ABSTRACTS ORAL PRESENTATIONS

O001
Bacterial Spore Germination: Still Surprises After All These Years
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Spores of Bacillales and Clostridiales species are formed in sporulation, and are metabolically dormant and resistant to just about everything. Spores of some species/strains are also causative agents of disease and food spoilage and may be potential bioweapons. While these spores can remain in their dormant, resistant state for many years, if given the proper stimulus, commonly nutritional or other signals that the spores' environment is conducive to growth, spores will rapidly germinate and return to vegetative growth. A significant number of proteins are required for spore germination, including: 1) proteins, termed germinant receptors, that recognize and respond to nutritional or other physiological "germinants"; 2) membrane proteins comprising a channel allowing the release of the spore core's huge depot (~ 25% of core dry weight) of dipicolinic acid (DPA) in a 1:1 chelate with Ca^{2+}; and 3) cortex-lytic enzymes that degrade spores' large peptidoglycan cortex, allowing the full hydration of the spore core, completion of spore germination and loss of spores' dormancy and resistance. Germination has been studied most with spores of *Bacillus subtilis* and models of spore germination have been largely based on the *B. subtilis* paradigm. However, and perhaps surprisingly, studies in recent years have found that while germination of spores of some Clostridiales species is very similar to that of *B. subtilis* spores, with other Clostridiales spores a number of rather large differences have been identified. In addition, recent work has also found that spores have short-term memory of stimulation by a variety of different germinants. After discussion of the model for germination of *B. subtilis* spores, this presentation will go through recently identified significant differences in the germination of spores of some Clostridiales, as well as evidence for spores' memory of germinant exposures.

O002
Bacterial Communication with the Brain
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Cell wall peptidoglycan is a universal pathogen associated molecular pattern (PAMP) recognized by innate immune Toll-like receptor 2 (TLR2) resulting in inflammation. During treatment of meningitis, cell wall components released by dying bacteria engage TLR2 and cause a burst of inflammation and neuronal cell death that contributes significantly to poor outcome. Investigation of the PAMP/TLR2 interaction and its downstream signaling has revealed many potential points for therapy but, overall, the inflammatory process is so multifaceted that even with combination therapies, an impact on outcome is difficult to demonstrate. Thus, we have embarked on a different approach: examining the possibility of initiating brain repair rather than preventing neuronal death could lead to strategies to prevent morbidity and mortality. We developed a new mouse model to investigate the effects of PAMPs circulating in the maternal bloodstream on the developing embryo. Cell wall traverses the placenta into the developing fetal brain but the fetal brain escapes inflammation and neuronal death characteristic of children and adults. Surprisingly, the fetal brain undergoes neuroproliferation leading to a 50% greater density of neurons in the cortical plate. Neuroproliferation requires TLR2 and is recapitulated in vitro by TLR2/6, but not TLR2/1 ligands. This novel TLR2 innate immune signaling induces the neuronal transcription factor FoxG1, a cornerstone of regulation of brain development. Thus, cell wall/TLR2 canonical signaling appears to be absent or repressed during fetal brain development and the fetal brain is a model of involvement of innate immunity in development of the architecture of the brain. However, changes in the cortical architecture are followed by abnormal cognitive behavior after birth, including features of autism. The bacterial cell wall-TLR2 signaling axis is a new participant and important modulator of microbial-host/embryo signaling with widespread consequences to brain functioning over time.

O003
Epidemiology of plasmids conferring carbapenem resistance in a multi-species KPC outbreak
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Whole-genome sequencing (WGS) of bacterial pathogens has become a valuable tool for molecular epidemiological studies. Within an outbreak, it can provide high-resolution insight into relationships between different isolates, enabling determination of which cases are linked, and potentially resolving transmission pathways. However, outbreaks driven by antibiotic resistance genes pose additional challenges, as resistance genes may mobilise between different host strains or species. In such cases, transmission pathways are linked to the evolutionary history of the resistance gene, rather than chromosomal host strain relationships, and it is less clear how to effectively utilise genomic data in these situations. One resistance mechanism of particular clinical concern is *Klebsiella pneumoniae* carbapenemase (KPC), which is encoded by bla_{KPC}. The bla_{KPC} gene is usually present within the 10 kb transposon Tn*4401*, which can be found on various plasmids. We have been investigating a long-term, multi-species outbreak of KPC-producing Enterobacteriaceae

from a large US hospital. Within this outbreak, we see very high diversity, at multiple genetic levels. There are many different host strains and species carrying *bla*$_{KPC}$, indicating frequent plasmid transfer to new host strains, as well as many different *bla*$_{KPC}$ plasmids, indicating frequent transposition of Tn*4401* to new plasmid backbones. Nevertheless, the Tn*4401* structure itself is almost universally present across outbreak isolates, leading to a view of the outbreak as being "transposon-driven".
In order to track the Tn*4401* transposon through the outbreak, we have developed a tool for identifying variation within, and genetic contexts of, transposable elements from short-read WGS data. By applying this to Tn*4401* across >1000 Illumina-sequenced outbreak isolates (derived from patient carriage/infection, as well as the hospital environment), we are able to quantify the extent of Tn*4401* transposition and micro-evolution. Because shared Tn*4401* variants and/or flanking sequences provide a marker for shared evolutionary history of *bla*$_{KPC}$, they can be used to define relationships across the outbreak from the perspective of the resistance gene, rather than relying on host chromosome-based phylogenetic approaches. In this way, we are able to track specific subgroups of isolates using these unique genetic signatures.
We have also utilised long-read sequencing to fully resolve plasmid structures in selected isolates, enabling deeper insight into plasmid transfer and evolution in these cases. For example, we have demonstrated *bla*$_{KPC}$ plasmid transfer from *Serratia marcescens* to an already KPC-positive *K. quasipneumoniae*, with a number of subsequent rearrangements resulting in the generation of several new Tn*4401* variants, as well as new Tn*4401* variant / plasmid combinations. This has highlighted additional complexities in inferring transmission pathways from genetic data, as repeated plasmid acquisition and homologous recombination can result in multiple origins for the resistance gene of interest in a given isolate.

O004
Quantifying environmental transmission of AMR
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Antimicrobial resistance (AMR) is a global concern, and the environmental dimension of resistance evolution and transmission is increasingly recognised as an important and understudied area of research. Many AMR bacterial infections are community acquired and the processes that lead to transmission of these organisms are largely unknown. Mobile resistance mechanisms are known to have evolved over evolutionary time in environmental bacteria indicating that the environment plays a critical role in emergence of AMR. Evidence also suggests that human behaviour and waste management practices have a significant effect on the evolution and ecology of microbial communities present in the environment. Most research to date has focused on environmental reservoirs of AMR rather than attempting to assess or quantify human exposure and transmission from soil, fresh and coastal waters. Efforts are currently being made to apply risk assessment methodologies to quantify exposure and transmission risk, however fundamental problems arise from the lack of dose-response relationship data available for opportunistic pathogen exposure and the potential for AMR gene transfer from environmental bacteria to the human microbiome. In addition, the rarity of genetic transfer events that lead to novel host / gene combinations makes assessing risk posed by novel resistance determinants inherently difficult to predict. Whilst estimating risk of infection by AMR opportunists is still problematic, exposure and transmission risk estimates can be made with colonisation rather than infection as an endpoint. Recent research on coastal bathing water exposure in the UK estimated millions of exposure events to 3rd generation cephalosporin (3GC) resistant *E. coli* occur each year, and a study of gut carriage of 3GC resistant *E. coli* in highly exposed water users demonstrated increased colonisation compared to controls. Further data will be presented on a targeted metagenomic approach to assess exposure risk to *E. coli* with multiple AMR genotypes.

O005
Using Nanopore to unravel the plasmidome content of 1644 Enterococcus faecium strains
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Introduction
Plasmids can facilitate the dissemination of multi-drug resistance via horizontal gene transfer of resistance genes. Therefore, tracking the transmission of plasmids is important to study the dissemination of antibiotic resistance. Reconstruction of plasmid sequences from short- read whole-genome sequencing (WGS) is error-prone, because in many cases the presence of repeated sequences prohibit to obtain complete plasmid sequences using short-read WGS. To obtain the full sequences of plasmids, hybrid assembly with long-read sequences has emerged as a new possibility. Here, we show that genomic structure information resolved by Oxford Nanopore Technologies can be used to train and test several popular machine learning classifiers for accurately predicting the plasmid content of a single species.
Methods
Our dataset consisted of short-read sequences of 1644 *E. faecium* isolates including samples from hospitalized patients (n>1100), non-hospitalized patients (n=135), pets (n=164) and farm (n=185). We sequenced 60 isolates using ONT sequencing and subsequently performed hybrid assemblies with Illumina short-read data. We mapped short-read based contigs (n= 10,421) from the 60 ONT isolates against complete chromosomal and plasmid sequences using bwa-mem and alignments were parsed using samtools to label each contig as chromosome- or plasmid- derived. Subsequently, we trained and tested several machine learning classifiers using pentamer frequencies as features. Area under the curve and precision-recall analysis were performed to select the best classifier. Resulting classifier (SVM) was benchmarked against other existing tools using an independent set of isolates We also evaluated its performance predicting all complete genome sequences available at NCBI RefSeq.
Results
Genomic structure inferred from complete genomes was used to train different classifiers and SVM was selected as best

classifier (AUC = 0.97, F-1 Score = 0.95) to predict plasmid sequences in *E. faecium*. SVM classifier outperformed other existing tools (Precision = 0.90, Genome fraction (~Sensitivity) = 0.84) in all the isolates from the independent set. Additionally, performance using complete genome sequences was outstanding (F1-score = 0.99) and no false positives were detected (Specificity = 1). SVM prediction in our collection of 1,644 isolates resulted in an average number of ~119 chromosome (2,632,470 bp) - and ~58 plasmid- (254,700 bp) derived contigs per isolate. Average posterior probability of the predicted chromosome- and plasmid- derived contigs corresponded to 0.95 versus an average posterior probability of 0.91 of predicted plasmid-derived contigs which indicated a high certainty in the plasmidome prediction.

Conclusions

We showed that genomic structure information resolved by ONT sequencing can be used to build a model capable to accurately classify plasmid sequences in *E. faecium*. We demonstrated the scalability of our model by accurately predicting the plasmidome of a large collection of *E. faecium* isolates with only short-read WGS available. Our model allows the assignation of a particular gene of interest as plasmid- or chromosome- encoded which will facilitate studying the dissemination of plasmid encoded antibiotic resistance in *E. faecium*. We anticipate that a similar methodology can be implemented in other species if a sufficient number of short-read and long-read data is available.

O006

Occurrence of antibiotic resistant bacteria along a wastewater chain

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Introduction: Antibiotic usage by humans can select for resistant (pathogenic) bacteria in the human gut, which are via faeces released into wastewater. To investigate whether antibiotic resistant bacteria (ARB) from wastewater end up in the environment, a sampling campaign was initiated to monitor the complete wastewater pathway up to the surface water. Three different bacteria; *Escherichia coli, Aeromonas spp* and *Klebsiella spp*, were tested for their abundance and antibiotic resistance (AR) patterns.

Methods: Water samples were collected every four weeks, two days in a row, from January 2017 until December 2017 in Sneek, The Netherlands. The locations included clinical (hospital and nursing home sewage) and non-clinical sources (urban sewage), influent and effluent from the wastewater treatment plant (WWTP), and surface waters with and without influence of WWTP effluent. The target bacteria were cultured on Tryptone Bile X-glucuronide medium (*E.coli*), Simmons Citrate Agar (*Klebsiella spp*), and Aeromonas agar base (*Aeromonas spp*). Five colonies per bacterial species and per sample were kept for further analysis. Antibiotic susceptibility testing was performed by disk diffusion. The antibiotics tested were selected based on AR data records from patients in Friesland in the last 5 years.

Results: In total, 211 samples were collected, from which 1027 *E. coli*, 868 *Klebsiella spp (K.pneumoniae and K.oxytoca)*, and 943 *Aeromonas spp* isolates were obtained. *Aeromonas spp* was most abundant, followed by *E.coli.* For all three species, the concentrations were highest in the samples directly taken at the source (hospital, nursing-home and urban sewage) and in the WWTP influent (7.5-9 $\log^{10}$ CFU/liter), while in the WWTP effluent the concentrations decreased to 5-5.5 $\log^{10}$ CFU/liter. Although the *Aeromonas spp* concentrations in the receiving surface waters were comparable to WWTP effluent (~5.2 $\log^{10}$ CFU/liter), *E.coli* and *Klebsiella spp* concentrations were lower in these samples, 4 $\log^{10}$ CFU/liter and 3.5 $\log^{10}$ CFU/liter, respectively. In the surface water without influence of wastewaterthe concentrations were lowest for all the tested species (4.5, 3, and 2.5 $\log^{10}$ CFU/liter for *Aeromonas spp, E.coli* and *Klebsiella spp* respectively). So far, 270 *Klebsiella spp* isolates have been tested for AR. Most resistant *Klebsiella spp* were found in hospital sewage (n=40); 17.5% was resistant to one antibiotic class, and 25% to two or more classes. The second-highest resistance ratio was found in nursing-home sewage (n=33; 24.2% and 3% against one and two antibiotic classes, respectively), followed by the WWTP influent (n=50; 20% and 2% resistance to one and four classes, respectively).

Conclusion: Wastewater is a source of resistant *E.coli, Klebsiella spp* and *Aeromonas spp* in the environment. Although hospital wastewater amounts to a small percentage of the total wastewater, it represents a point source of resistant *Klebsiella spp*. An in-depth characterization, e.g. by genome comparative analysis of the isolates from different locations, can help in confirming the role of wastewater as origin of resistant bacteria in the environment.

O007

Detection, quantification and typing of HEV RNA in food products on the Dutch market

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The incidence of hepatitis E virus (HEV) infection in the Netherlands has increased recently. In most cases, HEV infections are asymptomatic; the virus may, however, be responsible for mild to fulminant acute hepatitis and also chronic hepatitis in immunocompromised patients. Domestic pigs and wild boars show high infection rates with HEV genotype 3 (gt3) sequences that are closely related to those detected in hepatitis E patients. The main transmission route(s) still need to be determined, but consumption of raw or undercooked porcine meat might be of great importance. HEV RNA has been detected in porcine liver, pork and pork products by several groups. The presence of infectious HEV was demonstrated in pork liver sausage and livers, although the test systems used are too complex for routine analyses. The recent increase in HEV gt3 infections warranted start of food analyses for the presence of HEV in food on the Dutch market.

To enable such monitoring, methods have recently been set up and validated, as an ISO standard for the detection and quantification of HEV in food is currently not available. The methods consisted of a matrix dependent virus extraction followed by nucleic acid extraction and RT-qPCR detection. Controls for extraction efficiency and RT-qPCR inhibition were implemented for each sample. Quantification of HEV RNA was performed using a linearized plasmid DNA with a modified target sequence. Positive samples were reanalysed using a nested typing RT-PCR designed by RIVM and

obtained fragments (493 nt) were sequenced to enable comparison to sequences reported in human cases. Results of ongoing monitoring studies will be presented and compared to findings in other studies reported. Results include those on monitoring of porcine blood products that are used as ingredients in meat productions. HEV RNA was detected in a high percentage in non-heated liquid products and were typed as HEV gt 3. Contamination levels were highest in liquid whole blood, plasma and fibrinogen, warranting heat treatments down-stream of the process to adequately inactivate any infectious HEV present. Further monitoring targeted a variety of pork products, including liver, liverwurst and liver pate of which the latter two are being heat treated in the industrial production process. HEV RNA was detected, quantified and sequenced in most liverwurst and liver pate samples, but also in some liver samples, showing a wide range of contamination levels with mostly HEV gt3 strains. In the tested pork chops and wild boar meat no HEV was RNA was detected. Methods for the analyses of HEV RNA in fermented or dry sausages were adapted to Szabo (2015), and preliminary results indicate the presence of quantifiable amounts of HEV RNA typed as gt3c in part of the samples tested. As these sausages are ready-to eat-products and have not undergone heat treatment processes, these might pose a risk for infection that is further investigated at this moment.

O008
Clinical implications of hepatitis E
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Hepatitis E virus (HEV) has a broad variety of appearances. Asymptomatic cases were described in immune competent individuals, as well as atypical benign illness causing fatigue, which is mostly self-limiting. On the contrary, on the tip of the iceberg, high mortality rates in pregnant women have been reported to be caused by HEV genotype 1 infections and HEV genotype 3 was found to be either the single agent or presented as an acute-on-chronic infection resulting in severe liver disease and liver failure. Besides infection of the liver, hepatitis E virus is associated to extrahepatic infections. Mostly neurological diseases like Guillain Barré and brachial neuritis have been reported. Due to this broad clinical picture, misdiagnosis may occur, leading to the wrong choices of treatment in transplantation patients. Therefore, application of good diagnostic tools – techniques and screening algorithms- are of utmost importance to provide the best clinical care. This presentation will highlight the most important findings of the last decade of HEV clinical research and the latest insights for clinical practice.

O009
Sources and risk factors for acute hepatitis E in the Netherlands
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Background
The number of hepatitis E patients has recently increased in the Netherlands, as in other European countries. Although swine are known as main reservoir for hepatitis E virus (HEV), causes of the (re-)emergence of HEV and exact transmission routes of HEV are currently unknown.
Methods
To identify risk factors for HEV exposure and infections in the Netherlands, two studies were performed among: (1) acute hepatitis E patients, and (2) blood donors. A questionnaire on potential risk factors for HEV exposure, health and socio-demographic characteristics was completed by:
381 patients with laboratory-confirmed acute symptomatic hepatitis E, enrolled through 23 medical microbiological laboratories (June 2015 - October 2017), and 1535 population controls matched for age, gender and region of residence. The IgM- and/or RNA-positive blood samples were submitted to the RIVM, where the samples were tested for HEV-RNA and were genotyped.
1562 healthy blood donors from all over the Netherlands (March - May 2016) aged 18-70 years, whose plasma samples were tested with Wantai EIA for anti-HEV IgG antibodies.
Results
Hepatitis E infection in the Netherlands was associated with consumption of traditional Dutch dry fermented raw pork sausages which are generally consumed sliced unheated on bread. According to multivariate analyses adjusting for age and gender:
Compared to population controls, patients with acute hepatitis E reported more comorbidity such as preexisting liver disease (aOR 3.0; 95%CI 1.5-5.7) or diabetes (aOR 2.4; 95%CI 1.6-3.8), and more often used immunosuppressants (aOR 2.8; 95%CI 1.7-4.6) or gastric acid inhibitors (aOR 2.3; 95%CI 1.7-3.2). Patients with acute hepatitis E were more likely than population controls to report consumption of “cervelaat” (aOR 2.1; 95%CI 1.5-2.9), “sliced sausage” (aOR 1.8; 95%CI 1.2-2.5), “farmer sausage” (aOR 1.7; 95%CI 1.2-2.4). Furthermore, population controls were more likely to never eat pork meat (aOR 0.2; 95%CI 0.1-0.5). Working with a septic tank was a risk factor (aOR 10.0; 95%CI 1.4-72.4), which was reported by 4 patients and 4 population controls. These aOR may alter slightly in our finishing analyses.
HEV-IgG-seroprevalence was 31% (648/2,100) and increased with age. The study population consisted mainly of healthy individuals with relatively few health complaints or underlying diseases. HEV-IgG-seropositive blood donors were more likely to report consumption of dry sausages called “cervelaat”, “fijnkost”, “salami” and “salametti” (combined aOR 1.5; 95%CI 1.2-1.9). Contact with contaminated water was a risk factor for seropositivity (aOR 2.5; 95%CI 1.5-4.4), which was reported by just 65 (4.2% of) blood donors.
Conclusions
Two studies show that several dry raw pork sausages are associated with HEV exposure and infections in the Netherlands. The prevalence and infectivity of HEV in these products should be investigated, as well as the production methods and possible origin of HEV-contamination within these sausages, e.g. small amounts of pork liver. Although not

frequently reported, contact with contaminated water was a risk factor.

O012

Microbial controls on the methane release from thawing permafrost

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The timing and radiative forcing of the permafrost carbon feedback (PCF) is not well understood. This is manifested in PCF projections of 0.05 to 0.39 °C temperature increases by the year 2300. Average modelled CH_4 contributes only ~16% of the PCF despite its high radiative forcing compared to CO_2. Short-term permafrost batch experiments also resulted in a minor CH_4 contribution to the PCF compared to CO_2 (#_ENREF_1). This contribution had long lag phases (#_ENREF_2) but on longer time scales equal amounts of CO_2 and CH_4 may be produced anaerobically. CH_4 concentrations and isotopic signatures in submarine permafrost sediments furthermore showed oxidation of CH_4 after it was released by permafrost thaw (#_ENREF_3). Large uncertainties in projected CH_4 release thus depend on the microbial response to permafrost thaw. Important questions include: i) what constrains methanogenic activity in recently thawed sediment and in permafrost and ii) are there unrecognized yet relevant microbial methane filters associated with sub-aquatic permafrost thaw?

Using Illumina sequencing, quantitative PCR, total cell counts and biomarker analysis in combination with stable isotope probing, batch experiments, geochemistry and statistics, we addressed these questions on permafrost cores from Canada, Alaska and Siberia of Holocene and Pleistocene origin that range from frozen to completely thawed permafrost sediments.

The low number of methanogenic cells is a primary initial constraint on CH_4 production in thawing permafrost. The methanogenic population size is thereby a function of carbon density and serves as a good predictor for the potential production of CH_4 given permafrost thaw. We suggest that thousands of years of exposure to permafrost conditions bred high-affinity methanogenic communities, since in most of our incubations they were unable to exhaust large concentrations of substrates or to substantially build up biomass, even after one year of incubation at moderate temperatures. Comparative sequencing analysis on incubations with weak versus strong responses to permafrost thaw will clarify community level constraints on microbial CH_4 production.

We further identified microbial communities responsible for anaerobic methane oxidation (AOM) in sediments undergoing sub-aquatic permafrost thaw. Specifically, ANME-2a/b and ANME-2d assemblage oxidize CH_4 in submarine permafrost and ANME-2d in thermokarst lake sediments. Calculated potential AOM consumes between 70 and 100% of the in-situ methane equaling up to 1.2 Tg C per year when extrapolated to the circum-arctic submarine permafrost distribution. We propose that AOM is active at around 0°C where subaquatic permafrost thaws which should be accounted for in permafrost carbon feedback models.

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O013

Smart residue amendments to improve greenhouse gas uptake by agricultural soil

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Concentrations of the three major greenhouse gases (GHG) carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) increased since the industrial revolution. An estimated part of ~50% and ~60% for CH_4 and N_2O, respectively, originates from agricultural practices. Organic fertilizers, generally applied to increase soil fertility in a more sustainable way, have been shown to stimulate CH_4 uptake from agricultural soils. This finding opens up possibilities to modulate fertilizer application targeted at minimizing GHG emissions. To tackle this challenge, we incubated an agricultural soil with different organic amendments (compost, sewage sludge, digestate, cover crop mixture), either as single application or in a mixture under different soil moisture concentrations and different amounts of organic amendment. GHG fluxes and in vitro CH_4 oxidation rates were measured continuously, while changes in organic matter and abundance of GHG relevant microbial groups (nitrifiers, denitrifiers, methanotrophs) were measured at the end of the incubation. While most GHG emissions were dependent on soil moisture and amount of organic fertilizers, several combinations of amendments led to promising reductions of CO_2, CH_4 and/or N_2O emissions compared to unamended soil. The best results could be obtained by the compost treatment, which even provide a better overall GHG balance than the unamended soil. However, compost is not very nutrient and N rich, therefore the combination of compost with one of the more N rich organic amendments (sewage sludge, digestate) provide a good balance between fertilization capability for plants and a moderate GHG emission. Both combinations emitted significantly less GHGs than the single amendments of either sewage sludge or digestate. Future research should focus on the interrelation of plants, soil, microbes and their impact on the global warming potential. Overall all three GHGs differ noticeably in their flux measurements, while CO_2 and N_2O showed a great variety through all treatments, CH_4 fluxes seemed stable over time and CH_4 uptake showed a clear increasing for all treatments trend over time. Like the CH_4 fluxes, total microbial abundance showed no differences before and after the incubation.

O014

The effects of warming on methane cycle microorganisms in Arctic thermokarst lake sediments

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Introduction Permafrost covers a quarter of the Northern Hemisphere land surface and contains half of the global belowground organic carbon stocks. According to IPCC climate models the mean annual air temperature in these Polar Regions is expected to rise 8 °C by 2100. This may lead to increased permafrost thaw and thermokarst formation. Elevated temperatures can alter microbial organic matter decomposition rates resulting in higher CO_2 and methane emissions. Here we investigated the effect of increased temperatures on microbial community structure and metabolic activity in the methane cycle of thermokarst lake sediments. Our aim was to retrieve information that could be used to better predict how warming will affect methane fluxes in the Arctic.
Methods Seven sediment cores were obtained from two thermokarst lakes in the region of Barrow, Alaska, USA. Cores were sliced and subsamples were homogenized and used for methanogenic and methanotrophic activity measurements at 4 and 10 ˚C, the current and predicted in-situ temperature. Lake sediments were amended with methanogenic and methanotrophic substrates and followed over a 270-days period to gain insights into direct and long-term temperature effects. Further enrichment cultures were performed to target novel methane cycle microorganisms. To reveal microbial species diversity, 16S rRNA genes and metagenomes were sequenced by Illumina MiSeq technology.
Results Methanogenic incubations showed that hydrogenotrophic methanogenesis is not a relevant pathway in this ecosystem. Methylotrophic and aceticlastic methanogens were, however, quite active. Aerobic methanotrophs were very active at both 4 and 10 °C. Our results showed an increase in both methane production and consumption at higher temperatures. 16S rRNA gene based phylogenetic analysis indicated a clear enrichment of *Methanosaetaceae* and *Methanosarcinaceae* on incubations with acetate and methylated amines respectively. Methylococcales were enriched in aerobic methanotrophic incubations along with other potential methylotrophs affiliated with Flavobacteriales and Methylophilales.
Conclusion In this study we found that microbial diversity is a good indicator for the metabolic potential of this ecosystem. A 6 °C temperature increase enhances both methanogenesis and aerobic methanotrophy rates. However, the response is less strong than can be expected solely based on the temperature effect on enzymatic activity. This study will help to better understand the processes behind global warming and to improve future climate projections and predictions, and it can be the basis for studying defined co-cultures of methane cycle microorganisms.

O015

Novel mesocosm set-up reveals methane oxidizers in submerged sphagnum moss as major methane filter in peatlands

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Wetlands are the largest natural source of methane emissions, emitting on average 164 gT yr^{-1} [1]. In peatlands methane emission is in general lower than the potential emission, due to the activity of methane-oxidizing bacteria that act as a microbial biofilter [2,3]. Until now, methane oxidation and production activity have been studied using peat soil slurries and net-production measurements in the field. In this study, we developed a novel mesocosm set-up that enabled exact control of methane input and monitoring of the dissolved methane throughout a peat column in a setting that closely resembled natural conditions. Two mesocosm set-ups were used in parallel: an experimental setup with a *Sphagnum* peat moss layer in peat water, and a control column containing only peat water. Over the 2-month course of the experiments, the methane oxidation rates increased by 50%. We were able to show that methane consumption only occurred in the peat moss layer and in the peat water, indicating that methane-oxidizing bacteria were only associated with the *Sphagnum* spp. FISH microscopy and qPCR of both 16S rRNA and pmoA genes, indicated that significant numbers of methane-oxidizing bacteria were present in and on the moss. Analysis of 16S rRNA amplicons revealed a diverse microbial community including type I and type II methanotrophs. Together these findings showed that our mesocosm setup can be used to study methane cycling in *Sphagnum* peat mosses under defined conditions which is an important step forward towards better understanding of the interactions between *Sphagnum* peat moss and its associated methanotrophic communities.

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O016

Characterization of a methanotroph hydroxylamine oxidoreductase: How methanotrophs achieve growth under high ammonia concentrations

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Aerobic methanotrophs make a living by oxidizing methane to CO_2. The first step in this process is the O_2-dependent oxidation of methane to methanol catalyzed by either a soluble (sMMO) or a particulate methane monooxygenase (pMMO). The latter is closely related to ammonia monooxygenase (AMO) from ammonia oxidizing bacteria (AOB), where it oxidizes ammonia to hydroxylamine. In AOB, hydroxylamine is further oxidized to nitric oxide by an octaheme hydroxylamine oxidoreductase (HAO). Due to the similarity between AMO and pMMO, both enzymes are capable of co-oxidizing the alternative substrate. Therefore, aerobic methanotrophs can also oxidize ammonia to hydroxylamine as a

side reaction. Hydroxylamine is a potent inhibitor of the methanol dehydrogenase (MDH), with a K_i of 12 µM, requiring rapid turnover to prevent interruption of the energy-yielding steps of methane oxidation. Interestingly, many methanotrophs encode an HAO-like protein, which is postulated to oxidize hydroxylamine and thus prevent inhibition of MDH.
So far the only characterized HAOs are from the AOB *Nitrosomonas europaea* and from the anaerobic ammonia oxidizing (anammox) bacterium *Kuenenia stuttgartiensis*. Both enzymes exhibit high sequence and structural similarity and perform the three-electron oxidation of hydroxylamine to nitric oxide. A diagnostic feature of HAOs is the presence of a P460 chromophore with a Soret maximum at 460 nm in the reduced spectrum. This redshift has been attributed to a tyrosine residue double-crosslinked to the active site heme, observed in the crystal structure of both enzymes. The crosslink is hypothesized to tune this octaheme cytochrome variant towards oxidative catalysis. Methanotroph HAOs show high sequence similarity with the studied HAOs, including the presence of the crosslinking tyrosine, suggesting a role in oxidation of hydroxylamine.
In this study, a hydroxylamine oxidoreductase from the methanotrophic bacterium *Methylacidiphilum fumariolicum* SolV was isolated and its catalytic properties are being investigated. In line with the aforementioned hypothesis, this enzyme possesses the diagnostic P460 chromophore supporting efficient oxidative catalysis. Similar to both studied HAOs, it is capable of oxidizing hydroxylamine while reducing bovine cytochrome *c* in a 1:3 stoichiometry, producing nitric oxide as the end product. This HAO homolog appears to enable *M. fumariolicum* SolV to grow in the presence of high ammonium concentrations that it encounters in the environment (up to 28 mM) by avoiding the buildup of hydroxylamine and its inhibitory effect on methanol respiration.

O018

Production of Chemicals by *Clostridium* sp in biorefineries

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The expected shortage of petroleum in the near future and concerns regarding the net increase of carbon dioxide emissions and environment pollution by fossil fuel combustion, have resulted in a search for sustainable sources for the production of transport fuels and chemicals. Biomass, CO_2 or syngas could provide sustainable alternatives as feedstock's for fuels and chemicals. The fermentative production of butanol, a C4 alcohol with multiple applications as fuel or solvent, carried out by various Clostridial species as part of the acetone-butanol-ethanol (ABE) process is an example of a process for with high potential for production of green fuels and chemicals. The ABE process has a long industrial history and is currently being re-introduced at commercial scale (Green 2011).
As in most biological processes for production of chemicals or fuels, there are important drawbacks that limit economic viability of the ABE process: low butanol yields from substrate, due to the formation of by-products and to the toxicity of butanol itself to the cultures that limits the end-concentrations achieved, and the high costs of the separation of the different products.
In this presentation, several strategies employed at WFBR to improve the economic viability and the understanding of the physiology and genetics of solvent-producing Clostridia will be shown, including metabolic engineering for the reduction of by-products formation and of sporulation.
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O019

Transcriptional response of Clostridium difficile to sub-inhibitory concentrations of antimicrobial compounds

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Antimicrobial exposure can alter transcription patterns, potentially leading to tolerance and/or increased resistance. In particular, DNA replication targeting inhibitors can alter gene expression of origin-proximal genes as a result of an altered origin (*oriC*):terminus (*terC*) ratio (1).
We performed an RNA-Seq analysis of *Clostridium difficile* exposed to 5 different antimicrobials at levels below the minimal inhibitory concentration. The most dramatic transcriptional response was observed for the *C. difficile*-specific DNA polymerase inhibitor 362E (N2-(3,4-Dichlorobenzyl)-7-(2-[1-morpholinyl]ethyl)guanine; MorE-DCBG) (2), which was highly effective against a diverse panel of 363 clinical isolates from our laboratory. Our results show that treatment with 362E leads to relative up-regulation of origin-proximal genes, relative down-regulation of terminus-proximal genes and an increased *oriC*:*terC* ratio. This effect was also observed for the broad-spectrum DNA polymerase inhibitor HPUra, but not for the other antimicrobials tested. Our ongoing work to validate the RNA-sequencing results using promoter-luciferase reporters and insight in the mechanism of transcriptional regulation will be presented.
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O021

From men to mice and back again - an innovation cycle with armed *Clostridia* as safe and specific weapons to fight cancer

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The worldwide incidence of cancer is huge and despite significant advances in recent years, overall 5-year survival rates remain poor for many cancer patients. There is therefore a pressing need for alternative strategies to the well-trodden paths of conventional therapies. Most solid cancers contain regions of necrotic tissue and the extent of necrosis is associated with poor survival. Despite the high frequency of tumor necrosis, particularly in advanced disease, no therapeutic modalities exist to explicitly address this clinical issue. In our alternative anti-cancer approach, we use the harmless anaerobic bacteria *Clostridium sporogenes*, that upon injection as spores, will germinate and thrive in these necrotic regions, providing cancer-specific colonisation, a totally natural exquisitely specific phenomenon. Upon concise development of the required technology, we stably “armed” *C.sporogenes* with a chemotherapy-activating gene (nitroreductase, NTR) that also enables the bacteria to be imaged by positron emission tomography. We term this approach Clostridial-directed Enzyme Prodrug Therapy (CDEPT). So, although we still use a chemical toxin to kill cancer cells, rather than allow this to affect the whole body, we use a non-toxic prodrug that is only converted to the active therapeutic within the cancer tissue. CDEPT thus offers a unique opportunity to turn a pathological feature associated with treatment failure into a precision therapy. In this presentation, the past, present and future of this approach will be discussed.

O022

Infant's immune response to vaccination after maternal pertussis vaccination

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Pertussis caused by the *Bordetella pertussis* bacterium, is a highly contagious respiratory illness and a major cause of infant morbidity and mortality, which is most severe in infants below the age of two months, too young to be vaccinated. Maternal pertussis vaccination induces high antibody titers in pregnant woman that could offer protection against clinical pertussis to these young infants from birth onwards during the first months of life through active transplacental transport of these maternal antibodies. However, maternal antibodies have been shown to interfere with infant responses to primary immunizations. We investigated the possibility to delay the first infant pertussis vaccine dose from 2 to 3-months of age and to reduce the primary series doses from 3 to 2 immunizations if used in combination with maternal vaccination.

A randomized controlled trial has been conducted in which 118 pregnant women either received a TdaP (Boostrix) vaccination during pregnancy (maternal-group), between 30 and 32 weeks of gestation, or within 48 hrs after giving birth (control-group). The infant DTaP-IPV-Hib-HepB schedule consisted for both groups of immunizations at 3, 5 and 11-months of age. Blood samples were collected at birth and 2 months of age and before and after primary and booster vaccinations.

Pertussis, tetanus and diphtheria specific IgG antibody titers in cord blood and in serum collected at age 2- and 3-months were significantly higher in infants of mothers that were vaccinated (n=58) during pregnancy than in infants of the control group (n=60). After primary vaccination mean titers increased for both groups but the response was significantly lower in the maternal compared with the control group. The booster response was comparable in both groups but absolute titers were still significantly lower in the maternal compared with the control group.

Our data show that children of mothers vaccinated during pregnancy, have significantly higher pertussis antibody levels at 3-month of age compared to 2-month (current age first vaccination) and 3-month-old children of non-vaccinated mothers. This implies that the first immunization can be postponed to the age of 3-months, although stilla significant reduction in antibody response to primary series pertussis antigens was observed. Whether this has clinical implications needs to be carefully monitored. Data on functional antibody and cellular memory responses are currently being analyzed.

O023

Vaccination against measles virus and respiratory syncytial virus

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Introduction

Measles virus (MV) and respiratory syncytial virus (RSV) are closely related enveloped viruses with unsegmented negatively stranded RNA genomes, and are transmitted via the respiratory route. However, their pathogenesis and clinical manifestations are very different. MV infection of non-immune humans almost always results in systemic virus replication leading to clinical measles. Measles causes immune suppression, and is frequently associated with complications like pneumonia or gastro-enteritis. However, after recovery the host is immune for life, and re-infections are rare. In contrast, RSV infections are associated with a wide spectrum of clinical signs, ranging from subclinical infection to severe bronchiolitis. Virus replication is restricted to the respiratory tract, and bronchiolitis in infants is caused by a strong inflammatory response in the airways with mucus overproduction and plugging of the small airways. Although several risk factors have been identified, it is poorly understood what drives development of severe disease. In contrast to measles, natural RSV infection does not induce protective immunity and re-infections are common.

A historical perspective on vaccination

During vaccine trials in the 1960s, experimental formalin-inactivated RSV vaccines (FI-RSV) predisposed infants to enhanced pulmonary disease upon subsequent natural RSV infection. FI-RSV induced an incomplete and unbalanced

type of immunity dominated by T-helper type 2 (Th2) cells, while specific cytotoxic T-cells were absent and antibodies were of low avidity. Pulmonary recall of these immune responses during natural infection resulted in bronchial hypersensitivity responses. Similar observations were also reported for MV: vaccination with FI-MV predisposed infants for enhanced disease upon natural MV infection, caused by Th2-mediated hypersensitivity responses and immune complex formation. It may be speculated that similar aberrant immune responses, elicited by either FI-RSV or FI-MV, result in distinct clinical syndromes, determined by the distribution of viral antigens in tissue upon natural infection with these viruses. Whereas safe and effective live-attenuated MV vaccines were developed fifty years ago, there is still no licensed RSV vaccine.

Measles and RSV vaccines: new developments

Despite their success, live-attenuated MV vaccines also have limitations, including problems with injections safety and cold chain dependence. Needle-free MV vaccination regimens were developed to address some of these issues, including aerosolized vaccines. Since live-attenuated virus vaccines typically contain a low antigenic dose, replication in the host is essential for induction of protective immune responses. We have shown that the virus needs to reach the lower respiratory tract to induce protective immune responses. Recent progress in the field of RSV research has resulted in accelerated development of candidate RSV vaccines, of which several are now being evaluated in clinical trials. Since the peak incidence of bronchiolitis is seen in infants 1-6 months of age, RSV vaccination strategies require maternal or perinatal immunization schemes.

Conclusion

The Global Vaccine Action Plan (GVAP), endorsed by the World Health Assembly in 2012, calls for the elimination of measles in at least 5 of the 6 WHO Regions by 2020. This will require enhanced vaccination and surveillance efforts. In parallel, RSV vaccines may finally become available for prevention of severe RSV disease in infants.

O024

Measles vaccination before 9 months of age results in impaired humoral immune responses

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During a measles outbreak in The Netherlands, children between 6-12 months old were offered an early measles, mumps and rubella (MMR) vaccination next to regular vaccination at 14 months of age. While this measure protects in outbreak situations, there are concerns about long-term duration of acquired immunity. Here we determined measles-specific serum IgG, neutralizing antibodies and IgG avidity for children who received their first measles vaccination (MMR-0) between 6-9 months (n=46) or 9-12 months (n=33) next to regular vaccination at 14 months (MMR-1). A control (n=43) group was only vaccinated at 14 months old. Before receiving regular vaccination at 14 months, children vaccinated at 6-9 months had lower measles-specific IgG and neutralizing antibody levels compared with children vaccinated between 9-12 months old ($p<0.0001$). 1 year later, children vaccinated between 6-9 months had significantly lower neutralizing and IgG antibody concentrations than children vaccinated between 9-12 months ($p=0.04$ and $p<0.0001$ respectively) and children vaccinated at 14 months old ($p<0.0001$). However, no clear differences in antibody avidity were observed. In conclusion, age of primary MMR vaccination affects measles-specific IgG and neutralizing antibody concentrations. These data show blunting of the humoral response when reducing the age of measles vaccination, which may affect long-term protection.

O025

Respiratory Syncytial Virus directly infects NK cells and affects anti-viral effector functions

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Respiratory syncytial virus (RSV) infection can lead to severe respiratory illness and is the major cause of hospitalization in infants under 1 year of age. During RSV infection in mice, increased numbers of natural killer (NK) cells are present in the lungs. There are contradictory reports about NK cell counts in humans during RSV infection and the putative role of these cells in RSV disease is ambiguous. NK cells reportedly reduce the viral load; however, accumulation of these cells can cause acute lung injury through the production of excessive levels of IFN-γ. We hypothesized that RSV and RSV-immune complexes interact with NK cells and affect their function, potentially leading to severe immunopathology. Interestingly, incubation of NK cells with RSV resulted in viral replication, as virus-encoded GFP could be detected by flow cytometry at 20h post infection. Additionally, incubation with RSV-immune complexes formed at sub-neutralizing antibody concentrations increased the percentage of infected NK cells up to 6-fold. Viability of the cells was not affected up to 48h post infection. Although replication could be detected, there appears to be no production of infectious viral particles. Infected NK cells produced high levels of IFN-γ, even in the absence of an external activating trigger. Antibody-dependent enhancement of infection led to even higher IFN-γ production. The IFN-γ response was lower in the presence of an infection inhibitor, or non-infectious RSV-immune complexes. Interestingly, strong IFN-γ production by RSV-infected NK cells was not accompanied by an enhanced killing capacity, as evidenced by the percentages of perforin-secreting cells upon stimulation.

We have shown for the first time that RSV can infect NK cells *in vitro* and that infection leads to enhanced IFN-γ production by these cells. We also show that the number of infected NK cells is increased in the presence of sub-neutralizing antibody concentrations. The observed increase in IFN-γ production, in the absence of increased perforin secretion, possibly contributes to the immunopathology present in infants with severe RSV disease. Future research will focus on assessing the mechanism(s) through which RSV affects these important anti-viral NK cell functions.

O029

The role of urine cultures in diagnosis and treatment of urinary tract infections in a long-term care facility: time for improvement

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Objectives: To assess the role of urine cultures in diagnostic reasoning and treatment of urinary tract infections (UTI) and to uncover the reasoning behind the utilization of urine cultures by physicians in a long-term care facility (LTCF)
Design: Retrospective assessment of urine cultures and antibiotic (AB) prescriptions for UTI during a 1,5-year period in a 370 bed LTCF. Prospective analysis of the physicians reasoning for ordering urine cultures using questionnaires.
Setting: A 370 bed non-academic LTCF
Participants: Thirteen physicians: 11 elderly care physicians and 2 junior doctors.
Results: A total of 803 antibiotic courses were prescribed tot 378 residents. 155 cultures were performed from urine samples of 135 residents, 66 of these cultures were accompanied by an AB prescription on the same day. There was a discrepancy between culture results and the actions taken in 75% of the cases. In these cases the initial antibiotic treatment was not adapted when either: the isolated micro-organism was resistatnt to the AB prescribed, or the culture was positive and no AB had been prescribed, or the resident was on antibiotic treament but het culture was negative. The most frequent reasoning for ordering a urine culture was 'to confirm the diagnosis UTI'.
Conclusion: In the majority of the cases antibiotic therapy was not adjusted, when urine culture results suggested that this was appropriate. And the physicians were erroneously convinced that UTI can be diagnosed by a positive urine culture.

O030

The Dordrecht model: local laboratory and municipal health service (MHS) for infection control in Dutch nursing homes.

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The Dordrecht model: local laboratory, and municipal health service (MHS) for infection control in Dutch nursing homes
Infection control in nursing homes and long term care facilities has been "neglected area" in the Netherlands for several decades. Infection control measures were mainly implemented in Dutch hospitals according to national guidelines since the late eighties of the last century (WIP). These guidelines were used as a template by medical microbiologists and infection control staff by installing a hospital infection prevention commission. An infection prevention commission consists of hospital staff members and hospital employees active in the field of infection control. Having an infection control commission is nowadays a legal obligation for Dutch hospitals.
Increasing governmental rules and demands for infection control are required for Dutch nursing homes since the last few years. Up till now, however, few financial resources have been offered.
In Dordrecht the regional laboratory for medical microbiology (RLM Dordrecht- Gorinchem) and the municipal health service (MHS) Zuid- Holland Zuid (GGD ZHZ) agreed to work close together as an (regional) infection prevention team to control spread of infectious diseases and epidemics in the region. In this tri- partite model nursing homes together with the regional MHS and regional laboratory for medical microbiology record their agreements on infection control. This Infection prevention team consists of the local MHS doctor for infectious diseases, the medical microbiologist and an infection control nurse take care for infection control in local nursing homes and other care facilities (e.g. Salvation Army houses). The members of the regional infection prevention team are also active in the local health care facilities.
The tri-partite model can be considered as a proven model for infection control in Dutch nursing homes. With still few money to spend on infection control and increasing governmental rules and demands for Dutch nursing homes the "Dordrecht model" has been proven effective during the last decade.

O033

HCV infection among HIV-negative MSM who use PreP to prevent HIV infection

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Background: Hepatitis C virus (HCV) has been recognized as an emerging sexually transmitted infection (STI) among HIV-positive MSM. However, HIV-negative MSM at high risk for HIV might also be at increased risk for HCV. We studied the HCV prevalence in HIV-negative MSM who start pre-exposure prophylaxis (PrEP) in Amsterdam. Phylogenetic analysis was used to compare HCV strains obtained from HIV-negative and HIV-positive MSM.
Methods: At enrolment in the Amsterdam PrEP demonstration project, HIV-negative MSM were tested for the presence of HCV antibodies and HCV RNA. If positive for HCV RNA, an HCV NS5B gene fragment (709 bp) was sequenced and compared with HCV isolates from HIV-positive MSM (n=223) and risk groups other than MSM (n=153),using phylogenetic analysis.
Results: Of 375 HIV-negative MSM enrolled in Amsterdam PrEP, 18 (4.8%, 95% confidence interval 2.9–7.5%) of participants were anti-HCV and/or HCV RNA positive at enrolment; 15 of 18 (83%) had detectable HCV RNA. HCV genotyping showed genotype 1a (73%), 4d (20%), and 2b (7%). All HCV-positive MSM starting PrEP were part of MSM-specific HCV clusters containing MSM with and without HIV.
Conclusion: HCV prevalence among HIV-negative MSM who started PrEP was higher than previously reported. All HIV-negative HCV-positive MSM were infected with HCV strains already circulating among HIV-positive MSM. The increasing overlap between

sexual networks of HIV-positive and HIV-negative MSM might result in an expanding HCV-epidemic irrespective of HIV-status. Hence, routine HCV testing should be offered to MSM at high risk for HIV, especially for those enrolling in PrEP programs.

O034

Persistence and risk of transmission of the hepatitis C virus NS5B S282T substitution in a HIV-positive man who has sex with men

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Background and Aims:
The hepatitis C virus (HCV) nonstructural protein 5B (NS5B) S282T is a resistance-associated substitution (RAS) which confers major resistance to sofosbuvir in vitro. This substitution has never been found in treatment-naïve patients because it is associated with a severe fitness loss and therefore usually not persists after cessation of treatment. Here, we report a case of a HIV/HCV coinfected patient with persistence of S282T upon treatment failure.
Method:
Clinical and laboratory data were extracted from patient records. For resistance analysis, PCR and Sanger sequencing of NS5B and NS5A was performed.
Results:
A 66-year-old HIV-infected man who has sex with men (MSM) acquired his fourth HCV infection with genotype 4d in September 2016. He reported an active sex life with multiple male partners at different locations across Europe and the USA. All previous HCV infections were successfully treated with diverse antiviral regimens. He initiated treatment with sofosbuvir/ledipasvir in November 2016, for a planned duration of 12 weeks. At treatment initiation, sequence analysis showed no RAS in NS5B, but P/T58L in NS5A. Twelve weeks after treatment initiation, presumably during treatment discontinuation, HCV RNA load was 7,570,000 IU/mL. Sequence analysis showed the S282T substitution in NS5B. In NS5A, 58L was still present. Hereafter, HCV RNA load levels fluctuated but increased again over time. At treatment failure the patient reported that he stopped taking sofosbuvir/ledipasvir at week 8 and restarted 2-4 weeks afterwards. The latter might have explained the HCV RNA load levels fluctuation. Remarkably, the S282T substitution persisted over time, even when HCV RNA levels were rising. Surprisingly, after this rise a superinfection with genotype 1a was found. This genotype was not detectable in earlier samples, using a subtype specific PCR. Sequence analysis did not show any RAS in genotype 1a. In July 2017, the patient initiated treatment consisting of sofosbuvir, elbasvir/grazoprevir and ribavirin, after which HCV RNA decreased quickly. Final treatment outcome is pending.
Conclusion:
In this HIV-infected MSM the NS5B S282T substitution remained detectable for >5 months after treatment for his 4th HCV infection, indicating that viral fitness was not compromised. Given the very high HCV RNA load at HCV superinfection combined with high-risk sexual behavior, onward transmission of a S282T strain, associated with sofosbuvir resistance, cannot be excluded.

O035

Chemosynthetic symbioses in marine bivalves: Getting to the bottom of microbe-host relationships

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Nutritional symbioses between bivalves and chemosynthetic bacteria form the basis of entire ecosystems such as deep-sea hydrothermal vents and cold seeps, and can contribute substantially to productivity in shallow marine habitats such as seagrass meadows. Animal 'microbiomes' can be highly complex, but chemosynthetic symbioses are extraordinarily specific: Most animal hosts associate with only one species of chemosynthetic bacteria. This makes them ideal for investigating how microbes influence animal function, development and evolution. My lab focuses on symbioses between marine clams of the family lucinidae and their intracellular sulfur-oxidizing symbionts. I will highlight how our work has helped to reveal new metabolic capabilities of the symbionts, including nitrogen fixation, their unexpected functions in host defense, and new potential mechanisms of communication with their hosts.

O036

Shellfish microbiome analysis: in search for tetrodotoxin producing bacteria

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In recent years, every summer the very potent neurotoxic Tetrodotoxin (TTX) is found in Dutch mussels and oysters from the Eastern Scheldt, in levels that make these shellfish inappropriate for human consumption. TTX is well-known as the compound that makes tropical puffer fish lethally toxic. TTX is produced by bacteria, and accumulates in their animal host. So far, a gene cluster involved in TTX biosynthesis has not been identified, and the microbial source for the Dutch TTX outbreak is unknown.
We have collected mussels and oysters during the 2017 TTX outbreak, and are using nanopore sequencing to identify potential TTX producing bacteria and their TTX biosynthetic gene clusters. We analysed the microbiome present in both TTX positive and TTX negative shellfish samples by 16S amplicon sequencing. In collaboration with colleagues from Vladivostok (Russia) we have sequenced the genomes of several TTX producing bacteria isolated from TTX positive toxic ribbon worms collected from the Sea of Japan. We are now analysing gene clusters from these bacteria that bear the hallmarks of TTX biosynthesis.

Together, this information should allow us to identify the bacteria in the Dutch shellfish that produce TTX. We will use this knowledge to study the ecology of these bacteria in the marine environment. This will allow us to monitor these bacteria on the shellfish production areas and predict TTX outbreaks, allowing the shellfish farmers to postpone their harvest until the TTX has been washed out of the shellfish again.

O037

Bacteria can help maggots to survive dangerous vegetables

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Introduction

The microbiomes of insects provide a source of untapped metabolic and biotechnological applications. We have unraveled a possible novel mechanism with which pest insects and plant pathogenic microbes deal with the natural defenses of cabbage plants. Plants of the cabbage family, including many of the most important food crops like broccoli and rapeseed produce toxic isothiocyanates (ITCs) upon tissue damage. Within the gut microbiota of a cabbage fly, a bacterial gene called *saxA* was found to provide resistance against certain ITCs. In this work we characterized key aspects of the SaxA protein family such as substrate specificity and the three dimensional structure.

Methods

Seven homologous SaxA amino acid sequences representing genes from plant and animal associated microbes were selected for heterologous expression in *E. coli*. After tag-purification, each protein was assayed for its hydrolytic capability on a mix of six readily available ITCs (methyl-, ethyl-, allyl-, phenyl-, benzyl-, and 2-phenylethyl isothiocyanate). Decrease of ITC content was monitored over a period of two hours by analyzing subsamples of the reaction mixture on GC-MS. The crystal structures of two SaxA proteins (Drgb3_SaxA and Vpar_0210) representing the novel SaxA family were resolved using X-ray crystallography. Size exclusion chromatography on a Superdex 75 column was used to determine the native protein stoichiometry of these two proteins.

Results

The effect that seven phylogenetically distinct SaxA proteins (with only up to 42% amino acid identity) had on six different isothiocyanates was very similar. Phylogenetic diversity thus seems not to be a determinant for substrate specificity in the isothiocyanate hydrolase protein family. Benzyl-ITC and 2-phenylethyl-ITC were particularly quickly turned over by all tested SaxA proteins. Notable structural features of SaxA included a hydrophobic active site with two Zn2+ ions coordinating water/hydroxide, and a flexible cap that is implicated in substrate recognition that covers the active site.

Conclusions

1-Different SaxA proteins exhibit similar preferences towards the tested ITCs, suggesting that they have not co-evolved with plant defenses to specialize in the breakdown of specific ITCs.
2-SaxA affinity for different ITCs seems to correlate to ITC size, hydrophobicity, and direct contact toxicity, but inversely with ITC volatility.
3-SaxA likely forms native dimers with outward-facing active sites that can be covered with a flexible loop that is implicated in substrate recognition.
4-ITCs are currently applied as crop loss preventatives and their anticarcinogenic properties are being investigated for application in medicine. We suggest that SaxA is a potential mitigator of these beneficial effects of ITCs, and should therefore be accounted for in future studies.

O038

Characterization of growth of lipid-dependent *Malassezia* yeast species, members of the skin mycobiome

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Introduction

The genus *Malassezia* belongs to the phylum Basidiomycota and comprises at least 14 established species. *Malassezia* species are lipid-dependent due to the lack of cytosolic fatty acid synthase required for the de novo synthesis of fatty acids (FAs). These yeasts are part of the microbiome of healthy humans representing 50% – 80% of the total skin fungi. They have also been associated with dermatological conditions like dandruff, seborrheic dermatitis, and pityriasis versicolor. They can systemic infections in patients such as neonates that receive intravenous lipid therapy. Pathogenicity of *Malassezia* has been related to several factor including the ability to produce enzymes such as esterases, lipases, lipoxygenases and proteases which enable growth on host skin and lead to changes in sebum (skin fat) composition.
The skin functions in the innate defense against pathogens due to its low water content, acidic pH, its microbiota, and antimicrobial lipids (i.e. free FAs). Understanding lipid dependency of *Malassezia* will help to understand how these yeasts establish themselves as part of the skin microbiota, which adaptation mechanisms are involved, and how, and whether, lipid metabolism impacts the shift to pathogenicity. The complex nutritional requirements of *Malassezia* have delayed the full comprehension of its lipid metabolism.
Reconstruction of the lipid-synthesis pathways of *Malassezia* species in silico predicted amongst others defects in the assimilation of palmitic acid in *M. globosa*, *M. sympodialis*, *M. pachydermatis*, and the atypical isolate of *M. furfur*, but not in *M. furfur*. These predictions were validated by physiological characterization in chemically defined media (MM) using different lipid sources.

Methods

Growth on FAs in liquid MM: Strains were first grown for 7 days at 33 °C in lipid-rich mDixon medium. To prevent subsequent growth in MM due to the presence of residual lipids we performed a two-phase growth in MM. First, cells were diluted into MM containing defined lipid sources. After 3 days, these cells were diluted again in fresh MM with lipid components. Growth was monitored for 7 days by determining OD600 and/or CFU by plating on mDixon plates.

Results
M. furfur could assimilate palmitic acid or oleic acid as well as all Tween variants. The atypical *M. furfur* strain could assimilate only Tween 80, Tween 20, and oleic acid. *M. pachydermatis*, *M. globosa*, and *M. sympodialis* were able to grow in the first step in MM but not in the second step in MM with any if the lipid sources. Only *M. furfur* was able to maintain growth in MM with palmitic acid. Both *M. pachydermatis* and atypical *M. furfur* could sustain growth in MM with a mixture of palmitic acid and oleic acid.
Conclusion
A new culturing method for *Malassezia* spp in chemically defined media was developed
Predicted assimilation defects of palmitic acid for *Malassezia* spp were confirmed
Palmitic acid is fungicidal for this subset of *Malassezia* spp but not for *M. furfur*.
FAs that induce lipid toxicity and do not affect the skin cells and microbiome harmony might have a therapeutic use.

O039
Development of cecal microbiota biodiversity during a broiler production round at two farms
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Introduction: Gut microbiota influence health and production performance of broiler chickens, therefore differences in performance between flocks and farms may be caused by differences in gut microbiota dynamics. However, there is a lack of fundamental knowledge of microbiota dynamics within and between broiler flocks and farms. Thus, the biodiversity of the cecal microbiota defined as the species richness, species evenness, and the compositional and phylogenetic structure was investigated longitudinally on two farms. At each farm, the flocks in two poultry houses were studied throughout one broiler production round.
Methods: The flocks on the two broiler farms showed excellent production performance, and no antibiotic treatments were applied during the study. Coccidiostatic drugs were standardly applied in the feed in all flocks. The two farms had a different feed supplier, but the broilers in the two houses within the same farm received the same feed and management. During one production round cecal content was collected at the chicks' day of arrival and on days 2, 7, 14, 21, 28, and 35. At each time point, cecal samples were collected from nine birds from each of the two poultry houses at each farm. In total, cecal content of 324 Ross 308 broilers was analyzed. The biodiversity of the cecal microbiota was assessed by 16S ribosomal RNA gene amplicon Illumina HiSeq sequencing. Alpha and beta diversity metrics, and multivariate analyses were applied to quantify the cecal microbiota biodiversity.
Results: The results showed that the percentage of operational taxonomic units (OTUs) shared between different flocks and between farms was highest at day 35. No difference was observed in species richness and species evenness neither between the farms nor between the flocks within a farm. From around the second week of age onwards, the number of observed OTUs, species richness and species evenness seemed to stabilize at both farms. Age of the broilers was found to most strongly affect cecal microbiota composition. Furthermore, unweighted UniFrac distance was slightly higher than the weighted UniFrac distance between the two farms, whereas the opposite trend was observed for corresponding Jaccard and Bray-Curtis distances that are not phylogenetically weighted. Taken together, this suggested that abundant taxa are more phylogenetically related compared to the less abundant taxa between the two farms.
Conclusion: 1. The development of cecal microbiota composition during a production round at two different farms was comparable. 2. In all flocks on both farms, the day within the production round had a similarly large effect on gut microbiota dynamics. 3. Although the broiler parent stock, diet, and environment were different, 52% of the OTUs were shared between the farms at 35 days.

O040
Host and Environmental Factors Affecting the Intestinal Microbiota in Chickens
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Introduction: The development of intestinal microbiota in poultry plays an important role in production performance, overall health and resistance against microbial infections. In studies to determine effects of feed interventions or antimicrobial drugs, the composition of intestinal microbiota is often evaluated using multiplexed sequencing of 16S ribosomal RNA gene amplicons. Both within and across studies, considerable variation of intestinal microbiota composition has been observed. This may be due to technical factors associated with sampling and sequencing, but a large part of this variation in microbiota composition may also be explained by different host characteristics and environmental factors. Without insight into the relevant factors determining the variation, inference in microbiota studies is error prone.
Method: To facilitate the improvement of design, reproducibility and interpretation of poultry microbiota studies, we have reviewed the literature on host characteristics and environmental factors that may act as confounding factors influencing the observed intestinal microbiota in chickens.
Results: A large effect on intestinal microbiota can be attributed to host-related factors, such as age, sex, and breed. The diversity of chicken intestinal microbiota increases most during the first weeks of life, and corresponding colonization patterns seem to differ between layer- and meat-type chickens. Also environmental factors, such as biosecurity level, housing, litter, feed access and climate affect intestinal microbiota composition. These host and environmental variables may not always be known or considered in the design of microbiota studies, but can have a large impact on study outcomes and interpretation of the data.
Conclusion: 1. Providing details on a broad range of host and environmental factors in articles and sequence data repositories are essential and will create opportunities to combine data from different studies for meta-analysis, which will

facilitate scientific breakthroughs towards nutritional and husbandry associated strategies to improve animal health and performance.

O043

Impact of bile acids on bacterial colonization

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The adult intestine harbors a dense and relatively stable microbial community. In contrast, neonates are born essentially sterile with the establishment of the microbiota starting upon rupture of the amniotic membranes. Since the most dramatic changes in bacterial density and composition are observed during early life, this phase might critically influence the ultimate microbial composition and the life-long maintenance of host-microbial homeostasis. An intimate interrelationship exists between the gut microbiota, hepatic bile acid synthesis, and bile acid absorption. We hypothesized that bile acids might be one of the influencing host factors, since hepatic bile acid synthesis initiates in a developmental stage-dependent manner shortly after birth.
Therefore, we characterized the development of the gut microbiota and the hepatobiliary system in parallel with the luminal bile salt compositions during the postnatal period. Our analysis included 16S rDNA V4 sequencing at various time points (1-8 weeks) and anatomical sites after birth (colon, small intestine). In addition, transcriptomic analysis of hepatocytes for the expression of hepatic genes engaged in bile salt homeostasis and bile salt signaling (qPCR), as well as targeted analysis of liver tissues for the quantity and composition of bile acids were performed (LC-MS).
Transcriptomic analyses demonstrate major alterations in hepatic bile synthesis factors and transporters during the postnatal period and early infancy. Moreover, we observed a rapid colonization of the neonate intestine, and increase in microbial richness combined with a major shift in composition during weaning. The microbiota composition was found to be highly individual directly after birth, but shifted towards a more homogenous pattern within one week. Small intestine and colon harbored a comparable microbiota composition during the pre-weaning period. Our results are consistent with the existence of selective host mechanisms that shape the initial, largely environment-dependent colonization pattern and ensure the development of a beneficial mature microbiota composition.

O046

Differences in the immune response of populations living in different geographical areas

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Several vaccines are known to perform less well in populations living in rural areas of the developing world than in populations residing in affluent countries, and studies are showing that environmental exposures can have a profound role in shaping immune responses to vaccines or incoming infections. Therefore, a full understanding of the immune cell composition in populations residing in areas endemic for diseases we are aiming to eradicate is imperative to successful vaccine development and control of infectious diseases.
To study this, we performed unbiased immune profiling of 20 African and 10 Indonesian adults, both with lifelong exposure to parasites, as well as 10 healthy European volunteers. The use of mass cytometry and our recently developed Cytosplore to analyse high-dimensional single-cell immunological data, allowed the identification of very distinct immune signatures in Europeans and in those living in areas where exposure to microorganisms and parasites is high. Significant differences were not only found in the adaptive immune cells but also in the innate cells such as γδ T cells and innate lymphoid cells. These differences generated by pre exposure to infectious agents, could help us to start to explain the geographic differences we see in responses to vaccines.

O049

Hepatitis E virus screening of Dutch blood donors

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Introduction: It has been known for several years that the incidence of autochthonous hepatitis E virus (HEV) genotype 3 infections in the Netherlands is high. Most gt3 infections are asymptomatic or cause minor acute illness, but in patients with immunosuppression infection may become chronic. Although use of blood products is probably a minor source of HEV infections compared with dietary exposure, HEV transmission via blood transfusion has been observed. Because of concerns about the safety of blood products, screening of blood donors was implemented in July 2017.
Methods: Blood donations were screened in pools of 24 samples using the Roche Cobas HEV test on the Cobas 6800 platform. Confirmation by PCR and serology was performed for all HEV RNA positive donations, and samples with sufficient load were genotyped. Results were compared with those from screening of a subset of plasma donations implemented in 2013. An estimate of the number of prevented transmissions was made based on the viral load distribution and the relation between viral dose and probability of transmission.
Results: During the first six months of screening, 107 of 211,059 donations (0.051%) tested HEV-RNA positive. Confirmation showed 100% of the positive indeed contained HEV RNA resulting in a specificity of 100%. 79 donations (73.8%) were anti-HEV IgG and IgM negative. All 36 isolates that could be genotyped were genotype 3 and clustered with HEV isolates from Dutch HEV patients and Dutch swine. The incidence of HEV infection in donations used for production of plasma products (tested in pools of 96 donations) showed strong fluctuations between 2013 and 2017. The highest incidence was observed in 2014 and 2015 when 0.116% of the donations tested HEV RNA positive. In 2017 10/24371 donations (0.04%) tested positive in the screening in pools of 96, with a strong drop during the second half of

the year. Based on the distribution of viral loads and the relation between the dose of virus transfused and the probability of infection we estimate that intercepting the 107 donations prevented 27.5 HEV infections in recipients. Also, we estimate that ~50% of donations remains undetected because of the screening in pools of 24, resulting in an adjusted incidence estimate of 0.10% (1:1000 donors) during the second half of 2017. Because of the low viral dose only three of the donations that were not intercepted are estimated to cause HEV transmission to the recipient.
Conclusion: The incidence of HEV infection among Dutch blood donors declined in the second half of 2017 but was stable at ~1 in 2000 donations during this period. This incidence is still among the highest in Western Europe. The majority of HEV positive donors presented with early infection. Because of the high incidence of HEV infection, screening blood donors for HEV RNA effectively contributes to the safety of blood products.

O050

IcTyping: rapid universal bacterial strain typing for real-time infection control

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Introduction
Bacterial strain typing is essential for the process of tracking and tracing the spread of bacterial strains. To contain outbreaks as they occur, a rapid typing technique is essential. To be effective on a larger scale, such a method should be cheap and applicable locally in any hospital. Data should be interchangeable between hospitals and should ultimately be comparable to other typing methods.
Here we demonstrate a universal bacterial strain typing approach and coined it infection control Typing, or icTyping in short.
Methods
The technology underlying icTyping is an amplified fragment length polymorphism (AFLP) approach. We have redesigned all steps of the process and combined it with an automated software pipeline for fully standardized data-analysis with minimal hands-on time. First, we created in-vitro reference databases for the species *Clostridium difficile* (CD), *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA), with strains collected in the VU medical center from 2011-2017. Secondly, we created an in-silico database of all available whole-genome sequences (WGS) of these species in public repositories and performed a virtual AFLP on all sequences. Next, all common fragments were defined per species in vitro and in-silico and fragments were matched. Here we demonstrate the setup and efficacy of this novel approach for the three bacterial species.
Results
In total 552 strains of CD, 1360 strains of SA and 534 strains of PA were used to build the in-vitro database (madelon?). Whole genome sequences of 954 strains of CD, 7968 strains of SA and 1675 strains of PA were retrieved from public repositories. icTyping showed highly comparable results between the two laboratories. Detected variation was almost entirely attributable to low-level background noise that could easily be adjusted for by the software. By linking in-vitro fragments to in-silico fragments, icTyping results can be directly compared to WGS results and via this route to all other typing methods.
Conclusion
icTyping is a rapid and reproducible typing technique that is applicable to any bacterial species. Data is comparable between laboratories and can be matched to WGS data. In conclusion, icTpying holds great promise as a cheap universal typing method that can be used locally in an acute setting and may be a valuable addition to WGS approaches.

O051

Low-level viremia during dolutegravir-containing regimens in two HIV-infected patients

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Background: Dolutegravir is a second-generation integrase-inhibitor with a proposed high genetic barrier to resistance. A decreased virological response was rarely observed in clinical trials and was limited to HIV-patients with prior failure on first-generation integrase-inhibitors with multiple mutations (in particular position 140/148) in *integrase*. As HIV-treatment guidelines for resource-limited settings are changing, global widespread use of dolutegravir is anticipated. Little information from clinical practice is available regarding relevant resistance mutations. We describe two HIV-patients who experience low-level viremia (HIV-RNA <1000 cp/mL) on a dolutegravir-containing regimen.
Methods: Clinical data were obtained from patient records. Genotypic (Sanger sequencing) and phenotypic analyses were performed. A template of *integrase* of the patient virus was introduced into an HxB2 backbone. Virus stocks were titrated. MT2 cells were infected with equal amounts of virus in presence of a 5-fold drug dilution. Drug sensitivity was determined in duplicate using an MTT assay and expressed as fold change (FC) in IC50 compared to HxB2wildtype.
Results: Patient 1 (Nigerian origin) presented in October 2014 with AIDS. Extensive resistance in *RT* indicated prior use of nucleoside reverse transcriptase inhibitors (NRTIs) and non-NRTIs. He started boosted darunavir, maraviroc and dolutegravir. After initial viral suppression, the viral load rebounded in August 2015 resulting in persistent low-level viremia (112-658 cp/mL) despite adequate drug levels. No new mutations were observed in *protease, RT* or *integrase*. Without evident neurological symptoms, the viral load in the cerebrospinal fluid (CSF) was 13100 cp/mL (plasma: 856 cp/ml). The resistance profile was similar to plasma, except for the presence of E92G in *integrase* in CSF, resulting in an FC for dolutegravir of 1.6/1.3 *in vitro*. Twice daily dosing of dolutegravir and addition of zidovudine resulted in sustained virological suppression in plasma and CSF.
Patient 2, diagnosed in Luxembourg in 1992, received dual therapy followed by several regimens. Sustained virological

suppression was never achieved despite good adherence due to add-on therapy. Early 2012 failure of a salvage regimen including raltegravir, maraviroc, etravirine, boosted darunavir and several NRTIs was observed. In addition to dual-tropic virus, extensive resistance in *RT*, *protease* and *integrase* (L74M-Q95R-T97A-Y143R) was observed. He switched to dolutegravir (BID), T20, etravirine, boosted darunavir and combivir, resulting in viral suppression within 1 month. In the following years he experienced episodes of low-level viremia. In September 2015 viremia increased to 1600 cp/mL and selection of additional mutations in *integrase* (V31I-H51Y-L74M-V75I-Q95R-T97A-T122S-E138K-Y143R-S147G-K156N) was observed. Although various prediction algorithms predict low-level resistance to dolutegravir, phenotypic analyses showed an FC for dolutegravir of 244/160 *in vitro*. New therapeutic options are needed.
Conclusion: Selection of additional mutations in CSF compared to plasma suggest viral replication in CSF. The central nervous system should be considered as a potential source of viral escape in patients with unexplained low-level viremia. In one patient selection of additional mutations in *integrase* resulted in remarkably high levels of resistance *in vitro* despite absence of mutations at position 148/140, and clinical failure. The European Society for translational Antiviral Research has set up a dolutegravir failure registry to gain a better understanding of dolutegravir resistance.

O052
Two years of experiences of the Netherlands Donor Feces Bank
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Introduction: Since 2016, the Netherlands Donor Feces Banks (NDFB) is facilitating the treatment of patients with multiple recurrences of *Clostridium difficile* infections (rCDI) with fecal microbiota transplantations.
Methods: An observational study was performed using a standardized approach of data collection and guidance of an expert team of medical microbiologists, infectious diseases physicians and gastroenterologists.
Results: Between March 2016 and December 2017, 260 of 472 donor candidates completed a questionnaire; 220 (85%) were excluded, mainly because age above 50 and an unhealthy BMI. Thirty nine (15%) donor candidates were invited for laboratory screening of blood and feces of which 15 (38%) passed this screening. Carriership of *Blastocystis hominis*, *Dientamoeba fragilis* and Multi Drug Resistant Organisms were the most observed exclusion criteria. Of 15 donors, 6 failed at a following screening test, which is performed every two months. Finally, 9 (3.5%) active donors were enrolled. Between March 2016 and December 2017, 79 patients were evaluated by our FMT expert team. Of 82 patients, 62 (76%) were considered as suitable candidates for FMT treatment and 20 (24%) were interpreted as patients with underlying bowel disease who concomitantly carried *C. difficile*. The mean age of the 62 FMT treated patients was 72 year, 58.6% was female, and the mean recurrence rate was 3.4 CDI episodes. The treatment was performed in 23 different hospitals with a success rate of 85%. Nine patients suffered a CDI relapse, of which 4 were associated with antibiotic use within one month after FMT. All 4 were successfully treated with anti-CDI antibiotics only. Two serious adverse events (SAE) of faecal vomiting were reported without further complications.
Conclusion: Only a low percentage (3.5%) of healthy volunteers is qualified as suitable feces donor. A high percentage (24%) for FMT requests was rejected after careful evaluation of a multidisciplinary expert team. The success rate of FMT for multiple recurrent CDI was 85%.

O053
Pitfalls of metagenomic next-generation sequencing for viral respiratory infections.
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Introduction
Metagenomic next-generation sequencing (mNGS) enables unbiased
detection of all potential pathogens. However, for standardization, library preparation, sequencing platform and post-sequencing bioinformatic analyses can still be considered a challenge. In this study, the pitfalls of mNGS for diagnosing viral respiratory tract infections were evaluated.
Methods
Respiratory samples, previously analysed using lab-developed multiplex real-time PCR , were subjected to mNGS. Aliquots of internal controls (equine arteritis virus and phocine herpesvirus 1) were added and subsequently nucleic acids were extracted using MagnaPure 96 with DNA and Viral NA small volume Kit (Roche, Almere, the Netherlands). Library preparation using the NEBNext Ultra Directional RNA Library Prep Kit was performed on the NA eluates. Ten million paired- end reads were obtained from both the Illumina HiSeq 4000 and Illumina Nextseq 500 sequencing systems. Reads were classified with the bioinformatic classification tool for microbes Centrifuge and visualized using Krona plots.
Results
The first challenge of mNGS for diagnosing respiratory viruses was the lack of an universal viral library preparation kit. Available kits were adjusted to detect both DNA and RNA viruses simultaneously. The NEBNext Ultra Directional RNA library Prep Kit contains poly A capture, rRNA removal and DNAse steps, resulting in a lower number of viral and bacterial reads, and therefore lower the sensitivity.
The second hurdle was the sequencing system. Using the Illumina Hiseq 4000 resulted in false positive viral reads due to index-hopping. Although the percentage of index-hopping was low, it was within the relevant range of the overall low percentages of viral (target) reads in the clinical samples. Index-hopping could be eliminated by using the Illumina Nextseq 500 sequencing platform.
Bioinformatic analyses posed other pitfalls. Standard Centrifuge settings, using the NCBI nucleotide database and standard classification algorithm, resulted in false positive and false negative results due to misclassification. The

nucleotide database contains a wide variety of unnotated viral sequences, including partial sequences, in contrast to the annotated sequences in the NCBI Refseq database, which resulted in a high specificity. The classification was further improved by changing the settings of the assignment of homologous reads to multiple species to unique assignment, in case of homology to the lowest common ancestor.

Conclusion

Optimal library preparation, sequencing platform and bioinformatic analyses were essential for optimal mNGS-based virus detection. After adjusting library preparation, diminishing index-hopping and optimizing the database and classification algorithm, sensitivity of mNGS was comparable to real-time PCR for respiratory viruses.

O054

Thirty cases of Enterovirus-D68 associated acute flaccid myelitis in Europe 2016; a case series and epidemiological overview.

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Introduction: Enterovirus D68 (EV-D68) is a respiratory virus that has gained interest since the large outbreak in Europe and the United States of America (USA) in 2014 and the concurrent rise in cases of acute flaccid myelitis (AFM) reported in the USA.

Methods: Through a European collaborative network including virologists and clinicians, 30 cases of EV-D68 associated AFM were identified, who were diagnosed during 2016 in 12 different European countries. Clinical and virological information was collected from these clinical cases.

In addition, information was gathered on EV-diagnostics and on notification regulations in the various European countries.

To compare the viral sequences, the working group members were asked to share samples or sequencing files of their EV-D68 cases (both of respiratory and AFM cases).

Results: The clinical presentation of the 30 European EV-D68 associated AFM cases resembled that of patients from other parts of the world with –mostly– young age at onset, occurrence of a respiratory prodromal phase and neurological manifestations such as asymmetric flaccid limb weakness, cranial nerve deficits and bulbar symptoms. Three out of 30 were adult cases.

Sixteen laboratories performed close to 22.000 tests on EV, with 2381 EV positive results and 416 EV-D68 positive results. The number of tests performed, the EV positivity rate and the EV-D68 positivity rate varied widely between the laboratories. 414 out of the 416 EV-D68 positive samples were respiratory specimens (99%).

Acute flaccid paralysis (AFP) is a reportable disease in Europe within the scope of polio eradication, but no clear regulations exist for non-polio AFP/AFM. Norway is the only country that introduced an official requirement of EV-D68 AFM notification in 2016, as outbreaks with severe viral disease are mandatory reported.

Sequence analysis showed that the 2016 strains analyzed so far cluster with EV-D68 subclade B3 within clade B1.

Conclusions: 1. For 2016, 30 EV-D68 related AFM cases were identified in Europe, which is most likely an underestimation of the true number of cases. 2. Case identification is dependent on awareness amongst clinicians, adequate viral diagnostics on respiratory samples, capability and capacity of laboratories to type EVs and on notification regulations. 3. Only by collaboration between various medical specialties (e.g. (pediatric) neurologists, virologists, pediatricians, infectious disease doctors and radiologists), in Europe and globally, the burden of EV-D68 associated AFM can be mapped out and progression can be made in diagnostics, treatment and prevention.

O057

Standardised diagnostics of CDI in The Netherlands and Europe

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There are numerous strategies for laboratory diagnosis of *Clostridium difficile* infection (CDI), but choosing the most optimal method continues to be challenging. First of all, there has been debate on which of the two reference assays, cell cytotoxicity neutralization assay (CCNA) or toxigenic culture (TC) should be considered the gold standard for CDI detection. Although the CCNA suffers most from suboptimal storage conditions and subsequent toxin degradation, TC is reported to falsely increase CDI detection rates as it cannot discern CDI patients from patients asymptomatically colonised by toxigenic *C. difficile*. As both reference tests are lengthy and laborious techniques, several rapid assays have been developed for CDI detection. These rapid assays fall into three broad categories: (1) enzyme immunoassays for glutamate dehydrogenase , (2) enzyme immunoassays for toxins A/B and (3) nucleic acid amplification tests detecting toxin genes. All three categories have their own limitations, being the inability to differentiate colonised patients from CDI patients or suboptimal specificity and/or sensitivity. Therefore, these rapid assays are not suitable for stand-alone use. Instead, the use of these rapid assays in multi-step algorithmic testing has now been recommended by international guidelines in order to optimize diagnostic accuracy. Notwithstanding these recommendations, testing methods between hospitals vary widely, which may impact reported CDI incidence rates. CDI incidence rates are also influenced by sample selection criteria, as several studies have shown that if not all unformed stool samples are tested for CDI, many cases may go undetected due to an absence of clinical suspicion.

O061

Pet and feeder rat-associated Seoul infections in the Netherlands

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Hantaviruses are the causing agents of two types of human disease: haemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Small mammals, mainly rodents, are the natural reservoir of hantaviruses, and transmission to humans is mainly associated with inhalation of aerosolized excreta. To date, Puumala (PUUV), Tula (TULV) and Seoul virus (SEOV) were reported to be present in their natural hosts in the Netherlands. However, until recently, all diagnosed human Hantavirus infections in the Netherlands were attributed to PUUV.
End 2016, a 28-year-old man presented at the hospital with fever, vomiting, diarrhoea and decreased liver function. The man worked at a rat farm and kept feeder rats at home; he mentioned that he regularly got bitten by these rats. Serological tests were negative for leptospirosis, but showed high antibody titres towards SEOV. Six out of ten of the patient's rats were PCR-positive towards SEOV. Whole genome sequencing showed the virus was 99% identical to the SEOV Cherwell strain associated with pet rats in England and Wales. In 2017, serological testing of three more patients indicated recent SEOV infection. All of these patients were frequently exposed to feeder or pet rats. Two of these patients were PCR-positive towards SEOV. A short 349 bp L-segment sequence from one of these patients was 100% identical to the Cherwell strain.
In conclusion, here we present the first four confirmed SEOV cases in the Netherlands. Moreover, our data indicate that SEOV is present in Dutch pet and feeder rats.

O062

Usutuvirus: a prelude to West-Nile Virus in the Netherlands? An overview.

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During the past decade, arboviruses have been expanding to and within Europe, exemplified once more by the outbreak with chikungunya virus in France and Italy in 2017, the first human cases with tick-borne encephalitis virus in the Netherlands in 2016 and the first human cases with Crimean-Congo hemorrhagic fever in Spain in 2016. In addition in 2016 Usutu virus (USUV), a mosquito-borne bird flavivirus, has rapidly expanded its geographic coverage in Europe in a multi-country outbreak (including the Netherlands) of multiple virus lineages in birds. In 2017, USUV caused once more mortality among black birds and great grey owls in the Netherlands.
Evidence is accumulating that USUV has zoonotic potential with clinical manifestation. Human clinical cases present with neurological signs, fever, rash, jaundice, or combinations thereof. Two recent studies in Italy indicated that human USUV infection may not be a sporadic event. USUV infections in patients with or without neurological impairments occurred more frequently than West Nile virus (WNV) infections in a four-year period in Italy while sero-prevalence among forestry workers was high. Acute USUV infections have been detected in blood donations in Germany and Austria in respectively 2016 and 2017, raising blood safety concerns.
The 2016 USUV outbreak in North-West Europe showed similarity to the explosive outbreak with the closely related WNV lineage 2, in Central Europe in 2008-2009 and in Greece in 2010 after a few years of limited local circulation. It has been speculated that the expanding emergence of USUV might be a prelude to the emergence of WNV, both with a similar avian-mosquito lifecycle and both being introduced to naïve regions via viremic migratory birds (humans are dead-end hosts for WNV and USUV). The viruses show overlap in bird and mosquito species involved in maintenance of virus circulation. Indeed Dutch *Culex pipiens* mosquitoes have been found competent for both USUV and WNV transmission. However, it has been argued that the endemic circulation patterns for both viruses are different with USUV showing patterns of steady circulation with incidental human infection while WNV circulation seems to occur in peaks with irregular human outbreaks.

O063

Yellow fever: re-emergence in the world and case in the Netherlands

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Yellow fever virus (YFV), a member of the genus *Flavivirus*, is known to be enzootic in Africa and South America, causing periodic outbreaks of disease in monkeys and humans. In 2016 there was a Yellow Fever (YF) upsurge in Angola and the Democratic Republic of the Congo, with more than 900 confirmed cases and about 130 deaths. From December 2016 to June 2017, a YFV outbreak was ongoing in South America, with cases in Brazil, Bolivia, Colombia, Ecuador, French Guiana, Peru and Suriname. In Brazil, the country with the highest incidence, 777 confirmed cases have been reported, with 261 deaths. Currently, Nigeria is facing a YFV outbreak. Mass vaccination campaigns using the highly effective live-attenuated YFV vaccine have been organized to limit transmission in these countries.
Monkeys are the main reservoir for YFV, with various mosquitoes serving as vectors to transmit the virus. This sylvatic (or jungle) cycle sporadically leads to human infection. Subsequent introduction of a viraemic human case to urban areas with high population densities can initiate an urban transmission cycle, in which *Aedes aegypti* mosquitoes transmit YFV from human to human. In Africa, the urban cycle is commonly involved; in South America however, the recent outbreak remained limited to the sylvatic cycle.
Suriname had not reported YF cases since 1972. Yet, in March 2017, a Dutch traveler who had visited Suriname for two weeks was referred to the University Medical Center Groningen because of high fever and signs of acute liver injury. During her visit she stayed in the capital of Suriname, Paramaribo, and she made several daytrips by boat and car, of

which two in the tropical rainforest. Because of the combination of fever, leukopenia, thrombocytopenia, liver injury and travel history, YF was included in the differential diagnosis. In four consecutive samples of 3-6 days post start of symptoms (dps), YFV-RNA was detected (Erasmus Medical Center Rotterdam and Bernhard Nocht Institute). On dps 3, the indirect immunofluorescence assay was negative for IgM and IgG against YFV. A convalescent sample of dps 6 was clearly positive for YFV IgM, with non-reactive IgG. Follow-up sampling showed RNA positivity in blood until dps 20 and in urine until dps 24. After a period of severe illness, including signs of hepatic encephalopathy, the patient's condition stabilized on dps 10. Currently, she is fully recovered.
Laboratory diagnosis of YFV infection relies heavily on molecular testing, as accurate diagnosis based on serology is complicated by cross-reactivity with other flaviviruses and the inability to discriminate between naturally acquired immunity and vaccine-acquired immunity. Increasing amounts of evidence point at other bodily fluids such as saliva, semen, or urine as complementary or alternative sample types for some flaviviruses.
In conclusion, YFV periodically emerges in (sub)tropic countries, which may lead to import cases in Europe. Urine sampling might provide a long window of opportunity to confirm YF diagnosis in suspected cases.

O064
New compounds against tuberculosis: targeting type VII secretion in Mycobacteria
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Introduction: *Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB) and estimated to be responsible for the death of 1.5 million people each year. The world is facing a steady rise in the number of tuberculosis cases with multi drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). Therefore, the discovery and development of novel drugs against mycobacteria is a major priority. However, development of new intervention strategies is not easy; *M. tuberculosis* is a persistent bacterium with an unusual and highly impermeable cell envelope that protects the bacterium from antibiotics. To secrete proteins across this cell envelope, mycobacteria use specialized secretion systems known as type VII secretion (T7S). Most virulent mycobacteria contain three different but homologous T7S systems that are essential for growth or virulence (ESX-1, ESX-3 and ESX-5). To prevent the rapid generation of antibiotic resistance it would be advantageous to block more than one essential T7S system of the tubercle bacteria with a single drug.
Results: Using a whole cell-based high throughput screening campaign we succeeded to identify a new class of small molecules that inhibit the secretion of ESX-5 substrates, while T7S-independent proteins were still produced and secreted. This compound and its derivatives showed no or low toxicity, while reducing significantly the bacterial burden in zebrafish infected with *M. marinum* and also in macrophages infected with *M. tuberculosis*. Interestingly, some of these inhibitors blocked secretion by a second ESX system (ESX-1).
Conclusion: We identified a new class of chemical compounds that show high anti-mycobacterial activity and could decrease generation of antibiotic resistance by targeting several essential secretion systems simultaneously.

O065
Seed endophytes and their potential as novel antimicrobial reservoirs
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Introduction:
Plant seeds can benefit from seed-associated microorganisms, of which seeds endophytes are of particular interest as they are transmitted from generation to generation. In our current research, we isolated culturable endophytic bacteria from onion seeds and assessed their antagonistic activity against different pathogens and PGPR (plant growth promoting rhizobacteria) properties. This study is to decipher: i) what are the novel antimicrobials produced by the endophytic bacteria against the antagonists; ii) what are the mode of action for the endophytic bacteria while interacting with the host plant. iiii) how do the endophytic bacteria affect the growth and development of the host plant,
Methods:
1). Isolation and antagonistic assays of isolated bacteria against a series of plant, food and human pathogens
2) Illumina sequencing of the isolated bacteria
3). Genome mining via Bagel3, AntiSMASH for potential novel antimicrobials
4) Chemical characterization of active antimicrobials via HPLC and MALDI analysis
Results:
Paenibacillus was isolated and tested for antimicrobial activities. It possessed a wide range of antagonistic activities against different fungal, oomycetal and bacterial pathogens, such as *Botrytis cinerea*, *Fusarium culmorum*, *Pythium ultimum*, *Pseudomonas syringae* pv. tomato DC3000, *Streptomyces scabies*, *Bacillus cereus* ATCC14579, *Pseudomonas aeruginosa* PAO1 and *Escherichia coli* ET8. Meanwhile, PGPR traits siderophore and protease productions were detected indicating the plausible biocontrol capacity. Different potential novel bacteriocins, NRPS and PKS-NRPS hybrids compounds were discovered. Chemical characterization revealed the production of known compound Fusaricidin. Further analysis of other novel antimicrobial compounds is ongoing.
Conclusion:
To sum up, we have isolated promising bacterial sources to discover novel antimicrobial compounds from endophytes, and to elucidate the mechanism of plant-microbe interaction, which can be applied in agricultural and/or industrial applications.

O066

Investigating bacterial spore germination and stress resistance

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Bacterial spores are ubiquitous in nature, can survive food preservation processes and subsequently can cause, upon outgrowth, food spoilage as well as safety risks. The risks are exacerbated by the heterogeneous germination and outgrowth behavior of isogenic spore populations. A major unknown factor is the inherently heterogeneous spore protein composition which is in part due to the sporulation conditions and media used. We show that in *Bacillus subtilis* the *kinA* kinase gene under control of an IPTG inducible promoter can help achieve highly homogeneous sporulation. KinA is at the basis of the phosphorelay cascade activating Spo0A and thereby triggering sporulation. Under these conditions the analysis of spore protein composition can be deconvoluted from the sporulation medium composition. Data shows that spores formed in rich media are more heat sensitive than spores made on a minimal defined medium.
Next, our studies focus on the characterization of the molecular mechanisms involved in normal spore development as well as germination and outgrowth. The results form a basis for the identification of spore proteins as putative antimicrobial targets. Data have been obtained for *Bacillus subtilis*, *Bacillus cereus* and *Peptoclostridium difficile*. For quantification we have deployed metabolic labeling with ^{15}N. Spores of all three species contain around 1000 proteins including glycolytic enzymes as well as enzymes involved in amino acid metabolism. Prominently present in the spore inner membrane are the germinant receptor proteins. Live-imaging and fluorescence microscopy at single spore level allowed us to analyze the presence of the 'germinosome', a cluster of germinant receptor proteins. In contrast to previous reports we observed using Pseudo Wide-Field reconstructed 3D images obtained from Structured Illumination Microscopy of GerD-GFP labelled cells ~40% of the spores with two and ~10% with three fluorescent foci. Similar observations were made with GerKB-mCherry suggesting the presence of more than one germinosome in around half of the spores in our crops studied. In addition we analyzed the spore germination process, outgrowth as well as spore intracellular pH dynamics using recently published improved pHluorin (IpHluorin). Germinosome identification using the fluorescent reporter protein fusions showed that *B. subtilis* spores contain 2 to 3 germination protein foci per spore. Its impact on models of spore germination is being assessed. While the data obtained from such tools offer novel insight in the mechanisms of bacterial spore awakening they can also be used to probe candidate antimicrobial compounds for inhibitory effects on the initial phases of spore germination in order to strengthen microbial risk assessment. We have shown that human thrombocidin derived amphipathic antimicrobial peptides, selected for possible future clinical application, target the spore membrane and lead in outgrowing cells to coalescence of regions of increased membrane fluidity likely causal of their antimicrobial action.
Finally, we evaluate the antimicrobial action of five small organic molecules showing activity against spores from the model organism *B. subtilis* and the food-born pathogen *B. cereus*. One compound prevents *B. cereus* spore germination. We are investigating patenting options and study the compound's potential for the food or medical sector.

O067

***Bacillus subtilis* 168 as heterologous production host for lantibiotics**

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Introduction

Lantibiotics are ribosomally synthesized antimicrobial peptides which are naturally produced by Gram-positive bacteria. Nisin is a well-known example which is produced by *Lactococcus lactis*. Lantibiotics are of great interest because of their strong therapeutic potential and the ability to use them as food preservative. Lantibiotics are initially synthesized as precursor peptides containing an N-terminal leader peptide that is important for maturation of the core peptide. A protease (e.g. NisP for nisin) is responsible for removal of the leader peptide thereby yielding the antimicrobial active core peptide.
An interesting option is to engineer microbes as production platforms for lantibiotics due to their ribosomal synthesis and post-translational modifications. *Bacillus subtilis* is an attractive candidate as microbial host for the commercial production of lantibiotics, because of its ability to grow to high cell densities amongst others. For that reason, we developed a lantibiotic expression system in *B. subtilis* 168 in which the genes encoding the subtilin modification and transport machinery (*spaBTC*) as well as different types of lantibiotic structural genes were integrated in the genome.

Methods

My research project includes the following techniques: general molecular cloning techniques, heterologous expression of lantibiotics by recombinant *B. subtilis* strains, antimicrobial activity tests to analyze the extracellular presence of antimicrobial peptides, and verification of the lantibiotic peptides by SDS-PAGE and mass spectrometry.

Results

A functional lantibiotic expression system was established in *B. subtilis* 168. We found that *B. subtilis* is able to produce and secrete antimicrobial active subtilin and flavucin by using the subtilin modification and transport machinery. However, several hurdles seemed to be associated with using *B. subtilis* 168 as production host. This included that product yields were limited and that extracellular serine protease activity of *B. subtilis* 168 caused removal of the leader peptide thereby yielding antimicrobial active peptides in the supernatant. In order to increase production yields and to prevent cytotoxicity towards the production host, we introduced our lantibiotic expression system into the genome-minimized *B. subtilis* 168 strain ''PG10''. PG10 lacks extracellular serine protease activity and therefore showed to be able to produce various lantibiotic precursor peptides differing in their core peptide and cleavage site. Antimicrobial active core peptides of subtilin, nisin and flavucin could be released by *in vitro* cleavage by either supernatant of *B. subtilis* 168 or by the serine protease NisP. Moreover, we overexpressed several extracellular serine proteases of *B. subtilis* and found that two of them also could be used for *in vitro* processing of presubtilin.

Conclusions
We have developed a functional heterologous expression system in *B. subtilis* for the production of lantibiotics.
By using a genome-reduced strain of *B. subtilis* 168 we achieved the production of precursor peptides which allows for higher production yields.
Different tools can be applied for *in vitro* processing of the precursor peptides.

O068
Gelatin nanospheres deliver vancomycin intracellularly into macrophages of zebrafish embryos and protect against *Staphylococcus aureus* infection
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Introduction
Intracellular infections are a notorious clinical concern. For instance, in biomaterial-associated infection, the presence of a biomaterial predisposes for infection not only because of biofilm formation on the materials, but also because of the ineffectiveness of host phagocytes to kill intracellular bacteria. Intracellular infections are very difficult to treat because of the low cell membrane penetration of many antibiotics. Therefore, drug delivery systems which can enhance the antimicrobial activity of antibiotics against intracellular infection are urgently needed.
Materials and Methods
Preparation of fluorescently labeled and vancomycin-loaded gelatin nanospheres
Acetone was added to purified gelatin (type B, Sigma-Aldrich) solution to allow the formation of gelatin nansopheres (GNs). Subsequently, the GNs were cross-linked with glutaraldehyde (Acros Organics). The suspension of GNs was then centrifuged, washed, and re-suspended in Milli-Q water. The labelling of GNs using DyLight™650 NHS ester was performed according to the manufacturer's protocol (ThermoFisher Scientific). A solution of BODIPY-FL®-conjugated vancomycin (ThermoFisher Scientific) was added to the suspension of DyLight™650-labelled GNs to form a suspension of vancomycin-loaded GNs (V-GNs). The suspension of V-GNs was incubated overnight to allow complete adsorption of the vancomycin to the GNs.
Microinjection of GNs into zebrafish embryos and visualization of cell-GNs interaction in vivo
The suspensions of GNs or V-GNs were injected into either the blood stream or tail muscle of 3 days old zebrafish embryos using a FemtoJet microinjector (Eppendorf) and a light microscope. Embryos of the transgenic zebrafish line mpeg1:Kaede or fms:mCherry expressing green or red fluorescent proteins in their macrophages, respectively, were used. Fluorescence and confocal microscopy were used to visualize the distribution of and macrophage response to injected GNs in embryos.
Treatment of S. aureus-infected embryos with vancomycin-loaded GNs
An inoculum of *Staphylococcus aureus* was injected into the bloodstream of 1 day old wild type embryos. At 2 h post infection, the embryos were treated with GNs loaded with vancomycin. Treatment with GNs alone or with free vancomycin served as controls. After treatment, survival of embryos was monitored daily until 7 days post infection. The differences in survival curves were statistically analyzed using Graphpad Prism 7.0.
Results
The distribution of injected GNs in embryos was dependent on the administration route, i.e. intravenous or intramuscular injection. With the assistance of GNs as carriers, increased cellular uptake of vancomycin by macrophages within zebrafish embryos was achieved, while no uptake of free vancomycin was observed without GN carriers. Moreover, treatment with vancomycin-loaded GNs resulted in higher survival of *S. aureus*-infected zebrafish embryos. Treatment with free vancomycin did not rescue infected embryos. These results indicate that GNs can deliver antibiotics with limited intracellular activity such as vancomycin into macrophages and enhance the efficacy of antibiotic treatment of intracellular infections.
Conclusions
1. Gelatin nanospheres are promise carriers to enhance the activity of antibiotics against intracellular pathogens such as *aureus*;
2. Gelatin nansophere-based delivery system may contribute to controlling biomaterial-associated infection and other intracellular infections.

O069
Streptomyces morphology engineering by manipulating the extracellular matrix and the effects on antibiotic production
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Many bacteria growing in a biofilm secrete polysaccharides as a cellular embedding material. These extracellular polymeric substances (EPS) influence how these organisms grow, communicate and develop. It also influence the resistance of many pathogens against antibiotics by both limiting diffusion and penetration. Streptomyces are multicellular filamentous bacteria that are best known for producing antibiotics and are also producers of multiple EPSs that influences the morphology of these organisms. The effect of EPS on antibiotic production is poorly understood. Industrial production of antibiotics usually occurs submerged in bioreactors. In liquid grown cultures the mycelia of some Streptomyces species adapt a free floating, dispersed morphology, while others aggregate into dense pellets. Streptomyces coelicolor, a type strain and an important host for heterologous antibiotic production, makes these dense pellets. The formation of pellets has classically been associated with antibiotic production. This is supported by studies comparing the smaller and larger pellets for production [1] or by comparing the proteome [2]. It has also been shown that

the core of pellets undergo programmed cell dead (PCD) [3], which has been linked to development and the regulation of the secondary metabolism.
Recently we discovered matA and matB which are required for the pelleting morphology in S. coelicolor [4]. With immunofluorescent imaging we showed that these genes are responsible for the catalysis of poly-1,6-N-acetylglucosamine (PNAG). Removal of these genes influences the growth of the organism and the timing of antibiotic production. Also the pattern of PCD was severely affected. Interestingly introduction of the matA and matB genes in non-aggregating streptomycetes induces the formation of pellets without exception. We introduced the genes into 25 non-aggregating natural isolates to assess if novel antibiotics could be activated. When pellet morphology was induced, an increase of PCD was observed and in some species LC-MS analysis revealed compounds that depend on the pelleting morphology.
This study unravels how the morphology of Streptomyces is controlled in a liquid grown environment and indicates that engineering of the EPS is a valuable strategy for the production and discovery of antibiotics.

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O071

Microbiota and immunoregulation in reproductive tissues

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General consensus held that infants emerge from a relatively sterile uterine environment and that neonatal immune responses develop after birth. However, evidence now indicates that the pre-natal environment plays an important role in shaping post-natal and even adult immunity. At the fetal-maternal interface a delicate balance is required between a tolerogenic (regulatory T cell/Th2) signature -to protect the fetus from harmful allo-recognition- and a pro-inflammatory (Th1/Th17) signature to counter infectious threats. The novel finding of a dedicated placental microbiome led us to hypothesize that this could be the missing link to provide the environmental cues for maintaining the balance between tolerance and inflammation in the local placental milieu, promoting pregnancy success and in utero development of neonatal immunity. Interference in the build-up of the microbiome by the use of for instance selected antibiotics may lead to reproductive disorders and repercussions for neonatal immunity. Our aim was to investigate whether different microbiota (commensal or non-commensal) can influence the uterine immune microenvironment.

We started by first studying the immunophenotype of lymphocytes isolated from reproductive tissues. Lymphocytes isolated from menstrual blood[1] (as a source of endometrial lymphocytes) and from decidua parietalis of term placentas of healthy women were immunophenotyped by 10-color flow cytometry. Secondly, endometrial lymphocytes and peripheral blood mononuclear cells (PBMC), as a control, were stimulated with different micro-organisms (*Escherichia coli*, *Candida albicans*, and *Fusobacterium nucleatum*). Cytokine production was measured by Luminex after 5 days.
Results show that endometrial derived T cells are mainly naïve in contrast to decidua parietalis derived T cells, which harbor significantly more effector and memory T cells. In addition, more regulatory T cells ($CD4^{+}CD25^{hi}$) were found in the decidua patietalis compared to endometrium, while the presence of Th1, Th2, and Th17 T cells did not differ[2].
Bacterial stimulation shows that in response to *E.coli* and *F.nucleatum* endometrial derived lymphocytes produce IL-10 and less pro-inflammatory cytokines (IL-6 and IL-1β) compared to PBMC. Both PBMC and endometrium derived lymphocytes produce pro-inflammatory cytokines (IL-17F, IL-22, and IL-13) in response to *C.albicans* stimulation.
In conclusion, we show that endometrium and placenta differ in immune cell composition and these differences support the notion of active immunoregulation during pregnancy. Stimulation with different micro-organisms suggests that *F.nucleatum* and *E.coli* (present in the placental microbiome) induce primarily a tolerogenic cytokine profile in endometrium, while *C.albicans* induces a more pro-inflammatory response. This experimental setup now offers unique opportunities to study the influence of other micro-organisms on the local uterine immune microenvironment.

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O072

Gastrointestinal function and microbiota development during the early life of preterm infants

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I will present the main findings of my nearly completed PhD research project regarding gastrointestinal function and microbiota development during the early life of preterm infants.
Preterm infants are prone to health complications during their early life, including infection, sepsis and necrotising enterocolitis. Health complications are mainly associated with organ immaturity and are therefore related to the infants' gestational age. Preterm infants are born with an immature gut and are therefore likely to experience feeding constraints,

raising a major challenge in meeting their nutritional needs. Functioning of the gastrointestinal tract, and of the microbiota residing therein, is of significance for pathogen resistance and for optimal metabolic- and immune performance. During early life, development of the gut microbiota coincides, and affects, development of the metabolic-, cognitive- and immune systems. At this time, the intestinal microbiota is dynamic and its development is highly susceptible to host- and environmental factors. A such, gut microbiota establishment is likely to be impacted in preterm infants, since they have an immature gut and are commonly exposed to caesarean section delivery, specific feeding regimens and antibiotics. Although gut microbiota development can be negatively impacted during early life, the developing microbiota also provides an opportunity to be targeted as means of therapeutic strategy. In light of this, it is important to understand how the preterm infant gastrointestinal tract is functioning, which microbes colonise, what the microbes are doing and how microbiota establishment is affected.
Research was performed using material obtained during a single-centre, observational study including 238 infants born between 24-42 weeks gestation admitted to the neonatal unit of Isala, Zwolle. Infants were followed during the first six postnatal weeks, during which clinical factors were documented, and faeces and gastric aspirates were longitudinally collected for microbiota analysis. Microbiota development was not only approached compositionally via the application of qPCR and 16S rRNA gene amplicon sequencing, but also functionally via metaproteomics through LC-MS/MS, shedding new light on gut function and microbiota development in preterm infants.

O073

Gut microbial composition and diversity in young children with recurrent asthma-like symptoms: a case-control study

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Introduction. Asthma is the most common chronic disease in childhood. Although asthmatic symptoms are common in preschool children, only 30% will eventually develop true asthma. The remaining children are asymptomatic at age 6, but suffer from transient, viral-associated wheeze. The ultimate objective of the Asthma DEtection and Monitoring (ADEM) study is to develop a non-invasive technique for the early diagnosis of asthma in young preschool children with recurrent asthma-like symptoms (wheezing). This could eventually result in an earlier and better treatment of childhood asthma. The aim of the present study is to investigate if the gut microbiome can differentiate between wheezing and non-wheezing preschool children, and can eventually be used to predict the development of true asthma at the age of 6 years.
Methods. In this prospective case-control study, 202 children with wheezing and 50 children without wheezing aged 2-3 were included. Children were followed up until the age of 6 years. Data obtained by standardized questionnaires on respiratory symptoms were collected. Faecal samples were collected and subjected to high-throughput sequencing of V3-V4 region of 16S rRNA gene for the analysis of microbial composition and diversity.
Results. Preliminary analysis shows that the relative abundance of certain microbial taxa significantly differs between the wheezing and non-wheezing group. No significant differences were found in microbial diversity. After taking into account confounding factors such as breastfeeding, both alpha- and beta-diversity of the microbiota were higher in the wheezing as compared to the non-wheezing group.
Conclusion. Differences in gut microbial composition and diversity were observed between children with wheezing and those without. Analyses are ongoing to examine whether the microbiome composition of children with wheezing at age 2-3 years can be used to predict which children will eventually develop asthma at the age of 6 years.

O076

Controlled human co-infection with pneumococcus and live attenuated influenza virus

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Nasal carriage and its control are important as both determinants of pneumococcal disease and population transmission. The immunological mechanisms that control pneumococcal carriage in humans remain unclear. Loss of this control following influenza infection is associated with secondary bacterial pneumonia during seasonal and pandemic outbreaks. Here, we used human infection challenge with pneumococcus to show that carriage induces early degranulation of resident neutrophils and recruitment of monocytes to the nose, leading to clearance of pneumococcal carriage. Prior nasal infection with live attenuated influenza virus induced inflammation that impaired innate function and altered genome-wide nasal gene responses to carriage. Levels of the cytokine IP-10 promoted by virus infection at the time of pneumococcal encounter predicted bacterial density. These findings provide novel insights in nasal immunity to pneumococcus and influenza-pneumococcal infection synergy.

O078

The human microbiome: education, communication, collaboration

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The complexity of the human microbiome is truly humbling. Thousands of different species are capable of colonising the human body, and it has been estimated that our microbes encode more than 10 million unique genes (which is around 500 times more than the human genome). There is also huge microbiome variation between different individuals, and between bodily sites within the same individual.
Regardless of underlying microbiome variation, under normal circumstances our resident microbes are considered to play a number of key roles in the maintenance of human health. Conversely, the microbiome can also be a driver of

disease. An emerging concept over the last decade has been that of “dysbiosis”, whereby the composition of the microbiota shifts from one that is generally benign or beneficial to host health to one that is deleterious for the host, and it is now clear that alterations in the microbiota are correlated with a whole range of diseases.
This has meant that public, academic and commercial interest in the microbiome has rocketed over the last decade. There is now a concerted effort, involving researchers around the world, to manipulate the microbiome for therapeutic purposes. There have been a number of encouraging advances, but much work remains to be carried out before we truly understand the role the microbiome plays, and how we might reproducibly alter it in beneficial ways.
A key challenge for educators, and those involved in knowledge exchange with the public, is therefore to try and communicate exciting advances, while cutting through the hype that surrounds this field of research. In my talk I will give an overview of current knowledge, and provide some examples that may be useful as teaching resources. I will also cover some of the major challenges that remain to be resolved in microbiome research, and highlight the importance of collaborations involving networks of scientists and clinicians with complementary skills in diverse areas such as microbiology, bioinformatics, immunology, systems biology, statistical modelling and chemistry to our attempts to overcome these challenges.

O079
REBLAB - a personal story about radically transparent science
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Social media has brought a lot of visibility to scientists: you can find many on Twitter gladly engaging in everyday conversation. We know where they go on vacation, what music they like or what papers they published, but rarely do they use any of those platforms to share their newest findings, straight from the lab. The way we communicate in science has remained hopelessly old-fashioned. Traditional publishers continue to place a disproportional financial burden on academic budgets, even with open access journals. What is the price we pay for the closed environment in which we work? What happens if you decide to put all your cards on the table?
Microbiologist and writer Rosanne Hertzberger shares her personal story and her new “open kitchen science” initiative: REBLAB. The aim of REBLAB is to publish every experiment, every method, every poster, every talk and every success or failure. Peer review is the silver standard of quality control, replication (or getting scooped) is the golden standard. Open kitchen science is not only a different communication and publishing strategy. It allows for alternative career paths in science. The vast majority of PhD students will leave academia for a job elsewhere, often completely quitting any efforts to contribute to their field. Alternative forms of communication, such as blogging, open science forums, or social media allow for a more low-key involvement of the growing group of highly educated and informed citizens that find themselves outside of the walls of academia.

O080
Emerging sampling and microbiota analysis tools that simplify and facilitate citizen science
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16S rRNA gene targeted microbial profiling is widely employed to determine the bacterial composition. This technology has become relatively mature and can be further employed for citizen science approaches and/or student projects. Success of the latter depends heavily on using proper sample collection and transport to the laboratory for analysis that is easy and robust to be used by the general public. At MyMicroZoo™, our objective is to provide simple and established protocols for collection, stabilization, and analysis of human derived samples, such as feces. To that end we evaluated sample storage and microbiota analysis methods.
Fecal samples were collected and aliquoted followed by direct DNA extraction or storage at -80C or in stabilizing agent. DNA extraction was performed using different methodologies. Bacterial composition was determined by 16S rRNA gene profiling. Mock communities were used to evaluate sample collection and DNA extraction as well as the outcome of composition analysis. While -80C freezing and storage in stabilizing agent showed highly similar profiles for samples analyzed after different storage times, profiles did differ between storage method. Both methods are suitable for storage of fecal samples, but the stabilizing agent enables sampling at the study participant’s home which facilitates recruitment of participants itself.
One of the more important aspects of citizen science projects is to involve participants in research and guide them through analysis results that would otherwise be only readily interpreted by an academic audience. Therefore, the results are visualized in our online platform where users can see time based analyses to see their own changes in bacterial diversity and composition as well as get information based on scientific literature about the bacteria that are found in their own samples. In addition, the users can compare their results with that of other (groups) of MyMicroZoo™ users.
In conclusion, MyMicroZoo™‘s microbiota analysis pipeline facilitates microbiota research in citizen science research project using an established and evaluated analysis. Furthermore, MyMicroZoo™ can provide the outcome of microbial profiling to the participants, securely and anonymously, to motivate participants and involve them more closely in the project.

O085
Mechanisms of mAb Protection against Ebola
N. Sullivan
Biodefense Research Section

Dr. Sullivan is a tenured Senior Investigator and Chief of the Biodefense Research Section at the Vaccine Research Center, a division of the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. Dr. Sullivan’s current

research is on the immunologic correlates and mechanisms of protection against infection by hemorrhagic fever viruses such as Ebola, Marburg, and Lassa. Dr. Sullivan and her team have developed highly effective vaccine strategies for Ebola virus infection in non-human primates. Her work on filovirus immunology and vaccine development is widely considered as one of the very best in the field despite the difficulties of conducting research under highly specialized BSL-4 containment conditions. More recently, Dr. Sullivan and her team discovered a potently protective monoclonal antibody, mAb114, from a human Ebola survivor that completely rescues Ebola-infected primates, even when given as a monotherapy several days after their Ebola exposure. Studies with the antibody demonstrated that the most potent mechanism of antibody protection is through high affinity, low pH-stable binding that blocks a critical Ebola interaction with its intracellular receptor, Neimann-Pick C1. This antibody will undergo Phase I clinical trials in 2018 to support stockpiling in preparation for use in future Ebola outbreaks.

O086

Type VI secretion system: From the discovery to the mode of action of a dynamic bacterial nanomachines

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Bacteria have evolved several nanomachines to deliver proteins from their cytosol to target cells. These nanomachines differ in their structure, regulation, secreted substrates and are essential for bacterial survival in different environments. In my talk, I will describe the journey from the discovery of the Type VI secretion system (T6SS) to the current understanding of its structure, dynamics and mode of action. I will explain how we used a combination of live-cell imaging, cryo-electron microscopy and genetics to show that T6SS works as a powerful speargun to force large proteins across cell envelopes of both eukaryotic and bacterial cells. I will provide evidence that T6SS structure is evolutionarily related to contractile phage tails and show that subcellular localization of T6SS assembly is in many bacteria regulated with a remarkable precision with implications for its function. I will discuss how T6SS influences bacterial pathogenesis, competition and horizontal gene transfer and thus impacts human health.

O090

Surgical site infections - Insights into practice

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Surgical site infections remain a significant problem, substantially contributing to surgical morbidity and mortality. In surgery involving the implantation of osteosynthesis material or prosthetic joints, infections can have devastating consequences. The preferred treatment strategy, removal of the implant material and extensive debridement, is sometimes difficult to achieve. In spinal surgery for instance, removal of infected osteosynthetic material is often unwanted because of the risk of instability. In August 2017, orthopedic surgeons of the Erasmus MC in Rotterdam perceived a high incidence in surgical site infections after spondylodesis surgery, and contacted the department of infection prevention. The incidence of deep and superficial surgical site infections after spondylodesis was calculated. In 2015, the incidence of these infections had been 5.3%; in 2016 and 2017 (up to August) the incidence was 9.2% and 8.0%, respectively. Details on these infections were analyzed. In 62.5% of infections, *S. aureus* was the causing microorganism. Spa typing showed that these isolates were unrelated to each other. Audits were performed during surgical procedures, which suggested several improvements. Furthermore, attempts were made to implement preoperative decolonization of *S. aureus* carriers with mupirocin nasal ointment and chlorhexidine body wash. This presentation focuses on the considerations to decolonize carriers only, instead of universal decolonization of patients. Also, practical issues on the implementation of this strategy will be discussed.

O091

A novel functional link between comammox and anammox bacteria

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Recently, *Nitrospira* bacteria were discovered as complete ammonia-oxidizing (comammox) microorganisms that convert ammonia all the way to nitrate by a single cell (van Kessel et al., 2015; Daims et al., 2015). In our study, comammox bacteria were enriched in a bioreactor, which was inoculated with biomass obtained from the anaerobic compartment of a recirculating aquaculture system biofilter. In the bioreactor, hypoxic conditions were maintained and the culture was supplied with aquaculture water supplemented with low concentrations of ammonia, nitrite and nitrate. The endproduct of the conversion of these nitrogenous compounds was dinitrogen gas. The culture was dominated by anaerobic ammonium-oxidizing (anammox) bacteria related to *Brocadia*, *Nitrospira* and denitrifying microorganisms. Surprisingly, FISH analysis showed colocalization of comammox *Nitrospira* and anammox bacteria in the same flocs. The tight clustering and low concentrations of substrates (oxygen, ammonia and nitrite) available indicate a functional link between these two types of microorganisms. In order to investigate the interactions between comammox and anammox bacteria in more detail, the culture was switched to defined mineral medium. Activity assays using labelled ammonia showed the production of single and double labelled dinitrogen gas, indicating that both anammox and comammox bacteria are consuming ammonia. These results also suggest that comammox *Nitrospira* in this culture perform partial ammonia oxidation to nitrite, which is subsequently used by anammox. To further investigate this, an aerobic culture was started from the original enrichment culture to selectively enrich comammox *Nitrospira*. Activity assays under different oxygen concentrations revealed that under low oxygen concentrations comammox *Nitrospira* started to accumulate nitrite instead of performing full oxidation of ammonia to nitrate. Since the nitrite will serve as co-substrate for anammox, these results demonstrate that comammox and anammox bacteria can be grown as co-operators rather than competitors under low oxygen conditions. Furthermore, these findings indicate that the combination of comammox and anammox

processes might be an interesting alternative for hypoxic N-removal from wastewater.

O092

A novel tool for the in situ detection of ammonia and methane oxidizing bacteria

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Introduction: Over the last two decades, metagenomics in combination with cultivation approaches resulted in the identification of novel groups of ammonia and methane-oxidizing microorganisms, highlighting that our understanding of the microbial biodiversity within the biogeochemical nitrogen and carbon cycles is still incomplete. However, when novel microorganisms are detected in metagenomic datasets, linking an inferred function to a specific microorganism remains challenging. Therefore, activity-based protein profiling (ABPP) protocols have been developed as a way of linking specific functions to proteins present in complex metaproteomes. These ABPP techniques often employ bifunctional enzyme probes featuring: (i) a reactive group that binds to the active site, thereby inhibiting and covalently labelling the enzyme and (ii) an ethynyl or azide group that can be subsequently used for the attachment of a reporter molecule (fluorophores, biotin) to the enzyme via a Cu-catalyzed alkyne-azide cycloaddition (‘click’) reaction.
Materials and methods: In this study we developed an ABPP-based protocol for the *in situ* fluorescent labelling of ammonia (AMO) and methane monooxygenases (PMO), using the alkyne 1,7-octadiyne (1,7OD) as a bifunctional ABPP probe. More specifically, the covalent binding of 1,7OD allows fluorescent labelling of the AMO and PMO enzymes via a ‘click’ reaction. For that reason, a number of pure and enrichment cultures, as well as environmental samples, were subjected to the ABPP-based protocol in order to verify its ability to specifically target ammonia and methane-oxidizing microorganisms.
Results: Using the above-described ABPP-based protocol, we were able to specifically label active ammonia and methane oxidizing bacteria. Furthermore, combination of this protocol with 16S rRNA-targeted fluorescence *in situ* hybridization permitted direct linking of these functional lifestyles to phylogenetic identification. Application of the method allowed the specific detection and visualization of novel key players of the ammonia and methane cycles, directly in wide variety of samples. More specifically, our ABPP-based protocol facilitated also the detection and identification of the novel complete ammonia oxidizers (comammox) belonging to the genus *Nitrospira*.
Conclusions: This study presents a novel technique for the reliable detection, visualization and identification of ammonia and methane oxidizing bacteria. Furthermore, we foresee this method as a valuable tool to identify novel ammonia- and methane-oxidizing microorganisms in complex environmental samples.

O093

Induction and Suppression of Secondary Metabolite Production in Competing Actinomycetes

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Actinomycetes are well known for the production of many antibiotics now used in the clinic. Little is known about the regulation of antibiotic production for these multicellular bacteria in nature. Actinomycetes live in diverse microbial communities in the soil where they compete for resources and space. Competition is mediated by secreted secondary metabolites, such as antibiotics, but these products are expensive to produce and, when produced by others, potentially harmful. The regulation and expression of these products is therefore predicted to be modified by interactions with coexisting species that represent possible threats. Both the induction of secondary metabolite production by potentially harmful competitors and the suppression of antibiotic production in other species are predicted to provide benefits during competitive interactions.

To gain insight into the dynamics of competition we studied interactions between 21 actinomycete strains isolated from a single soil sample from the Himalaya and three well-characterized lab strains. During asocial assays a colony was grown alone before its inhibitory capacity against each of the 24 strains was tested. Next, we conducted pairwise social assays in which two colonies were grown in close proximity (designated focal and modifier colonies) to enable them to interact through diffusible molecules before we examined their inhibitory capacity against each of the 24 strains. We then compared the outcome of asocial and social assays to reveal the extent of social modification.
Our results show that induction is widespread and occurs more commonly that suppression (59% and 13% respectively). All 24 strains can induce and suppress at least three others. All 24 strains can be induced, while only two thirds of strains can be suppressed. These responses are mainly determined by the identity of the focal strain, indicating that these strains can be induced or suppressed by the presence of a modifier regardless of its identity. We next tested whether these responses are influenced by resource environment by examining these interactions in the presence of both high (0.5%) and low (0.05%) concentrations of glycerol as the sole carbon source. Overall, we did not detect any significant differences between the high and low resource concentration. However, at the level of individual strains we identified clear differences in induction and suppression across media. Finally, we used multilocus sequence typing to test if these responses are related to the phylogenetic distances between the actinomycetes. Our results show that inhibition was negatively correlated with genetic distance between the focal and target strain, indicating that strains are more likely to inhibit closely related competitors. There was no correlation between phylogeny and induction or suppression, indicating that these interactions evolve faster than the divergence times between strains. Consistent with this highly related strains differ markedly in their influence on and their response to others.
Our results show that competitive interactions are modified with high frequency and that they are not phylogenetically determined, suggesting that these competitive responses evolve rapidly. These results highlight the diverse ways in which interference competition mediated by secondary metabolites may be regulated in nature.

O094

An expanded dissimilatory sulfur cycle in the anoxic and sulfidic Black Sea revealed by gene-centric metagenomics

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Introduction

The Black Sea is the largest anoxic and sulfidic basin on the planet. The waters are stratified, resulting in a permanent anoxic zone stretching over several tens of meters, which separates the shallow 50 to 80-meters-thick oxic surface layer and the up to 2100-meters-deep sulfidic zone. The water column can be divided into well-separated redox zones dominated consecutively by a microbially driven nitrogen, manganese and sulfur cycle. This makes the Black Sea a convenient model system to study 1) the biogeochemistry and microbiology of the ancient Proterozoic ocean, which was largely anoxic and/or sulfidic, and 2) the anoxic/sulfidic zones in the modern-day ocean, which are rapidly expanding. In anoxic zones the sulfur cycle does not dominate redox chemistry, but is still active *cryptically*, i.e. without accumulation of sulfide (<0.1 µM) due to direct re-oxidation. The sulfur cycle is driven by dissimilatory sulfate reduction, producing sulfide which is re-oxidized through a complex network of biotic and/or abiotic reactions involving sulfur cycle intermediates (SCIs) such as elemental sulfur and thiosulfate. Currently, which microorganisms and pathways mediate these reactions in anoxic/sulfidic zones is only superficially understood.

Methods

In February 2016, the Black Sea was sampled at 15 different depths spanning the oxic, anoxic and sulfidic zone. We measured the redox potential and the oxygen, sulfide and thiosulfate concentrations. Additionally, metagenomics sequence reads were obtained by shotgun-sequencing DNA from biomass collected on filters with a pore size of 0.3 µm. The reads of all 15 samples were cross-assembled together, resulting in scaffolds, enabling the reliable detection of full-length genes using Hidden Markov Models (HMMs). The quantitative distributions of genes over the different depths were inferred from coverage values obtained by back-mapping the reads per sample onto the scaffolds, and normalized according to the average distribution of 41 prokaryotic single-copy household genes.

Results

We analyzed the distribution of over 40 different dissimilatory sulfur genes using available and newly constructed HMMs. All essential genes of the canonical dissimilatory sulfate reduction pathway were detected in the anoxic zone, along with genes for sulfur oxidation. Oxidative dissimilatory sulfur genes were found to be most abundant in the upper sulfidic zone, coinciding with previously recorded maxima in sulfide oxidation and inorganic carbon fixation. A high coverage was found for several putative dissimilatory (bi)sulfite reductase alpha subunits (*dsrA*), implying the involvement of novel lineages of microorganisms. The coverage for these *dsrA* genes correlated with the coverage of *dsrEFH* genes, which are diagnostic for oxidative metabolism. Concentrations of thiosulfate peaked in the deep sulfidic zone, showing a correlation with the distribution of genes encoding for the alpha subunit of thiosulfate/polysulfide/sulfur reductase.

Discussion/Conclusion

Our results have expanded the knowledge of the Black Sea sulfur cycle. We found support for the presence of a cryptic sulfur cycle as demonstrated in other anoxic zones, and for the involvement of novel microorganisms. Although our metagenomics approach is limited to identifying the genetical potential, and not the (transcriptional) activity of microorganisms, this approach has been demonstrated to be more explanative then previous -omics approaches.

O095

How do coal degrading methanogens make biogas? Unravelling central metabolism of *Methermicoccus shengliensis*

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Methane is the second most important greenhouse gas on earth. The main source of methane emission into the atmosphere are methanogenic archaea emphasizing the importance of those organisms for the global carbon cycle. Although methanogens have been studied for more than 111 years [1], a novel methanogenic pathway was recently discovered: the thermophilic methanogen *Methermicoccus (M.) shengliensis* is able to use a large variety of methoxylated aromatic compounds as substrates for methane generation [2-4]. Despite the significance and novelty of this unique archaeon a detailed analysis of its metabolism is still missing. Here, we used transcriptomic and proteomic methods to investigate the response to growth on methoxylated aromatics along with enzymological characterization of new methyltransferase enzymes. The transcriptomic analysis revealed a gene cluster highly expressed under growth on the methoxylated compound trimethoxybenzoate. The encoded enzymes are most likely essential for methoxydotrophic methanogenesis. Four genes were chosen for heterologous expression in *E. coli* and subsequent purification by affinity chromatography. Unravelling the ability of this methanogen to convert methoxylated compounds to methane is indispensable for future applications of *M. shengliensis* in coal bed methane production or biodegradation of oil.

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O096

Phenotypic and genetic heterogeneity among isolates of A. fumigatus from humans, dogs and the environment

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Introduction

Aspergillus fumigatus is a ubiquitous saprotrophic fungus and opportunistic pathogen. In most cases, the inhalation of spores is harmless due to efficient clearance, but in humans with immunodeficiency invasive pulmonary aspergillosis (IPA) can develop which has a high mortality rate. This fungus can cause also non-invasive infections in humans and animals, for example sino-nasal aspergillosis (SNA) in otherwise immunocompetent dogs. A factor that could influence the infection process is the inherent isolate variability within this fungal species. Within this context we have compared different isolates of *A. fumigatus* using genetic and phenotypic methods in order to find clues about the features that determine the virulence of *A. fumigatus*.

Methods

We have compared a set of isolates of *A. fumigatus* from A) humans with IPA (29 isolatesfrom 9 patients) ,B) dogs with SNA (27 isolates form 9 patients) C) environmental isolates (27 isolates) with reference strains. Azole resistance was determined by microdilution assay antifungal susceptibility testing and tandem repeats in the promotor region of the *cyp51A* gene. Sequencing of calmodulin (*CaM*), beta-tubulin(*benA*) and mating type genes (*MAT1-1 and 1-2*) and microsatellite (STRAf) analysis were performed to detect genetic differences between isolates. Plating on different media was performed to observe differences in macro and micromorphology. mRNA was isolated and used in RNAseq analysis.

Results

We show that dogs with SNA are infected by one genotype only whereas human patients are infected by various genotypes. Only in the selected set human isolates and one of the indoor and outdoor environmental isolates with azole resistant were detected. Interestingly, phenotypic analysis indicated that the isolates from dogs are most variable in growth speed and morphology as compared to human and environmental isolates, for example presence of “white isolates” from the SNA samples could be an indication of host driven evolution. Transcriptomic studies on SNA plaques directly derived from dog patients indicate lower expression of genes involved in the central regulatory sporulation pathway (*brlA-abaA-wetA*) as well as notorious variability of gene expression among different isolates.

Conclusions

A notable variation in asexual development, colony size, and colony color was observed in the strains isolated from dogs suffering from SNA. We conclude that:

Isolates from dogs with SNA were phenotypically more diverse that their environmental and human counterparts.

Each dog with SNA is infected by one single genotype of *fumigatus* in contrast to human patients who carry mixed infections

Reduced asexual reproduction, as observed in 11 out of 27 dog isolates can be an adaptation to increase fitness in the host

Within-host adaptation in dogs involves amongst others down regulation of the sporulation pathway

The basis of this heterogeneity might be due to genomic differences or epigenetic variations that occur during the infection process in dogs. RNA-seq and genomic analysis of the strains studied can provide clues about the origin of these observations.

O097

Staphylococcal superantigen-like protein 13 activates neutrophil via Formyl Peptide Receptor 2

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Introduction: *Staphylococcus aureus* is a common Gram-positive human pathogen that causes variety of diseases ranging from mild soft tissue infections to more serious life-threating systemic infections. *S. aureus* secretes a variety of proteins that target the innate and adaptive immune system and facilitates successful colonization and infection. Therefore, unraveling the nature of these immune evasion molecules contributes to a better understanding of the pathological processes underlying infectious and inflammatory diseases. And lead to new options in treatment and prevention.

Methods and Results: A high throughput phage display selection strategy combined with Next generation sequencing was used to identify secreted *S. aureus* proteins that interact with immune components, especially immune cells. In a phage selection on human neutrophils a secreted protein was selected that was identified as Staphylococcus Superantigen-Like protein 13 (SSL13).

SSL13 belongs to a family of 14 proteins with structural similarity to Staphylococcal superantigens but lack a functional T-cell receptor binding domain and therefore exhibit no superantigenic activity. It was previously reported that several SSL members (SSL5, SSL7 and SSL10) are involved in host immune responses. Genetic analyses of 88 clinical *S. aureus* strains revealed that the gene encoding SSL13 is carried by 100% of *S. aureus* strains but it has not been functionally characterized. We confirmed SSL13 is produced *in vivo* as antibodies against this protein can be detected in human serum.

SSL13 was expressed and purified in an *E. coli* expression system. Cell binding experiments on purified human immune cells showed binding of SSL13 to neutrophils and monocytes but not lymphocytes. In contrast to other previously characterized SSLs, that inhibit immune functions, cell activation experiments conducted at 37°C identified SSL13 as a strong activator of neutrophils. Receptor inhibition experiments using a set of inhibitory proteins showed SSL13 activates neutrophils via the formyl-peptide receptor 2 (FPR2). Moreover, a cell migration assay showed SSL13 acts as a

chemoattractant, induces degranulation and oxidative burst in neutrophil. As with many other staphylococcal immune evasion proteins that show a high degree of human specificity SSL13 was not able to efficiently activate mouse neutrophils hampering *in vivo* experiments in animal models.
Conclusion: In contrast to the known SSLs immune evasion molecules, SSL13 is a chemoattractant and a neutrophil activator that acts via the FPR2. Therefore, SSL13 is a unique SSL member not belonging to the immune evasion class, but is a pathogen alarming molecule sensed by the FPR2.

O098

Macrophage Galactose C type lectin (MGL) interacts with *Staphylococcus aureus* and coagulase negative staphylococci through wall teichoic acid modifications

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Introduction:
Staphylococcal species are common members of the microbiota. Especially *Staphylococcus aureus* is associated with skin pathology such as skin and soft tissue infections and atopic dermatitis. Dendritic cells (DCs) in the dermis of the skin are critical cells in coordinating subsequent immune responses and maintaining skin homeostasis. DCs residing in skin and lymph nodes express the calcium-dependent pattern-recognition receptor Macrophage Galactose C-type lectin (MGL). It recognizes terminal α- and β-N-acetylgalactosamine (GalNAc) that is present on various pathogens, including *Campylobacter jejuni* and *Fasciola hepatica*, but also on certain types of cancer cells.
Wall teichoic acid (WTA) is a conserved and abundant component of the Gram-positive cell wall with important functions for bacterial physiology, infection biology and phage-mediated horizontal gene transfer. *S. aureus* clonal complex 5 strains (represented by PS187) and several species of coagulase-negative staphylococci express WTA that is composed of poly-glycerolphosphate decorated with GalNAc residues, which is mediated by the glycosyltransferase TagN. The aim of our study was to explore whether this conserved staphylococcal WTA epitope could be recognized by MGL and thereby control immune activation or modulation.
Methods:
Binding of recombinant human MGL (rhMGL), mouse homologue MGL2 (mMGL2) and FITC-labeled plant lectins wheat germ agglutinin (sWGA) recognizing GlcNAc (N-acetylglucosamine) and soy bean agglutinin (SBA), specific for GalNAc to staphylococcal strains (*S. aureus* PS187 WT, PS187D*tagN*, PS187D*tagN* + pTagN, PS187 D*tagN* + p*S.lugdunensis*TagN, USA300; *S. carnosus*, *S. capitis*, *S. epidermidis*, *S. lugdunensis*, *S. pseudointermedius*, *S. saprophyticus*, *S. simulans*) was performed using flow cytometry. Specificity of the interactions was verified by addition of soluble GalNAc or glucose. DCs were differentiated from primary human monocytes (isolated from buffy coat or fresh blood) in presence of IL-4 and GM-CSF. Bacterial binding by DCs was assessed by incubation with FITC-labeled *S. aureus* strains in different bacteria to cell ratios and analyzed by flow cytometry.
Results:
rhMGL interacted with *S. aureus* PS187 in a TagN- and GalNAc-dependent manner. Interaction was not human-specific as binding was also observed with mMGL2. No interaction was observed with coagulase negative strains despite the presence of *tagN*. However, plasmid overexpression of CoNS *tagN* in PS187 D*tagN* mutants conferred rhMGL binding, suggesting that our culture conditions do not induce *tagN* expression in CoNS. *S. aureus* PS187 bound to DCs in a concentration-dependent manner, which was partially dependent on WTA GalNAc expression.
Conclusion:
Our data show that human MGL and mouse MGL2 interact with *S. aureus* through GalNAc present on WTA and this interaction contributes to binding with human DCs. Coagulase-negative staphylococci are also able to interact with hMGL under culture conditions that induce expression of TagN. Follow up studies aim to determine the cytokine profile of DCs after stimulation with PS187 or *tagN* mutant bacteria to assess the possible impact on immune system activation. Moreover study focusing on impact of GalNAc and TagN expression in CoNS will be performed and will include experiments using skin model.

O099

***Mycobacterium bovis* BCG does not export PE_PGRS and PPE-MPTR proteins and induces an impaired antigenic repertoire**

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Introduction
Mycobacterium tuberculosis uses different ESX/Type VII secretion (T7S) systems to transport proteins important for virulence and host immune responses. We recently reported that secretion of T7S substrates belonging to the mycobacteria-specific PE and PPE proteins of the PGRS (polymorphic GC-rich sequences) and MPTR (major polymorphic tandem repeat) subfamilies required not only a functional ESX-5 system but also a functional PPE38/71 protein for secretion (Ates et al 2018 Nature Microbiol.). Inactivation of *ppe38*/*71* and the resulting loss of PE_PGRS/PPE-MPTR secretion were linked to increased virulence of *M. tuberculosis* strains.
Results/methods
Here, we investigated the occurrence and effect of *ppe38/71*-deletions (Δ*ppe38/71*) in different *M. tuberculosis* complex (MTBC) members, with a main focus on those that have deleted the region of difference 5 (RD5). This region, encompassing the phospholipase-encoding genes *plcABC* and the adjacent *ppe38-71* genes, is also absent from *Mycobacterium bovis* and *M. bovis* BCG (BCG) strains. We show, by immunoblotting and immunological techniques, that

BCG therefore is unable to secrete the plethora of PE_PGRS and PPE-MPTR proteins, a phenotype that can be restored by introduction of the *M. tuberculosis ppe38*-locus. Epitope mapping of the PPE-MPTR protein PPE10, resulted in the identification of two highly immunogenic epitopes, showing that this class of proteins can be important in inducing $CD4^+$ T-cell responses against *M. tuberculosis*. These newly identified epitopes were used to monitor T-cell responses in splenocytes from recombinant BCG/*M. tuberculosis* immunized mice, confirming that PPE10-specific immune-induction is dependent on ESX-5/PPE38-mediated secretion and therefore that BCG is unable to induce such responses. Surprisingly, restoration of PE_PGRS/PPE-MPTR secretion in recombinant BCG neither altered global antigenic presentation or activation of innate immune cells, nor protective efficacy in two different mouse vaccination-infection models.

Conclusion/Discussion

Here, we show that a predicted total of 89 PE_PGRS/PPE-MPTR surface proteins are not exported by BCG, due to a Δ*ppe38/71*-associated secretion defect. Importantly, many of these proteins were reported to be important immune modulators and/or virulence factors. These proposed functions are put into perspective by our results, which show that restoration of secretion does not significantly alter the activation of innate immune cells, antigenic presentation to $CD4^+$ T- cells, or protection against *M. tuberculosis* in mouse models. This unexpected finding stimulates a reassessment of the immunomodulatory properties of PE_PGRS/PPE-MPTR proteins, some of which are contained in vaccine formulations currently in clinical evaluation.

O100

An unusual prohibitin regulates malaria parasite mitochondrial membrane potential

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Introduction

Proteins of the stomatin/prohibitin/flotillin/HflK/C (SPFH) family are membrane-anchored and perform diverse cellular functions in different organelles. Malaria parasites harbour four SPFH proteins: the conserved prohibitin 1, prohibitin 2, stomatin-like protein, and an unusual prohibitin-like protein (PHBL).

Methods

We investigated the SPFH proteins of the murine malaria model parasite *Plasmodium berghei*, using advanced experimental genetics to fluorescently label the proteins and generate gene deletion mutants. The resulting recombinant parasite lines were analyzed throughout the entire complex life cycle in mammalian and mosquito host using fluorescence and electron microscopy and flow cytometry.

Results

The SPFH proteins localize to the parasite mitochondrion, and systematic gene targeting suggests essential functions for the three conserved SPFH proteins during blood infection. In contrast, *PHBL* was successfully ablated, but its absence impaired asexual parasite propagation and virulence. Strikingly, *PHBL*-deficient parasites fail to colonize the *Anopheles* vector due to a specific arrest during ookinete development *in vivo*. We show that this arrest correlates with the depolarization of the mitochondrial membrane potential ($\Delta\Psi_{mt}$).

Conclusion

Our results underline the importance of SPFH proteins in the regulation of core mitochondrial functions and suggest that fine-tuning of $\Delta\Psi_{mt}$ in malarial parasites is critical for the colonization of the definitive host.

O101

Identification of a mycobacterial PE-protease that cleaves secreted surface proteins

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Introduction

Mycobacteria are best known for their pathogenic family member *Mycobacterium tuberculosis*, which still causes 1.7 million deaths annually.

Mycobacteria have a unique cell wall that requires a specialized secretion systems, known as Type VII secretion systems. There are 5 different Type VII secretion systems, named esx-1 to esx-5. ESX-5 is only present in slow-growing mycobacteria, such as the pathogenic *M. tuberculosis* and *M. marinum*. These pathogenic mycobacteria secrete amongst others a class of proteins named after the conserved motif found in their N-terminus: the PE proteins. *M. tuberculosis* has 99 PE proteins, most of which are secreted through ESX-5 and are present on the cell surface or in the extracellular environment. However, only very little is known about the function of these PE-proteins. LipY is the only PE protein with a clear function, as it acts as a lipase on the bacterial cell surface. We used a LipY- OVA albumin (OVA) fusion construct to further study secretion of PE proteins in *M. marinum*.

Methods and Results

To study secretion of PE-proteins, a fusion construct consisting of the PE-domain and linker domain of LipY and OVA (LipY-OVA) was expressed in *M. marinum*. LipY-OVA was detectable on the cell surface, but was only at very low levels present in the culture supernatant. To increase secretion of LipY-OVA, mutant constructs were made by error-prone PCR. Subsequent screening for secretion mutants by double filter assay and immunoblotting allowed us to identify a hypersecreting mutant that lacked the complete LipY linker domain (LipY-$OVA_{\Delta LD}$). This hypersecreting mutant was used to further study the secretion of PE proteins. We screened a transposon library of *M. marinum* expressing LipY-$OVA_{\Delta LD}$ for mutants that showed increased secretion on double filter assay. Hypersecreting mutants were analyzed by ligation mediated-PCR to identify the gene that was disrupted by the transposon. Three independent mutants appeared to have a disruption in the same gene. Here we show that this gene encodes for a PE-protease. This PE-protease not only cleaves LipY-OVA, but also endogenous PE proteins. Furthermore, the PE-protease cleaves itself as was shown by *in*

vitro translation and by overexpression of the PE-protease and an active site mutant in *Escherichia coli* and *M. marinum*.

Conclusion

The discovered PE-protease is the second PE protein with a clear function to date. This protease shows both auto-cleavage and cleavage of endogenous PE proteins. This protease is the first protein that has been shown to process PE proteins on the cell surface of mycobacteria. These results contribute to a further understanding of the role of PE proteins in mycobacteria.

O102

Fatty acid processing affects survival of mycobacteria

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Introduction

Mycobacterium tuberculosis causes tuberculosis, a chronic and wasting disease. One of the characteristics of this disease is that an *M.tuberculosis* infection can go unnoticed for decades. Such a latent infection can reactivate and lead to active disease later in life. During such a latent infection, *M. tuberculosis* withstands host-derived attacks by forming so-called persister cells. These persisters are characterized by a non- (or slowly-) dividing, drug resistant phenotype, which makes them challenging to treat. One of the hallmarks of persister cells is the presence of intracellular lipid bodies which are produced using host-derived lipids. The goal of this project is to decipher the mechanism of mycobacterial lipid body formation and unravel the role of these structures in persister cell formation and functioning. To address this we analyzed mutants having an altered lipid response.

Methods

M. marinum was used as a model organism. A transposon bank was generated and exposed to different fatty acid sources. The transposon bank was plated on minimal medium agar plates with specified fatty acids as carbon source, analyzed by flow cytometry to determine ability to form lipid bodies and exposed to liquid minimal medium with specified fatty acids as carbon source to determine viability.

Results

Wt *M. marinum* appeared not to be able to survive on long-chain fatty acids (C16:0 and C18:0), that were esterified to polyethoxylated sorbitan, whereas other long-chain fatty acids (C12:0 and C18:1) did not interfere with survival. C18:1 was even efficiently used as carbon source and resulted in enhanced growth. Importantly, loss of viability by the long-chain fatty acids was reverted in specific *M. marinum* mutants. These transposon mutants either involved the transport of fatty acids from the exterior or fatty acid processing. These mutants are now further analyzed to uncover the pathway of mycobacterial lipid body formation.

Conclusion

We observed variation in fatty acid processing and the subsequent effect on mycobacterial viability. We therefore hypothesize that environmental (host) fatty acids can have both a positive or detrimental effect on mycobacteria.

O104

The effect of antibiotic exposure on resistance development and plasmid transfer

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The threat of antibiotic resistance to public health is generally recognized. Worldwide more than half of all antibiotics are applied for other purposes than treating infections in humans, raising livestock being quantitatively the largest non-human usage. Exposure of microbes to non-lethal concentrations of antibiotics invariably causes development of and selection for resistance. In agriculture, flocks and groups of animals are treated by administering antimicrobials through the drinking water or by mixing it in the feed. As a consequence dosing cannot be as precise as when individual patients swallow pills or are given a drug intravenously. Two main mechanism contribute to generation of resistance upon exposure to non-lethal levels of antimicrobials: 1) adaptation of cellular mechanisms followed by mutations and 2) horizontal transfer of resistance genes. De novo development of resistance is rapidly induced by exposure to non-lethal levels of drugs that still allow some growth. When growth reaches (almost) normal levels again, the concentration can be increased and the process repeated. Initially the expression of between 100 to 250 enzymes is either up or down regulated, increasing the minimal inhibitory concentration (MIC) by a factor of around 10. Further increases of the MIC to 100 to 10,000 times the wildtype MIC are accompanied by one or several mutations and sometimes genetic rearrangements, depending on the bug/drug combination. Simulation of patient treatment in chemostats showed that this process can explain the development of resistance observed in patients in intensive care units. Rates of transfer of plasmids containing resistance genes vary by a factor of 10^9, depending on several factors that are presently poorly understood. While high levels of antimicrobials almost always prohibit transfer or strongly reduce the rate, exposure to low levels can either stimulate or slow down, depending on the plasmid. The difference between the two mechanisms for the acquisition of resistance may not be as clear-cut as it seems. Acquisition of resistance to amoxicillin resulted in a 60-fold multiplication of a chromosomal DNA segment, followed by formation of a transferable element separate from the chromosomal DNA that codes for high-level resistance to amoxicillin. From the practical point of view regarding patient treatment and prevention of avoidable resistance, the conclusion should be that exposure to non-lethal concentrations of antimicrobials should be avoided whenever possible. For patient treatment the optimal protocol would aim to provide the highest dose the patient can endure for the shortest time that achieves the therapy objectives.

O105

Within host-adaptation of *Bordetella pertussis* under vaccine immune pressure

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Introduction

Pertussis, or whooping cough, is a highly contagious respiratory diseases caused by the Gram-negative bacterium *Bordetella pertussis*. The bacterium infects the mucosal layers of the respiratory tract of children and adults of all ages, and was responsible for high mortality and morbidity rates before the widespread implementation of whole cell pertussis vaccines (wPs). Despite the high effectiveness of wPs, safety concerns led to the replacement of wPs with acellular pertussis vaccines (aPs). These vaccines consist of one to five purified pertussis proteins, including pertussis toxin (Ptx), filamentous hemagglutinin (FHA), pertactin (Prn), and fimbriae (Fim). Worryingly, there is an increase in pertussis observed in several countries that have switched to aPs. Adaptation of the bacterium relative to vaccine strains is one of the causes and a recent adaptation involves the non-expression of pertactin (Prn). Here, we have investigated the efficacy of aP vaccines with and without Prn and whole cell pertussis (wP) vaccines using a mouse model. Furthermore, we studied the impact of vaccination and strain variation on FHA expression in the nose and lungs and the antibody response to strain variants.

Methods

Naïve and vaccinated mice were challenged with two Prn-producing (Prn^+) and two Prn-deficient (Prn^-) strains and colonization was investigated in the nose and lungs. FHA expression was determined by Luminex and Western blot. Genetic phase variation was studied using a Ligase Detection Reaction. Antibody and complement deposition on *B. pertussis* was studied by flow cytometry.

Results

We show that aP vaccines prevent infection of the lungs but fail to reduce colonization in the upper respiratory tract. In contrast, wP vaccines significantly reduce bacterial load in both the nasopharynx and the lungs. We found that *B. pertussis* isolated from the respiratory tract variably expressed FHA, which was due to phase variation in a homopolymeric G-track in the *fhaB* gene. Phase variation was strongly enhanced in *B. pertussis* isolated from the lower respiratory tract, but not in the upper respiratory tract, suggesting differences in immune pressure and/or essentiality of FHA between these two anatomical niches. Importantly, we found that phase variation of FHA was particularly selected for in Prn-negative *B. pertussis* strains following vaccination with FHA-containing acellular pertussis vaccines, but not after vaccination with wP. Opsonization experiments show that Prn-negative strains bind less antibody and complement than Prn-positive strains. Furthermore, downregulation of FHA leads to significant further reduction of antibody and complement deposition.

Conclusion

This study reveals a novel mechanism by which *B. pertussis* can escape clearance by vaccination and suggests that the efficacy of acellular pertussis vaccines against Prn^- strains may be further reduced due to downregulation of FHA. This suggests that immunity to *two* of the *three* pertussis vaccine antigens used in the Netherlands may be compromised.

O106

Diverse evolutionary pathways to colistin resistance in *Klebsiella pneumoniae* and their impact on fitness and virulence characteristics.

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Introduction: *Klebsiella pneumoniae* has emerged as an important cause of multi-drug resistant nosocomial infections, leading to a resurgence of the use of the last-resort antibiotic colistin. Colistin exerts its antimicrobial effect through electrostatic interaction with the negatively charged lipid A moieties of lipopolysaccharides, thereby destabilizing the outer membrane of Gram-negative bacteria.

Methods: To study the development of colistin resistance in *K. pneumoniae*, we used four clinical, colistin-susceptible strains and evolved these *in vitro* to high-level resistance by culturing in the presence of increasing concentrations of colistin. Evolutionary trajectories towards colistin resistance were mapped through whole genome sequencing of evolving populations. Whole genome sequencing of axenic isogenic colistin-susceptible/resistant strains pairs was performed to determine SNPs, indels, and movement of Insertion Sequence (IS) elements. Growth fitness, lipid A composition, susceptibility to human serum and human antimicrobial peptide LL-37, and membrane permeabilisation were determined.

Results: All colistin-susceptible *K. pneumoniae* strains readily evolved high-level resistance to colistin. Resistance was associated with mutations in genes encoding the PhoPQ two-component system, the LPS-assembly protein LptD, the regulator of LPS biosynthesis YciM, and the integration of an IS5 element in the promotor region of the genes encoding the two-component system CrrAB. None of the strains had identical mutations. Sequencing of population during *in vitro* evolution towards resistance showed the existence of selective sweeps of advantageous mutations through the populations. In the resistant isolates, lipid A modifications were observed, including hydroxylation, palmitoylation, and addition of L-4AraN. One of the strains was negatively affected in its fitness by resistance. Permeabilisation of the outer and inner membrane was significantly decreased upon exposure to LL-37 and colistin in the resistant isolates versus the susceptible parental strains. Three strains exhibited decreased susceptibility towards LL-37, whilst susceptibility to

complement-mediated killing in serum increased for two strains, while the other strains were unaffected.
Conclusion: These results highlight the relative ease by which *K. pneumoniae* can evolve colistin resistance, through multiple evolutionary trajectories, with variable effects on virulence characteristics, and without appreciable fitness costs.

O107

Carbapenemase producing Enterobacteriaceae in the Netherlands, 2014-2017

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Introduction: Carbapenem resistance mediated by mobile genetic elements has emerged worldwide and has become a major public health threat. Therefore, Dutch medical microbiology laboratories are requested to submit carbapenemase-producing *Enterobacteriaceae* (CPEs) to the National Institute for Public Health and the Environment as part of a national surveillance system. Here, we report on the surveillance data from 2014-2017.
Methods: Meropenem MICs and species identification of all submitted isolates were confirmed by Etest and Maldi-ToF. The presence of ten carbapenemase-encoding genes was determined by PCR. Carbapenemase production was assessed phenotypically by the Carbapenem Inactivation Method (CIM). Of all submitted CPEs, one species/carbapenemase gene combination per person per year was subjected to next-generation sequencing (NGS) via Illumina HiSeq, to identify the carbapenemase allele. Genetic relationships for *Klebsiella pneumoniae* and *Escherichia coli* were examined by whole-genome Multiple Locus Sequence Typing (wgMLST). Patient characteristics were collected by a web-based questionnaire.
Results: 1,556 suspected CPE isolates were received (2014: n=276, 2015: n=382, 2016: n=424, 2017: n=474). 761 (2014: n=84, 2015: n=197, 2016: n=198, 2017: n=282) were confirmed as CPE (CIM positive) and 559 of these were analysed by NGS (2014: n=54, 2015: n=147, 2016: n=157, 2017: n=201).
The predominant CPE species were *K. pneumoniae* (n=360, 47%) and *E. coli* (n=212, 28%). Of all CPEs, 723 (95%) carried a carbapenemase encoding gene, mostly *bla*OXA-48like (n=342, 47%), *bla*NDM (n=205, 28%) and *bla*KPC (n=102, 14%).
NGS analyses showed that *bla*OXA-48 was indeed predominant, found in 39% of all isolates (216/560). Additionally, alleles like *bla*NDM-6 and *bla*NDM-7 were identified that have not been reported previously in the Netherlands.
WgMLST allowed identification of clusters based on a cut-off of 50 genes. For *K. pneumoniae*, 25 clusters were observed ranging in size from 2 to 35 isolates and for *E. coli*, 17 clusters consisting of 2 to 10 isolates were identified. All these clusters contained isolates from multiple submitters.
Conclusions: The number of submitted CPEs in the Netherlands is increasing and *bla*OXA-48 is the predominant carbapenemase allele. We also observed alleles that have never been described in the Netherlands before. WgMLST identified multiple clusters in different healthcare centres. We are now studying epidemiological data to understand possible transmission pathways.

O108

Quantitative assessments of resistance gene reservoirs based on pan-genome analyses

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Antibiotic resistance is a global health threat, increasingly rendering antibiotic therapies ineffective. Resistance to antibiotics can occur either by mutations or horizontal gene transfer, the latter allowing clinical pathogens to acquire resistance genes from human commensals or environmental bacteria. We set out to categorize resistance gene occurrences in human, animal and environmental reservoirs, and assess the mobility characteristics of these resistance genes. By grouping resistance genes into clusters of identical genes, we can assess whether identical resistance genes reside in different species or even in different genera, ranking transfer events of resistance genes. Next, we can identify the resistance genes in different reservoir resistomes, and how such reservoirs overlap, thereby speculating on the origins of resistance genes currently encountered in the human population.
We use the Comprehensive Antibiotic Resistance Database to annotate resistance genes in the genome repository PATRIC using default settings, after which we cluster all sequences with CD-HIT (sequence identity threshold set to 100% and query coverage to 80% of the sequence length). Genic metadata is used to assign resistance genes to the reservoirs wastewater, soil, water, cattle, chicken, pig, food, pets and other.
From 117,850 bacterial genomes, we collect 1,003,750 resistance genes, which are collapsed to 67,134 clusters, each containing identical genes. We identify that 5,971 multi-species resistance gene clusters, and 2,770 multi-genus resistance gene clusters, mapping extensive resistance gene transfer. Some species share identical resistance genes with hundreds of other species, such *as Klebsiella pneumoniae* and *Escherichia coli*. We find resistance genes with identical representative sequences present in over a 100 distinct bacterial species, suggesting extensive gene transfer over large phylogenetic distances. These results aid to identify the most frequently shared resistance genes between bacteria, and the bacteria most frequently engaged in resistance gene transfer.
Next, we assign clusters to three resistome categories: 1) the **unique** human resistome consists of resistance genes present only in human isolates, 2) the **promiscuous** resistome, consisting of resistance genes present in human isolates as well as isolates from multiple other reservoirs, and finally 3) the **restricted** resistome consists of resistance genes from human isolates and a single additional reservoir, suggestive of possible transmission routes.
In conclusion, we identify the resistance genes most frequently transferred between bacterial species and genera, and recognize the resistance genes that are shared between different animal and environmental reservoirs, suggestive of distinct transmission routes. These data aid in prioritizing resistance genes impacting human health.

O109

Reconstruction of plasmids carrying carbapenemase encoding genes using multiplexed nanopore sequencing on a MinION

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Introduction: Carbapenemase producing Enterobacteriaceae (CPE) pose an increasing health threat in the world. Most carbapenemase encoding genes are located on large plasmids that can be transferred between bacterial strains of the same or of different species in the environment or within persons. To assess possible transfer of such plasmids, detailed characterization of plasmids is essential. Due to the presence of repeating sequences, such as IS-elements, it is virtually impossible to reconstruct these large resistance plasmids using short Illumina reads. Therefore, we validated the use of long-read sequencing on the nanopore platform to reconstruct entire plasmids containing carbapenem resistance genes.
Methods: Genomic DNA was isolated by incubating bacterial suspensions in a lysis mix, followed by proteinase K treatment and ethanol precipitation. DNA shearing was performed using covaris g-TUBEs, after which 12 samples per run were barcoded using the ligation sequencing kit and subjected to 48 hours sequence runs on a MinION using the MinKNOW software. Basecalling was performed using Albacore 2.0.1 and a single FASTA file was extracted from the FAST5 files using Poretools 0.5.1. In Canu version 1.4, contig files were created and loaded into CLC genomic workbench where Illumina sequencing data were used for polishing and further analyses.
Results: Validation experiments showed a consistent N50 for isolates subjected to DNA shearing, while unsheared DNA yielded a wide range of N50. Prolonged end-repair incubation time did not improve read length. Comparison of the assembled MinION data from two CPE isolates with the assemblies obtained by PacBio data, demonstrated near identical sequences. A third isolate could not be assembled into a single contig with either PacBio or MinION data. All subsequent isolates were sequenced using sheared genomic DNA and standard end-repair incubation time.
In the first optimized multiplex-run, complete plasmids carrying carbapenemase encoding genes ranging from 16 kb to 163 kb, could be reconstructed for 11 of 12 the isolates. Four isolates carried *bla*$_{\text{VIM-1}}$ containing plasmids, two carried *bla*$_{\text{OXA-48}}$ and the remaining isolates *bla*$_{\text{KPC-2}}$, *bla*$_{\text{KPC-3}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-5}}$ and *bla*$_{\text{OXA-181}}$. The four *bla*$_{\text{VIM-1}}$ isolates all carried the same plasmid and this was also true for the *bla*$_{\text{OXA-48}}$ isolates. Illumina data of CPE isolates from the national surveillance were mapped against five of the individual plasmids from this run. Mapping of the *bla*$_{\text{VIM-1}}$ plasmid against 40 *bla*$_{\text{VIM-1}}$ isolates resulted in five matches in three different *Enterobacteriaceae* species. The *bla*$_{\text{KPC-3}}$ plasmid was not found in the other 27 *bla*$_{\text{KPC-3}}$ isolates; the *bla*$_{\text{NDM-5}}$ plasmid was found in 22 of 48 isolates (three species) and the *bla*$_{\text{OXA-181}}$ plasmid was found in 23 of 30 isolates (two species). For mapping against the *bla*$_{\text{OXA-48}}$ plasmid, Illumina reads of a subset of isolates were used and the plasmid was found in 44 of the 68 isolates (six species).
Conclusion: We showed that multiplexed nanopore sequencing can be used to reconstruct large resistance plasmids. The technology is now validated and implemented in our laboratory, making it possible to create a plasmid database to track and compare plasmids carrying carbapenemase encoding genes in isolates collected for the Dutch surveillance of CPE.

O110

Ceftibuten plus amoxicillin-clavulanic acid for oral treatment of urinary tract infections with ESBL producing *E. coli* and *K. pneumoniae.*

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Introduction
Oral therapeutic options are limited for complicated urinary tract infections (UTIs) caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Orally available 3rd generation cephalosporins combined with clavulanic acid have been proposed for such infections, since clavulanic acid may protect the partner cephalosporin from hydrolysis by ESBLs, but clinical data is lacking.
Methods:
In this retrospective observational case-series, the clinical and bacteriological effect was evaluated of oral treatment with ceftibuten 400mg QD plus amoxicillin-clavulanic acid 625 mg TID for 14 days in 10 patients with a UTI and suspicion of pylonephritis caused by an ESBL producing micro-organism, co-resistant to ciprofloxacin and co-trimoxazole. Since other oral antibiotics were deemed not to reach adequate tissue levels and to avoid intravenous antibiotics, ceftibuten plus amoxicillin clavulanic acid was offered if (1) the clinical condition allowed outpatient treatment and (2) disc diffusion sowed that the urinary isolate was susceptible to cefotaxime plus clavulanic acid (30-10 microgram) and ceftazidime plus clavulanic acid (30-10 microgram) using the CLSI zone diameter breakpoints for cefotaxime and ceftazidime. MIC's were determined using Vitek-2. Phenotypic ESBL confirmation was performed using combination discs. Genotypic ESBL confirmation was performed with PCR and microarray. The sensitivity to ceftibuten was determined using a 30 microgram disc. Sensitivity to ceftibuten plus amoxicillin plus clavulanic acid was determined by adding an amoxicillin-clavulanic acid disc (20-10 microgram) adjacent to the ceftibuten disc, and measuring the ceftibuten zone diameter on the line perpendicular to the line between the disc centers. Clinical data were collected from patient records and correspondence. Bacteriological cure was defined as absence of the pretreatment micro-organism in the first follow-up culture obtained within 3 months after treatment.
Results:
A total of 10 patients (5 women) with UTI were evaluated in 2016 and 2017. Six patients were from outpatient hospital care, and 4 were from primary care. Median age was 74,5 years (range 34-82 years). Pretreatment urinary cultures yielded 7 *E. coli* and 3 *K. pneumoniae* isolates producing an ESBL: CTX-M-1 group (n=8), CTX-M-9 group (n=1), SHV-4 plus CTX-M-9 group (n=1). All isolates were resistant to cefotaxime and resistant (n=9) or intermediate susceptible (n=1) to ceftazidime. In contrast, all isolates were susceptible to ceftibuten with a disc zone ranging from 25 mm to 32 mm

(S≥23mm according to EUCAST). Addition of an amoxicillin-clavulanic acid disc extended the ceftibuten zone by 2-8 mm. Three isolates were susceptible to amoxicillin-clavulanic acid. All patients experienced clinical cure. From 8 patients follow cultures were obtained. Bacteriological cure was observed in all 8 patients.

Conclusions:

Based on the results of this small case-series, ceftibuten plus amoxicillin-clavulanic acid may be an option for oral treatment of UTI's caused by ESBL producing *E. coli* or *K. pneumoniae*. Although adding amoxicillin-clavulanic acid increased the susceptibility of the ESBL isolates to ceftibuten *in vitro*, the clinical value of this addition remains to be determined.

O111

Genomic comparisons of antimicrobial resistance in canine and human *S. pseudintermedius*

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Introduction:

Staphylococcus pseudintermedius is a major pathogen in dogs and can occasionally be found in human infections. In dogs *S. pseudintermedius* is the major cause of pyoderma and is often multidrug resistant (MDR), including methicillin resistant. In humans *S. pseudintermedius* is an opportunistic pathogen found in elderly and immunocompromised patients and is primarily methicillin susceptible. Antibiotic resistance and in particular methicillin resistance in *S. pseudintermedius* is mediated by genes very similar to those of *S. aureus*, leading to a risk of transmission of resistant bacteria between humans and dogs and of resistance genes between *S. pseudintermedius* and *S. aureus.*
This study compares the genomes of *S. pseudintermedius* isolates from canine and human infections, focussing on resistance gene content and clonal distribution.

Methods:

Fifty methicillin-resistant *S. pseudintermedius* (MRSP) isolates from dogs, 56 methicillin-susceptible *S. pseudintermedius* (MSSP) isolates from dogs and 26 *S. pseudintermedius* isolates from humans were sequenced; including four isolates (1 human, 3 canine) from the same household. The resistance genes and sequence types were identified using the batch upload pipeline from the Center for Genomic Epidemiology (CGE) .

Results:

All but one of the 50 canine MRSP isolates were MDR; ST 71 was the dominant sequence type (ST), followed by ST 45 and ST 258. From the 56 canine MSSP isolates, 18 were MDR; no dominant ST was found among this selection. From the 26 human isolates, 11 were MDR and one was MRSP; ST 241 was the dominant ST among the human isolates . The human isolate and two out of three dog isolates from the same household also belonged to ST241. This sequence type was only found in MSSP from humans, and in the dogs from this household.

Conclusions:

1. The number of MDR isolates among human *S. pseudintermedius* isolates was higher than previously expected.
2. The clonal distribution seems to differ between MRSP and MSSP from dogs and between MSSP from dogs and humans.

O112

Novel approaches to unlabel VRE-suspected labelled patients

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Introduction

During a large vancomycin resistant *Enterococcus faecium* (VRE) outbreak in The Netherlands thousands of patients were labelled as *VRE-suspected* as a result of possible exposure to VRE. Some of these labels were removed following culturing results, but for financial reasons most patients were not cultured. To also unlabel those uncultured patients, we investigated two approaches: 1) unlabelling those patients that passed a timeframe that exceeds the duration of VRE carriage; 2) assessing the risk of exposure by establishing the VRE incidence on wards during the timeframe a patient resided there based on culturing information of contemporary residents.

Methods

Rectal specimens obtained during the outbreak and as part of various extensive follow-up schemes were cultured using an enrichment broth with 16mg/l amoxicillin. Subsequently, a chromogenic agar was incubated for 48 hours and suspect colonies were inoculated on a blood agar plate and characterised with MALDI-TOF and a vancomycin e-test if *Enterococcus spp.* were detected. To analyse the duration of infection, a patient was considered *VRE-positive* if VRE was detected in at least one culture. A person was considered *VRE-negative* if a set of at least five cultures within a period of 10 days were all negative, and no future samples were ever measured as positive.

Results

To establish the duration of infection, we performed a survival-type analysis of 432 instances where we were certain of the VRE status of a previously *VRE-positive* patient without ongoing exposure to VRE (see Figure). One year after testing a patient *VRE-positive*, 12% of the patients remained positive; one individual still tested positive after >2.5 years. We additionally assessed the likelihood that individuals labelled as *VRE-suspected* were indeed infected with VRE when culture data was unavailable by using the information of VRE test results of contemporary patients in the wards that the patient stayed in. Using this information and conservative criteria, we removed the label for 4% of labelled patients.

Conclusion

Removing patients of the label *VRE-suspected* after a year is not advisable, given that even without ongoing exposure 12% of the patients were still positive. The VRE incidence data on different wards may be a better approach to this issue, and the flexibility in the setting of thresholds for the maximum allowed incidence on a ward to be considered low-risk enables tailored usage for each situation.

O114

Artilysins, enzyme-based antibacterials derived from bacteriophages

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A recent portfolio overview of the antibacterial development pipeline has highlighted bacteriophage-encoded endolysins as an emerging class of antibiotics with the highest potential. These enzymes are produced at the end of the lytic cycle of bacterial viruses (bacteriophages) in the infected host cell. They degrade the peptidoglycan layer of the cell, resulting in osmotic lysis and dispersion of the newly produced phage particles. Addition of purified endolysins to Gram-positive cells results in a rapid killing of multi-drug resistant strain with a low probability to provoke resistance development. Fusion of a peptide with specific physicochemical properties to either the N- or C-terminus expands the applicability of endolysins drastically. Cationic and amphipathic peptides with outer membrane permeabilizing activity have demonstrated to transfer the endolysin moiety across the outer membrane, resulting in a quick lysis of Gram-negative bacteria. Recombinant fusion with similar cationic peptides also increases the enzymatic and bactericidal activity of endolysins acting against Gram-positive species due to an increased affinity for the cell wall. This class of engineered endolysins are coined 'Artilysin®s'. The progress in the field during the last decade has now yielded a multitude of (engineered) endolysins against many major Gram-positive and Gram-negative pathogens. Many endolysins have a modular structure, comprising a cell wall-binding domain and an enzymatically active domain. Domain swapping efforts have allowed to engineer endolysins with desired and improved properties. We expect that we are only at the beginning of the synthetic biology of modular endolysins, which bears the potential to produce customized antibacterials against any bacterial pathogen.

O115

Genome instability conditions phage adaptation, host-switching and evolution

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The concept of 'biosphere dark matter' is attributed to (bacterio)phages, viruses of bacteria that comprise the most vast and diverse reservoir of genetic information. Many microbiologists, ecologistsand molecular geneticists debate the role of phages in microbial population dynamics and biogeochemical re-generation. Many others debate on the origin of phages, proposing different models for their genesis, nature and evolution. Herein, foreign DNA elements (of evolutionary distant virions) were used to study the phage modular genome organization, phage evolution, and host-switching. In more detail, we question how gene function and essentiality, host-specificity, or adaptation is influenced by these unfamiliar elements. To study evolutionarily-driven questions, we proposed a universal phage genome shuffling method for the generation of chimeric virions. Using *Escherichia coli* phages as a model, a Myoviridae, a Siphoviridae, and a Podoviridae were used to create the first reported chimeric phage comprised of elements of the three major families of tailed phages (*Caudovirales*). These virions display distinct features when compared to their parental phages. Using next-generation sequencing (Oxford Nanopore) we were able to unravel these rearrangements for the recombinant phage ØChi3. About 65% of the phage genes were derived from the parental myophage (mostly contributing to the host-recognition structures), 29% from the siphophage (tail-structure), and 7% from the podophage (capsid elements). When studying the stability of different lineages we observed genome re-organization leading to host-switching, and more strikingly to evolutionary dead-ends, where the recombination of the foreign elements have led to genome instable copies driving these to extinction. Our findings support the evidence of genomic flexibility and adaptability attributed to phages, in accommodating and re-arranging novel information from foreign elements, such as other phages (hybrids) or DNA pieces from transposable mobile genetic elements. In addition, we herein report the first proof of inter-family gene exchange in phages, posing the hypothesis of exchangeable protein modules, and protein evolution among microbial populations.

O116

Phages and CRISPR-Cas9: new antimicrobial approaches

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Since the emergence and spread of antimicrobial resistant mechanisms is much faster than the discovery or development of novel antibiotics (AB), alternative antibacterial strategies, including bacteriophages (briefly: phages) may have a significant impact for future clinical applications. In our ongoing studies, we have isolated and characterized phages against the six most important bacterial pathogens, collectively addressed as "ESKAPE" (i.e. *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter cloacae*). The genome similarity of newly isolated lytic phages to those already deposited in public databases ranged from 50% for *A. baumannii* phages up to 98% for *P. aeruginosa* phages. Growth/kill dynamics as well as anti-biofilm activity demonstrated the antibacterial potential of most phages. In addition, an enhanced bacterial suppression could be achieved when combining phages with certain AB against which the bacteria are otherwise resistant when applied alone. Apart from conventional phage therapy, we pursue some additional anti-bacterial

strategies involving phages or products encoded by them. This includes preventive approaches, such as removal of bacterial pathogens from critical hospital surfaces prior to the event of a nosocomial infection, the suppression of horizontal transfer of AB-resistance genes across bacterial species or even phage-mediated reversal of antibiotic resistance. In some of these regards, lysogenic phages can be helpful. These phages can act as an efficient delivery system of the CRISPR-Cas9, which can target any desired gene. Our recent studies have proved that the CRISPR-Cas9 system when appropriately designed for targeting a resistance gene was efficient in reducing β-lactam resistance while preserving cell viability and partially the β-lactam plasmid integrity after edition. By inactivating the resistance gene, the bacteria not only lose their resistance phenotype due to the gene edition but also can not transfer a functional resistance gene to other bacteria. This talk will point at the value of lytic as well as lysogenic phages in favor of human health.

O117

Resistance of group A streptococcus to human phospholipase A2-IIA uncovers a missed structural modification on streptococcal Lancefield antigens

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Background: Human Group IIA-secreted phospholipase A_2 ($sPLA_2$-IIA) is a potent cationic antimicrobial protein that is present in plasma and tissue fluids. The importance of $sPLA_2$-IIA in host defense against Gram-positive pathogens, including Group A streptococcus (GAS), has been demonstrated in animal models. Compared to other Gram-positive bacteria, GAS is remarkably resistant to $sPLA_2$-IIA activity. This human-specific commensal is an important cause of infection-induced mortality, resulting in the death of 500,000 people globally each year. Previous research has demonstrated that mutation of the enzyme sortase A (srtA), which links LPXTG-containing proteins to the cell wall, renders GAS 30-fold more susceptible to $sPLA_2$-IIA killing. We set out to uncover additional mechanisms conferring GAS resistance towards $sPLA_2$-IIA.
Methods: We screened a random GAS mutant library generated by transposon insertion to identify resistant and susceptible mutants by NextGen sequencing. To confirm the involvement of identified genes, we generated specific deletion mutants through allelic exchange mutagenesis. The bactericidal effect of $sPLA_2$-IIA was assessed using classical plating assays using recombinant active $sPLA_2$-IIA. Biochemical assays and phylogenetic analysis were performed to unravel the molecular mechanism behind the observed $sPLA_2$-IIA susceptibility phenotype.
Results: GAS transposon library screening identified both susceptible and resistant GAS mutants. Hits that identified resistant mutants clustered in three adjacent genes (*gacH-J*) within the Group A Carbohydrate (GAC) biosynthesis locus. The GAC is a cell wall anchored polysaccharide that contributes to GAS virulence. Indeed, the *gacH* deletion mutant was approximately 10-fold more resistant to $sPLA_2$-IIA compared to wild-type GAS. GacH is annotated as a phosphoglycerol transferase similarly to lipoteichoic acid synthase (LtaS). However, phylogenetic analysis of Gram-positive species clearly identified two distinct clades of enzymes, represented by LtaS (*Staphylococcus aureus*) and GacH (GAS). Furthermore, biochemical analysis of purified GAC revealed that GacH is responsible for incorporation of glycerolphosphate in the GAC.
Conclusion: Our findings pinpoint to a yet unrecognized structural modification of the GAC, i.e. glycerolphosphate, justifying a thorough redetermination of Lancefield antigen structures in species expressing a GacH homologue. Furthermore, the presence of glycerolphosphate has important biological consequences since it provides a negative charge to the bacterial cell wall, which impacts interaction with positively-charged host molecules such as $sPLA_2$-IIA. Finally, since the GAC has been investigated as a possible GAS vaccine antigen, our structural findings also have consequences for vaccine design.

O118

Adaptation of *Bordetella pertussis* to the respiratory tract

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Introduction

Bordetella pertussis, a Gram-negative bacterial pathogen, causes whooping cough in susceptible humans. Genotypic and phenotypic changes in circulating *B. pertussis* strains are thought to contribute to the resurgence of whooping cough. Knowledge about *B. pertussis* gene expression patterns is mainly derived from *in vitro* studies and has primarily focussed on the global virulence regulon BvgASR. For instance, all current pertussis vaccines are based on *B. pertussis* cultured during virulent (Bvg+) *in vitro* conditions. In contrast, there is a paucity of studies investigating bacterial gene expression in the respiratory tract. To gain insight into the basic mechanisms by which *B. pertussis* adapts to the local host environment during infection, we studied *B. pertussis* gene expression in the mouse respiratory tract and compared this to *in vitro* Bvg-regulated gene expression. Finally, we studied the impact of vaccination with membrane proteins isolated from virulent (Bvg+) and avirulent (Bvg-) on intranasal challenge with *B. pertussis*.

Methods

Naïve C57BL/6 mice were infected intranasally with *B. pertussis.* After three and seven days, bacteria were harvested from the nasopharynx and lungs and analysed by microarrays. *In vivo* bacterial gene expression was then compared to gene expression differences observed in response to *in vitro* modulation of the BvgASR system. Membrane fractions from Bvg+ and Bvg- *B. pertussis* cultures were isolated and used to vaccinate mice. Mice were then challenged intranasally with B1917 and bacterial load in the nose and lungs was determined. To determine whether antigens

expressed during the Bvg- and Bvg+ phase were recognized during infection, immunoblot analysis was performed with serum from infected mice.

Results

Approximately 30% of all bacterial genes were found to be differentially expressed during infection as compared to *in vitro* conditions. This approach identified several novel potential vaccine antigens that were exclusively expressed *in vivo*. Significant differences in expression profile and metabolic pathways were identified between the upper and the lower airways. We found high expression of several Bvg-repressed (Bvg-) genes *in vivo* and mouse vaccination experiments using Bvg- culture-derived outer membrane proteins demonstrated protection in the lungs. Subsequent immunoblot analysis suggest that both Bvg phase-dependent and phase-independent proteins are expressed and immunogenic during infection.

Conclusion

B. pertussis adapts to the mouse airways by changing its gene expression profile. We identified distinct expression patterns of virulence-associated genes between *in vivo* and *in vitro* conditions, as well as temporal and spatial expression differences within the respiratory tract. This study provides novel insights into the *in vivo* adaptation of *B. pertussis* and may facilitate the improvement of pertussis vaccines.

O119

Virulence associated gene 8 of *Bordetella* pertussis enhances contact system activity by inhibiting the regulatory function of C1esterase inhibitor.

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Bordetella pertussis is a Gram-negative bacteria and the causative agent of whooping cough. Whooping cough is currently re-emerging worldwide and therefore still poses a continuous global health threat*. B. pertussis* expresses several virulence factors that play a role in evading the human immune response. One of these virulence factors is Virulence associated gene 8 (Vag8). Vag8 is a complement evasion molecule that mediates its effects by binding to the complement regulator C1 esterase inhibitor (C1-INH). This regulatory protein is a fluid phase serine protease that not only inhibits the complement system but is also involved in contact system regulation. Activation of the contact system results in the generation of bradykinin, a pro-inflammatory peptide. Here, the activation of the contact system by *B. pertussis* was explored in detail. We demonstrate that recombinant as well as endogenous Vag8 enhanced contact system activity by binding and inactivating C1-INH. Moreover, we show that *B. pertussis* itself is able to activate the contact system. This activation was dependent on Vag8 production as a Vag8 knock-out *B. pertussis* strain was unable to activate the contact system in our experimental setups. These findings show a previously overlooked interaction between the contact system and the respiratory pathogen *B. pertussis.* Activation of the contact system by *B pertussis* may contribute to its pathogenicity and virulence*.*

O120

Human dendritic cells internalize *Staphylococcus aureus* more efficiently than *Staphylococcus epidermidis*, but the level of the ensuing T-cell proliferation predominantly depends on superantigens

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Introduction: *Staphylococcus aureus* and *Staphylococcus epidermidis* are related species which can cause acute and subacute infections, respectively. Differences in human adaptive immune responses to these two bacteria are not well understood. Dendritic cells (DCs) have an important role in the control and regulation of adaptive T-cell responses. Therefore, we aimed to compare the ability of *S. aureus* and *S. epidermidis* strains to induce DC activation and subsequent antigen-specific $CD4^+$ T-cell proliferation, and to investigate the underlying mechanisms.

Methods: Human monocyte-derived DCs were stimulated with different strains of *S. aureus* and *S. epidermidis*. The level DCs activation and antigen presentation was assessed. Next we analyzed the ability of DCs having phagocytosed *S. aureus* or *S. epidermidis* to induce antigen-specific $CD4^+$ T-cell proliferation.

Results: We show that *S. aureus* was internalized more effectively than *S. epidermidis* by DCs, but that both species were equally potent in activating DCs as evidenced by similar induction of DC maturation marker expression and antigen loading on MHC-II molecules. Differences in the level of ensuing DC-induced T-cell proliferation were attributed to the capacity of *S. aureus* strains to produce superantigens (SAgs). *S. aureus* strains not harbouring SAg genes and *S. epidermidis* induced similar low levels of T-cell proliferation.

Conclusion: Taken together, *S. aureus* and *S. epidermidis* do not differently affect DC activation and ensuing antigen-specific T-cell proliferation, unless a strain has the capacity to produce SAgs.

O121

Visualization of *Haemophilus influenzae* sialic acid theft from primary human bronchial epithelial cells and selective inhibition with sialic acid-based inhibitor abrogates serum resistance

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Introduction

Non-typeable *Haemophilus influenzae* (NTHi) is a commensal organism part of the upper respiratory tract microbiome, but can become an opportunistic pathogen, mainly in children and elderly. For instance, NTHi infections are found in

more than 50% of children suffering from otitis media, an inflammatory disease of the middle ear that affects 65-300 million people globally and is a major cause of hearing loss. In addition, NTHi is found in the majority of patients with acute exacerbation of chronic obstructive pulmonary disease, a leading cause of death worldwide.
The switch of NTHi from a symbiotic colonizing bacterium to an opportunistic pathogen is associated with an increased resistance to serum-mediated killing. A key virulence factor in this process is the uptake and presentation of sialic acid sugars on the bacterial cell surface. The metabolic pathway for sialic acid utilization by NTHi is therefore a promising therapeutic target.

Methods

NTHi was grown in defined RPMI medium with or without sialic acid SiaNAc or sialic acid analogues SiaNAz or SiaPoc. For inhibition of sialic acid incorporation, sialic acid-based inhibitor SiaNAc-3F$_{ax}$ was added to defined RPMI medium containing sialic acid.
Sialic acid incorporation into the lipooligosaccharide (LOS) was visualized by using sialic acid analogues SiaNAz or SiaPoc and detection by reacting them to biotin-alkyne or -azide, using copper-catalyzed-alkyne-azide-cycloaddition (CuAAC) followed by staining with fluorescent streptavidin and flow cytometry or Western blotting with streptavidin-HRP.
Primary human bronchial epithelial cells (PHBEC) were grown on air-liquid interface on transwells in order to differentiate them into a tight multicellular mucus-producing cell layer. NTHi bacteria were grown 24 hours on the apical side of PHBEC and sialic acid transfer from PHBEC to NTHi was visualized by reacting the bacteria to biotinalkyne CuAAC followed by staining with fluorescent streptavidin and flow cytometry.
Resistance to serum-mediated killing was determined by incubating the bacteria 1 hour with 20% pooled human serum at 37°C.

Results

Incorporation of sialic acid analogues SiaNAz or SiaPoc into the LOS of NTHi was dose dependent and required a functional sialic acid transporter because no labelling was detected for the siaP transporter mutant. Incorporation of sialic acid was inhibited with sialic acid-based inhibitor SiaNAc-3F$_{ax}$ in a dose-dependent manner with an IC50 in the high nanomolar range.
We were able to visualize sialic acid transfer from PHBEC to NTHi, which was dependent on neuraminidase activity and was also efficient inhibition with sialic acid-based inhibitor SiaNAc-3F$_{ax}$.
Inhibition of sialic acid incorporation by sialic acid-based inhibitor SiaNAc-3F$_{ax}$ abolished resistance to serum-mediated killing, indicating that bacterial virulence was abrogated.

Conclusion

We show that potent sialic acid-based glycotools can be used to visualize or inhibit sialic acid utilization in NTHi. By using PHBEC, we demonstrated that sialic acids were released by neuraminidases and subsequently taken up by NTHi. Moreover, we demonstrated that pharmacological inhibition of NTHi LOS sialylation abolished the resistance to serum-mediated killing. Given the crucial role of sialylation in NTHi infections, these tools enable the development of NTHi-specific therapies.

O122

Prevalence of sIgE to alpha-gal among patients with a tick bite or erythema migrans in a retrospective study in the Netherlands, 2007-2008

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Introduction: In the Netherlands, national surveys among general practitioners (GPs) have shown that the number of tick bite consultations tripled in the period between 1994-2009 from 191 per 100.000 inhabitants to 564 per 100.000 inhabitants. In the last survey, conducted in 2014, the incidence for tick bites decreased to 488 consultations per 100,000 inhabitants. Recent studies have estimated that yearly approximately 1.5 million inhabitants of the Netherlands are bitten by a tick and are therefore at risk for developing Lyme borreliosis. Besides Lyme borreliosis, these people are also at risk for other tick-borne diseases or developing a red meat allergy.
In the last 10 years, various studies have suggested an association between a tick bite and red meat allergy. Red meat allergy is caused by the development of specific immunoglobulin E (sIgE) antibodies against the carbohydrate galactose-α-1,3-galactose (alpha-gal) and only develops in those patients who have been predisposed. Alpha-gal is present in the cell membrane of most mammalian species, except for humans and higher primates. As ticks use mammals as hosts, such as mice and dear, alpha-gal of these mammals may remain in their saliva and gastrointestinal tract. When, subsequently, a human gets bitten by a tick, transfer of alpha-gal occurs. The immune system can respond to this by producing sIgE and this can be triggered by the intake of alpha-gal containing meat.
In 2017, the first Dutch patient was described who had presented with generalized urticaria, which appeared approximately two hours after the consumption of red meat. This allergy was linked to tick-bites and the patient was diagnosed with the “alpha-gal syndrome”. Red meat allergy is relatively new and not much is known regarding its occurrence among tick bitten patients in the Netherlands.
Methods: We will investigate the prevalence of sIgE against alpha-gal among patients who presented at the GP with a tick bite and/or erythema migrans (EM) in 2007 and 2008. The GPs were all located in a high risk area for tick bites. Blood was drawn at the time around the initial visit at the GP and three months later. The patients also filled out a questionnaire at both time points inquiring about symptoms, antibiotic treatment, history of tick bites and Lyme borreliosis. As a control group, we selected sera derived from PIENTER-2, a cross-sectional seroprevalence survey conducted in the Netherlands in 2006 and 2007. Sera will be analysed with the ImmunoCap 250® analyser (Thermo Fisher Scientific/ Phadia AB, Uppsala, Sweden) by using nGal-alpha-1,3-Gal (alpha-GAL/bovine thyroglobulin, Thermo Fisher Scientific).
Results/ conclusion: In total, 314 patients with a tick-bite and/or EM and 300 controls were included. Their serum samples will be subjected to alpha-gal-specific IgE in the beginning of 2018, and risk factors will be assessed. The

results of this study will be presented at the meeting.

O123

Metabolic complementarity within microbial communities from global ecosystems

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The majority of microbial communities consist of thousands to tens of thousands of species, all of which compete for available resources within an ecosystem. This competition sets a boundary on their co-existence as resources are available in a finite supply and form. However, species may interact through complementary metabolisms, that is, one species utilizes what another produces, expanding the niche space of an ecosystem. Metabolic complementarity of two species can then be quantified by the extent of this metabolic overlap, or specifically, by the number of complimentary reactions that these species have. Of particular interest are complimentary metabolisms that may lead to the production of greenhouse gases, such as microbes that make recalcitrant organic matter available to other microbes that then release CH_4, CO_2 or N_2O. To identify the extent of metabolic complementarity in global microbial communities, we surveyed genome-centric metagenomic studies and identified how metabolic complementarity varies between ecosystems. In initial surveys, we find the highest metabolic complementarity of microbial communities in marine, freshwater, brackish, and built environments and the lowest in engineered, extreme, and animal ecosystems. To identify key drivers of metabolic complementarity, we further disentangle the relationship of ecosystem properties (i.e., temperature, latitude/longitude), metagenome and community characteristics (i.e., average genome size, assembly quality, and community richness, dominant metabolic pathways) with the degree of metabolic complementarity. Understanding the drivers of complementarity in species' metabolisms may provide insight into the ecological rules that promote functional redundancy in microbial communities.

O124

Environmental surveillance of *Francisella tularensis* holarctica in the Netherlands

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Tularemia is an emerging zoonosis with a human, animal and environmental component, which is caused by the Gram-negative bacterium *Francisella tularensis*. The bacterium is able to infect a range of animal species and infection of humans may lead to serious disease.
Human infections occur through contact with infected animals, ingestion of food or water, insect bites or exposure to aerosols or water. *F. tularensis* may persist and replicate in aquatic reservoirs where the bacterium can be hosted by free-living protozoa.
In the Netherland, tularemia was absent for over 60 years until in 2011 an endemic patient was diagnosed, followed by 17 cases in the period since. The apparent re-emergence of tularemia could be caused by changes in reservoirs or transmission routes. In 2015, environmental surveillance following a tularemia epizootic among hares in Friesland revealed presence of *F. tularensis* DNA in surface water and sediments. Insights into the distribution of *F. tularensis* may help explain the re-emergence of tularemia and address the relevance of detecting Ft in surface water for infection risks. We performed environmental surveillance of *F. tularensis* in surface waters in the Netherlands by using two approaches. Firstly, samples were obtained from routine monitoring -not related to tularemia- at 127 locations that were visited between 1 and 8 times in 2015 or 2016. Secondly, sampling efforts were performed after reported tularemia cases (n=8) among hares or humans with an environmental link in the period 2013-2017. *F. tularensis* DNA was detected at 22 out of 127 randomly selected surface water locations (17%) in different parts of the country, included three brackish or saltwater locations. At most of these positive locations DNA was not detected at each time point and levels were very low, but at two locations contamination was clearly higher. When sampling followed a tularemia case, *F. tularenis* DNA was detected in at least one water sample for 7 out of 8 such instances. This was true even though the period between suspected infection and sample collection was at least 4 weeks and the exact location of infection was unknown. By using a protocol tailored for amplification of low amounts of DNA from environmental samples, 10 gene targets were sequenced and identified *F. tularensis holarctica* subclades B1 and B4 at two locations.
Environmental surveillance independent of tularemia cases showed that various surface waters harbor *F. tularensis*. However, it is not possible to infer or predict infection chance based on these *F. tularensis* DNA levels, due to several factors such as a limited samples, variable time periods between cases and sampling and absence of information about viability and infectivity based on qPCR. Nevertheless, our case-related data support the feasibility of tracking a particular strain as source of infection. A link between environmental source and case could be substantiated by genotyping, which was shown to be possible directly on water samples with only low levels of *F. tularensis*.By applying these techniques to materials from patients or animals, it will be possible to establish a link between tularemia cases and environmental samples.

O125

Microbiota of the female urogenital tract - no evidence for a distinct urinary microbiome

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Introduction: The urogenital microbiota is increasingly being recognized as a significant factor in female reproductive health. Recently, culture based analysis has yielded to next generation sequencing (NGS) of 16S rDNA amplicons for investigation of the vaginal microbiota. NGS-based analysis has concomitantly found its way into analysis of almost every bodily compartment in search of bacteria. This had led to claims of the existence of a distinct urinary microbiota. This is in contrast with the traditional view that the urinary bladder is sterile unless an active infection is ongoing. All other

bacteria detected in urine are in this view considered contaminants introduced during passage through the urethra.
Methods: To investigate this issue, we analysed vaginal swabs and urinary samples of 297 otherwise healthy women attending reproductive clinics for IVF or IVF/ICSI treatment. All samples were analysed using IS-pro, a 16S-23S profiling technique validated for microbiota analysis in clinical routine, in addition to NGS of 16s rDNA . IS-pro has the additional advantage that it performs well in low bacterial load and high bacterial load samples alike, which may be problematic with NGS. Resulting data was analysed for correlations in presence and abundance of microbiota between samples, respectively.
Results: Firstly we compared results obtained through NGS and IS-pro with each other. Analysis regarding the NGS and IS-Pro profiles and the abundance of bacteria in vaginal samples were found to be highly similar, with a median Pearson's R squared of 0,97. A similar correlation of urine samples resulted in a R squared of 0,83. This indicates a high level of similarity between NGS and IS-pro results when investigating relative abundance of microbiota in samples. Secondly, we compared microbiota profiles found in vaginal and urinal samples of the same patient with each other. Pearson's R analysis of vaginal samples and urine samples from individual patients characterized by IS-pro or NGS demonstrated a significantly lower correlation (0,78 and 0,32, respectively). DNA load comparisons showed significantly lower amounts of DNA before and after PCR amplification in urine samples compared to vaginal samples. NGS analysis of urine was probably negatively impacted by low DNA yields. Relatively high read numbers of likely contaminants were found.
Conclusions: IS-pro and NGS yielded highly similar results for vaginal microbiota analysis. For urine, similarity was lower, probably due to low DNA yield. For IS-pro, vaginal and urine microbiota were highly similar within individuals, with the main difference being a much lower load in urine. A small number or urine samples showed a high bacterial load. This was caused by E.coli DNA. Our data pleads against the existence of a urinary microbiome. Rather it is a dilution of the vaginal microbiota that is picked up passage of the urethra.

O126

Novel bacterial and human methanethiol oxidases widely distributed in the biosphere and involved in extra-oral halitosis

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The oxidation of methanethiol (MT) is a significant step in the global sulfur cycle. MT is an intermediate of bacterial metabolism of globally significant organosulfur compounds including dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS), which have key roles in marine carbon and sulfur cycling. In aerobic bacteria, MT is degraded by a methanethiol oxidase (MTO) but the enzymatic and genetic basis of MT oxidation have remained poorly characterized. We have identified for the first time the MTO enzyme and its encoding gene (*mto*X) in the DMS-degrading bacterium *Hyphomicrobium* sp. VS [1]. MTO is a soluble periplasmic, homotetrameric metalloenzyme that requires Cu for enzyme activity. Genes orthologous to *mto*X exist in many bacteria able to degrade DMS, other one-carbon compounds or DMSP, notably present in marine ecosystems. The detection of mtoX orthologues in diverse bacteria, environmental samples and its abundance in a range of metagenomic datasets all point to this enzyme being widely distributed in the environment and playing a key role in global sulfur cycling. MTO is a member of a distinct clade of the Selenium-binding protein (SBP56) family for which no function has been reported. The human homolog, Selenium binding protein1 (SELENBP1), has been associated with several cancers, but its exact role was unknown. We have shown that the human SELENBP1 is a methanethiol oxidase, related to MTO of bacteria, converting methanethiol to hydrogen peroxide, formaldehyde and hydrogen sulfide, an activity previously not known to exist in humans [2]. Bi-allelic nonsense, splice site and missense mutations in its encoding gene, SELENBP1, were found in five patients from three families with chronic blood-borne halitosis, a unpleasant cabbage-like smelling breath. Increased levels of methanethiol and its methylated product dimethylsulfide are the main odorous compounds in their breath and as such responsible for the malodor syndrome. Patient fibroblasts cell lines from the patients showed reduced amounts of SELENBP1 protein as and well as a deficiency of MTO enzymatic activity. The activity could be restored by lentiviral-mediated expression of the wild-type SELENBP1 gene. A knockout mouse line showed the same biochemical characteristics. In addition to the environmental impact of MTO, our data define a novel inborn error of metabolism in humans caused by MTO-deficiency leading to a malodor syndrome.

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O127

Mucolytic activity of *Akkermansia muciniphila* enables human milk oligosaccharides degradation

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Introduction and objectives: *Akkermansia muciniphila* is a member of the intestinal microbiota specializes in mucus degradation. It is found to be abundant in healthy mucosa while it is inversely correlated to intestinal disorders such as IBD, Crohn's disease, ulcerative colitis and appendicitis, rendering *A. muciniphila* a potential biomarker for a healthy intestine. The enzymes that *A. muciniphila* harbours to degrade mucin can target similar structures in Human Milk Oligosaccharides (HMOs). This could explain the presence of this organism in the early life intestine. The aim of this study is to evaluate the ability of *A. muciniphila* to degrade HMOs.
Materials and Methods: *A. muciniphila* was grown on basal medium supplemented with human milk or HMOs as sole carbon and nitrogen source. Microbial growth was determined by counting the colonies formed (CFU/ml) in Brain Heart Infusion (BHI) supplemented with 5% crude mucin agar plates while the SCFAs' production and sugars degradation were analysed by HPLC. The whole proteome of *A. muciniphila* in human milk environment was characterised by LC-MS/MS. *A. muciniphila's* enzymes were expressed in *E. coli* and the ability of the cells' lysates and the purified enzymes to degrade HMOs structures was tested using specific enzymatic assays. *A. muciniphila* grown in mucin was used as reference for aforementioned techniques.
Conclusion: *A. muciniphila* showed survival of its cells even after 96 hours of incubation in human milk leading at the same time in release of significant amounts of acetate, propionate and succinate. On top of this, monomeric sugars were released in the media due to degradation of disaccharides that are present in human milk, implying *A. muciniphila* fosters enzymes that are able to consume HMOS. Proteome analysis showed upregulation of proteins that are involved in transmembrane transporter activity in *A. muciniphila* grown on human milk compared to mucin. Furthermore, key-mucin degrading enzymes (α-L-fucosidase, β-galactosidases, exo-α-sialidases) appeared to be expressed in both mucin and human milk conditions. The purified enzymes, revealed ability of these enzymes to degrade human milk oligosaccharides structures. The capacity to survive in the early life environment by degrading and consuming human milk components might be beneficial for *A. muciniphila* during initial colonization before reaching the mucosal layer in the intestine.

O128

The polygonal cell shape and surface protein layer of anaerobic methane oxidising *Methylomirabilis* bacteria

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Methylomirabilis bacteria perform anaerobic methane oxidation coupled to nitrite reduction via an intra-aerobic pathway, producing carbon dioxide and dinitrogen gas. These Gram-negative bacteria possess an atypical polygonal cell shape with sharp ridges that run along the cell length. Previously, a putative surface protein layer (S-layer) was observed as the outermost cell layer of these bacteria.
We hypothesised that the putative S-layer of *Methylomirabilis* bacteria is the determining factor for their polygonal cell shape. We isolated this S-layer from the cells and through LC-MS/MS identified a 31 kDa candidate S-layer protein, DAMO632COR_00855, which had no homology to any other known protein. Antibodies were generated against a synthesized DAMO632COR_00855 protein and used in immunogold localisation to verify its identity and location. Both in thin sections of *Methylomirabilis* cells and in negative stained enriched S-layer patches, the immunogold localisation identified DAMO632COR_00855 as the S-layer protein. Using (cryo)electron tomography and sub-tomogram averaging we observed that the S-layer had a hexagonal symmetry with a centre-to-centre spacing of 10.5 nm. Each pore of the S-layer was surrounded by six electron densities and each electron density was bridged with four adjacent electron densities, forming a complex network.
With this study we characterised the S-layer of *Methylomirabilis* bacteria and showed that it indeed might be responsible for the polygonal shape of these cells. Further experiments will aim to investigate the physical properties of the S-layer and its role in the polygonal cell shape.

O131

Novel pneumococcal virulence factors identified with the zebrafish embryo infection model

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Background: Bacterial meningitis is a severe disease in both children and adults. *Streptococcus pneumoniae* is the most important cause of meningitis and carries a high rate of morbidity and mortality. Here, we performed a high-throughput genomic screen on our extensive library of *S. pneumoniae* clinical isolates to identify new putative virulence factors and substantiated their role in a zebrafish embryo pneumococcal meningitis model. This combined approach resulted in the identification of two genes involved in the peptidoglycan synthesis as new pneumococcal virulence factors involved in the pathogenesis of meningitis.
Materials/methods: Whole genome sequencing was performed on strains isolated from patients with pneumococcal meningitis. Genes associated with mortality were identified. Knock-out mutants in two of these genes (*SPD_DB* and *SPD_DH)* were generated and their growth characteristics were determined. We tested these mutants in a zebrafish

embryo model of pneumococcal meningitis. Zebrafish embryos (n=60 per group) were infected in the caudal vein or hindbrain ventricle with wild-type *S. pneumoniae* D39 or *S. pneumoniae* D39 mutant strains. Survival analyses were performed; we also assessed the competitive index between wild-type and mutant strains in the zebrafish.
Results: The genes identified were *SPD_DB* and *SPD_DH;* both genes are involved in peptidoglycan synthesis. Knockout mutants of these two genes showed strong attenuation in the zebrafish meningitis model after injection in the hindbrain ventricle or caudal vein. The *in vitro* growth rates of the knockout mutants were similar to those of the parent strain. The competitive index of the two mutant strains over the wild-type strain was smaller than one; this indicated that the mutant strains had a growth disadvantage in the zebrafish infection model.
Conclusions: We identified two new putative pneumococcal virulence genes by high-throughput genomic screen of strains isolated from adult patients with meningitis. Mutants in these two genes showed attenuation in the zebrafish embryo meningitis model. Our results suggest an important role for peptidoglycan metabolism in the pathogenesis of pneumococcal meningitis.

O132
Evolving epidemiology of bacterial meningitis in The Netherlands
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The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) receives nationwide isolates from patients with bacterial meningitis. During the last 5 years the incidence of bacterial meningitis according to the number of CSF isolates received by the NRLBM, varied between 1.6 and 1.8 cases per 100,000 inhabitants. The 5 most common species among the CSF isolates were *Streptococcus pneumoniae* (48%), *Neisseria meningitidis* (12%) *Haemophilues influenzae* (9%), *Streptococcus agalactiae* (7%) and *Escherichia coli* (6%). This distribution did not alter much during the period 2013-2015.
However, during the last quarter of 2015 the incidence of *N. meningitidis* invasive disease started to increase from 0.43/100,000 in 2014 (n=73) to 0.59/100,000 in 2015 (n=89), 0.80/100,000 (n=136) in 2016 and 1.2/100,000 (n=204) in 2017. This increase was mainly due to an increase of cases due to meningococci with the serogroup W polysaccharide capsule (MenW). The proportion of MenW meningococci increased from 1% in 2014 to 10% in 2015, 34% in 2016 and 41% in 2017. The incidence of MenW increased from 0.02/100,00 to 0.05/100,00, 0.29/100,00 and 0.47/100,00 in 2010-2014 (average n=4), 2015 (n=9), 2016 (n=50) and 2017 (n=83), respectively. In 2017, the incidence highest in children younger than 5 years of age (1.0/100,000; n=8) and 15-24 year olds (0.83/100,000; n=16). The case fatality rate was 14% (11/78) and was highest in 15-24 year olds (4/16; 25%).
Of 135 MenW strains isolated in the period 2015-2017, 119 (90%) had finetype P1.5,2:F1-1. MLST deduced from whole genome sequences of these isolates revealed that these were all strains belonging to clonal complex 11. In the UK an national outbreak of MenW disease occurred, with an increase of the number of cases from 19 in 2008–09 to 176 in 2014–15. Cluster analyses showed that these MenW:cc11 isolates were closely related to MenW:cc11 isolates from cases in the UK and presumably originated in South America. In addition, cluster analyses of core genome sequences showed that MenW:cc11 evolved over time.
In conclusion, in the Netherlands, the incidence of MenW disease continues to increase. The disease mainly affects children <5 years, adolescents and young adults, and shows a high case fatality rate. In 2018, MenC vaccination will be replaced by MenACWY vaccination in infants and MenACWY vaccination will be introduced in 13-14 year olds. Surveillance will continue to evaluate whether vaccination should be extended to other age cohorts.