12:00 - 12:15 Improving the reconstruction of antibiotic resistance-plasmids in Escherichia coli

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Introduction
Escherichia coli is the most prevalent resistant pathogen in Europe. Antibiotic resistance genes (ARG) in E. coli are frequently encoded by plasmids, mobile genetic elements that can be horizontally transferred. Given their relevance in the dissemination of resistance, we need to identify and track E. coli plasmids in a precise, fast and accessible manner.
Recently, we benchmarked the performance of six software that reconstruct plasmids using Illumina short-read data. The best performing tool, MOB-suite, correctly reconstructed only 28% of E. coli ARG-plasmids. Here, we present an improved method for reconstructing E. coli plasmids using Illumina data.

Methods
We developed plasmidEC, a tool for classifying assembled contigs into plasmid- or chromosome-derived. PlasmidEC combines the output from three different classification tools implementing a majority voting system. Plasmid-predicted contigs are later binned into individual components based on their sequencing coverage and assembly graph connectivity by using gplas.
We benchmarked our method (plasmidEC_gplas) against MOB-suite, by applying both tools to a dataset of 199 E. coli complete genomes. Resulting predictions were aligned against the original plasmids, and evaluated using the metrics recall and precision.

Results
When reconstructing ARG-plasmids (n=96), recall values obtained with plasmidEC_gplas (median=0.82, IQR=0.55-0.93) surpassed those of MOB-suite (median=0.33, IQR=0.11-0.81). Precision values were almost identical.
For plasmids carrying multiple resistance genes (n=66), plasmidEC_gplas correctly binned all ARGs into a single prediction in 60.6% (n=40) of the cases, while MOB-suite achieved this in 42.4% (n=28). Both tools detected a similar number of plasmid-derived ARGs.

Discussion
PlasmidEC_gplas outperforms MOB-suite at reconstruction of ARG-plasmids in E. coli. Differences in recall values suggest that information from the assembly graph can improve the correct binning of ARG-plasmids. Overall, these results show that assembly graph exploration with information-rich features can be used for plasmid predictions from short-read data if long-read sequences are not available.

14:30 - 14:45 Two-fold greater hazard of 90-day mortality following hospitalisation with SARS-CoV-2 versus other common respiratory virus infections

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Introduction
Mortality following infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been estimated to be higher than mortality following influenza. Elsewhere, we report that mortality rates for patients admitted with infections with influenza, respiratory syncytial virus (RSV), rhinovirus and human metapneumovirus (hMPV) were comparable (see other abstract at this meeting). Here, we compared mortality and ICU stay in patients admitted with COVID-19 to patients admitted with infection with one of these other respiratory viral agents.

Methods
This retrospective cohort study included all adult patients admitted following infection with a respiratory virus, as determined by RT-PCR, between July 1st 2017 and July 1st 2021. Respiratory viruses included were influenza A and B, RSV, hMPV, rhinovirus and SARS-CoV-2. Primary outcome was 90-day mortality. A Cox proportional hazards regression model was fitted to assess the covariate-adjusted association between mortality and respiratory virus (i.e. SARS-CoV-2 versus other common respiratory viruses). Secondary outcome measure was proportion of patients admitted to the intensive care unit (ICU).

Results
In total, 1002 patients were admitted following a SARS-CoV-2 infection and 2224 following infection with other respiratory viruses. Patients with a SARS-CoV-2 infection were younger (median 68 years versus 72 years, $p < 0.01$) and covariate-adjusted hazard ratio for 90-day mortality was 2.3 (95 % CI: 2.0 – 2.8, $p < 0.001$). ICU admission occurred in 22 % patients with a SARS-CoV-2 infection compared to 9 % of patients with an infection with other respiratory viruses.

Conclusion
A SARS-CoV-2 infection was associated with a twofold increase in the risk of cumulative death compared with infection by other respiratory viruses. Patients with COVID-19 had a twofold greater risk of ICU admission. These findings can be used to tailor infection control measures for COVID-19 patients.

11:15 - 11:30 A Falciformispora senegalensis Grain Model in Galleria mellonella larvae

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[1]*ErasmusMC*

Introduction :
Eumycetoma is a chronic granulomatous subcutaneous infectious disease, characterized by the formation of grains. It can be caused by different causative agents. Madurella mycetomatis and Falciformispora senegalensis are the two most common causative agents of black-grain eumycetoma. Grains cannot be induced in vitro, however for M. mycetomatis we previously demonstrated that grains can be formed in the invertebrate Galleria mellonella. To be able to determine if the formation of grains and therapeutic efficacy are similar in different eumycetoma causative agents, it is necessary to develop additional grain models for different pathogens. In this study we determined if larvae of the greater wax moth Galleria mellonella could be used to induce grain formation for F. senegalensis.
Methods :
F. senegalensis strain CBS132257 was selected and four different inocula were used to infect G. mellonella larvae, ranging from 0.04 mg F. senegalenis/larvae to 10mg F. senegalensis/larvae. Larvae were monitored for 10 days. Survival was monitored and grain formation was monitored by macroscopy and histology.
Results :
Like M. mycetomatis, most most larvae were still alive at day 10 when injected with 0.04 mg/larvae. However at 10 mg/day almost all larvae died within the 10 day observation period. At the higher inoculum, grains were formed within 4 hours after infection. The grains produced in the larvae resembled those formed in patients. Therapeutic response is currently investigated.
Conclusion :
In conclusion, the F. senegalensis grain model in G.mellonella larvae described here could serve as a useful model to study the grain formation and therapeutic responses towards antifungal agents in the future.

15:15 - 15:30 Immunogenicity of mRNA-1273 COVID-19 vaccination in PLWH after inadequate primary vaccination response

Drs. Marlou Jongkees[1], K.S. Hensley[1], C.H. Geurts van Kessel[3], R.D. de Vries[4], J.G. den Hollander[6], E.F. Schippers[5,7], H.S. Ammerlaan[8], W.F.W. Bierman[9], M. van der Valk[10,11], M.A.H. Berrevoets[12], A.H.W. Bruns[13], R. Soetekouw[14], E.M. Leyten[15], K.C.E. Sigaloff[16], M.G.A. van Vonderen[17], C.E. Delsing[18], J. Branger[19], C. Rokx[1], A.H.E. Roukens[5], B.J.A. Rijnders[1], K. Brinkman[2]

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A single prime-boost COVID-19 vaccination cycle led to lower antibody levels in people living with HIV (PLWH) compared to healthy controls, with a substantial proportion of inadequate responders. Here, we evaluated whether an extra vaccination could induce measurable immune responses in this population.

In a nationwide prospective cohort study (COVIH, n=1148) all PLWH with inadequate antibody response to primary vaccination (≤300 BAU/mL Trimeric Spike IgG, Liaison) were invited for an mRNA-1273 (100mcg) vaccination (n=165). The anti-SARS-CoV-2 Trimeric Spike IgG response 4 weeks later was assessed as primary endpoint. Secondary endpoints include clinical biomarkers for antibody responses, reactogenicity and the induction of SARS-CoV-2-specific neutralizing antibodies and T-cell responses targeting circulating variants.

Seventy PLWH with inadequate antibody responses (41 after primary vaccination with ChAdOx1-S, 25 after BNT162b2, 4 after Ad26.COV2.S) were included. The mean antibody level directly pre-vaccination was 34.2 BAU/mL (95% CI 24.0-77.5), including 7 with undetectable serological responses. Their median age was 64 years [IQR 61-67], 87.1% was male, median current and nadir CD4+ T-cells were 650/µL [IQR 454-933] and 230/µL [IQR 160-350], and 94.3% had HIV-RNA <50 copies/ml on cART. Median time between completing the primary vaccination schedule and the extra vaccination was 170 days [IQR 153-187].
In total, 97.1% (66/68) of the PLWH responded well to extra vaccination (>300 BAU/ml). The mean increase in antibody level was 4428 BAU/ml (95% CI 3369-5487). The responses were comparable after primary vaccination with ChAdOx1-S and BNT162b2, 3890 BAU/ml (95% CI 2974-4806) and 4939 BAU/ml (95% CI 7384-2493) respectively, p=0.36. We found no significant differences in antibody responses in relation to baseline CD4+ T-cell count (<500, >500/mm3), age groups (<65, >65 years) or time post-primary vaccination.

An additional mRNA-1273 vaccination substantially increased antibody levels in PLWH with inadequate antibody response to primary vaccination, regardless of initial vaccine type or HIV characteristics.

16:15 - 16:30 Elucidating the importance of aspartic proteases of pathogenic mycobacteria

Aniek Meijers[1], Dr. MJ Burggraaf[1], Prof. dr. S Nejentsev[2,3], Dr. CP Kuijl[1], Prof. dr. W Bitter[1,4]
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Mycobacterium tuberculosis is the world's leading bacterial infectious agent, responsible for 1.5 million deaths annually. The absence of an effective vaccine and the rise of multi-drug resistant M. tuberculosis strains emphasize the need to identify novel targets for prevention and intervention strategies. Recently, we identified a novel aspartic protease, PecA (PE_PGRS35) on the bacterial cell surface, which is secreted by the specialized type VII secretion system. PecA of the pathogenic strain Mycobacterium marinum was shown to be responsible for the processing of secreted PE_PGRS proteins, including itself. Specifically, PecA appears to remove the PE domain of these proteins, which contains the type VII secretion motif. Interestingly, the M. marinum ΔpecA strain exhibited reduced bacterial outgrowth following infection in zebrafish larvae as compared to wildtype M. marinum infection.
Both M. marinum and M. tuberculosis contain two additional predicted aspartic proteases with similar secretion domains (PE_PGRS16 and PE26). The exact function of PecA, PE_PGRS16 (PecB) and PE26 (PecC) are studied using their single, double and triple mutant strains generated by CRISPR-Cas9, as well as complementation strains with the active protease and the active site mutant in M. marinum and M. tuberculosis. Proteomic analysis on secreted surface-associated protein fractions revealed many putative substrates for PecA, including members of the abundant PE and PPE protein families. In addition, analysis of semitryptic peptides resulted in the identification of a potential consensus cleavage site. PecB and PecC also appear to process specific substrates, including PE and PPE proteins, although to a lesser extent than PecA. Furthermore, the role of these aspartic proteases are evaluated during macrophage and zebrafish infection.

12:15 - 12:30 Sonication of vascular grafts and endografts in patients treated for vascular graft infection improves the microbiological yield

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Introduction
Vascular graft and endograft infection (VGEI) is a severe complication associated with high mortality and requires extensive antimicrobial treatment alongside surgical intervention. The diagnosis of VGEI is often challenging. For the definitive microbiological diagnosis, sonication of vascular grafts may increase the microbiological yield of these biofilm-associated infections. The objective of this study was to determine whether sonication of explanted vascular grafts and endografts results in a higher diagnostic accuracy compared to conventional culture methods and aids in clinical decision making.

Methods
We performed a prospective diagnostic study comparing conventional culture with culture obtained by sonication of explanted vascular grafts in patients treated for VGEI. Explanted grafts and endografts were cut in two equal halves and were either sent for sonication or conventional culture. Microbiological-, radiological-, biochemical- and clinical criteria based on the MAGIC case definition of VGEI were used for definitive diagnosis. Fifty-seven vascular graft or endograft samples of 36 patients (four reoperations; 40 episodes) treated for VGEI were included; 34 episodes were diagnosed with VGEI. The relevance of sonication cultures was assessed by expert opinion to determine the clinical impact on decision making.

Results
The sensitivity of conventional culture and sonicate culture were identical (73.5% for both). Specificity was 66.7% and 83.3%, respectively. The contamination rate was identical in both methods (sonication culture 13%, conventional culture 14%). However, sonication culture detected clinically relevant microorganisms that went unnoticed by conventional culturing in 9 out of 57 samples (16%, 8 patients) and provided additional relevant information regarding growth densities in another 11 samples (19%, 10 patients).

Conclusion
Sonication of explanted vascular grafts and endografts: (1) improves the microbiological yield and (2) aids in the clinical decision making for patients with a suspected VGEI compared to conventional culture alone.

12:45 - 13:00 Inferring the Heimdallarchaeial origin of eukaryotes

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Introduction: The origin of eukaryotes is considered one of the major transitions in evolution, which took place through the symbiotic merger between an archaeon and an alphaproteobacterium. A recently discovered group, named Asgard archaea, has taken the spotlight as the probable closest archaeal relatives of eukaryotes. Yet, the limited genomic diversity available for Asgard archaea prevented a robust conclusion on the exact phylogenetic identity of the last eukaryotic common ancestor.
Methods: We have analysed metagenomes from globally distributed samples and consequently expanded the genomic sampling of Asgard archaea. Using these new genomes, we analysed two separate gene marker datasets, and applied state-of-the-art phylogenomic approaches to tackle composition-based artefacts, and explore the effects of taxon sampling, data recoding, and removal of fast-evolving sites. We then used gene tree/species tree reconciliation approaches to estimate modes of evolution within Asgard archaea.
Results: Comparing the newly obtained Asgard archaeal genomes with recently published data, we identified two novel major clades, which we name Gefion- and Idunnarchaea. Our phylogenomic results robustly placed eukaryotes within the Heimdall-Idunnarchaeota complex, and indicated a likely origin within the Heimdallarchaeota. Gene-tree/species-tree reconciliation approaches indicated significantly higher levels of gene duplication and lower levels of gene loss than other archaea. Finally, we scrutinized the genomic reconstruction of ancestral Asgard archaeal lineages to refine the gene content and metabolic potential of the last archaeal ancestor of eukaryotes, finding a likely mesophilic heterotrophic lifestyle, and access to a diverse set of eukaryotic proteins for vesicular trafficking, membrane remodeling, N-glycosylation and informational processes.
Conclusion: We infer a robust phylogenomic placement of eukaryotes within the Heimdall-Idunnarchaeota complex (1), and identify features characterising the Asgard archaeal ancestor of eukaryotes (2). This work provides key insights into the prokaryote-to-eukaryote transition and the emergence of eukaryotic cellular complexity.

14:15 - 14:30 Impact of COVID-19 lockdown on lung transplant recipients: decline in overall respiratory virus infections is associated with stabilization of lung function.

Drs A.E. de Zwart[1], dr. A. Riezebos-Brilman[2], Prof. dr. G.A. Lunter[3], drs. E.C.U. Neerken[1], dr. C.C. van Leer-Buter[4], Prof. dr. J.W.C. Alffenaar[5], drs J.P. Gemert[1], dr. M.E. Erasmus[6], dr. C.T. Gan[1], Prof. dr. H.A.M. Kerstjens[1], dr. J.M. Vonk[3], Dr. E.A.M. Verschuuren[1]

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Introduction: In April 2020 COVID-19 lockdown measures were instigated leading to a dramatic drop in non-COVID respiratory virus infections (RVI). This provided a unique situation to assess the impact of RVI incidence on annual FEV1 decline, episodes of temporary drop in lung function (TDLF) suggestive of infection and chronic lung allograft dysfunction (CLAD) in lung transplant recipients (LTR).

Methods: All lung function tests (LFT) of LTR transplanted between 2009-April 2020 were used from post-transplant baseline onward. LFT were censored after COVID-19 infection. Weekly RVI counts from the virology department defined RVI incidence over time. TDLF was defined as sudden, reversible FEV1 drop compared to previous 4 values (any TDLF≥10%, severe TDLF≥20%). Annual FEV1 decline was estimated using linear mixed effects models with separate estimates for pre-lockdown (2009/2020) and during lockdown (2020/2021). Effect modification by TDLF frequency of individual LTR (two subgroups, split at median) and RVI incidence was tested. Rates of CLAD and TDLF were analysed over time.

Results: 479 LTR (12,775 LFT) were included. Annual FEV1 change pre-lockdown was -114ml, while during lockdown FEV1 did not decline: +5ml (difference: $p<0.001$). RVI pressure significantly affected FEV1 level (an increase in weekly RVI-count of 10 leading to a 7ml lower FEV1 ($p<0.001$). The frequent TDLF subgroup showed faster annual FEV1 decline compared to infrequent TDLF (-150ml vs. -90ml; $p=0.003$). During lockdown, we found significantly lower odds for any TDLF (OR 0.53, $p=0.008$) and severe TDLF (OR 0.34, $p=0.005$) as well as a numerically lower CLAD incidence (OR 0.53, $p=0.060$). Effect modification by RVI pressure indicated an association between the events and RVI.

Conclusion: During the lockdown the strong reduction in RVI coincided with markedly less FEV1 decline, less TDLFs and possibly less CLAD. Effect modification by RVI incidence suggests an important role for RVIs in lung function decline in LTR.

17:00 - 17:15 Isolation of Persister Cells of Bacillus subtilis and Determination of Their Susceptibility to Antimicrobial Peptides

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Persister cells are growth-arrested subpopulations that can survive possible fatal environments and revert to wildtypes after stress removal. Clinically, persistent pathogens play a key role in antibiotic therapy failure, as well as chronic, recurrent, and antibiotic-resilient infections. In general, molecular and physiological research on persister cells formation and compounds against persister cells are much desired. In this study, we firstly demonstrated that the spore forming Gram positive model organism Bacillus subtilis can be used to generate persister cells during exposure to antimicrobial compounds. Interestingly, instead of exhibiting a unified antibiotic tolerance profile, different number of persister cells and spores were quantified in various stress conditions. qPCR results also indicated that differential stress responses are related to persister formation in various environmental conditions. We propose, for the first time to the best of our knowledge, an effective method to isolate B. subtilis persister cells from a population using fluorescence-activated cell sorting (FACS), which makes analyzing persister populations feasible. Finally, we show that alpha helical cationic antimicrobial peptides SAAP-148 and TC-19, derived from human cathelicidin LL-37 and human thrombocidin-1, respectively, have high efficiency against both B. subtilis vegetative cells and persisters, causing membrane permeability and fluidity alteration. In addition, we confirm that in contrast to persister cells, dormant B. subtilis spores are not susceptible to the antimicrobial peptides.

15:00 - 15:15 Investigating SARS-CoV-2 breakthrough infections per variant and vaccine type

Dr. Jozef Dingemans[1], Dr. Brian M.J.W. van der Veer[1], Koen M.F. Gorgels[2], Dr. Volker Hackert[2], Audrey Y.J. Hensels[2], Dr. Casper D.J. den Heijer[2], Prof. Dr. Christian J.P.A. Hoebe[1,2], Dr. Lieke B. van Alphen[1], Prof. Dr. Paul H.M. Savelkoul[1]
[1]Maastricht UMC+, [2]South Limburg Public Health Service

Background

SARS-CoV-2 infections that occur in fully vaccinated individuals are reffered to as “breakthough infections” and have been frequently reported despite the high efficacy of the currently available vaccines against severe disease. In this study, we investigated 378 breakthrough infections recorded between January and July 2021 to study wether particular variants and vaccine types are more frequently associated with infections in fully vaccinated individuals. In addition, we studied 157 breakthrough infections from December 2021 to January 2022 when the Omicron variant became dominant.

Methods

SARS-CoV-2 isolates from fully vaccinated individuals were whole genome sequenced via the Oxford Nanopore Technology and the frequency of their genotypes was compared to that of circulating community lineages. A Kruskal-Wallis test was performed to compare median age, time of positive test post 2nd dose, and CT value between groups (symptoms or vaccine types). Fisher’s exact test was performed to investigate the relationship between SARS-CoV-2 genotype and symptoms or vaccination status.

Results

Although the proportion of breakthrough infections was relatively low and stable when the Alpha variant was predominant, the rapid emergence of the Delta variant lead to a strong increase in breakthrough infections, with a higher relative proportion of individuals vaccinated with Oxford-AstraZeneca or J&J/Janssen being infected compared to those immunized with mRNA-based vaccines. Interestingly, symptomatic individuals harbored significantly higher SARS-CoV-2 loads than asymptomatic vaccinated individuals. Furthermore, there was a significantly higher representation of the Omicron variant compared to the Delta variant in individuals who received a booster vaccine vs non-boostered but fully vaccinated indiviuals.

Conclusions

Altogether, these results indicate that the emergence of the Delta variant might have lowered the efficiency of particular vaccine types to prevent SARS-CoV-2 infections in the period from January to July 2021, while the Omicron variant showed enhanced breakthrouh infections in boostered individuals compared to the Delta variant.

11:30 - 11:45 Streamlined CRISPR genome engineering in wild-type bacteria using SIBR-Cas

Mr. Constantinos Patinios[1], Dr. S Creutzburg[1], A Arifah[1], B Adiego-Pérez[1], E Gyimah[1], Dr. C Ingham[2], Dr. S Kengen[1], Prof. J van der Oost[1], Dr. R Staals[1]
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CRISPR-Cas is a powerful tool for genome editing in bacteria. However, its efficacy is dependent on host factors (such as DNA repair pathways) and/or exogenous expression of recombinases. In this study, we mitigated these constraints by developing a simple and widely applicable genome engineering tool for bacteria which we termed SIBR-Cas (Self-splicing Intron-Based Riboswitch-Cas). SIBR-Cas was generated from a mutant library of the theophylline-dependent self-splicing T4 td intron that allows for tight and inducible control over CRISPR-Cas counter-selection. This control delays CRISPR-Cas counter-selection, granting more time for the editing event (e.g. by homologous recombination) to occur. Without the use of exogenous recombinases, SIBR-Cas was successfully applied to knock-out several genes in three wild-type bacteria species (Escherichia coli MG1655, Pseudomonas putida KT2440 and Flavobacterium IR1) with poor homologous recombination systems. Compared to other genome engineering tools, SIBR-Cas is simple, tightly regulated and widely applicable for most (non-model) bacteria. Furthermore, we propose that SIBR can have a wider application as a simple gene expression and gene regulation control mechanism for any gene or RNA of interest in bacteria.

17:00 - 17:15 Type VII Secretion System Substrates mediate Vitamin B12 Transport in Mycobacteria

B. Izquierdo Lafuente, Prof. Dr. W. Bitter[1,2], Dr. A. Speer[2]
[1]VU, [2]Amsterdam UMC

Introduction
Vitamin B12 (B12) is a complex molecule that participates as a cofactor in important metabolic pathways. Mycobacteria present three enzymes that relied on this vitamin. One of these enzymes is MetH, a methionine synthase that catalyzes the conversion of L-homocysteine into L methionine. In addition, vitamin B12 can also regulate gene expression through a B12 sensitive riboswitch. This riboswitch inhibits transcription of metE that encodes for an alternative B12-independent methionine synthase. We explored the use of this enzyme pair to identify vitamin B12 transport proteins in mycobacteria. Understanding cell envelope transport processes in mycobacteria could help in the identification of antibiotics with improved transport efficiencies.
Results
We constructed a metH mutant, which was sensitive to vitamin B12 and demonstrated that Mycobacterium marinum, a commonly used surrogate of Mycobacterium tuberculosis, can use exogenous B12. Vitamin B12 is a bulky and water-soluble molecule, thus we speculated that outer membrane proteins could mediate its transport. Interestingly, mycobacteria present a second vitamin B12-sensitive riboswitch located upstream of a ppe gene. PPE proteins are substrate of the Type VII secretion family and some are outer membrane proteins involved in nutrient uptake. We then created a mutant of the ppe gene in the metH mutant background, which was resistant to B12. Cell fractionation experiments showed that the PPE was soluble and not detectable in the envelope fraction, suggesting a different role on B12 uptake. To identify other proteins required for vitamin B12 transport, we selected mutants that were resistant to exogenous vitamin B12 in the metH mutant strain. The analysis showed that other type VII substrates were responsible for this process. These new factors are now being analyzed in detail.
In summary, our data indicate that vitamin B12 can be scavenged by M.marinum and this transport is dependent on substrates of Type VII secretion system.

11:45 - 12:00 Revealing shifts in the Dutch Neisseria meningitidis population structure through whole-genome sequencing over the past sixty years

Boas Van Der Putten[1], S Man-Bovenkerk[1], A Arends-van 't Klooster[1], I de Beer-Schuurman[1], W Bril-Keijzers[1], C Burger[1], W Freudenburg-de Graaf[1], A van der Ende[1], NM van Sorge[1]
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Introduction
Vaccination against Neisseria meningitidis serogroup C from 2002 and a tetravalent conjugate polysaccharide vaccine covering serogroups ACWY from 2018 has significantly reduced meningococcal meningitis incidence. Currently, N. meningitidis serogroup B is the main cause of invasive meningococcal disease in the Netherlands. Neither of the licensed recombinant protein vaccines against serogroup B is included in the national immunization program. Using whole-genome sequencing, we aimed to identify shifts in the meningococcal population structure and predict MenB vaccine coverage over a 60-year period.

Methods
From the collection of the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM), 2,231 invasive N. meningitidis isolates were selected for whole-genome sequencing: 1,582 from the period 1959-2010 and the remaining 649 from 2015 onwards. Lineages, antigenic profiles, and vaccine coverage were predicted in silico through BIGSdb.

Results
After quality control, 2,149 N. meningitidis isolates were included. The majority (61%) were serogroup B and the median patient age was 6 years. Lineages which are currently very rare, were dominant in the past, such as clonal complex (CC) 8 in the late 1960s and early 1970s or CC1 in the late 1970s. Although CC11 has been prevalent throughout this 60-year period, it has displayed at least three different serogroups (B, C and most recently W). Predicted coverage of a recombinant protein vaccine against serogroup B meningococci (Bexsero) has strongly fluctuated over the years, ranging from 75% (late 1970s) to 95% (late 1980s). Currently the Bexsero coverage against all MenB cases is estimated to be 78%.

Conclusion
The past sixty years showed rapid shifts in the meningococcal population structure. Consequently, predicted MenB vaccine coverage fluctuated over time. This is particularly relevant in light of the current restrictive measures due to the COVID-19 pandemic, which may have caused perturbations in the N. meningitidis population structure.

12:30 - 12:45 Organic matter controls methane emissions through anaerobic methane oxidation in Amsterdam canal sediments

Koen Pelsma[1], NAGM van Helmond[2], WK Lenstra[2], MSM Jetten[1], CP Slomp[2], CU Welte[1]
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Methane (CH4) is increasingly recognised as one of the most critical greenhouse gases to tackle in the coming decades. With a global warming potential 86 times that of carbon dioxide (CO2) and a record atmospheric concentration of 1.9 ppm it is paramount to identify CH4 sources and sinks. Estuarine and freshwater ecosystem are implicated to emit disproportionate amounts of CH4 relative to their surface cover. The Amsterdam canals have been identified as human-impacted urban environment where there is little methanogenic potential. We hypothesised that these canals might accommodate anaerobic methanotrophy (AOM) in the sediment negating a large part of the CH4 being produced in the sediment. By combining in situ measurements, biogeochemical analyses and microbiological techniques we demonstrated that AOM occurs in the deeper layers of the canal sediment starting at 10 cm depth. Batch incubations with a suite of terminal electron acceptors revealed that an organic matter substitute, graphene oxide, stimulates AOM activity. Sulfate-, nitrate-, and ferrihydrite-amended incubations did not yield AOM activity. Geochemical analysis of the sediment cores showed a large fraction of solid Fe(II) confirming its minor contribution as electron acceptor for AOM in the canals. Remarkably, unamended control incubations yielded significant CH4 consumption based on 13C-CO2 measurements. We attribute this activity to the 6 weight-% organic matter present in the sediment. 16S rRNA amplicon sequencing identified ANME2a-2b as the most likely taxa performing AOM. Our data suggests that organic matter is the dominant electron acceptor for AOM in urban freshwaters.

12:45 - 13:00 Rapid spread of the Delta variant in bars and nightclubs after lifted infection control measures in the Netherlands

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
On June 26, 2020, the restriction of maintaining 1.5 meter distance in venues like bars and restaurants in the Netherlands was lifted, nightclubs and festivals were also reopened. Attendees had to show a corona entree ticket to prove completed vaccination, recovered from COVID-19 (valid for 180-days) or tested negative for SARS-CoV-2 (valid for 36hours). About two weeks later, the number of reported SARS-CoV-2 cases increased about 20-fold. Here, we investigate outbreaks in bars and nightclubs in a largely unvaccinated population during a time period when the Delta variant became dominant.
Methods
Epidemiological investigation was performed by linking each case who had visited a venue in the 7 days prior to symptoms by the source and contract tracing team. Whole genome sequencing (WGS) was performed on outbreak and surveillance samples using nanopore sequencing.
Results
Sixteen venues were included in this study with number of cases ranging between 10-98. In total, 348 (314 visitors and 34 employees) of 468 (74%) cases that visited any venue in the region of South Limburg were linked to these sixteen venues.
WGS was successful in 136/154 of selected samples and all were the Delta variant, except for one who had a mixed-infection with both Alpha and Delta variants. Phylogenetic analysis showed 8 outbreak clusters linked to multiple venues per cluster, mostly those with a corona entree ticket and dance facilities. Many of these cases (65%) had an epidemiological link with a minimum of 2 cases visiting a location at a similar moment. Afterwards, these genotypes were frequently observed in regional genomic surveillance. No difference in viral load between fully, partially or non-vaccinated cases was observed.
Conclusions
Lifting restrictions on catering industry venues with a corona entree ticket in a largely unvaccinated population leads to a surge in COVID-19 cases and facilitates spread of new (sub)variants.

12:45 - 13:00 Epidemiological and genomic characterization of a Streptococcus suis outbreak in Thailand caused by an emerging zoonotic strain with acquired multi-drug resistance

Mr Jaime Brizuela[1,2], Medical Doctor R Kajeekul[4], Mr B van der Putten[1], Mr T Roodsant[1,2], Prof. C Schultsz[1,2], Dr. A Kerdsin[3]

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Introduction: Streptococcus suis is an emerging zoonotic swine pathogen which causes severe infections in humans. Human infections are restricted to a limited combination of S. suis clonal complexes (CC) and serotypes. In northern Thailand, S. suis is the leading cause of bacterial meningitis in adults. In April 2021 an outbreak amongst ~500 attendants of a religious ceremony, with 19 confirmed cases, and 2 deaths occurred in the province of Nakhon Ratchasima. We characterized the outbreak using whole genome sequencing.

Methods: Cases were confirmed through positive blood cultures and antimicrobial susceptibility testing was performed against a broad range of antibiotics (CLSI). Illumina MiSeq short read sequencing was performed on 15 outbreak and 7 post-outbreak isolates. Complementarily, 9 outbreak isolates were sequenced with Oxford Nanopore Technologies long read sequencing. Reference mapping and core-genome alignment were used for phylogenetic analysis. The pangenome was reconstructed and the acquisition of mobile genetic elements (MGE) was investigated.

Results: The outbreak was traced back to the consumption of raw pork products and was caused by S. suis serotype 2 with a novel sequence type (ST), belonging to the emergent zoonotic clade CC233/379 which originated in Thailand. Reference mapping revealed that all outbreak strains were identical at a genomic level. Core-genome alignment using a global collection of 1703. suis genomes suggested that outbreak and post-outbreak isolates had acquired the serotype 2 capsule through recombination. The outbreak strain had reduced susceptibility to penicillin and was resistant to erythromycin, clindamycin, chloramphenicol and tetracycline due to acquisition of multiple antimicrobial resistance (AMR) genes via MGEs, and key amino acid substitutions.

Conclusions: An outbreak of septicaemia and meningitis with high mortality was caused by a serotype 2 S. suis strain from a novel ST. The strain became multi-drug resistant by obtaining several MGEs containing AMR genes as well as critical residue substitutions.

17:15 - 17:30 Efficacy of ertapenem, gentamicin, fosfomycin and ceftriaxone for the treatment of anogenital gonorrhea: a randomised non-inferiority trial (the NABOGO trial)

Dr. Alje Van Dam[1,2], Drs M De Laat[1], Dr V Jongen[1], Dr T Heijman[1], Dr CM Wind[3], Dr A Boyd[1], Drs J De Korte-Elenbaas[1], Prof MF Schim van der Loef[1], Prof HJC De Vries[1,4]
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Background
Neisseria gonorrhoeae (Ng) causes gonorrhoea, a common sexually transmitted infection. Emerging strains resistant to first-line ceftriaxone threaten Ng management. Hence, alternative treatments are needed. We evaluated the efficacy of ertapenem, gentamicin and fosfomycin as alternative treatments for anogenital Ng.
Methods
At the Centre for Sexual Health in Amsterdam we included adults 18 years or older, with anorectal or urogenital gonorrhea in a randomized controlled, double-blind, non-inferiority trial (three experimental- and one control-arm). Participants were randomized (1:1:1:1) to receive: intramuscular (IM) 500mg ceftriaxone (control arm), IM 1000mg ertapenem, IM 5mg/kg gentamicin (maximum 400mg), or 6g fosfomycin orally. The primary outcome was the proportion of participants with a negative nucleic acid amplification test of the predefined primary infected site, 7-14 days after treatment. The primary analysis was per-protocol (i.e. excluding those lost to follow-up). The modified intention-to-treat analysis included all randomised patients with anogenital gonorrhoea. Non-inferiority was established if the lower 95% confidence interval for difference between experimental and control arms was greater than -10%.
Findings
Between September 2017 and June 2020, we assigned 346 participants to ceftriaxone (n=103), ertapenem (n=103), gentamicin (n=102), and fosfomycin (n=38). The fosfomycin arm was terminated early after interim analysis revealed <60% efficacy. In the primary per-protocol analysis, all patients (93/93) in the ceftriaxone, 99% (86/87) in the ertapenem, 93% (79/85) in the gentamicin, and 12% (4/33) in the fosfomycin arm cleared Ng [risk difference, ertapenem versus ceftriaxone, -0.01(95%CI:-0.08,0.05); gentamicin versus ceftriaxone -0.07(95%CI:-0.16,-0.01)]. In mITT analysis risk differences were -0.08 (95%CI:-0.17, 0.003) for ertapenem versus ceftriaxone, -0.11 (95%CI -0.21, -0.04) for gentamicin versus ceftriaxone. We observed a higher proportion of patients with ≥1 adverse event in the ertapenem or fosfomycin versus ceftriaxone arm.
Interpretation
Single-dose 1000mg ertapenem is non-inferior to single-dose 500mg ceftriaxone in gonorrhea treatment. Ertapenem is a potential effective alternative for anogenital Ng infections.

12:00 - 12:15 Microbial glycosyl hydrolase activity and cross-feeding in the gut mucosa

Msc Maryse Berkhout[1], Dr CM Plugge[1], Dr C Belzer[1]
[1]*Wageningen University*

Introduction
A mucus layer protects the intestinal epithelium from contact with gut microbes. The outer mucus layer attracts specific gut microbiota of which several bacteria can degrade the mucin glycoproteins that constitute the mucus. Mucin glycan degradation is a complex process that requires a broad range of extracellular glycan degrading enzymes, as mucin glycans are intricate and diverse molecules. Consequently, it is hypothesised that microbial mucin breakdown requires concerted action of various enzymes in a network of multiple mucosal residents.

Methods
We reviewed previous studies for carbohydrate-active enzymes (CAZymes) that are potentially involved in mucin glycan degradation. Next, we constructed a consortium of primary mucin degraders (PMD) and listed their relevant CAZymes. We constructed phylogenetic trees to infer evolutionary relationships between these PMD CAZymes. Furthermore, we reviewed microbial cross-feeding interactions in the gut mucus.

Results
Our PMD consortium consisted of mucin degraders Akkermansia muciniphila, Bacteroides spp., Ruminococcus spp. and Bifidobacterium spp. and contained 832 CAZymes of interest of 20 different enzyme families. All enzyme categories needed for mucin glycan degradation were represented: galactosidases, hexosaminidases, fucosidases and sialidases. Most of these CAZymes are hypothesised to be extracellular, so their products might initiate cross-feeding. Additionally, we provided further evidence for mucin-driven cross-feeding interactions by reviewing in vitro and in vivo studies.

Conclusion
We summarise the evidence for the current hypothesis that mucin glycan degradation occurs in a network of cooperating human gut mucosal microbiota.
1. Previous research demonstrated cross-feeding on mucin between mucin glycan degraders and partners, which results in the production of beneficial compounds.
2. The mucin degraders in the PMD consortium possess different palettes of CAZymes, which suggests that they complement each other.
3. Many of the CAZymes in our consortium are hypothesised to be extracellular, so the products of these enzymes could be available to other mucosal residents.

16:45 - 17:00 Modifications of phoQ sensitizes Klebsiella pneumoniae to IgM-induced complement mediated killing

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The rising numbers of multi-drug resistant bacteria is of great concern to healthcare worldwide. Special attention goes out to ESKAPE pathogens, including the Gram-negative bacterium Klebsiella pneumoniae, which has shown a large increase in β-lactam resistance over the last decades.
Central to the human immune defence against Gram-negative bacteria is the complement system, a network of interactive proteins that can directly kill bacteria via formation of the membrane attack complex (MAC). This multi-protein ring structured pore inserts into the outer membrane and permeates it. While lipopolysaccharides (LPS) are crucial for the protection against complement, LPS is also a target of several antibiotics including colistin, which upon binding destabilizes the Gram-negative cell envelope.

We wondered how development of colistin resistance in K. pneumoniae would affect bacterial sensitivity to MAC, and found that colistin resistant (ColR) mutations could sensitize K. pneumoniae to complement-mediated killing in human serum. Specifically, we showed that the mutations in phoQ (generated during an in vitro colistin evolution experiment) enabled the classical complement pathway (driven by antibodies) to be active on the ColR strain, thereby sensitizing it to be killed by MAC. Next, we demonstrated that the phoQ mutations specifically allowed IgM, but not IgG, to bind to the ColR strain and drive formation of bactericidal MAC pores.

Together this indicates that, due to the phoQ mutations, an epitope for IgM becomes available on the cell surface of the ColR strain that is important for the formation of bactericidal MAC pores. These findings demonstrate that mutations inducing antibiotic resistance can have an opposite effect on complement sensitivity and bactericidal clearance via the immune system.

16:15 - 16:30 Culture-based versus empirical antibiotic prophylaxis to prevent infectious complications in men undergoing transrectal prostate biopsy: a randomised, non-blinded multicenter trial

Mrs Sofie Tops[1], Dr. E. Kolwijck[1,2], Dr. E. Koldewijn[3], Dr. D.M. Somford[4], Drs. F. Delaere[5], Drs. M.A. van Leeuwen[6], Dr. A.J. Breeuwsma[7], Drs. T.F. de Vocht[8], Drs. H.J.H.P. Broos[9], Drs. R.A. Schipper[10], Dr. M.G. Steffens[11], Dr. S. Teerenstra[12], Dr. M.C.A. Wegdam-Blans[13], Drs. E. de Brauwer[14], Drs. W. van den Bijllaardt[15], Drs. A.C.A.P. Leenders[2], Dr. J.P.M. Sedelaar[16], Prof. dr. H.F.L. Wertheim[1]

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Introduction: A global rise in ciprofloxacin-resistant rectal flora has caused an increase of infections after transrectal prostate biopsy (PB) where ciprofloxacin is generally used as prophylaxis. Alternatives must be sought as these infections can be severe and even lead to sepsis. Rectal culture-based antibiotic prophylaxis is a plausible, alternative strategy. We assessed the impact of culture-based prophylaxis on infectious complication rates in men undergoing transrectal PB.

Methods: In this non-blinded randomised trial (NCT03228108), we enrolled patients from 11 Dutch hospitals undergoing transrectal PB. Patients were 1:1 randomized to receive empirical prophylaxis with oral ciprofloxacin (control group; CG) or prophylaxis based on rectal swab culture result (intervention group; IG). Primary outcome was any infectious complication within seven days post-biopsy. Secondary outcomes were infectious complications within 30 days, and bacteremia and bacteriuria within seven and 30 days post-biopsy. For primary outcome analysis, the Chi-square test stratified for hospital was used.

Results: Between April 4, 2018 and July 30, 2021, 1538 patients were enrolled. Data from 1288 patients (83.7%) (CG: 652 and IG: 636) were available for analysis. Ciprofloxacin-resistant rectal flora was detected in 15.4%. Infection rates within seven days post-biopsy were 4.3% (CG) and 2.5% (IG) (p-value: 0.08; difference: -1.8%; 95% CI -0.004-0.040). In the CG, infections occurred in 2.4% of the patients with prophylaxis-sensitive rectal flora and 14.7% of the patients with prophylaxis-resistant rectal flora. Infection rates within 30 days were 5.7% (CG) and 4.7% (IG) (difference: -1.0%; 95% CI -0.016-0.35). Bacteremia was observed in 1.4% (CG) and 0.2% (IG) of the patients (difference: -1.2%; 95% CI 0.001-0.026). Bacteriuria occurred in 3.2% (CG) and 1.7% (IG) of the patients (difference: -1.5%; 95% CI: -0.004-0.032).

Conclusion: Culture-based prophylaxis contributes to the reduction of infectious complications after transrectal PB, importantly also with regard to more severe infections like bacteremia.

14:45 - 15:00 SARS-CoV-2 viral load distribution reveals that viral load increases with age and is associated with hospital and ICU admission

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Background: We studied the SARS-CoV-2 viral load distribution in different age categories, and the association between initial viral load and hospital and ICU admission.
Methods: All nasopharyngeal (NP) and oropharyngeal (OP) swabs from patients tested via SARS-CoV-2 RT-PCR between March 1, 2020 and August 1, 2021, predominantly in the Public Health Services regions Kennemerland and Hollands Noorden, were included. All tests were performed in a single large regional laboratory, allowing comparisons of Cp-values (indicating viral load) between samples.
Results: In total, 678.511 samples were collected, of which 53.366 tests (7.9%) derived from 51.484 unique patients were positive for SARS-CoV-2. These samples revealed a clear relation between age and SARS-CoV-2 viral load, with especially children aged <12 years showing lower viral loads than adults (β: -0.03, 95CI% -0.03 to -0.02, p<0.001), independent of sex and/or symptom duration. In a subgroup analysis 20.207 SARS-CoV-2 positive patients were included, of whom 310 were hospitalized within 30 days of their positive test. When patients were categorized in three SARS-CoV-2 viral load groups, the high viral load group (Cp < 25, n=7.618) was associated with an increased risk of hospitalization compared to the low viral load group (Cp > 30, n=4.050) (OR [95%CI]: 1.57 [1.11-2.26], p-value=0.012), adjusted for age and sex. An even more pronounced association was observed for ICU admission (OR [95%CI]: 7.06 [2.15-43.57], p-value=0.007).
Conclusions: SARS-CoV-2 viral load increases with age. As rapid antigen tests are less sensitive than PCR, these results suggest that SARS-CoV-2 antigen tests have lower sensitivity in children than in adults. Higher initial SARS-CoV-2 viral load is associated with an increased risk of hospital and ICU admission. This emphasizes the additional value of reporting SARS-CoV-2 viral loads to identify patients who are at highest risk of adverse outcomes and may therefore benefit from more intensive monitoring or therapeutic intervention.

14:45 - 15:00 Mycobacteria naturally form viable wall-deficient cells that are undetectable by conventional diagnostics

MSc Noortje Dannenberg[1], MSc T. Weijers[1], Dr. V. J. Carrion Bravo[1,2], Prof. Dr. H. P. Spaink[3], Prof. Dr. T. H. M. Ottenhoff[4], Prof. Dr. A. Briegel[1], Prof. Dr. D. Claessen[1]
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The cell wall is considered essential for most bacteria and the enzymes involved in cell wall synthesis are therefore among the prime targets of effective antibiotics. Considering the importance of the cell wall, it is surprising that under specific stressful conditions some bacteria transiently shed their cell wall to form cell wall-deficient cells. These cells are insensitive to cell wall-targeting antibiotics, are more competent for DNA uptake and can revert to their walled state when the stressful conditions have ended. Recent observations suggest a link to chronic infections, during which such wall-deficient Escherichia coli cells could be isolated from clinical recurrent urinary tract infection samples. This raises the question whether similar cells are also formed by other pathogens, such as mycobacteria, responsible for devastating diseases such as Tuberculosis and leprosy. Here we show that a wide range of mycobacterial species, including Mycolicibacterium smegmatis, Mycobacterium avium and mycobacterial clinical and endophytic isolates, are able to naturally form cell wall-deficient cells and that this formation is stimulated by the presence of cell wall-targeting agents. Confocal microscopy and cryo-transmission electron microscopy confirm that these cells contain DNA but lack their cell wall. We furthermore show that these cells are viable and can revert to a walled state. Importantly, conventional diagnostic media used for detection of mycobacteria do not sustain these cells, perhaps indicating that such cells have been largely overlooked in clinical settings.

15:00 - 15:15 Integrated surveillance of SARS-CoV-2 and other human respiratory viruses in a public testing facility in Utrecht to monitor co-circulation in a susceptible population (ongoing study)

Dr. Nienke Plantinga[1], Dr. M.C.J. van Lanschot[2], Dr. S.F.H. Raven[2], Dr. G. Boland[1], Dr. S.F.T. Thijsen[3], E. Fries[1], T.O. Siksma[1], M. Mostert[3], Dr. R. Schuurman[1], Dr. L.M. Hofstra[1]
[1]University Medical Center Utrecht, [2]Municipal Health Service, [3]Diakonessenhuis

Introduction: Public health measures targeted at SARS-CoV-2 impact the circulation of other respiratory viruses. Pre-pandemic surveillance in GP practices is hampered by public SARS-CoV-2 testing facilities. This ongoing study aims to evaluate integrated surveillance of SARS-CoV-2 and other respiratory viruses in a public testing facility.

Methods: Respiratory surveillance was set up within an public testing facility for SARS-CoV-2. Community-dwelling (a)symptomatic persons provided verbal informed consent for completion of a questionnaire, and additional respiratory pathogens testing on residual material from swabs taken for SARS-CoV-2 RT-qPCR (Allplex Seegene). Daily, a random subset was tested for sixteen respiratory viruses in three multiplex realtime PCRs (Seegene). Data were analysed at weekly intervals and reported nationally (RIVM). The Medical Research Involving Human Subjects Act was considered not applicable by the local ethical board.

Results (will be updated in case of presentation at the Scientific Spring Meeting 2022): Between week 40-47 of 2021, 2,055 subjects consented and completed the questionnaire; 1,102 subjects were tested for viral pathogens. The weekly median age ranged from 27 to 39 years. The percentage of individuals with respiratory symptoms declined from 98.5%(week 40) to 58.9%(week 47). The weekly prevalence of any respiratory pathogen including SARS-CoV-2, ranged from 70.6% in week 40 to 32.6% in week 46, and SARS-CoV-2 prevalence from 2.2%(week 40) to 18.6%(week 46). Overall rhinoviruses were detected most frequently (29.7%), followed by SARS-CoV-2 (8.0%) and Parainfluenzavirus (5.5%, mostly type 4). Influenzavirus was detected in 3.1% of participants, all but one cases occurred in October.

Conclusion: Integrated respiratory viral surveillance within public testing facilities is feasible and informative. Prevalences may be affected in response to e.g. measures to prevent SARS-CoV-2 transmission and testing policies. Population characteristics facilitate interpretation. Together with pre-pandemic surveillance in GP practices, these instruments may inform policy makers and hospitals for adequate preparedness during the COVID-19 pandemic.

15:15 - 15:30 The C2H2 transcription factor SltA is involved in conidial germination and hyphal elongation in Aspergillus fumigatus

Mr Tim Baltussen[1], PhD N Van Rhijn[2], PhD J Dijksterhuis[3], Professor P.E. Verweij[1], Professor M.J. Bromley[2], Associate Professor W.J.G. Melchers[1]

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Introduction

Aspergillus fumigatus is a filamentous saprophytic fungus that produces multinucleate tubular cells termed hyphae. This highly polar extension of the tip helps A. fumigatus to penetrate and invade blood vessels and tissue which results in invasive aspergillosis (IA). Before the fungus grows in a highly polarized manner, the conidium breaks dormancy and the reactivated cell expands isotropically before it undergoes localized expansion of the cell membrane which leads to a tubular outgrowth. Potential regulators of germination and early growth remain largely unexplored.

Method

We selected fourteen transcription factors (TFs) upregulated during germination. TF null mutants were generated in the parental strain MFIG001 (WT). We utilized bright-field, fluorescence microscopy and Scanning Electron Microscopy to examine conidial germination of the TF null mutants and WT temporally (0 to 16h). RNA-seq was performed using a TF null mutant (sltA) and WT strain (0 – 16h)

Results

We observed a markedly distorted hyphal elongation morphology in the ΔsltA mutant that is not apparent in WT strain and other TF null mutants used in this study. The ΔsltA mutant had a germination rate almost two times higher after 6 h compared with the WT and ΔsltArec strain. The ΔsltA mutant showed hyperbranching and tip splitting of the hyphae which is caused by a dysregulation of the Spitzenkörper. After 72 h the ΔsltA strain showed reduced colony growth on Aspergillus Minimal Medium when compared with the WT and ΔsltArec strains. However, when exposed to cell wall stress agents the relative colony size increased in the ΔsltA strain compared with WT and ΔsltArec strain.

Conclusion

We observed a role for transcription factor sltA in cell wall biosynthesis and membrane stability. Additionally, sltA is important for hyphal growth direction. Altogether, we identified a role for sltA in germination and tubular growth of the hyphal tip.

12:30 - 12:45 Semiautomated surveillance of deep surgical site infections after colorectal surgeries – A multicentre external validation of two surveillance algorithms

Msc Janneke Verberk[1], MSc Tjallie van der Kooi, MD PhD David Hetem, Nicolette Oostdam, Mieke Noordergraaf, PhD Sabine de Greeff, Prof. dr. Marc Bonten, PhD Maaike van Mourik
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Introduction
Automated surveillance methods increasingly replace or support conventional (manual) surveillance as the latter is labour-intensive and vulnerable to subjective interpretation. The aim of this study was to validate two previously developed semiautomated surveillance algorithms to identify deep surgical site infections (SSI) in patients undergoing colorectal surgeries in Dutch hospitals.
Methods
In this observational cohort study, four hospitals selected colorectal surgery patients between 2018-2019 based on procedure codes, and extracted routine care data from electronic health records. Per hospital, a classification model and regression model were applied independently to classify patients into low- or high probability of having developed deep SSI. High probability patients need manual SSI confirmation, low probability records are assumed no deep SSI. Sensitivity, positive predictive value (PPV), and workload reduction were calculated compared to conventional surveillance.
Results
672 colorectal surgery patients were included, of whom 28 (4.1%) developed deep SSI. Both surveillance models achieved good performance: after adaptation to clinical practice, the classification model had 100% sensitivity and PPV ranged from 11.1-45.8% between hospitals. The regression model had 100% sensitivity and 9.0-14.9% PPV. With both models, less than 25% of records needed review to confirm SSI. For the regression model more complex data management skills were required, partly due to incomplete data.
Conclusion
In this independent, external validation both surveillance models had good performance. However, the classification model is preferred above the regression model because of source data availability and less complex data management required. Next step is implementation in infection prevention practices and process workflows.

12:00 - 12:15 Spatial separation of ribosomes and DNA in Asgard archaeal cells

Dr. Burak Avci[1], Dr. J Brandt[3], Mrs. D Nachmias[4], Prof. Dr. N Elia[4], Prof. Dr. M Albertsen[3], Prof. Dr. TJG Ettema[1], Assoc. Prof. Dr. KU Kjeldsen[2], Prof. Dr. A Schramm[2]
[1]Wageningen University And Research, [2]Aarhus University, [3]Aalborg University, [4]Ben-Gurion University of the Negev

The origin of the eukaryotic cell is a major open question in biology. Asgard archaea are the closest known prokaryotic relatives of eukaryotes, and their genomes encode various eukaryotic signature proteins, indicating some elements of cellular complexity prior to the emergence of the first eukaryotic cell. Yet, microscopic evidence to demonstrate the cellular structure of uncultivated Asgard archaea in the environment is thus far lacking. We used primer-free sequencing to retrieve 715 almost full-length Loki- and Heimdallarchaeota 16S rRNA sequences and designed novel oligonucleotide probes to visualize their cells in marine sediments (Aarhus Bay, Denmark) using catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH). Super-resolution microscopy revealed 1–2 µm large, coccoid cells, sometimes occurring as aggregates. Remarkably, the DNA staining was spatially separated from ribosome-originated FISH signals by 50–280 nm. This suggests that the genomic material is condensed and spatially distinct in a particular location and could indicate compartmentalization or membrane invagination in Asgard archaeal cells.

11:30 - 11:45 Uncovering the role of Bathyarchaeota in freshwater sediments using genome-guided cultivation

Dr. Patricia Geesink[1], Prof. Dr. T. J. G. Ettema[1]
[1]*Wageningen University and Research*

Archaea represent one of the two primary domains of life and have been shown to inhabit almost every environment on Earth. In the past decades, sequencing-based studies have uncovered a wide diversity of previously unknown archaeal lineages in a variety of habitats. However, the majority of archaeal phyla lacks cultivated representatives and information on their potential role in the environment is solely predicted based on genomic information. One example of an ubiquitous, yet uncultivated archaeal phylum are Bathyarchaeota. While found in a variety of environments, including freshwater and marine habitats, information on the role of Bathyarchaeota in the environment is limited. Here, we sampled ten sediment cores from Lake Erken (Sweden) for metagenomic sequencing as well as cultivation work in order to elucidate the diversity and lifestyle of Bathyarchaeota in freshwater sediments. With a relative abundance of up to 23% members of the Bathyarchaeota are dominating the microbial community in Lake Erken sediments. The analysis of 17 high-quality metagenome assembled genomes (MAGs) that were reconstructed from Lake Erken indicates a widespread potential of Bathyarchaeota to break down different carbohydrates including chitin, cellulose and mannan. By using information on their genomic potential, enrichment cultures of Bathyarchaeota were set up and monitored over a period of two years. With Bathyarchaeota increasing up to a relative abundance of 29%, our enrichments and the response of Bathyarchaeota to different carbon sources allows us draw conclusions on the metabolic potential of these elusive archaea in freshwater sediments. The high abundance, diversity and ability to degrade a wide range of complex carbon sources highlights the importance of Bathyarchaeota in lake sediments and potentially global carbon turnover. The combination of genome-resolved metagenomics and classical cultivation work lay the foundation for further attempts to isolate and study Bathyarchaeota using high-throughput cultivation techniques.

14:30 - 14:45 Investigating changes in the membrane lipid composition induced by high hydrostatic pressure in a piezotolerant Marinifilaceae bacteria isolated from the Black Sea

Anandi Tamby[1], Subhash Yadav[1], Dr. Diana X. Sahonero-Canavesi[1], Dr. Nicole Bale[1], Dr. Laura Villanueva[1,2]
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The lipid membrane is a dynamic frontier between the internal cell and external stimuli, protecting the cells from potential stressors. Extremophiles have developed lipid adaption strategies to maintain membrane integrity under extreme conditions. The deep-sea is characterized by high hydrostatic pressure (HHP), a variable which is known to induce changes in lipid composition, including increased unsaturation and branching in the fatty acids component of lipids. However, HHP is normally correlated with low temperature in the deep sea as well as very specific nutrient availability, and hence the effect of HHP alone on microbial membrane lipids has not been fully constrained. Here, we analyze the membrane lipid composition of the mesophile piezotolerant Bacteroidetes Labilibaculum euxinus isolated from the Black Sea at 2,000 meters depth. Analysis of the intact polar lipids in L. euxinus revealed the presence of phosphorous-free lipids, notably ornithine and capnine lipids. Preliminary results show that L. euxinus modulates their relative abundance when subjected to HHP and to different phosphate concentrations, revealing a potential cross-adaptive strategy to both HHP and low phosphate availability. We are currently experimenting further with cultures under both phosphate limitation and HHP to fully elucidate the role phosphorous-free lipids play in HHP adaption. Previously identified genes linked to ornithine lipid biosynthesis are absent from the L. euxinus genome, hinting at the presence of an alternative biosynthetic pathway.
Keywords: hydrostatic pressure, cross-adaptation, extremophile, marine microbiology, anaerobic microbiology, piezophily, membrane lipids, ornithine lipids, Black Sea

14:15 - 14:30 Choosing sodium ions over protons – towards understanding the differences in molecular adaptations of aerobic and anaerobic alkaliphiles

Ir. Sam de Jong[1], Prof. dr. i.r M.C.M. van Loosdrecht[1], Dr. D.G.G. McMillan[1]
[1]*Delft University Of Technology*

Alkaliphiles are adapted to live in a proton scarce environment, resulting in difficulties with respiration and other proton-coupled transmembrane processes. A simple adaptation would be to increase the internal pH, to maintain a proper proton motive force, something some archaea do. A downside to altering the internal pH is having to adapt all internal proteins. Bacteria therefore solely adapt the membrane and the proteins governing processes over the membrane. Still, a clear distinction exists within alkaliphilic bacteria. Aerobic alkaliphilic bacteria prefer to use protons in the membrane energy processes, whilst anaerobic alkaliphiles often rely on sodium ions. The obvious explanation is that oxygen respiration can overcome the thermodynamic boundaries of proton pumping against the inverted gradient, yet the exact adaptations at a molecular level are unclear. To elucidate this enigma, we subjected an alkaliphilic bacterium that harbours both sodium and proton pumping proteins, the facultative thermoalkaliphile Caldalkalibacillus thermarum TA2.A1, to a range of conditions - from aerobic to anaerobic – in a chemostat system. We analysed adaptations in the membrane proteins, cell wall and the membrane itself.

Adaptations in the cell wall and the membrane can be regarded as a general adaptation for alkaliphiles. In C. thermarum, no difference was found for either the cell wall charge, nor for the membrane lipid content over the whole oxygen availability spectrum. The rather more plausible hypothesis for the divergence can be found in the governing of transmembrane processes itself, with particular emphasis on the switch between using protons or sodium ions. This we studied with membrane proteomics. C. thermarum chooses to modulate the level of various proton pumping proteins of the electron transport chain and sodium pumping transporter proteins as a function of oxygen availability. Summarizing, this works towards elucidating the evolutionary divide between aerobic and anaerobic alkaliphiles.

12:15 - 12:30 Methanogenic archaea use a bacteria-like methyltransferase system to convert methoxylated aromatic compounds

Julia Kurth[1,2], O Lemaire[3], M Jetten[2], T Wagner[3], C Welte[2]
[1]*Wageningen University,* [2]*Radboud University,* [3]*Max Planck Institute for Marine Microbiology*

Methoxylated aromatic compounds are components of lignin and coal and are very abundant on Earth. However, the conversion of these compounds has previously only been described for bacteria and not for archaea. Only recently the methanogenic archaeon Methermicoccus shengliensis was shown to be capable to convert methoxylated aromatic compounds, also called methoxydotrophic growth [1]. In a recent study we showed that M. shengliensis uses an O-demethylation/methyl transfer (Mto) system that is more related to that of acetogenic bacteria than the methyl transferase system of methylotrophic archaea [2]. With biochemical approaches we were able to show that the methyl group is transferred from the methoxylated compound to the corrinoid protein MtoC by the O-demethylase MtoB. We further got strong evidence by activity assays and protein crystallization that MtoA transfers the methyl group to tetrahydromethanopterin instead of coenzyme M, which differs from the conventional methanogenic methyl-transfer systems. This most likely leads to an altered energy metabolism and redox (im)balance during methoxydotrophic growth. In summary, methoxydotrophic methanogenesis differs regarding its methyl transfer and C1 metabolism from other methanogenesis pathways and might play an important role in anoxic subsurface environments.

[1] Mayumi, D., Mochimaru, H., Tamaki, H., Yamamoto, K., Yoshioka, H., Suzuki, Y., et al. (2016) Methane production from coal by a single methanogen. Science 354: 222–225.
[2] Kurth, J.M., Nobu, M.K., Tamaki, H., de Jonge, N., Berger, S., Jetten, M.S.M., et al. (2021) Methanogenic archaea use a bacteria-like methyltransferase system to demethoxylate aromatic compounds. ISME J: doi.org/10.1038/s41396-021-01025-6.

15:00 - 15:15 Expanded diversity of anoxic marine methylotrophy: physiological capabilities of a non-canonical methylotroph on a range of methylated compounds

Msc Peter fischer[1,2], dr L Villanueva[2,3], prof dr D.Z. Sousa[1]
[1]Wageningen University & Research, [2]Royal Netherlands Institute for Sea Research, [3]Utrecht Uniersity

In anoxic marine sediments, methylated compounds such as methanol have long been considered to be non-competitive substrates for methanogens. However, evidence from metagenomic data suggest that bacteria capable of methylotrophy, such as sulfate reducing microorganisms, might co-exist with methylotrophic methanogens in sulfate-rich sediments. Nevertheless, there is a lack of culture representatives to prove this hypothesis. To this end, we have sampled euxinic Black Sea sediments, in which 16S rRNA amplicon analysis has previously shown the presence of methanogenic archaea, with the aim to isolate and describe non-methanogenic methylotrophs. Sampled sediment was incubated with methanol as sole energy and carbon source, using 2-bromoethanesulfate to inhibit methanogenic activity. Cultures were subsequently transferred after depletion of methanol to obtain a highly enriched culture over 24 months. 16S rRNA amplicon sequencing was performed at the start and mid-point of enrichment to determine the community composition. In addition, the complete metagenome of the 24-month enriched culture was sequenced to infer the metabolic potential of the microorganisms present. This culture was composed predominantly (88% based on sequencing depth) of a sulfate reducing microorganism of the genus Desulfosporosinus. Furthermore, three fermentative organisms, two Firmicutes of unknown lineage and one member of the phylum Bacteroidetes of the genus Labilibaculum were present. A methylotrophic, sulfate reducing bacterium, strain P130, was isolated that was closely related to Desulfosporosinus nitroreducens. Further physiological tests confirmed methylotrophy of methanol, glycine betaine, choline, dimethylethanolamine, methylethanolamine and n-methyltaurine under in situ temperature and salinity.
Conclusions:
1. Utilization of methylated compounds in Black Sea sediments, and potentially in other marine anoxic sediments, is more complex than previously thought;
2. At least one representative sulfate reducing microorganism is capable of methylotrophy under Black Sea sediment in-situ temperature and salinity;
3. This representative organism might be able to compete with methanogens for common methylated substrates in marine sediments.

11:45 - 12:00 The value of acetoclastic methanogens in the energy metabolism of the syntrophic butyrate oxidizer Syntrophomonas wolfei

Maaike Besteman[1], A Doloman[1], DZ Sousa[1]
[1]*Wageningen University & Research*

Introduction: Anaerobic digestion (AD) converts organic waste into biogas by the activity of a complex microbial community. Microbial interactions are vital to this community, including the ecological relationship between fatty-acid oxidizing bacteria and methanogens that finalize the AD chain by producing methane. The thermodynamically-dependent syntrophic relationship between fatty-acid oxidizers and hydrogenotrophic methanogens has been extensively studied. However, the effect of acetoclastic methanogens, and their potential for lowering the thermodynamics barriers of fatty-acid oxidizers by decreasing acetate concentration, is less well understood. Here we studied syntrophic interactions in predefined bi-and tri-cultures containing the butyrate degrading syntroph Syntrophomonas wolfei, the hydrogenotrophic methanogen Methanospirillum hungatei and, only in tricultures, the acetoclastic methanogen Methanothrix soehngenii.

Methodology: Bi-and tri-cultures were set-up using anaerobic basal medium supplemented with 20 mM butyrate. Butyrate consumption and acetate production were measured with HPLC; H2 and CH4 was measured with GC. Individual species in the co-cultures were monitored with quantitative PCR. The transcriptome of exponentially-grown co-cultures was sequenced to compare gene expression patterns in the absence or presence of the acetate scavenger M. soehngenii.

Results & conclusion: Growth of the tri-cultures was overall faster than of the bi-cultures, which more often had extended lag-phases. However, after lag-phase, rates of butyrate consumption by S. wolfei were identical. Methane production and acetate production and consumption matched stoichiometric predictions. The comparative transcriptomics revealed high similarity in transcripts between the conditions with and without acetate scavenger. However, in tri-cultures, the formate dehydrogenase complex of S. wolfei was significantly higher expressed ($p < 0.001$). This difference in the syntrophs energy metabolism could explain the tri-cultures stability.

The present study confirmed that the presence of acetoclastic methanogens benefits the energy conservation of syntrophs and lays the groundwork for future research. Future work could explore the co-cultures stability and the syntrophs energy conservation mechanisms in greater detail.

16:30 - 16:45 What is the role of rifampicin within the standard regimen for M. avium? A hollow-fibre study

Jelmer Raaijmakers[1], J.A. Schildkraut[1], Dr. J van Ingen[1]
[1]*Radboudumc*

Background
Mycobacterium avium complex (MAC) bacteria are the most frequent causative agents of nontuberculous mycobacterial pulmonary disease (NTM-PD) worldwide. Rifampicin is currently recommended for the treatment of MAC-PD alongside azithromycin and ethambutol. Rifampicin has poor in vitro activity against MAC, but is thought to prevent the emergence of macrolide resistance. We evaluated the contribution of rifampicin within the standard therapy of MAC-PD in an intracellular hollow-fibre model.

Materials
In an in vitro hollow-fibre experiment, epithelial lining fluid pharmacokinetic profiles of either the recommended 3-drug (rifampicin, ethambutol, azithromycin) or a 2-drug (ethambutol, azithromycin) treatment was simulated. THP-1 cells infected with M. avium ATCC 700898 were exposed to these regimens for 21 days. On day 0, 3, 7, 14 and 21, samples were drawn to determine bacterial- and THP-1 cell densities. Bacterial samples (intra- and extracellular fractions) were inoculated on drug-free and azithromycin (8x MIC) containing 7H10 agar plates. At day 0 and 21, samples were drawn to determine antibiotic concentrations and to generate complete pharmacokinetic profiles.

Results
Both the 3-drug and 2-drug therapies were initially able to maintain bacterial stasis for up to 3 days for both the intra- and extracellular bacterial fractions. After the initial stasis, a rapid regrowth towards the growth control was observed for both therapies from day 7 onwards. This coincided with emergence of a macrolide-resistant subpopulation in both treatment arms. The THP-1 cell concentration ($\pm 8 \cdot 10^5$ cells/ml) remained static over time.

Conclusions
Rifampicin did not add pharmacodynamic effect to a regimen of azithromycin and ethambutol and it was unable to suppress the emergence of macrolide resistance. This questions its role in the currently recommended MAC-PD regimen, particularly in milder manifestations. The findings of current study should be confirmed in clinical trials.

16:45 - 17:00 In vitro effectiveness of contact lens solutions available on the Dutch market against Acanthamoeba species

Anna Randag[1,2], L. de Kroon[3], H. Otten[4], C. Arias Claro-Handgraaf[3], B. Schimmer[5], T. Kortbeek[5], J. van Rooij[1,2], F.F. Stelma[3]

[1]The Rotterdam Eye Hospital, [2]Rotterdam Ophthalmic Institute, [3]Radboud University Medical Center, [4]Visser Contactlenzen, [5]National Institute for Public Health and the Environment (RIVM)

Introduction

Acanthamoeba keratitis, a potentially severe corneal infection, is almost universally associated with contact lens use in developed countries. Contact lens solution manufacturing protocols, however, lack testing of anti-amoebic activity. This study aimed to investigate the relative effectiveness of contact lens solutions available on the Dutch market against Acanthamoeba species.

Methods

The effectiveness of 13 multiple purpose solutions (MPS), two hydrogen-peroxidase solutions (HPS) and one povidone-iodine based solution (PIS) against both trophozoites and cysts of Acanthamoeba castellanii and Acanthamoeba polyphaga was determined. The Spearman Karber (SK) log reduction method and the XTT colorimetric assay were used to calculate the effectiveness at the manufacturer's minimum recommended disinfection time (MMRDT) and after eight hours (presumed overnight exposure).

Results

At MMRDT, one (MPS) solution showed an SK log reduction of > 3.0 against A. castellanii trophozoites. Two additional MPS and both HPS reached this threshold after eight hours. SK log reduction values for A. polyphaga trophozoites were between 1 and 3 at all time points. Using the XTT colorimetric assay, two MPS and both HPS showed > 90% reduction in metabolic activity of A. castellanii trophozoites, after both MMRDT and eight hours. For A. polyphaga five out of 13 MPS, both HPS and the PIS showed a reduction of 90% activity after eight hours (MMRDT not available). Cysts were resistant against all solutions.

Conclusion

Following the manufacturer's guidelines of minimum recommended disinfection times, few solutions provide sufficient effectiveness against Acanthamoeba trophozoites and none against cysts. The variation in results between the two Acanthamoeba strains tested indicate species variation in susceptibility. The results underline the importance of adequate hygiene when handling contact lenses.

15:15 - 15:30 Respiratory virus surveillance during the SARS-CoV2 pandemic winter season 2021/2022 in the Amsterdam region

Dr. Brenda Westerhuis[1], R Vigeveno[1], D Amarthalingam[1], M Admiraal[1], F Zethof[1], R Brunst[1], dr. S Bruisten[1], dr. M van der Lubben[1]
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SARS-CoV2 infections dominated the 2020-2021 winter season and measure taken to prevent Covid-19 diminished circulation of other respiratory viruses including influenza viruses and respiratory syncytial virus (RSV) profoundly. Lack of prior exposure may contribute to off-season circulation and more severe infection. In the summer of 2021 there was an early increase of RSV cases in week 24, which resulted in increased hospitalization of infants. In addition, for influenza viruses vaccine mismatches may also contribute to a possibly severe upcoming influenza season. Since testing facilities only test for SARS-CoV2 reliable data on circulation of other respiratory viruses from national surveillance systems is scarce. To monitor respiratory viruses in the Amsterdam-region during the autumn/winter season 2021-2022 we included nose-throat swabs from two groups that were obtained for SARS-CoV-2 testing. 1) Anonymized randomly selected samples from the GGD Amsterdam testing facilities were also tested for influenza-A/B viruses, RSV-A/B, and rhinovirus (RT-qPCR). 2) Samples send in by general practitioners and nursing homes were additionally tested for influenza-A/B viruses(Aptima®SARS-CoV-2/FluAssay)
Results from group 1 showed that the first influenza cases occurred in week 41 (around 3%) but cases decreased in week 46 (<1%), in line with increasing SARS-CoV2 cases and subsequent implementation of restrictive measures. Similarly, the highest number of rhinovirus infections were detected around week 39 (35%) and declined thereafter. Low numbers RSV A/B infections were present during the whole period (0-3%). Samples from group 2 demonstrated a similar low level (<1%) influenza virus circulation. To date no large influenza virus outbreaks occurred and the ongoing large scale surveillance will contribute to early detection of cases allowing prompt action to prevent further spread. The low level circulation of influenza viruses currently seen in The Netherlands as compared to France and Sweden might be due to Dutch restrictive measures implemented for the SARS-CoV2 Omicron variant.

16:00 - 16:15 Composition and stage dynamics of mitochondrial complexes in Plasmodium falciparum

Felix Evers[1]
[1]*Radboudumc*

Our current understanding of mitochondrial functioning is largely restricted to traditional model organisms, which only represent a fraction of eukaryotic diversity. Malaria parasites from the genus Plasmodium harbor only a single, indispensable mitochondrion with a minimalistic mitochondrial genome and lacking recognizable orthologues to many canonical mitochondrial proteins. Additionally, this organelle appears to differ dramatically in function across different life-cycle stages. Thus, the pathogenic asexual stages rely on the respiratory chain only to cycle ubiquinone for pyrimidine biosynthesis, while for the transmissible gametocytes efficient and functional oxidative phosphorylation is essential to colonize the insect host. We applied complexome profiling to map the inventory of protein complexes across the asexual and sexual blood stages of Plasmodium falciparum. We identified remarkably divergent composition and clade-specific additions of all respiratory chain complexes. Furthermore, we showed that respiratory chain complex components and linked metabolic pathways are up to 40-fold more prevalent in gametocytes, while glycolytic enzymes are substantially reduced. Utilizing electron microscopy, we found that underlining this functional switch, ultrastructure between these stages differs dramatically with cristae being exclusively present in gametocytes. Taken together these findings reveal peculiar new biology and fascinating insights in the evolution of eukaryotic respiration and how the malaria parasite has adapted to different environmental challenges at the level of organellar structure and multiprotein complexes.

12:30 - 12:45 Harnessing patient derived microphysiological system to study host-microbe interactions

Dr. Jianbo Zhang[1]
[1]*University of Amsterdam*

Gut microbiome has emerged as a key factor in human diseases including infectious diseases. Lacking, however, is an in vitro platform that can recapture the interaction of human colonic and bacterial cells under physiologic microenvironment. Here, we established a GuMI physiome platform that can maintain key features of this microenvironment including mucosal barrier, oxygen gradient, nutrient feeding, and flow. Co-culture of organoids-derived human colon epithelia and oxygen-sensitive commensal bacteria indicate that GuMI can maintain the growth of obligate anaerobes without compromising the barrier function for up to four days. RNA sequencing analysis revealed that GuMI recapitulates cell responses to hypoxia and several gut commensals predominant in human fecal microbiota. We further successfully incorporated innate immune cells including dendritic cells into GuMI and were able to long-term co-culture. Multiplex cytokine assays suggested that the presence of innate immune cells activates the systemic immune responses to commensals. In summary, GuMI physiome platform faithfully recapitulate colon mucosal microenvironment and can be a useful tool to study interactions of host mucosa with microbiome and pathogens.

16:30 - 16:45 The scavenger receptor LOX-1 – a novel receptor for Staphylococcus aureus wall teichoic acids on endothelial cells

Esther Lehmann[1], Dr Jessica Slavetinsky[1], Dr Rob van Dalen[1], Prof. Dr. Andreas Peschel[1], Dr. Christopher Weidenmaier[1]
[1]*University Of Tübingen*

Stapyloccoccus aureus is an opportunistic pathogen that asymptomatically colonizes around 30 percent of the human population and is the most prevalent isolate from patients with endovascular infections. Bacteria from these blood stream infections frequently disseminate to peripheral organs, causing further subsequent acute or chronic infections. The complex multifactorial process of adherence of S. aureus to the vascular surfaces and subsequent dissemination into the underlying tissue is not yet fully understood. Mechanistically, adhesion to the endothelial cells is the first crucial step for the initiation and progression of endovascular infections. The important role of protein adhesins in the bacterial binding to the vascular lining has been demonstrated. Recently, wall teichoic acid (WTA), a glycopolymer adhesin and major surface component of S. aureus, was shown to mediate binding to human epithelial cells through interaction with host scavenger receptors.
Here, we identify the scavenger receptor LOX-1 as a novel endothelial WTA receptor, facilitating S. aureus adhesion. We analysed the interaction of S. aureus with LOX-1 using bacterial adhesion- and WTA ligand-binding-assays. Binding of S. aureus to human umbilical vein endothelial cells (HUVECs) was inhibited by preincubation with the natural LOX-1 ligand OxLDL or anti-LOX-1 antibodies. In agreement with this finding, S. aureus mutants deficient in WTA synthesis (ΔtarO) showed reduced binding to HUVECs. Importantly, binding of the mutant could not be further reduced by pre-blocking of the receptor. Direct interaction of S. aureus with LOX-I via WTA could be confirmed in an adhesion model using a LOX-1 transfected cell line, as well as by in vitro binding experiments with immobilized purified WTA. Thus, LOX-1 is a receptor for WTA-mediated adhesion of S. aureus to the endothelial lining, potentially providing a new mechanism for S. aureus dissemination into peripheral organs.

11:15 - 11:30 CRISPR and Caspase teaming up

Ir. Sam Van Beljouw[1]
[1]*Tu Delft*

Many prokaryotes carry CRISPR-Cas systems, which use CRISPR RNA-bound effector proteins to detect viral nucleic acids. Type III CRISPR-Cas immunity is widespread in prokaryotes and is generally mediated by multisubunit effector complexes. These complexes recognize complementary viral transcripts and can subsequently activate other immune proteins. We describe an odd member of the type III CRISPR-Cas family, namely the type III-E effector from Candidatus “Scalindua brodae” (Sb-gRAMP). Sb-gRAMP is natively encoded by a single gene with several type III domains fused together and cleaves single-stranded RNA at two defined positions six nucleotides apart. Intriguingly, Sb-gRAMP physically combines with the caspase-like TPR-CHAT peptidase to form the CRISPR-guided caspase (Craspase) complex, suggesting a potential mechanism of target RNA–induced protease activity to gain viral immunity by host suicide.

12:00 - 12:15 Long read compared to short read next generation sequencing of the 16S-23S rRNA region for identification of bacterial species in clinical samples; a pilot study. (Cancelled)

Dr. Mirjam Kooistra-smid[1,2], E. van Zanten[1], G.J. Wisselink[1], Dr. R.F.J. Benus[1]
[1]Certe, [2]University of Groningen, University Medical Center Groningen

Introduction
The use of next generation sequencing (NGS) in diagnostic medical microbiology is increasing as these methods overcome limitations of culture and 16S rDNA Sanger sequencing. NGS of the 16S-23S rRNA region (16S-23S NGS) enables identification with a high taxonomic resolution of bacteria in polymicrobial infections. Here we present a pilot study comparing long read sequencing (LRS) with short read sequencing (SRS) of the 16S-23S rRNA region for the identification of bacterial microorganisms in clinical samples.

Methods
Twenty clinical samples were subjected to 16S-23S NGS using SRS (NexteraXT 600-V2 kit, sequencetime 56 hours; MiSeq) and LRS (ONTSQK-LSK109, sequencetime 2 hours, MinION). The correct assignment of bacterial species was performed using de novo assembly or mapping to the RRN database (Benítez et. al, 2017) for short and long reads, respectively; this was followed by BLASTN using the NCBI database. Contigs and consensus sequences with an abundancy of ≥5% were used for the assessment of bacterial identification concordance.

Results
Using SRS, in total 53 contigs were assembled, with a mean length of 2657 bp; in 15/53 (28%) contigs the length of the contig was >3900 bp. LRS yielded in total 46 consensus sequences with a mean length of 3949 bp; in 37/46 (80%) sequences the length was >3900 bp. SRS detected bacterial species in 19/20 samples; a single bacterial species and polymicrobial communities (2-9 species) were identified in 9 and 10 samples, respectively. Using LRS in 17/20 samples bacterial species were detected; a single bacterial species and polymicrobial communities (2-8) were identified in 7 and 10 samples, respectively.
Bacterial identifications using LRS was concordant with SRS for 31/36 (86%) species.

Conclusion
Long read sequencing of the 16S-23S rRNA region enables accurate identification of bacterial species in complex samples. Moreover, compared to short read sequencing, the turnaround time is shortened by 54 hours.

14:45 - 15:00 Methylocalor cossyra CH1, gen. nov. sp., a novel gammaproteobacterial methanotroph isolated from hot soil of Pantelleria Island

Mrs Changqing Liu[1], Dr Carmen Hogendoorn[1], Stijn Peeters[1], Daniël Verhagen[1], Theo A. van Alen[1], Dr Arjan Pol[1], Professor Huub J. M. Op den Camp[1]
[1]*Radboud university*

Volcanic and geothermal systems are extreme environments characterized by low pH, high temperature and high gas emission. These gases include CO2, CH4, H2S and H2, which provide a suitable niche for chemolithoautotrophic microbial communities. Here, we reported the isolation and characterization of thermoacidophilic Methylococcaceae, strain CH1, isolated from the geothermal soils of the Favara Grande on Pantelleria (Italy). The genome of strain CH1 was sequenced and based on the phylogenetic analysis, the isolate seems to represent a novel gammaproteobacterial genus and species within the family Methylococcaceae, for which we proposed the name of "Candidatus Methylocalor cossyra CH1". The genome of strain CH1 has two complete pmoCAB operons and two single pmoC genes encoding the membrane-bound methane monooxygenase. For methanol conversion, mxaFJG and xoxFJ encoding calcium and lanthanide-dependent methanol dehydrogenases, respectively, are present. In addition, strain CH1 is able to fix nitrogen gas via the nif DHK-encoded nitrogenase. Transcriptomic analysis of chemostat-grown cells on CH4/NH4+ and CH4/N2 revealed differences in gene expression. With NH4+ as a nitrogen source, the genes encoding hydroxylamine oxidoreductase (HaoA) and cytochromes involved in the electron transfer chain were upregulated. Under nitrogen fixing conditions, clusters of genes associated with nitrogen fixation such as nifDHK and nifW. The latter gene encodes a protein with three ferredoxins that may provide the low potential reductants needed for nitrogen fixation. In addition, a soxR gene cluster encoding "Superoxide Response protein" was upregulated. The physiology of strain CH1 was investigated and growth was observed at pH values ranging from 3.5 to 7 (optimal, pH 6), and at temperatures ranging from 30–60 °C (optimal, 50 °C). Moreover, strain CH1 had a Ks for methane of 7 µM. To our knowledge, this new strain is the first isolated thermoacidophilic gammaproteobacterium from hydrothermal habitats, expanding our knowledge of their ecological role in methane cycling.

11:45 - 12:30 SkewDB: A comprehensive database of GC and 10 other skews for over 30,000 chromosomes and plasmids

bert hubert[1]
[1]*Individual*

Introduction
GC skew denotes the relative excess of G nucleotides over C nucleotides on the leading versus the lagging replication strand of eubacteria and some archaea. While the effect is small, typically around 2.5%, it is robust and pervasive. GC skew and the analogous TA skew are a localized deviation from Chargaff's second parity rule, which states that G and C, and T and A occur with (mostly) equal frequency even within a strand. Different bacterial phyla show different kinds of skew, and differing relations between TA and GC skew. GC skew tells us something about bacterial replication strategies & their history. Anomalous skews can highlight interesting research subjects. The research behind this abstract is in press with Nature Scientific Data. A preprint is available on https://skewdb.org/gcskew-article-sd.pdf and on BioRXiv.

Methods
This article introduces an open access database (https://skewdb.org) of GC and 10 other skews for over 30,000 chromosomes and plasmids. Further details like codon bias, strand bias, strand lengths and taxonomic data are also included. This data is based on NCBI sequences and annotations.

Results
The 30,000 chromosomes show clear differences between phyla. Specifically for Firmicutes, it is shown for the first time that GC skew has an intrinsic strand bias, while TA skew derives solely from codon bias. Furthermore, hundreds of anomalous bacteria are uncovered with replication strands that differ vastly in length or in bias, indicating possible interesting biological mechanisms.

Conclusion
Having a database of 11 kinds of skews for 30,000 DNA sequences:
1) delivers biologically relevant conclusions on prokaryotic replication 'out of the box', and
2) can guide wet lab research into bacterial replication
3) can aid the formation of hypotheses on the still slightly mysterious origin of GC skew

16:00 - 16:15 Breaking resistance of methicillin-resistant Staphylococcus aureus to human Group IIA-Secreted Phospholipase A2 and daptomycin

MRS Marieke Kuijk[1], PhD V. P. van Hensbergen[2], Prof. N. M. van Sorge[1,3]
[1]Amsterdam UMC, [2]UMC Utrecht, [3]Amsterdam UMC

Methicillin-resistant Staphylococcus aureus (MRSA) has been classified as a high priority pathogen by the World Health Organization underlining the high demand for new therapeutics to treat infections. Group IIA-secreted Phospholipase A2 (hGIIA), a positively-charged enzyme that hydrolyzes bacterial membrane phospholipids, is among the most potent bactericidal human proteins against Gram-positive bacteria, including S. aureus. We set out to determine hGIIA-resistance mechanisms of MRSA to identify possible drug targets by screening the Nebraska Transposon Mutant Library (1,920 arrayed mutants) using a sublethal concentration of recombinant hGIIA. The screen identified lspA as a novel hGIIA resistance gene. lspA encodes the lipoprotein signal peptidase LspA, catalyzing the final step in lipoprotein maturation. We confirmed the role of lspA via a markerless lspA deletion mutant as a new hGIIA resistance determinant in both in vitro assays and a hGIIA-transgenic mouse infection model. Increased susceptibility was associated with faster and increased cell wall penetration of hGIIA. Moreover, deletion of lspA also increased susceptibility to daptomycin, a last-resort antibiotic to treat MRSA infections. Exposure of MRSA wild-type to the LspA-specific inhibitors globomycin and myxovirescin A1 induced a lspA-mutant phenotype with regard to hGIIA and daptomycin killing. Based on analysis of >26,000 S. aureus genomes, we show that LspA is extremely sequence conserved, suggesting that LspA inhibition could be applied universally. The role of LspA in hGIIA resistance was not restricted to MRSA specific since Streptococcus mutans and Enterococcus faecalis were also more hGIIA-susceptible after lspA deletion or LspA inhibition, respectively. Overall, our data suggests that pharmacological blocking of LspA may disarm Gram-positive pathogens, including MRSA, to enhance clearance by innate host defenses and synthetic antibiotics such as daptomycin.

12:15 - 12:30 ICP1 bacteriophage therapy reduces Vibrio cholerae infection in the zebrafish larvae model

Msc Adam Sidi Mabrouk[1], Dr J. S. Depelteau[1], Prof.dr. A. H. Meijer[1], Prof.dr. A. Briegel[1]
[1]*University Of Leiden*

Cholera continues to pose a major challenge to human health. Treatments which directly target the causative agent, Vibrio cholerae, are limited to the use of antibiotics. However, treatment of the disease with antibiotics will eventually lead to resistant strains. Therefore, it necessitates the need for alternative treatments, such as the use of bacteriophages. While clearance of V. cholerae with the use of bacteriophages has been performed on several animal models, none of these models are natural hosts of V. cholerae. Here, we use a combination of microbiological and imaging methods to investigate the effects of ICP1 bacteriophage predation of V. cholerae in vitro and in vivo. In vitro, we demonstrate that ICP1 is able to predate V. cholerae in several types of media. In all cases, this results in the development of resistance to the bacteriophage infection. Using the natural host model, Danio rerio, we show that treatment with ICP1 effectively clears V. cholerae colonization in gnotobiotic and conventionalized zebrafish larvae. In both of these models, resistance does not appear at least 7 hours post ICP1 exposure. Taken together, this study contributes to the fundamental knowledge necessary to develop a potential bacteriophage therapy for V. cholerae infection.

14:00 - 14:15 Incidence of COVID-19-associated pulmonary aspergillosis (CAPA) is not increased in patients with COVID-19 treated with corticosteroids, interleukin-6 inhibitors or a combination.

Drs Rebecca van Grootveld[1,2], Drs NAF Janssen[3,4,5,6], Dr MT van der Beek[2], Drs M Ergün[4,7], Dr K van Dijk[8], Drs C Bethlehem[9], Dr S Stads[10], Drs J van Paassen[11], Dr SHW van Bree[12], HT Kranen[13], Drs M Kuindersma[14], Dr A Beishuizen[15], Prof Dr PE Verweij[1,4,7], Dr JA Schouten[16], CAPA2.0 study group[2,3,4,7,8,9,10,11,12,13,14,15,16]

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Introduction

Invasive pulmonary aspergillosis has been reported in patients with COVID-19 admitted to the intensive care unit (ICU) requiring ventilatory support from the beginning of the COVID-19 pandemic. Since the mainstay of treatment for COVID-19 has changed and now consists of the immunosuppressive agents dexamethasone (since September 2020) and interleukin-6 inhibitors (tocilizumab/sarilumab, anti-IL-6) (since January 2021) an increased incidence of COVID-19 associated pulmonary aspergillosis (CAPA) was expected.

Methods

A retrospective, observational, multicentre study was performed from September 2020-April 2021 and included patients admitted to the ICU who had undergone diagnostic procedures including broncho-alveolar lavage sampling, non-bronchoscopic lavage sampling, blood galactomannan testing and/or Aspergillus PCR testing. Clinical data were collected about demographics, comorbidities, COVID-19 treatment, CAPA diagnosis and outcome. Patients were classified based on 2020 ECMM/ISHAM consensus criteria. Patients with CAPA were compared to patients without CAPA. Total number of COVID-19 patients admitted to the ICU in the research period was also collected.

Results

From nine medical centres, 618 patients were included of a total number of 1760 COVID-19 patients admitted to the ICU. CAPA incidence in all COVID-19 patients admitted to the ICU was 14.4%. Incidence of CAPA in our cohort was 40.9% (8 proven including 5 with documented tracheobronchitis, 200 probable, 45 possible). No remarkable differences were observed in comorbidities except for diabetes mellitus, which occurred more often in patients with CAPA 32.4% vs 23.3%(p=0.012). Presence of EORTC/MSGERC risk factors was similar in both groups, as was treatment with corticosteroids, anti-IL-6 or a combination. Increased mortality at ICU discharge was observed for patients with CAPA 51% vs 38.6%(p=0.002) and was highest for patients with proven CAPA(7/8).

Conclusion

CAPA incidence and mortality in patients with CAPA in this large multicentre cohort is comparable to previous reports in literature. Treatment with anti-IL-6 or corticosteroids/anti-IL-6 was not a risk factor for CAPA.

15:15 - 15:30 Sulfated Glycosaminoglycan-like Polymers are Present in an Acidophilic Biofilm from a Sulfidic Cave

Mr. Stefan De Bruin[1], prof.dr. DZ Sousa[2]
[1]Delft university of technology, [2]Wageningen University and research

Extracellular polymeric substances are an important structural component in anaerobic granular sludge. Microorganisms are embedded inside the biofilm by self-produced extracellular polymers. These polymers can attract water and have hydrogel forming properties which aid in the stability of biofilms. Sulfated glycosaminoglycans (sGAG) are negatively charged extracellular polymeric substances (EPS) that occur in biofilms from various environments. Yet, it remains unclear whether these polymers are acquired from the external environment or produced by microbes in the biofilm.
To analyze the possibility of microbially produced sGAG, we measured the presence of sGAGs using colorimetric, spectroscopic and enzymatic methods in an acidophilic biofilm devoid of contamination. Samples were collected from Sulfur Cave in Puturosu Mountain (Romania).
A maximum of 55.16 ± 2.06 µg sGAG-like polymers were recovered per mg of EPS. Enzymatic treatment with chondroitinase ABC resulted in a 26.3 ± 7.9 % decrease of the mass of the polymers, suggesting the structure of the recovered sGAG is similar to chondroitin. Subsequent FT-IR analysis of these polymers revealed absorbance bands at 1212 cm-1, 1167 cm-1 and 900 cm-1, indicating a possible presence of polysaccharides and sulfate. Analysis of genomic sequences closely related to those predominant in the acidophilic biofilm, contained genes coding for sulfotransferase (an enzyme needed for the production of sGAG), which supports the hypothesis of microbial synthesis of sGAGs within the biofilm.
A clear presence of sGAG-like polymers were found in the acidophilic biofilm collected from Sulfur Cave. The presence of sGAG-like polymers in this biofilm show a possibility of microbial biofilms to produce sulfated polymers internally.

11:30 - 11:45 Next-generation sequencing for routine identification of nontuberculous mycobacteria.

MSc J.A. Schildkraut[1], MSc J.P.M. Coolen[1], BSc H. Severin[1], PhD W.J.G. Melchers[1], MD, PhD W. Hoefsloot[2], Professor, MD, PhD H.F.L. Wertheim[1], MD, PhD J. van Ingen[1]
[1]Radboudumc, [2]Radboudumc

Introduction:
Currently, Nontuberculous mycobacteria (NTM) are identified using line probe assays or (multi)gene sequencing. Because these methods target small genomic regions, exact identification to species level is often not possible due to the high level of genetic similarity. We introduce a Next-generation sequencing (NGS) workflow that identifies mycobacteria to (sub)species level. In addition, we provide deeper insight into the NTM phylogeny in relation to clinical manifestation.

Methods:
Two sets of clinical isolates sequenced from June to December 2020 (n=206; validation set) and January and June 2021 (n=237; clinical set) were subjected to NGS. All short-read sequence data was analysed using a custom pipeline called MyCodentifier. NGS short-read data is filtered, QC checked, and matched against a series of custom databases created based on the phylogeny as presented by Tortoli et. al. Lastly, in-depth phylogenetic analysis was performed using the NGS-WGS data and was correlated to clinical manifestation.

Results:
In the validation set we observed 98.6% (n=203) agreement between the line-probe assay and the NGS-hsp65 method. For the clinical set (n=237) the NGS-WGS method correctly identified 98.7% (n=234) of isolates to species complex level, compared to NGS-hsp65. By performing in-depth analyses, we identified M. marinum species that belonged to a cluster not represented by the Tortoli et. al. phylogeny. In-depth phylogenetic analysis of Mycobacterium avium complex isolates confirmed mis-classification of two species (M. timonense and M. bouchedurhonense) and identified subclusters within M. avium. No correlation between subclusters and clinical manifestation was observed.

Conclusion:
We are able to perform routine NGS to identify NTM (sub)species with high accuracy. By performing additional in-depth phylogenetic analyses we identified unique subspecies and were able to combine them to clinical manifestation. Currently, no correlation between genotype and clinical representation was found, however, this NGS workflow paves a way for more personalized healthcare in the near future.

12:30 - 12:45 Reversible bacteriophage resistance by shedding the bacterial cell wall

MSc. Veronique Ongenae[1,2], Reversible bacteriophage resistance by shedding the bacterial cell wall Adam Sidi Mabrouk[1,2], Reversible bacteriophage resistance by shedding the bacterial cell wall Marjolein Crooijmans[1,2], Reversible bacteriophage resistance by shedding the bacterial cell wall Daniel Rozen[1], Reversible bacteriophage resistance by shedding the bacterial cell wall Ariane Briegel[1,2], Reversible bacteriophage resistance by shedding the bacterial cell wall Dennis Claessen[1,2]
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The cell wall plays a central role in protecting bacteria from some environmental stresses, but not against all. In fact, an elaborate cell envelope may even render the cell more vulnerable, since it contains molecules and structures that bacteriophages recognize as the first step of host invasion. Bacteriophages are highly abundant in the environment and a major threat for bacteria. Therefore, bacteria have evolved sophisticated defense systems to withstand phage attacks, like CRISPR/Cas, restriction-modification or abortive infection. However, some bacteria are known to be able to shed the bacterial cell wall in response to several environmental stressors.We hypothesized that wall-deficient bacteria may be temporarily protected against phages, since they lack the essential entities that are necessary for phage binding and infection. To test this hypothesis, three model organisms (Streptomyces, E. coli and B. subtilis) were inoculated in osmoprotective medium and infected with bacteriophages at MOI=1. Cryo-electron tomography was used to give us a detailed overview of the interaction between phages and wall-deficient bacteria. In this research, we describe a previously unknown mechanism by which mono- and diderm bacteria survive infection with diverse lytic phages. Phage exposure leads to a rapid and near complete conversion of walled cells to a cell wall-deficient state, which remain viable in osmoprotective conditions and can revert to the walled state. While shedding the cell wall dramatically reduces the number of progeny phages produced by the host, it does not always preclude phage infection. Altogether, these results show that the formation of cell wall-deficient cells prevents complete eradication of the bacterial population and suggest that cell wall-deficiency may limit the efficacy of phage therapy, especially in highly osmotic environments or when used together with antibiotics that target the cell wall.

12:45 - 13:00 Nitrogen cycle bacteria significantly impact the nitrogen balance of common carp (Cyprinus carpio L.)

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[1]Radboud Universiteit, [2]Radboud Universiteit

Introduction.
Aquaculture is one of the largest and fastest growing food producing sector worldwide. The most sustainable method of aquaculture is recirculating aquaculture systems in which fish are kept at high densities in closed basins. Due to the high fish density and protein-rich diets, ammonia, the main nitrogenous waste product of fish, can reach high concentrations. Fish excrete most ammonia via their gills. Ammonia is toxic, so reducing water ammonia concentrations is of key interest in aquaculture.
Recently, it was shown that excreted ammonia is converted into dinitrogen gas (N2) in carp gills through the combined activity of ammonia-oxidizing and denitrifying bacteria. At the moment, the contribution of these bacteria to the nitrogen metabolism of the fish is unknown.
Methods.
A feeding experiment was performed to investigate the impact of nitrogen cycle bacteria on the nitrogen balance of common carp and measured the N2 production rates of individual fish. Additionally, we investigated the gill microbiome of carp using molecular detection methods and 16S rRNA amplicon sequencing.
Results.
We discovered that approximately 30% of the nitrogen intake was unexplained by excretion and assimilation. N2 production rates were in the same order of magnitude as the unexplained nitrogen, which indicates that the 'missing' nitrogen can be explained by bacterial symbionts. The presence of ammonia-oxidizing bacteria in carp gills was confirmed with both 16S rRNA sequencing and ammonia monooxygenase A PCR.
Conclusion.
The conversion of ammonia to N2 by symbiotic N-cycle bacteria can explain the reduced ammonia excretion of carp. The discovery of this symbiosis between fish and microorganisms sheds new light on the nitrogen handling by fish and can help make aquaculture more sustainable.

17:00 - 17:15 Virological characteristics of Delta and Omicron vaccine breakthrough infections in Health Care Workers from Dutch two academic medical centers

MSc Marc Shamier[1], A Tostmann[2], S Bogers[1], B.L. Haagmans[1], R Molenkamp[1], J Rahamat-Langendoen[1], N Van Der Geest-Blankert[3], C Van Rossum[2], M McCall[2], H Wertheim[2], M.P.G. Koopmans[1], C.H. Geurtsvankessel[1]
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Introduction: Despite the high transmissibility of the SARS-CoV-2 Omicron variant (B.1.1.529) due to escape from vaccine-induced neutralizing antibodies, vaccination remains an important pillar in the COVID-19 pandemic, as complete vaccination plus a booster dose continues to provide protection against hospitalization for omicron infections. In this study we investigated how viral shedding varies between vaccinated and unvaccinated health care workers infected with the Delta (B.1.617.2) and Omicron variants.
Methods: We compared the virological characteristics of SARS-CoV-2 infections in healthcare workers (HCWs) of two tertiary care centers in the Netherlands (Erasmus MC, Rotterdam and Radboudumc, Nijmegen). These included 161 Delta vaccine breakthrough infections; 34 primo Delta infections in unvaccinated hosts; and more than 200 breakthrough infections with the omicron variant ¥. Virus culture was performed on all samples collected in the Erasmus Medical Center by inoculating Calu-3 (ATCC HTB-55) cells.
Results: Comparing first positive nasopharyngeal samples of breakthrough infections and primo infections with the Delta variant, we found similar mean Ct-values (24.6 and 25.4 respectively, p=0.68). Furthermore, we found no difference in culture positivity (72.2% versus 76.5%, p=0.65), which is an indicator of viral shedding. The average Ct-value for HCWs infected with the Omicron variant was 24.3. HCWs with symptomatic infections had lower Ct-values than HCWs with asymptomatic infections, corresponding to higher viral loads (median Ct-value 23.2 versus 26.7 p=0.022 [Delta]; and 22.7 versus 29.8 , p<0.001 [Omicron]).
Conclusion: We found no difference in viral shedding between vaccinated and unvaccinated health care workers with infections with the Delta variant. Furthermore, HCWs with Omicron infections had similar viral loads to HCWs with Delta infections, implying that other factors than viral load may be responsible for the increased transmissibility of this variant. Although vaccination continues to provide protection against severe disease, vaccinated individuals can still acquire infection and carry infectious virus.

¥Additional inclusions expected

14:00 - 14:15 Multiheme protein complexes catalyzing hydrazine formation and oxidation in anaerobic ammonium oxidizing bacteria

Dr. Wouter Versantvoort[1], Dr. C. Ferousi[2], Dr. A. Dietl[3], Dr. M. Akram[3], Dr. W. Maalcke[1], Dr. R. Schmitz[4], Dr. S. Lindhoud[5], Prof. Dr. Ir. M. Jetten[1], Dr. J. Keltjens[1], Dr. T. Barends[3], Dr. J. Reimann[1], Dr. B. Kartal[6], Prof. Dr. L. Van Niftrik[1]

[1]Microbiology, Radboud University, [2]Industrial Biotechnology & Catalysis, NTUA, [3]Biomolecular mechanisms, MPI for medical research, [4]Biogeochemistry and pollutant dynamics, ETH, [5]Laboratory of Biochemistry, Wageningen University and Research, [6]Microbial Physiology, MPI for Marin Microbiology

Anaerobic ammonium oxidizing (anammox) bacteria are chemolithoautotrophic microorganisms that make a living by converting nitrite and ammonium to dinitrogen gas, with the rocket-fuel hydrazine as a unique free intermediate. Anammox bacteria are ubiquitous in both natural and engineered ecosystems where they contribute substantially to the release of fixed nitrogen from that environment. The anammox reaction, taking place in a dedicated organelle termed anammoxosome, starts with the reduction of nitrite to nitric oxide by nitrite reductase. This nitric oxide is subsequently utilized by hydrazine synthase to oxidize ammonium, forming hydrazine. In the final step, hydrazine is oxidized to dinitrogen gas by hydrazine dehydrogenase, releasing four low potential electrons. These electrons are shuttled through a menaquinone-dependent respiratory chain to maintain the proton motive force required for ATP synthesis, before they are recycled in the first two steps of the anammox reaction. All three steps of the anammox reaction are catalyzed by dedicated multiheme protein complexes, which have been purified to homogeneity from native biomass of the anammox bacterium Kuenenia stuttgartiensis. Here, the purification and characterization of these three protein complexes will be presented. Their activity is confirmed in vitro using both colorimetric and mass spectrometry based enzymatic assays. Lastly, the crystal structures of these complexes have been determined, showing large multimeric states for both the putative nitrite reductase and the hydrazine dehydrogenase and allowed an initial prediction of the catalytic mechanism of hydrazine formation.

15:15 - 15:30 Screening of anti-tuberculosis Compounds using a Zebrafish Infection Model identifies a novel Aspartyl-tRNA Synthetase Inhibitor

Eva Habjan[1]
[1]*VU University medical center*

Novel anti-tuberculosis compounds are urgently needed to fight the emergence of multi-drug resistant Mycobacterium tuberculosis (Mtb) isolates. One of the current drug discovery challenges is finding the compounds and scaffolds with convincing in vivo activity.
In this work, we exploited and adapted the medium-throughput capabilities of the zebrafish embryo infection model, using Mycobacterium marinum as a surrogate for Mtb. Using a representative set of clinically established drugs, we showed that this infection model is predictive and selective for antibiotics that can be administered orally. Subsequently, the zebrafish infection model was used to test 240 compounds from the anti-TB hit library for their toxicity and in vivo activity. From this set, 14 compounds showed a significant reduction of bacterial burden when tested in the zebrafish infection model. One of the most active compounds was the tetracyclic compound TBA161, which was studied in more detail. Analysis of spontaneous resistant mutants revealed point mutations in aspS (rv2572c), encoding an aspartyl-tRNA synthetase. The target was genetically confirmed, and molecular docking studies propose possible binding of TBA161 in a pocket adjacent to the catalytic site. Direct comparison of TBA161 with published aspS inhibitors demonstrates the superiority of the newly identified compound.
This study identified a novel anti-mycobacterial inhibitor that targets the essential enzyme AspS within the protein translation pathway and shows excellent activity in the zebrafish infection model. Moreover, it demonstrates the importance of incorporating early in vivo models in the drug discovery pipeline, accelerating the drug discovery route.

12:30 - 12:45 Prevalence and persistence of antibiotic resistance determinants in the gut of travellers returning to the UK - a metagenomics study

Dr Timothy Dallman[1], Dr G Godbole[2,3,4], Dr M Brown[2,3,4], Dr R Behrens[2,3,4]
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Introduction
The gut microbiota constitutes an ideal environment for the selection, exchange and carriage of antibiotic resistance determinants (ARDs) and international travel has been identified as a risk factor for acquisition of resistant organisms. Here we present the GutBack study, a longitudinal metagenomic analysis of the gut resistome in travellers to high-risk countries.
Methods:
Fifty volunteers, recruited at a travel clinic in London provided stool samples before (pre-travel), immediately (post travel) and six months after their return (follow up) to a high-risk destination. Faecal DNA was extracted and metagenomic sequencing performed on an Illumina HiSeq yielding a median of 37 million non-host reads data per sample. The resistome was profiled using the Resfinder database and taxonomic profiling performed using Metaphlan3.
Results:
A significant increase in abundance and diversity of resistome was observed after travel. Significant increases in abundance were seen in antimicrobial genes conferring resistance to first line antibiotics including Aminoglycosides, Sulphonamides and Trimethoprim as well increased abundance in ARDs conferring resistance to clinically important Macrolides and third generation cephalosporins. There was a significant association in increased resistome abundance if the participant experienced diarrhoea during travel or took antibiotics, but these two variables were co-correlated. The resistome abundance returned to pre-travel levels by the 6-month sample point but there was evidence of persistence of several ARDs. Increase resistome abundance was correlated with an increased abundance of Escherichia genus.
Conclusion:
Foreign travel remains a significant risk factor for acquisition of microbes conferring resistance to multiple classes of antibiotics largely mediated to exposure to taxa from Escherichia genus. Episodes of diarrheal disease are positively correlated with an increase in resistome abundance.

17:15 - 17:30 Role for a lytic polysaccharide monooxygenase in cell wall remodeling

Mr Xiaobo Zhong[1]
[1]*Leiden University*

Peptidoglycan is a major constituent of the bacterial cell wall and an important determinant for providing protection to cells. Besides peptidoglycan (PG), many bacteria synthesize other glycans that become part of the cell wall. Streptomycetes grow apically, where they synthesize a glycan that is exposed at the outer surface, but how it gets there is unknown. Here we show that deposition of the apical glycan at the cell surface depends on two key enzymes, the glucanase CslZ and the lytic polysaccharide monooxygenase LpmP. Activity of these enzymes allows localized remodeling and degradation of the PG, and we propose that this facilitates passage of the glycan. The absence of both enzymes not only prevents morphological development, but also sensitizes strains to lysozyme. Given that lytic polysaccharide monooxygenases are commonly found in microbes, this newly identified biological role in cell-wall remodeling may be widespread.