (S. aureus) is a Gram-positive coccus that causes a wide range of community- and hospital-acquired systemic infections. Gene deletion or complementation is a commonly applied strategy to elucidate the role of a gene in disease pathogenesis. Uptake of foreign DNA by S. aureus, an essential step for genetic manipulation, is a bottleneck in this process and requires electroporation. Methods to improve S. aureus transformation would accelerate the process of mutant construction and potentially expand the range of transformable species or strains. We investigated whether specific cell wall modifications affect S. aureus transformation efficiency.
Methods
S. aureus strain RN4220 and RN4220 ΔtagO were made electrocompetent by standard and well-described procedures. One µg of pth100-GFP plasmid, resulting in green-fluorescent staphylococci upon successful transformation, was used to transform S. aureus by electroporation. Transformed cultures were recovered and plated on agar plates containing antibiotics.
Results
We observed that a S. aureus tagO knockout was more transformable than the isogenic wild-type strain. TagO knockout strains are devoid of wall teichoic acid (WTA), a peptidoglycan-anchored glycopolymer comprising between 30-70% of the cell wall mass. Tunicamycin is an antibiotic that specifically inhibits TagO at lower concentrations. Addition of 0.4 and 1 µg/ml tunicamycin during generation of S. aureus competent cells resulted in 200-400 GFP-positive colony forming units (cfu), whereas no colonies were visible in the absence of tunicamycin. Addition of tunicamycin to the TagO knockout did not improve transformation. This suggests that the effect of tunicamycin is occurring through inhibition of WTA biosynthesis and not through off-target effects.
Conclusion
Our data show that tunicamycin exposure during growth of S. aureus RN4220 improves transformation of S. aureus through electroporation. We are currently investigating whether this effect is also observed in other S. aureus strains and even other Gram-positive species that express TagO-homologues.

P115 Stapling of peptides potentiates the antibiotic treatment of Acinetobacter baumannii in vivo

Ms. Gina Schouten[1], Felix Paulussen[1], Oscar P Kuipers[2], Wilbert Bitter[1], Tom N Grossmann[1], Peter Van Ulsen[1]
[1]Amsterdam Umc, [2]Rijksuniversiteit Groningen

The rising incidence of multidrug resistance in Gram-negative bacteria underlines the urgency for novel treatment options. One promising new approach is the synergistic combination of antibiotics with antimicrobial peptides. However, the use of these peptides is not straightforward, often they are not stable and sensitive to proteolytic degradation, which greatly limits their clinical potential. One approach to increase stability is to apply a hydrocarbon staple to the antimicrobial peptide, thereby fixing them in an α-helical or β-sheet conformation, which renders them less exposed to proteolytic activity.

In this work we applied several different hydrocarbon staples to two previously described peptides shown to act on the outer membrane, L6 and L8, and tested their activity in a zebrafish embryo infection model using a clinical isolate of Acinetobacter baumannii as pathogen. Although some staples had a negative effect on the activity of the peptides, we could show that the introduction of a specific hydrocarbon staple to the peptide L8 improved its in vivo potentiating activity, without affecting other characteristics, such as in vivo antimicrobial activity, toxicity or haemolytic activity.

In conclusion, stapling of antimicrobial peptides is an interesting approach to improve their in vivo activity

P116 Nitrifying consortia support complex microbial communities in oxygen- and nitrogen-limited bioreactors

Dr. Garrett Smith[1], Dr. Maartje van Kessel[1], Dr. S. Lücker[1]
[1]*Radboud Universiteit*

One critical step of Wastewater Treatment (WWT) is nitrification, during which microbes perform ammonia oxidation followed by nitrite oxidation, or complete ammonia oxidation (comammox). A major constraint on the ecological success and physiology of the nitrifiers is thought to be substrate concentrations, but often multiple types of nitrifiers co-occur in WWT systems, and their interactions with each other and heterotrophs are not well understood. Two laboratory-scale bioreactors were inoculated with WWT biomass and incubated in tandem: the first fed with medium containing 1 mM ammonium and <3.1 µM dissolved oxygen, and the second receiving the nitrogen-depleted effluent and ~177 µM dissolved oxygen. Both genome- and gene-centric analyses of integrated long- and short-read metagenomes was used to examine the composition and reconstruct the metabolic capabilities of the microbial communities. Twelve high-quality, even circular, recovered genomes showed that, while typically multiple types of nitrifiers inhabit both bioreactors, ammonia-oxidizing Nitrosomonas and canonical nitrite-oxidizing Nitrospira prevailed under oxygen limitation while comammox Nitrospira dominated under nitrogen limitation. Ammonia oxidation marker genes were more numerous than the recovered genomes represent, and 10 divergent sequences were identified in the nitrogen-limited bioreactor that may be involved in the degradation of alkanes rather than nitrification. Unexpectedly, heterotrophs accounted for >70% of the communities, including fungi, and poorly characterized taxa with uncertain metabolic capabilities that may include recycling oxidized nitrogen to ammonia or nitrite, complicating predictions of nitrification performance of the reactors. Despite relatively minimal nitrogen inputs and no exogenous organic carbon sources, (1) reactor communities remained diverse and contained multiple nitrifying taxa, but did select for different dominant populations, and (2) extensive heterotrophic communities were involved in coupling oxidation of organic compounds to potential reduction of oxidized nitrogen to nitrite or ammonium, and thus recycling carbon and nitrogen. Further work will focus on reconstructing metabolic networks supporting these communities.

P118 Natural variation in presence and sequence of Staphylococcus aureus wall teichoic acid glycosyltransferases affects immune recognition

Sara Tamminga[1], S. Völpel[2], K. Schipper[1], Prof. Dr. T. Stehle[2], Dr. Y. Pannekoek[2], Prof. Dr. N.M. van Sorge[2]
[1]Amsterdam UMC, [2]University of Tübingen, [3]Amsterdam UMC

Staphylococcus aureus (S. aureus) is a leading cause of skin and soft tissue infections and (hospital-acquired) systemic infections. The S. aureus cell wall is decorated with wall teichoic acids (WTAs), which are cell wall-anchored glycopolymers with important roles in nasal colonization, endocarditis, and antibiotic resistance. WTAs consist of a polymerized ribitol phosphate (RboP) chain that can be glycosylated with N-acetylglucosamine (GlcNAc) by three glycosyltransferases: TarS, TarM, and TarP. TarS and TarP modify WTAs with β-linked GlcNAc on the C-4 (β1,4-GlcNAc) and the C-3 position (β1,3-GlcNAc) of the RboP subunit, respectively. TarM modifies WTA with α-linked GlcNAc at the C-4 position (α1,4-GlcNAc). Importantly, these WTA glycosylation patterns impact immune recognition and clearance of S. aureus. Previous studies suggest that tarS is near-universally expressed within the S. aureus population, whereas a smaller proportion co-express either tarM or tarP. Here we dissected the presence and genetic variation of tarS, tarM, and tarP in a collection of 25,132 S. aureus genomes within the PubMLST database. Over 99% of isolates contained tarS, with 36% and 7% of isolates co-expressing tarM or tarP, respectively. Co-expression of tarS/tarM or tarS/tarP was correlated to specific S. aureus clonal complexes (CCs). We also found 26 isolates (0.1%) that contained all three glycosyltransferase genes. Interestingly, we identified tar alleles with natural mutations in critical residues of the enzymes or with early stop codons, which may affect their function. To assess this experimentally, several tar variants were used to complement S. aureus tar-negative strains and WTA glycosylation was analyzed using specific monoclonal antibodies and human Langerin. Overall, our data provide more insight into the genetic diversity of the three WTA glycosyltransferases and demonstrates how this natural variation can affect S. aureus immune recognition by both the innate and adaptive immune system.

P119 Staphylococcal Protein A binding of secretory IgA antibodies limits nasal colonization by Staphylococcus aureus

Dr. Rob Van Dalen[1], E. Lehmann[1], S. Alber[1], M. Harms[1], Dr. C. de Haas[2], Prof. dr. A. Peschel[1]
[1]University Of Tübingen, [2]UMC Utrecht

Introduction: The human nasal microbiome is an important factor in human health. Importantly, it hosts opportunistic pathogens such as Staphylococcus aureus, which is a major risk factor for infections. The human host uses several mechanisms to control its nasal microbiome, including the production of secretory IgA (sIgA) antibodies. In the gut, these antibodies were shown to be able to both support colonization of commensal bacteria as well as facilitate removal of invasive pathogenic species through various mechanisms. However, the effect that sIgA has on the nasal microbiome is currently unknown.
Methods and Results: Initial flow cytometry experiments on the specificity profile of nasal sIgA indicated that it interacts with S. aureus in an epitope-independent manner. Instead, it was dependent on Staphylococcal Protein A (SpA) in the bacterial cell wall. SpA can bind antibodies by their Fc tail, as well as bind VH3-type antibodies by their F(ab) domains. Since SpA is unable to bind IgA-Fc, we speculated that SpA binds VH3-type sIgA in a F(ab)-mediated manner. Using S. aureus SpA mutants, SpA-fractionated sIgA pools and recombinant IgA antibodies we could indeed show this interaction to take place and cause agglutination of S. aureus cells. This raised the question whether sIgA binding of S. aureus is beneficial for nasal colonization or instead limits it. In a cotton rat nasal epithelial cell adhesion model, sIgA attenuated epithelial adhesion by S. aureus, suggesting it to be a host strategy that controls S. aureus nasal colonization.
Conclusion: We show (1) for the first time F(ab)-mediated binding of VH3-family sIgA antibodies, and (2) that the presence of these antibodies in the nose could limit S. aureus colonization through interaction with SpA. This highlights nasal sIgA as a potentially interesting target for anti-colonization strategies against S. aureus.

P120 Within-host genetic variation in Neisseria gonorrhoeae over the course of infection

Drs J De Korne-Elenbaas[1], Dr SM Bruisten[1], Prof HJC De Vries[1,3], Dr. Alje Van Dam[1,2]
[1]Public Health Service, [2]Amsterdam UMC, [3]Amsterdam UMC

Knowledge of within-host genetic variation informs studies on transmission dynamics. We studied within-host genetic variation in Neisseria gonorrhoeae over the course of infection, and compared this to genetic variation across different anatomical locations and between hosts . Isolates were obtained during a clinical trial, and isolates from consecutive time points reflected persistent infections after treatment failure. We compared sequence types and recombination unfiltered- and filtered core genome SNP distances between 65 within-host isolate pairss from the same anatomical location over time obtained with a median interval of 7 days and 65 isolate pairs across different anatomical locations at one time point. Isolates with different MLST, NG-Sequence Typing for Antimicrobial Resistance (NG-STAR) and NG-Multi Antigen Sequence Type (NG-MAST) STs had a median of 1466 recombination filtered SNPs, whereas a median of 1 SNP was found between isolates with identical STs or with only a different NG-MAST. The threshold for indistinguishable isolates was set at <10 recombination filtered SNPs, showing that NG-MAST might erroneously discriminate between isolates that are indistinguishable based on SNPs. Comparable extents of genetic variation were observed over time and across different anatomical locations, indicating that antibiotic pressure and the host immune response did not trigger genetic variation during the studied infection period. Instead, genetic variation was mainly caused by recombination events, concentrated in genomic regions encoding hypervariable proteins. Ultimately, comparisons between 228 isolate pairs from different participants showed indistinguishable isolates in 6.5% of pairs, suggesting between-host transmission. Because within- and between-host isolates can be indistinguishable based on SNPs, additional epidemiological data and participant reported data are always needed to differentiate within-host persistence from between-host transmission.

P121 Analysis of erythromycin resistant Campylobacter coli from slaughter pigs and veal calves in the Netherlands using whole genome sequencing

MSc M. Van der Most[1], dr. M.S.M. Brouwer[1], F.L. Harders[1], A. Kant[1], A. Van Essen-Zandbergen[1], J.J. Testerink[1], drs. Y. Geurts[1], dr. K.T. Veldman[1]
[1]*Wageningen Bioveterinary Research, Wageningen University & Research*

Introduction
Due to new legislation, from 2021 onwards, the mandatory monitoring of antimicrobial resistance in zoonotic and commensal bacteria in livestock, includes Campylobacter jejuni and C. coli obtained from veal calves and C. coli from slaughter pigs. Since macrolides are the first-choice drugs for the treatment of campylobacteriosis in humans, erythromycin resistant Campylobacter isolates from veal calves and slaughter pigs, were analyzed using whole genome sequencing to determine the mechanism of resistance.

Methods
Annually at least 170 Campylobacter isolates obtained from caecal samples of both veal calves and slaughter pigs, are screened for antimicrobial susceptibility to a mandatory panel of antimicrobials using broth microdilution according to EUCAST guidelines. Retrospectively, all Campylobacter isolates collected in the first four months of 2021 with high resistance to erythromycin (Minimum Inhibitory Concentration: ≥ 512 mg/L), were sequenced with Illumina Hiseq short read sequencing. The assembled genomes were analyzed with Resfinder, Pointfinder, starAMR, abricate and sraX to unravel the molecular basis of the erythromycin resistance.

Results
From January to April 2021 a total of 90 C. jejuni and 170 C. coli isolates were tested for antimicrobial susceptibility. As a result 2.6% (3/116) and 31,5% (17/54) of the C. coli isolates from slaughter pigs and veal calves respectively were highly resistant to erythromycin. For all 20 isolates the phenotypic resistance to erythromycin was confirmed by molecular diagnostics. A A2075G substitution in 23S ribosomal RNA was found in all the erythromycin resistant isolates. No other mutations or genes associated with erythromycin resistance were detected.

Conclusion
The mandatory monitoring of antimicrobial resistance in Campylobacter from veal calves and slaughter pigs in the Netherlands, resulted in the detection of C. coli isolates resistant to erythromycin in both animal species due to a A2075G substitution in 23S ribosomal RNA. Importantly, no transferable macrolide resistance genes were detected.

P122 Case report of a mixed SARS-CoV-2 infection of Alpha and Delta variant in an outbreak investigation

Dr. Brian Van Der Veer[1], Dr. Jozef Dingemans[1], Msc Koen Gorgels[2], Dr. Volker Hackert[2], Dr. Casper den Heijer[2], Dr. Lieke van Alphen[1], Prof. Dr. Christian Hoebe[1,2], Prof. Dr. Paul Savelkoul[1]
[1]Maastricht University Medical Centre+, [2]South Limburg Public Health Service

Background
In June 2021 many restrictions like maintaining 1.5 meter distance were lifted and large gatherings were reopened. Attendees had to show a corona entree ticket that included a complete vaccination, COVID-19 recovery (valid 180 days) or negative test for SARS-CoV-2 (valid 36 hours).Two weeks later number of cases increased nearly 20-fold. Hence, outbreak investigation was initiated in which a potential mixed SARS-CoV-2 infection was observed in whole-genome sequence (WGS) analysis. Here, we describe a case report of the individual with a confirmed mixed infection.

Methods
WGS was performed with the Oxford Nanopore platform and data was analyzed with the MACOVID v2.0.1. pipeline. The sample of the potential mixed infection was repeated, including RNA isolation, to confirm the observed mixed bases.

Results
The individual of this case report was a female 20 years of age with a positive SARS-CoV-2 PCR-test (Ct-value 25). This individual was infected with the Alpha-variant but >15 positions showed mixed bases with an average occurrence 18.1% (SD 9.8%) and were confirmed in the repeated sample. In addition to the mixed bases, deletions that typically occur in the Delta-variant were observed. A new consensus sequence was constructed using all minor variant mutations, SNPs and deletions, that confirmed that the minor variant was the Delta-variant. This particular Delta-variant was not linked with any of the clusters in the outbreak investigation.

Conclusions
In the outbreak investigation we observed a mixed infection that consisted of 82% Alpha and 18% Delta-variant but was not linked with any of the outbreak clusters. Though this is the only mixed infection we have observed, these mixed infections could have an impact on for example monoclonal antibody treatments. In addition, SARS-CoV-2 might recombine as two variants could be present in the same cells potentially leading to more virulent strains.

P123 IMPACT OF CULTURE-ENRICHMENT ON RANKED PNEUMOCOCCAL SEROTYPE CO-PRESENCE DETERMINED USING MOLECULAR DIAGNOSTIC METHOD OF QUANTITATIVE PCR

B.a. Janieke Van Veldhuizen[1], M.S. W.R. Miellet[2], dr. A.J. Wijmenga-Monsuur[1], prof. dr. E.A.M Sanders[1], dr. N.Y Rots[1], dr. R. Mariman[1], PhD, DVM K. Trzcinski[2]
[1]National Institute for Public Health and the Environment (RIVM), [2]University Medical Centre Utrecht

BACKGROUND
We investigated the impact of culture-enrichment on the rank of serotypes when detected quantitatively in nasopharyngeal samples from toddlers co-carrying multiple pneumococcal strains.

METHODS
Pneumococcal serotypes were detected in nasopharyngeal swabs collected in a cross-sectional study from children aged <5 years using conventional culture method with Quellung serotyping followed by testing DNA extracted from all bacterial growth harvested from Sheep Blood-7 gentamicin plates in serotype-specific qPCRs covering 28 serogroups. Next, we quantified DNA extracted from uncultured samples with serotype-specific qPCRs and compared serotype ranks before and after culture-enrichment.

RESULTS
Co-presence of multiple serotypes was detected in 36 (5.5%) of 658 nasopharyngeal samples. The number of serotype carriage events detected by any method in these 36 samples was 81 (2-5 per sample) while the number of strains cultured and serotyped with Quellung was 41 (1-2 per sample). One of these 41 strains was of a serotype (24F) not covered with serotype-specific qPCRs. For the majority of samples (n=22 or 61.1%) the serotype ranks in uncultured and culture-enriched samples matched. A shift in the serotype rank was observed in 19.4% of the samples (7 of 36) whereas in another 8.3% (3 of 36) the discordance was due to fewer serotypes detected in the DNA of uncultured samples. In 8.3% of the samples (3 of 36) none of the serotypes detected with qPCRs matched the conventional culture with Quellung.

CONCLUSION
While increasing sensitivity of carriage detection, culture-enrichment has limited impact on the rank of serotypes present in nasopharyngeal swabs and can therefore be used to provide insight into co-carriage dynamics.

P124 Air-liquid culture system of primary epithelial cells could serve as highly differentiated model to investigate host-pathogen interactions at the epithelial site of the respiratory tract

Esther Van Woudenbergh[1,2], N van Heerbeek[3], J Honings[4], G den Hartog[1], MI de Jonge[2]
[1]Rijksinstituut Voor Volksgezondheid En Milieu, [2]Radboud Institute for Molecular Life Sciences , [3]Radboud University Medical Centre, [4]Radboud University Medical Centre

The respiratory epithelium is the first cellular layer in the human body that encounters airborne pathogens. Some pathogens, such as Streptococcus pneumoniae reside as a commensal at a certain region of the upper respiratory tract, while they can cause disease when they disseminate to lower respiratory regions. When studying host-pathogen interactions it is important to use a model system that closely mimics the natural conditions of the respiratory tract to investigate its properties, and role during viral and bacterial infections. For this purpose, we obtained primary epithelial cells from the nose (n=7), nasopharynx (n=3), larynx (n=2) and bronchiae (n=4) from different donors (8 male, 9 female; 17-64 years old). At this moment, we cultured primary human nasal epithelial cells from four different anatomical locations of the nose on an air-liquid interface and tracked the epithelial differentiation for seven weeks via immunofluorescence, and haematoxylin and eosin staining. After two weeks of differentiation the cells started to form a tight epithelial layer where the epithelial cells expressed ZO-1, a tight junction marker. Ciliated cells as well as goblet cells, and secretory cells started to appear after one week of differentiation and became more abundant over time. The length of the cilia increased up to five weeks of differentiation, and organized ciliar beating could be observed. Most strikingly, the thickness of the epithelial layer, length of the cilia, and abundance of the various cell types differed between the anatomical locations within the nose, which could also partly be due to donor-to-donor variation. Preliminary data shows that these differences are even more striking between the epithelia originating from different regions of the respiratory tract. In future research, we will use this in vitro primary respiratory epithelial cell model from different regions of the upper respiratory tract to investigate host-pathogen interactions at the epithelial site.

P125 Preferred immunogenic regions in surface-associated antigens of Staphylococcus aureus strains implicated in bovine mastitis

Msc Elias Vera Murguia[1], Dr Yaremit Mora Hernandez[1], Dr Natacha Monge Gomes do Couto[2], MSc Anastasia Revtova[1], Dr Jan Maarten van Dijl[1], Dr John W. A. Rossen[3], Dr Girbe Buist[1]
[1]Universitair Medish Centrum Groningen, [2]University of Bath, [3]University of Utah

Mastitis is an infection of the mammary gland that is commonly associated with S.aureus. Within the dairy industry approximately 11% of milk production is affected due to mastitis. In addition, bovine-mastitis is a risk for human health due to possible ingestion of bacterial toxins/antibiotic residues upon treatment. Vaccination of cows against S.aureus antigens is a promising approach to prevent bovine-mastitis, but effective vaccines aren't available. In this study, a reversed vaccinology approach, from in silico to the functional analyses with mastitic-cow sera, was applied to pinpoint domains of selected S.aureus surface proteins to identify highly immunogenic candidate antigens.

Methods
A combination of bioinformatic and immunological approaches were used to identify conserved immunogenic determinants of S.aureus mastitic isolates, focusing on cell surface-exposed proteins. Conserved potential immunogenic epitopes of 24 proteins were selected using antigenicity plots in the CLC-workbench. Further the B-cell epitope prediction tool Bepipred was used to compare those conserved predicted epitopes with the epitopes that showed conserved antigenicity. IsaA, Sle1, Aly and LytM surface proteins and their domains were purified to test their antigenicity with sera from infected cows to evaluate their immunogenic potential.

Results
20 conserved surface proteins were identified in the genome sequences of 63 S.aureus mastitis isolates. Using the CLC-pipeline and Bepipred, the respective sequences were aligned and, based on antigenicity and surface accessibility, conserved epitopes were identified in the selected surface proteins. Further, immunological assays using sera from twelve cows with mastitis revealed IgG responses against the four cell surface located cell wall hydrolases and their separate subdomains.

Conclusion
A bioinformatic pipeline was established to identify strictly conserved potentially immunogenic epitopes from surface proteins in S.aureus strains implicated in bovine-mastitis. The immunological assays highlight differential antibody response of sera from mastitic-cows to particular staphylococcal antigens, including cell wall hydrolases, some representing potential candidate vaccine targets.

P126 NO-forming nitrite reductases in the anaerobic ammonium oxidizer Kuenenia stuttgartiensis

Femke Vermeir[1], Wouter Versantvoort[1], Laura van Niftrik[1]
[1]*Radboud University*

Anaerobic ammonium-oxidizing (anammox) bacteria play a vital role in the loss of fixed nitrogen in nature as well as in sustainable wastewater treatment plants. These intriguing bacteria are the only organisms known to use toxic hydrazine (N2H4) as a free metabolite, making them highly interesting from a scientific and industrial point of view. N2H4 is produced as an important intermediate in the anammox reaction of which the one electron reduction of nitrite (NO2-) to nitric oxide (NO) by nitrite reductase is the first step. Interestingly, within the different anammox genera there is no universal nitrite reductase expressed carrying out this reaction. For instance, in Kuenenia stuttgartiensis there are three possible candidates: a membrane bound nitrite reductase, the soluble HAOr and the soluble NirS. Having multiple nitrite reductases could be an advantage for the bacterium, such as the possibility to utilize different electron donor pools or localize nitrite reductases in different parts of the cell. In this project, different NO-forming nitrite reductases are isolated directly from native K. stuttgartiensis biomass to shine light on this puzzling distribution of these enzymes and their role in the anammox reaction. Initially, membrane proteins were separated from soluble proteins and NO production from NO2- was measured using gas chromatography mass spectrometry (GCMS) for both fractions. Strikingly, both membrane proteins and soluble proteins produced similar amounts of NO. Fast protein liquid chromatography on the soluble proteins resulted in two distinct protein fractions producing NO from nitrite. Thus, K. stuttgartiensis seems to utilize all three putative nitrite reductases at its disposal to provide NO as a substrate for N2H4 synthesis.

P127 Effectiveness of commonly used contact lens disinfectants against SARS-CoV-2

MSc J.M.J. Veugen[1,2,3,4], Prof. dr. R.M.M.A. Nuijts[1,2,5], Dr. F.J.H.M. van den Biggelaar[1,2], Dr. M. Gijs[1,2], Prof. Dr. P.H.M. Savelkoul[3,6], Dr. P.F.G. Wolffs[3,4], Dr. M.M. Dickman[1,2]

[1]Maastricht University Medical Center+, [2]Maastricht University, [3]Maastricht University Medical Center+, [4]Maastricht University, [5]Zuyderland Medical Center, [6]Amsterdam Infection and Immunity Institute, Amsterdam UMC, Vrije Universiteit Amsterdam

Introduction: Insufficient contact lens (CL) hygiene may result in microbial keratitis among wearers. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was detected in conjunctival swabs of coronavirus disease 2019 (COVID-19) patients. The purpose of this study was to assess the effect of commonly used CL disinfectants against SARS-CoV-2, in particular the effect of the rub-and-rinse step on disinfection efficacy.
Methods: The efficacy of five disinfectant solutions was tested in the presence and absence of CLs. Three types of unused CLs (hard gas permeable, soft hydrogel, and soft silicone hydrogel) and worn silicone hydrogel CLs were tested. CLs infected with SARS-CoV-2 were disinfected at various times, with and without rubbing and rinsing, per manufacturer's instructions. Reverse transcription-polymerase chain reaction (RT-PCR) and viability PCR were applied to detect SARS-CoV-2 RNA and viral infectivity of SARS-CoV-2, respectively.
Results: When disinfectant solutions were used according to manufacturer's instructions, no SARS-CoV-2 RNA could be detected. When CLs were disinfected without the rub-and-rinse step, SARS-CoV-2 RNA was detected at almost each time interval with each disinfectant solution tested, for new and worn CLs. By applying disinfectant solutions directly to SARS-CoV-2 containing samples (in the absence of CLs), viable SARS-CoV-2 was detected in all disinfectant solutions except Menicon Progent.
Conclusions: Disinfectant solutions effectively disinfect SARS-CoV-2 from CLs if manufacturer's instructions are followed. The rub-and-rinse regimen is mainly responsible for disinfection.

P128 Linking the longitudinal development of the piglet nostril microbiome to methicillin-resistant Staphylococcus aureus carriage as a strategy to identify nasal probiotic strains.

Msc. Abel Vlasblom[1], Dr. S. Patel[2], Dr. C. Huseyin[2], Dr. M. Claesson[2], Dr. D. Crespo-Piazuelo[3], Dr. L. Wadja[3], Dr. P.G. Lawlor[3], Dr. K.M. Verstappen[1], Dr. A.C. Fluit[4], Msc M.R.C. Rogers[4], Dr. J.A. Wagenaar[1,5], Dr. A.L. Zomer[1], Dr. B. Duim[1]

[1]Utrecht University, [2]University College Cork, [3] Animal & Grassland Research and Innovation Centre, Teagasc, [4]University Medical Centre Utrecht, [5]Wageningen university and research

Introduction

The reservoir of livestock associated methicillin-resistant Staphylococcus aureus (LA-MRSA) in pig farms forms a potential zoonotic risk for society. Here we present the results of a European project, and its pilot, that aimed at investigating the development of the porcine nasal microbiome, while identifying negatively associated bacteria suitable for interventions reducing LA-MRSA. We successfully described the developing nasal microbiome, and isolated strains currently tested for efficacy against S. aureus in vivo.

Methods

In a pilot study, nasal swabs were obtained (n=104) from eight piglets from two sows (NLD). For the complete study, swabs were obtained from 252 piglets from 36 sows from 9 farms in 3 countries (IRL, GER, NLD), from birth till ten weeks (n=4032) in duplicate for species isolation and DNA extraction. DNA was used for S. aureus specific qPCR, V3-V4 16S rRNA gene, and tuf gene sequencing. Amplicon Sequence variants (ASVs) negatively associated with S. aureus qPCR counts were identified by rmcorr and mixed models. Species assigned to the ASVs were referenced against literature for probiotic suitability. Strains of interest were identified from samples belonging to the same piglets (MALDI-TOF) and screened for safety, following the EFSA guidance, in vitro (phenotypical antimicrobial resistance testing) and in silico (whole genome sequencing, accurate taxonomy, antimicrobial resistance genes, virulence factors).

Results

We identified 54 species negatively associated with S. aureus. Literature investigation shortened the list to 15 candidates, predominantly consisting of lactic acid bacteria (LAB) and species closely related to the Staphylococcus genus. The isolation effort followed by in vitro and in silico safety and efficacy studies yielded three suitable strains with qualified presumption of safety (QPS) status. These were consequently utilized in an in vivo probiotic experiment.

Conclusion

Investigation of longitudinal samples from the porcine nasal microbiome resulted in putative probiotic LAB strains currently tested in vivo.

P129 Genetic characteristics of methicillin-resistant Staphylococcus argenteus isolates collected in the Dutch national MRSA surveillance from 2008-2021

Ing. S. Witteveen[1], Dr. ing. A. P. A. Hendrickx[1], Ing. A. de Haan[1], Dr. D. W. Notermans[1], Ing. F. Landman[1], Ing. M. G. van Santen-Verheuvel[1], Dr. S.C. de Greeff[1], Prof. dr. E. Kuijper[1], Dr. N. M. van Maarseveen[2], Dr. S. Vainio[3], Dr. ing. L. M. Schouls[1], The Dutch CPE, CPPA and MRSA Study Group
[1]National Institute for Public Health and the Environment (RIVM), [2]Rijnstate Hospital, [3]St Antonius Hospital

Background: Staphylococcus argenteus can cause health issues and the capacity to cause infections is similar to S. aureus. S. argenteus can harbor antibiotic resistance genes and a variety of virulence factors analogous to methicillin-resistant S. aureus (MRSA). The aim of this study was to genetically characterize a collection of 54 isolates obtained in the Dutch national MRSA surveillance from 2008 until 2021, which appeared to be methicillin-resistant S. argenteus (MRSarg).

Methods: MALDI-ToF was used to identify the S. argenteus isolates. Next-generation sequencing (NGS) and SeqSphere were used to design an in-house wgMLST scheme for S. argenteus. MLST, wgMLST, ResFinder, PlasmidFinder, SCCmecFinder and VirulenceFinder analyses were performed on the NGS data.

Results: We collected 54 isolates from 47 persons submitted as MRSA that were identified as MRSarg. NGS analysis revealed the MRSarg isolates carried mecA either in Staphylococcal Chromosomal Cassette mec (SCCmec) type IV or in SCCmec subtype IVc(2B) which also carried the trimethoprim resistance gene dfrG. The isolates harbored a blaZ-containing plasmid and phage located immune-modulating genes scn and sak genes. Nine of the 47 unique isolates carried enterotoxin encoding genes. wgMLST revealed distribution of the Dutch MRSarg isolates in 5 distinct genogroups, comparable to internationally retrieved MRSarg sequences from NCBI and SRA databases. In one person there was long-term persistence of MRSarg up to 834 days. Lastly, there were eight genetic clusters of MRSarg isolates obtained from people living in close proximity, suggesting transmission.

Conclusion: We show that MRSarg is circulating in the Netherlands. Although MRSarg is distinct from MRSA, it has a similar population structure and carries similar resistance and virulence genes. To assess the prevalence and genetic characteristics, the Dutch national MRSA surveillance has since 2022 been temporarily expanded to include other methicillin-resistant members of the S. aureus complex, including S. argenteus and S. schweitzeri.

P130 Outcomes of inconclusive real-time polymerase chain reaction results in detection of severe acute respiratory syndrome coronavirus 2.

Dr C Rondaan[1], Dr L Gard[1], Dr C van Leer-Buter[1], Prof. dr. H.G.M. Niesters[1], Dr Xuewei Zhou[1]
[1]*University Medical Center Groningen*

Introduction: Inconclusive test results for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be misinterpreted as positive results with all due consequences. Goal of the current research was to evaluate outcomes of real-time polymerase chain reaction (RT-PCR) results with inconclusive results.

Methods: The University Medical Centre Groningen is a tertiary university hospital. Since the start of the pandemic, all admitted patients are routinely screened for SARS-CoV-2, irrespective of symptoms. For employees, testing is performed in case of symptoms or contact with an infected person. Results of respiratory samples from our in-house RT-PCR assay (targeting the E-gene) the Alinity M RT-PCR (Abbott, from October 2020) and Xpert Xpress SARS-CoV-2 (Cepheid, from June 2020) from April 2020 up to January 2021 with a cycle threshold (Ct) value with 34 (in-house and Xpert Xpress) c.q. 36 (Alinity M) or higher were defined as inconclusive.

Results: A total of 46.733 samples were analysed using in-house and Alinity M SARS-CoV-2 RT-PCR, of which 372 (0.80%) were found to have an inconclusive result. A repeat sample was available for 225 of the 372 samples (60%). Of these, 194 were negative (86%), 11 again inconclusive (5%) and 20 positive (9%). Eighteen out of 20 persons with a positive result were employees, 15 of whom (83%) had a link to an infected person. Inconclusive results detected with the Xpert Xpress (n=45 of 3603), were related to individuals with a known history of SARS-CoV-2 infection (n=29, 64%).

Conclusion: Using in-house and Alinity M RT-PCR, the majority of inconclusive results are negative upon repeated testing. Positive results on repeat testing were from employees with a link to a known infected person. An inconclusive result with the Xpert Xpress mostly reflected a history of SARS-CoV-2 infection. This observational study provides information assisting decision making when incurring inconclusive SARS-CoV-2 RT-PCR test results.

P131 Treponema pallidum strains among women and heterosexual men in Amsterdam, the Netherlands and Antwerp, Belgium between 2014 – 2020

Helene Zondag[1], MD Florine H. Jiwa[1], Dr. Irith de Baetselier[3], Dr. Sylvia M. Bruisten[1,2]
[1]Public Health Service (GGD) Amsterdam, [2]Amsterdam Institute for Infection and Immunity, [3]Clinical Reference Laboratory

Treponema pallidum subspecies pallidum (TPA), the bacterial pathogen causing syphilis, is found globally and remains steadily prevalent. In high income countries, men who have sex with men (MSM) are disproportionately affected by the syphilis epidemic. Limited information is available on the prevalence and TPA strain variation among women and heterosexual men. This study aimed to identify TPA strains within the men who have sex with women (MSW) and women and to compare them to the TPA strain distribution among MSM.

Between 2014 and 2020, 41 TPA positive samples were collected from 17 women and 24 heterosexual men, of which 31 were from the Sexual Health Centre (SHC) in Amsterdam and 9 from the SHC in Antwerp. TPA was characterized using the TPA multi-locus sequence typing method based on the partial amplification and sequence analysis of three genetic regions; tp0136, tp0548 and tp0705. In addition, the 23S rRNA loci were checked for macrolide resistance associated mutations (MRAM).

A total of 30/41 (73%) samples derived from 12 women and 18 MSW were typed. This resulted in 9 distinct allelic profiles, of which one was new. The most prevalent allelic profiles were 1.3.1 (40%) followed by 1.1.1 (17%). Among samples from women, exclusively SS14 lineage strains were found and less allelic profile variety compared to MSW. TPA strains from the Nichols lineage were found in 5/18 (28%) samples from MSW. The presence of a MRAM could be determined in 30/41 samples of which 90% contained a MRAM (25/30 A2058G, 2/30 A2059G).

The TPA strains among the MSW population were similar to the strain distribution among MSM and more heterogenous than the strain distribution among women. The most prevalent TPA strains and overall percentage of Nichols-like strains is similar to previous studies in Amsterdam focusing on MSM.