O001

Campylobacter releases high levels of the inflammatory metabolite ADP-heptose that activate pro-inflammatory responses in intestinal epithelial cells

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Campylobacter releases high levels of the inflammatory metabolite ADP-heptose that activate pro-inflammatory responses in intestinal epithelial cells.

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The Gram-negative bacterium Campylobacter jejuni is a major cause of foodborne disease in humans. After infection, C. jejuni rapidly colonizes the mucus layer of the small and large intestine and induces a potent pro-inflammatory response characterized by the production of a large repertoire of cytokines, chemokines, and innate effector molecules, resulting in (bloody) diarrhea. However, the virulence mechanisms by which jejuni causes this intestinal response are still largely unknown. Recently, a highly conserved bacterial-derived intracellular metabolic intermediate of the lipopolysaccharide biosynthesis, ADP-heptose, was found to stimulate potent pro-inflammatory responses in mammalian cells via the receptor ALPK1, indicating that this molecular represents a novel pathogen-associated molecular pattern (PAMP). Here we show that various ampylobacter species release a potent pro-inflammatory compound into the culture media, which subsequently activated NF-kB-mediated pro-inflammatory response including the induction of IL-8, IL-6 and CCL2 in a manner independent of Toll-like Receptor and Nod-like Receptor signaling. Chemical characterization, inactivation of the heptose-synthesis pathway by the deletion of the hldE and gmhB gene, and in vitro synthesis confirmed that the secreted compound is ADP-heptose. Finally, C. jejuni infection of intestinal epithelial cells resulted in the activation of immune responses through the released ADP-heptose. Our results show that Campylobacter, unlike most other Gram-negative bacteria, releases high levels of the inflammatory metabolite ADP-heptose into its environment, suggesting that this virulence mechanism could play an important role during Campylobacter infection in humans

O002

Iron sheet piles are protected against corrosion by a consortium of anaerobic microorganisms

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Introduction: Iron sheet piles are widely used in flood protection, dike construction and river bank reinforcement. Sheet pile corrosion leads to gradual deterioration and often makes early replacement necessary. The formation of natural deposit layers on sheet piles can prevent degradation and significantly increases the lifespan. However, little is known about the mechanisms of natural protective layer formation. Here we studied the microbial diversity of deposit layers on iron sheet piles at the Gouderak pumping station in Zuid-Holland, the Netherlands to 1) provide more insights into the microbial processes that steer corrosion processes, and to 2) investigate the feasibility and potential of microbial corrosion protection in the field.
Methods: DNA was extracted from five deposit layers, surrounding soil samples and top soil samples using a lab-optimized PowerSoil protocol. To gain insight in microbial species diversity, 16S rRNA genes and metagenomes were sequenced by 454 Pyrosequencing and Illumina MiSeq technology. High-quality draft genomes were obtained by using an optimized in-house metagenome binning pipeline. Soil physicochemical parameters and organic matter content were determined and correlated with microbial diversity parameters.
Results: Deposit layers showed clear enrichment of Methanobacteriaceae, Coriobacteriales and Syntrophobacterales. Syntrophobacterales play an important role in interspecies electron transfer during degradation of organic acids in methanogenic communities (Cheng et al., 2013, PLOS ONE). Methane-producing Methanobacter species can metabolize iron which initially leads to mild corrosion but could stimulate the formation of a carbonate-rich deposit layer on the long term. The role of Coriobacteriales in syntrophic methanogenesis has been recently hypothesized with studies on electromethanogenic biocathodes (Kobayashi et al., 2017, Genome Announc.). Their high relative abundance in our dataset underscores their potential role in iron corrosion protection.
Conclusion: By combining microbial diversity analyses with environmental physicochemical parameters we could reconstruct the microbial community composition of CPLs. The high relative abundance of Coriobacteriales underscores the need to further study their potential role in iron corrosion protection. Our study provides valuable insights in microbial diversity during iron corrosion protection and enables

the development of novel strategies for in-situ screening of iron sheet piles.

O003

MUC1 is a receptor for the Salmonella SiiE adhesin that enables apical invasion into enterocytes

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MUC1 is a receptor for the Salmonella SiiE adhesin that enables apical invasion into enterocytes

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Introduction: The bacterial pathogen Salmonella enterica is one of the most common causes of human foodborne infection affecting millions of people worldwide each year. The cellular invasion machinery of the enteric pathogen Salmonella consists of a type III secretion system (T3SS) with injectable virulence factors that induce uptake by micropinocytosis. To establish infection, Salmonella needs to cross the mucus layer and invade intestinal epithelial cells from the apical surface. However, the apical surface of intestinal epithelial cells is covered with a defensive barrier of large glycosylated transmembrane mucins. These large proteins prevent contact between the Salmonella type III secretion needle and the host plasma membrane thereby preventing invasion. MUC1 is the most extensively studied transmembrane mucin and is highly expressed at mucosal surfaces including the stomach and the intestinal tract. The Salmonella giant adhesin SiiE that is secreted by the TISS and encoded by Pathogenicity Island 4 (SPI4) has been shown to be a key factor in apical invasion of polarized intestinal epithelial cells. A specific receptor for SiiE that enables Salmonella apical entry has not been identified. In this study, we investigate the role of transmembrane mucin MUC1 during Salmonella invasion into intestinal epithelial cells.
Methods: Confocal microscopy was used to visualize the interaction between Salmonella Enteritidis and MUC1. Crispr/Cas9 technique was applied to knock out MUC1 in human intestinal cell line HT29-MTX. Gentamicin protection assay was performed to determine the number of recovered Salmonella after invasion. Optimized western blot for large glycoprotein was developed and used to compare the MUC1 expression level in different intestinal cell lines. Enzymatic removal assay was used to identify the sugar on MUC1 that interacts with Salmonella.
Results:We observed that Salmonella is capable of apical invasion of intestinal epithelial cells that express the transmembrane mucin MUC1. Knockout of MUC1 in HT29-MTX cells or removal of MUC1 sialic acids by neuraminidase treatment reduced Salmonella apical invasion but did not affect lateral invasion that is not hampered by a defensive barrier. A Salmonella deletion strain lacking the SiiE giant adhesin was unable to invade intestinal epithelial cells through MUC1. SiiE-positive Salmonella closely associated with the MUC1 layer at the apical surface, but invaded Salmonella were negative for the adhesin.
Conclusion: We demonstrate for the first time that the transmembrane mucin MUC1 facilitates Salmonella apical entry into intestinal epithelial cells. MUC1 serves as a receptor for the Salmonella giant adhesin SiiE.

O004

Small molecule inhibition of the human microbiome-derived pro-oncogenic toxin BFT

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Introduction and Objective: The human intestinal microbiome plays an important role in inflammatory bowel disease (IBD) and colorectal cancer (CRC) development. One of the first discovered bacterial mechanisms involves Bacteroides fragilis toxin (BFT), a metalloprotease encoded by Enterotoxigenic Bacteroides fragilis (ETBF) that causes disintegration and inflammation of the colon wall, leads to cancer in susceptible mice, and is enriched in the mucosa of IBD and CRC patients. Exposure to BFT is therefore considered to be an important microbiome-derived risk factor for CRC.
Materials and Methods: Based on the idea that similar binding sites can bind similar ligands, the in silico structure-based pharmacophore fingerprint comparison method KRIPO was used to screen the Protein Data Bank (www.rcsb.org) for proteins with a structurally similar binding site as BFT-3. Subsequently,

molecular docking (MOE 2013.080226) was used to select the compounds that interact with the zinc atom in the BFT-3 active site. Top candidates were tested in a HT29/c1 CRC cell culture model measuring epithelial morphology change, E-cadherin cleavage (a marker for cell disintegration) and IL-8 secretion; three complementary read-outs of BFT activity. The direct interaction of BFT with the inhibiting compounds was confirmed with a Thermal shift assay (TSA).
Results and DiscussionBy applying two complementary in silico drug design techniques, drug repositioning and molecular docking, we predicted potential BFT inhibitory compounds. After testing 12 top candidates on HT29/c1 cells, the existing drug chenodeoxycholic acid (CDCA), currently used for treating gallstones, cerebrotendinous xanthomatosis, and constipation, was found to significantly inhibit BFT-induced epithelial responses including epithelial morphology changes, E-cadherin cleavage and IL-8 secretion. The inhibiting effect was observed with all three BFT-isoforms (BFT-1, BFT-2 and BFT-3) at a concentration of 85 and 175 μM CDCA ($p< 0.001$). The inhibition of BFT's action resulted from a direct interaction between CDCA and BFT, as confirmed by an increase in the melting temperature of the BFT protein by CDCA at 85 and 175 μM CDCA (ΔTm 1.10, and ΔTm 2.10, $p < 0.0001$, 1-Way ANOVA).
We found that CDCA, a natural occurring primary bile acid, effectively inhibits BFT activity in three complementary assays targeting different parts of the cellular response to BFT. An advantage of CDCA as a potential drug is that it is a natural bile acid present in the human gut and is already being used as an oral treatment. This would simplify the transition from in vitro to in vivoand finally clinical studies, mainly because CDCA has already passed several toxicity tests. This research was the first step in finding a drug that targets BFT and can be used as (preventative) treatment for ETBF-related CRC. In vivoexperiments are required to investigate the BFT-inhibitory effect of CDCA on tumorigenesis and inflammation in the colon and rectum.
Conclusion: Together, this shows the enormous potential of in silicodrug discovery to combat harmful human and microbiome-derived proteinaceous compounds and more specifically suggests a potential for retargeting CDCA to inhibit the pro-oncogenic toxin BFT.

O005

Identification of protective antigens by exploring the natural niche of Streptococcus pneumoniae

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Introduction
treptococcus pneumoniaeis a bacterial pathogen and a major cause of morbidity worldwide. It can cause mild infections leading to otitis media and sinusitis, but also more severe and invasive diseases such as pneumonia, meningitis and sepsis. Pneumococcal conjugate polysaccharide vaccines (PCVs), containing up to 13 different polysaccharides, have been very successful in preventing pneumococcal infections. However, there are over 95 different pneumococcal serotypes and non-vaccine serotypes have begun to replace vaccine-type serotypes in pneumococcal disease. Novel vaccines consisting of broadly protective protein-based antigens represent a promising approach to complement or replace existing intervention strategies against pneumococcal disease.
Our aim is to understand pneumococcal colonization in the nasopharynx and characterize the proteins that are expressed under these nutrient limited conditions to identify novel vaccine antigens. Since divalent cations are essential for survival of . pneumoniae, we determined their concentration in human nasal fluid. This allowed us to design an in vivo-mimicking culture medium that we used for comparative proteomics of bacteria grown under in vivo-mimicking and standard conditions.

Methods
Nasal fluid of healthy adults was collected and the concentration of Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+} was determined by induction-coupled plasma mass spectrometry (ICP-MS). A standard chemically defined medium (CDM) was adjusted to nasal divalent cation concentrations (in vivo-mimicking (IVM)-CDM). Shot gun proteomics was performed on whole cell lysates of two strains: BHN100 (19F) and BHN418 (6B) in logarithmic phase, grown in CDM and IVMCDM (n=3). Proteins >1.5-fold up-regulated in IVM-CDM versus CDM with a q-value <0.05 for both strains and surface exposed (psort-b) were considered as potential vaccine antigens.
Results
We measured only trace amounts of freely available divalent cations in nasal fluid, except for magnesium and calcium. . pneumoniaecultured in IVM-CDM showed different growth kinetics compared to standard CDM. Proteome analysis identified 59 and 38 proteins as down- and up- regulated in IVM-CDM vs CDM, respectively, in both strains. Significant differences were observed for pyrimidine, purine

and pyruvate metabolism. Based on the proteomics data we selected nine surface-exposed, conserved candidate antigens up-regulated under in vivo-mimicking conditions.
Conclusions
We show that adjusting the divalent cation concentrations in culture medium to levels observed in nasal fluid strongly affected the proteome and metabolic activity of the pneumococcus. Using this medium, we discovered nine conserved, surface-exposed candidate protein antigens. Mouse studies are ongoing to determine whether these protein antigens protect against colonization by . pneumoniae.

O006

Proteases C1r and C1s affect binding of C1q to certain human IgG subclasses

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The complement system is an important effector mechanism for antibody-mediated clearance of microbes and altered host cells. When antibodies bind a target cell surface, the first complement protein C1 activates a proteolytic cascade that results in labeling of cells for phagocytosis, attraction of leukocytes and direct cell death via lytic pores. The large C1 complex consists of the recognition protein C1q and associated tetramer of serine proteases $C1r_2C1s_2$. C1q consists of a 'bunch' of six polypeptide chains that together bind to IgM or Fc domains of clustered IgGs. Following Ig binding, C1q activates the associated $C1r_2C1s_2$ proteases to cleave C4.

While interactions between C1 and Ig molecules are believed to depend solely on the globular heads of C1q, we here found that association of C1q to certain Ig subclasses is influenced by the $C1r_2C1s_2$ proteases. Using a flow cytometry-based assay model with human monoclonal IgGs recognizing antigen-coated bead surfaces, we observe that C1q binding onto IgG1 and IgG2, but not IgG3, is strongly enhanced by $C1r_2C1s_2$ tetramers. While removal of $C1r_2C1s_2$ (either via EDTA or C1 inhibitor) did not influence C1q binding to IgG3-coated surfaces, it led to dissociation of C1q from IgG1 and IgG2. Furthermore, addition of purified recombinant $C1r_2C1s_2$ to empty C1q increased its binding to IgG1 and IgG2, but not IgG3. Thus, even though $C1r_2C1s_2$ do not interact directly with Ig-Fc, they influence C1q interactions with surface-bound IgG1 and IgG2.

We hypothesize that these proteases induce conformational differences in C1q. Furthermore, we believe that this protease-dependent C1q binding explains why we observe a disconnection between C1q detection and downstream complement activation on bacterial surfaces in human serum. Altogether these basic insights into mechanisms of antibody-dependent complement activation and regulation will improve our understanding of (un)wanted complement activation processes.

This research was supported by an ERC starting grant (#639209 to S.H.M.R.)

O007

Whole cell pertussis vaccination-induced antibodies efficiently block bacterial adherence to respiratory epithelial cells

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Introduction
Pertussis, or whooping cough, is caused by the Gram-negative bacterium Bordetella pertussis. This highly contagious respiratory disease affects children and adults of all ages and was responsible for high infant mortality and morbidity before the widespread implementation of a whole cell pertussis vaccine

(wP) in 1954. Due to safety concerns, the wP vaccine was replaced by an acellular pertussis vaccine (aP) in 2005, consisting of a limited number of purified pertussis protein antigens. Although aP vaccination is highly effective against severe morbidity and death in infants, studies have suggested that aP-induced immunity wanes rapidly, prevents severe disease but not infection, and is less effective than vaccination with wP.
During the large-scale pertussis vaccination efficacy trials of the 80s and 90s, ELISA-based serology was used as the primary immunological endpoint for vaccination. These studies demonstrated that aP vaccines induced much higher levels of IgG in serum than wP vaccines. Since aP vaccines are considered to be less effective than wP vaccines, the role of antibodies towards protection against infection has been questioned. Antibodies can provide protection against pathogens through various mechanisms, including the neutralization of toxins and antibody-dependent complement-mediated killing. One of the first steps during the colonization process is the adherence of B. pertussisto the respiratory epithelium. In this study, we therefore established a novel bacterial adherence inhibition assay to determine the ability of antibodies to block adherence.
Methods
B. pertussiswas labeled fluorescently through conjugation. Labeled bacteria were pre-incubated for 1 hour at 37°C with human sera from infants primed with wP or with the WHO international standard pertussis antiserum (NIBSC 06/140) as a control, which contains known quantities of antibodies induced by acellular pertussis vaccination. The bacteria were then added to A549 cells, centrifuged to synchronize the bacterial attachment and incubated for 1 hour at 37 °C + 5% CO_2. After extensive washing to remove unbound bacteria, inhibition of bacterial adherence was examined by measuring the fluorescence using a fluorometer.
Results
Antibodies induced by both wP vaccination and aP vaccination significantly reduced bacterial adherence to airway epithelial cells. However, B. pertussisadherence was significantly more inhibited by wP-induced antibodies than aP-induced sera. Pertussis specific PT and FHA titers after wP vaccination correlate well with inhibition of adherence.
Conclusion
Antibodies induced by wP vaccination are better in preventing bacterial adherence than aP-induced antibodies. This assay can be used to determine the functionality of serological responses to (improved) vaccination and B. pertussiscolonization.

O008

Tumor promotion or inhibition? The effects of individual gut microbes on colorectal cancer cell proliferation

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Tumor promotion or inhibition? The effects of individual gut microbes on colorectal cancer cell proliferation
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Introduction and Objective: Colorectal cancer (CRC) is the third leading cancer in the Netherlands. Certain bacteria and/or their secreted molecules such as strains of treptococcusspp., Fusobacteriumspp., B. fragilis(ETBF) and Escherichiaspp. increase cell proliferation of cancer cells, whereas others, such as Bifidobacteriumspp. and Lactobacillusspp., decrease proliferation. Nevertheless, with over 1,000 bacterial species living in our guts, their roles in cell proliferation and CRC remain unclear. Therefore, our aim is to identify cancer promoting or inhibiting effects of microbiota members with a particular focus on species that are enriched or depleted in the human CRC microbiome.
Materials and Methods: We conducted a literature survey to select bacteria that were enriched or depleted in CRC. Selected strains were grown under anaerobic conditions until stationary growth phase. Bacteria-free supernatants (secretomes; n=154) and inactivated intact bacteria (cell wall-attached factors; n=145) were applied to five colon cancer cell lines (Caco-2, HT29, HCT15, HCT116, SW480) and one non-cancerous cell line (HEK293T). Cell metabolic activity was measured in a high-through-put assay. Bioinformatic analysis revealed which secretomes and cell-wall anchored factors play a

significant role in promotion or inhibition of CRC cell proliferation and which genes are potentially associated to the observed effects.
Results and Discussion: Secretomes of several strains from Bacteroidaceae, Enterobacteriaceae, Erysipelotrichaceaeand Tannerellaceaesignificantly increased the overall metabolic activity of CRC cells compared to non-cancerous cells ($p<0.05$), while the secretomes and inactivated bacteria from Fusobacteriaceae, Lachnospiraceaeand Clostridiaceaedecreased metabolic activity ($p<0.05$). Clustering the effects of bacterial strains and their secretomes on human CRC and non-cancerous cells showed that in many cases the effects were strain-dependent and cell line-dependent. Nevertheless, our bioinformatic analysis revealed a strong taxonomic signal. While strains from the same family such as Fusobacteriaceaetend to have similar average effects on cell lines, specific strains stand out exhibiting stronger enhancing/inhibiting effects than the average observed for its family. This suggests that specific strains may possess or lack certain proteins or cell wall-attached factors explaining why they stand out. In order to identify these proteins we ranked bacterial families by their effects on cells lines and found genes that are potentially associated with the observed enhancing/inhibiting effect ($p<0.01$).
Conclusion: We identified bacterial strains that affect cancer cell growth via secretomes and cell wall-attached factors, linking them with potential genes that explain this effect. Our findings reveal a consistency in the inhibiting/enhancing effects within families and thus, clear taxonomic signatures.

O009

Viral discovery using metagenomics in a clinical setting; the rediscovery of Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome

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INTRODUCTION
Metagenomic shotgun sequencing not only has the potential to detect rare pathogens, but also to discover new species. However, the detection of new pathogens in clinical samples is challenging due to their low abundance within very large datasets that are generated using next generation sequencing. To improve our metagenomic shotgun methods in regards to virus discovery, we added Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS) sequence reads to raw sequence data of clinical samples. In this study we learn how these reads would be classified by metagenomic tools using old reference genomes, how we could extract the unassigned reads and how to build genome contigs that would lead to the discovery of a new virus.
METHODS
Two clinical samples that were negative for various RNA and DNA viruses by means of multiplex real-time PCR were sequenced on the Illumina NextSeq 500 platform utilizing a shotgun metagenomic approach. The raw data containing over 10 million sequence reads per patient, was spiked with 1,000 or 20,000 in silicogenerated reads of the MERS and SARS corona viruses. Applying mapping tool BWA and metagenomic classification tools like Centrifuge, all reads were assigned to species in different domains. For the viral species we used a special reference index file of all virus genomes available in Refseq, excluding all coronavirus genomes submitted to Genbank after 2003, the discovery year of SARS. All the unassigned reads were assembled into new genomic contigs using SPAdes. The larger contigs were blasted against the nucleotide database to see if we could trace back the original virus species, and additionally how the virus contigs resemble other (corona) viruses from before 2003.
RESULTS
Utilizing metagenomics classification tools, only 10-30, and 100-200 sequence reads of the respectively 1,000 and 20,000 spiked MERS and SARS reads were assigned to corona viruses from before 2003, leaving a large mystery for the majority of MERS and SARS reads. From all the remaining unassigned reads thousands of contigs were assembled, of which approximately 100 contigs were well-covered and contained over 500 nucleotides. Of these large contigs, <25 contigs blasted to the MERS or SARS genome with most having an identity of 99-100%. Parts of these genome contigs had the greatest resemblance to the pre-2003 Coronaviridae genomes and not to any other specific (viral) species.
CONCLUSION
We were able to trace back our spiked-in MERS and SARS reads with great accuracy. Parts of the larger built viral contigs resembled Coronaviridae species prior to 2003. In the future, such a finding could indeed indicate the presence of a new virus in a clinical sample. Our method and results provided insight for further developing a sensitive virus discovery workflow for highly divergent pathogens, in addition to an accurate metagenomic classification analysis. With the new era of metagenomic shotgun sequencing being implemented in clinical settings, the identification of both known and novel pathogens directly in clinical patient samples by means of a catch-all metagenomic assay will be paradigmatic.

O010

Unravelling the role of plasmid sequences in Enterococcus faecium

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Introduction:
Enterococcus faeciumis a gut commensal frequently present in the intestine of mammals but also listed on the priority list of the WHO as a human opportunistic pathogen. Plasmids are autonomous extra-chromosomal elements which can act as major drivers of variation and adaptation in bacterial populations. We undertook a holistic approach to observe the role of plasmids in the dissemination of antimicrobial resistance genes and host adaptation in E. faecium.
Methods:
Our dataset consisted of 1,644 E. faeciumisolates whole-genome sequenced (WGS) by Illumina NextSeq/MiSeq including samples from hospitalized patients (n > 1,100), non-hospitalized patients (n = 135), pets (n = 164) and farm (n = 185) animals. Complete genome sequences were obtained from a selection of 62 isolates using Oxford Nanopore Technologies. The plasmidome content of non-long read sequences isolates (n = 1,582) was predicted using mlplasmids (posterior probability > 0.7, length > 1,000 bp). Differences in the number of plasmid-predicted sequences were determined using a Kruskal Wallis Test. Pairwise Mash distances (k = 21, s = 1,000) were used to establish a plasmidome phylogeny (bioNJ algorithm) and define plasmid populations based on plasmid k-mers. To disclose the contribution of plasmid sequences in host adaptation, we conducted a bootstrap approach (100 iterations) to randomly select pairwise distances from isolates belonging to either the same host or different hosts and use one way-ANOVA test for statistical analyses. Enrichment analysis of plasmid orthologous genes between hosts was performed using a combination of Prokka, Roary and Scoary.
Results:
We obtained 305 complete plasmid sequences ranging from 1.93 to 293.95 kbp (median = 15.15 kbp, mean = 53.48 kbp). Long-read sequenced isolates from hospitalized patients (n = 43) contained a significantly higher number of plasmids (mean = 5.70) and their cumulative plasmid length was also larger compared to other hosts (mean = 308.01 kbp). Furthermore, the number of predicted plasmid sequences in our entire collection was significantly higher in hospitalized patients (mean = 276.16 kbp) compared to other isolation sources. Clustering of predicted plasmid sequences revealed 9 different plasmid populations which were overrepresented in: i) hospitalized patients (6/9), ii) poultry (1/9), iii) dog (1/9) and iv) co-occurrence of pig and non-hospitalized patients (1/9). Plasmid-gene enrichment analysis showed that plasmids of hospitalized isolates were enriched with an aacA-aphDor a bacteriocin-like protein (bacA)among others. Plasmids of pig isolates were associated with a set of copper resistance genes. Pairwise distance comparison between hosts revealed that contribution into host adaptation mediated by plasmid sequences was significantly higher compared to chromosomal sequences.
Conclusions:
Plasmidome content of hospitalized patients differed in size and content compared to other hosts. Hospitalized isolates were associated with 6 different plasmid configurations suggesting different routes of plasmid adaptation in the hospital environment.Presence of bacteriocin-like or copper resistance genes among OG enriched groups unravelled widely disseminated plasmid-mediated mechanisms contributing to host adaptation.Plasmid sequences have a major contribution in adaptation compared to other genomic components. This suggests that plasmids are regulated by complex ecological constraints rather than physical interaction between hosts.

O011

A single gene enables phytopathogenic Pectobacterium to overcome the chemical defense system of Arabidopsis thaliana

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Introduction
Plants of the Brassica family, including Arabidopsisand many common vegetables, produce toxic isothiocyanates (ITCs) to defend themselves against pathogens. Despite this defense, plant pathogenic microorganisms like Pectobacteriumcause large yield losses of these crops. The bacterial gene axAwas

previously found to encode isothiocyanate hydrolase that could degrade isothiocyanates in vitro.
Objectives
The goal of this research was to determine whether axAis a virulence factor for overcoming the chemical defense system of Brassica plants with A. thalianaas model plant.
Methods
The axAgenes of P. carotovorumSCC1 and P. carotovorumNCPPB3384 were deleted using homologous recombination. ArabidopsisCol-0 leaves were infected with Pectobacteriumwildtype and ΔsaxAmutants, and the area of tissue maceration was measured. The experiment was repeated with Arabidopsis myb28/29plants that are impaired in isothiocyanate production. The related species P. polarishas two genomic copies of axAwhich were deleted sequentially. The effect of single and double axAdeletions on growth performance was tested by measuring optical density in minimal medium supplemented with Arabidopsisleaf extract. Slices of turnip (Brassica rapasubsp. rapa) were infected with P. polariswildtype and mutants, and area of infection was measured based on discoloration.
Results
Wildtype P. carotovorumreadily infected both A. thalianaCol-0 as well as A. thalianamyb28/29plants. The P. carotovorumΔsaxAmutants were impaired in their ability to infect Col-0 leaves, yet showed a similar phenotype on myb28/29plants as the P. carotovorumwildtype. Furthermore, the phenotype of the P. carotovorumΔsaxAmutants on Col-0 plants could be restored with a complementation plasmid containing axAfrom either source organism. Single gene deletion of either axAgene in P. polarisdid not change the growth of this organism in ITC-rich Arabidopsisextracts. The axAdouble mutant, however, did not grow in this extract. All mutants and wildtype P. polarisshowed similar growth patterns in the presence of Arabidopsis myb28/29extracts which contains no ITC.
Conclusion
A single gene, axA, imparts plant pathogens with the ability to overcome the isothiocyanate-based defenses found in Arabidopsisand many of the most common vegetable crops.

O012

Serum IgM binding to phosphorylcholine of non-typeable Haemophilus influenzae increases complement-mediated killing

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Introduction
Non-typeable Haemophilus influenzae(NTHi) colonizes the human upper respiratory tract without causing disease symptoms, but it is also a major cause of upper and lower respiratory tract infections in children and elderly, respectively. NTHi expresses various molecules, including phosphorylcholine (PCho), in their lipooligosaccharide (LOS) that contribute to its virulence. Incorporation of PCho is under control of phase-variation, which is a stochastic process dependent on slipped-strand mispairing of tandem repeats present in the gene and leads to switching the coding region in frame (phase-on) or out of frame (phase-off).The presence of PCho in the LOS of NTHi increases adhesion to epithelial cells and is an advantage for the bacterium enabling nasopharyngeal colonization. However, when PCho is present in the LOS of NTHi, it is also recognized by acute phase protein C-reactive protein and PCho-specific antibodies, both potent initiators of the classical pathway of complement activation. In this study, we determined the contribution of PCho-specific IgM in complement-mediated killing of NTHi.

Methods
Three hundred nineteen NTHi strains collected from the oropharynx/nasopharynx (n=153), middle ear fluid (n=109) or from the lower respiratory tract (n=57) were included in this study. The presence of PCho on the bacterial surface was determined by TEPC-15 mAb binding by flow cytometry. Binding of complement C3, complement C5b9 complex, IgG, IgM and CRP were determined by flow cytometry. C5b9 pore formation was determined by a Sytox green diffusion assay. Complement-mediated killing was determined in human serum or plasma.

Results
PCho detection by TEPC-15 was higher for NTHi strains collected from the nasopharynx/oropharynx compared to NTHi strains collected from the lower respiratory tract, which was associated with increased binding of IgM and IgG to the bacterial surface. Complement-mediated killing of a PCho-positive NTHi strain was largely dependent on PCho-specific IgM. The levels of PCho-specific IgM varied in plasma from 12 healthy individuals and higher PCho-specific IgM levels were associated with increased complement-mediated killing of a PCho-positive NTHi strain.

Conclusion
In conclusion, incorporation of PCho in the LOS of NTHi primes the bacterium for IgM binding resulting in complement-mediated killing. Therefore, switching PCho phase-off might be beneficial in situations where PCho-specific antibodies and complement are present.

O013

Identification of mycobacterial PE protease PecA that cleaves secreted surface proteins and plays a role in virulence

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Introduction
Mycobacterium tuberculosisis the causative agent of tuberculosis, a severe lung disease causing more than 1.5 million deaths each year. Mycobacteria have a unique cell wall that requires specialized secretion systems. These systems are known as ESX-1 to ESX-5. ESX-5 is only present in pathogenic mycobacteria, such as M. tuberculosisand M. marinum. Through ESX-5, these mycobacteria secrete a large class of proteins characterized by their N-terminal motif: the PE proteins. Although M. tuberculosishas 99 of these proteins, very little is known about their exact function and role in virulence. Here we studied PE protein secretion and identified a novel PE protease, PecA, in M. marinum. PecA processes other PE proteins, including the known triacylgylocerol lipase LipY and itself.
Methods and results
A fusion protein containing the PE domain of LipY and a fragment of Ovalbumin (LipY-OVA) was expressed in M. marinum. Secretion of LipY-OVA was visualized by double filter assay. After creating a transposon library in M. marinumexpressing LipY-OVA, mutants were screened for altered secretion. Three independent mutants containing a transposon in pecAshowed increased secretion. In silicomodeling suggested that PecA was an aspartic protease, marked by its active site containing two DTG/DSG motifs. And indeed, western blot analysis showed that PecA was required for LipY-OVA and LipY processing. By N-terminal sequencing we identified the cleavage site of PecA in LipY.
Since PecA is a PE protein itself, we also analyzed processing of PecA. PecA showed autocleavage in M. marinum, E. coliand in vitro. Next we showed that a class of endogenous PE proteins, the PE_PGRS proteins, were also processed by PecA, showing that PecA has a broad class of substrates. Importantly, the PGRS phenotype of a ΔpecAstrain could be complemented with pecAfrom both M. marinumand M. tuberculosis, but not by a catalytic dead form of PecA. Thus, the protease activity of PecA is responsible for the processing of PE_PGRS proteins in M. marinum.Most likely, PecA has a similar function in M. tuberculosis.
Last, we analyzed the role of PecA in vivoin our zebrafish embryo model. Zebrafish larvae were infected with wild type, ΔpecAand complemented M. marinumstrains. Infection was analyzed 5 days post infection by microscopy. The ΔpecAstrain showed an attenuated phenotype which could be complemented by reintroducing pecA, but not by the catalytic dead pecA.
Conclusion
1. PecA is a PE protease that cleaves LipY, other PE_PGRS proteins and itself in M. marinum.
2. Processing by PecA is dependent on its catalytic activity.
3. The phenotype of M. marinumΔpecAcan be complemented with pecAfrom both M. marinumand M. tuberculosis.
4. M. marinumΔpecAshows attenuation in a zebrafish embryo infection model.
5. Concluding, PecA is the first protein that has been shown to process PE proteins on the cell surface of mycobacteria and to have a role in vivoinfection.

O014

Sensory neurons detect pathogens in the cerebrospinal fluid and improve survival during bacterial meningitis

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Background: Cerebrospinal fluid (CSF) is a complex fluid bathing the nervous system whose composition changes with physiological states. A singular class of sensory neurons surrounding the central canal and contacting the CSF (CSF-cNs) has recently been shown to detect changes in CSF

flow, pH, and osmolarity. Although conserved in vertebrates, the physiological relevance of such sensory neurons remains unknown. Here, we investigated whether CSF-cNs could be recruited when pathogens infiltrate the CSF during meningitis.
Methods: CSF-cNs were isolated from the transgenic Tg(pkd2l1:Gal4, UAS:GFP)zebrafish line with GFP-labelled CSF-cNs with fluorescence activated cell sorting (FACS). Transcriptome analysis was performed. To test whether CSF-cNs could detect pathogen invasion in the CSF, we used a zebrafish model of bacterial meningitis where bacteria colonizes the CSF, including the central canal. We injected 2 days post fertilization (dpf) Tg(pkd2l1:GCaMP5G)larvae with treptococcus pneumoniaeor Listeria monocytogenesin the hindbrain ventricle and monitored calcium activity in CSF-cNs with fluorescence time-lapse imaging over 24 hours. In addition, we performed ablation of CSF-cNs or silencing of CSF-cNs in zebrafish and performed infection survival experiments to study the role of these sensory neurons in bacterial meningitis (n=60 per group per experiment, performed in triplicate).
Results: We identified a plethora of proteins and peptides (e.g. secretogranins, urotensin-related peptides and somatostatins) involved in innate immunity and CSF biomarkers of meningitis specifically expressed and secreted by CSF-cNs (203 genes specifically expressed in CSF-cNs in total; 43 genes (21%) involved in innate immunity). In response to the . pneumoniaeor L. monocytogenescolonization in the CSF, a subset of CSF-cNs exhibit large and long-lasting calcium transients indicative of sustained burst firing. Such massive CSF-cN activation was mimicked by injection of the bitter compound denatonium but not by the bacterial toxin pneumolysin nor by lipopolysaccharides. This activation was also not seen in other spinal cells such as motor neurons and ependymal radial glia. Survival experiments in zebrafish larvae, in which CSF-cNs were ablated or genetically silenced, show that the proteins and peptides released by CSF-cN actively contribute to survival and recovery during bacterial meningitis (Kaplan-Meier survival analysis; P=0.0002 and P=0.0081 respectively).
Conclusion: 1. We demonstrate that sensory neurons contribute to detection of pathogen invasion in CSF. 2. Sensory neurons suppress meningeal infections by releasing peptides involved in innate immunity and confer a survival benefit during meningeal infection.

O015

Synthetic wall teichoic acid provides new insights into the systemic and mucosal human antibody repertoire for Staphylococcus aureus glycotypes

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taphylococcus aureus is an important cause of disease in the community and the healthcare settings. Arguably the most immunogenic antigen of . aureusis the abundant cell surface component wall teichoic acid (WTA). The most common form of . aureusWTA consists of a poly-ribitol-phosphate (RboP) chain, which can be modified by three glycosyltransferases (TarS, TarP and TarM) that attach N-acetylglucosamine (GlcNAc) to different positions or in different linkages to RboP. Previous studies have indicated that human serum contains different amounts of antibodies that recognize these specific WTA-GlcNAc modifications. Dissection and structural characterization of WTA-antibody interactions are challenging; WTA expression and modification are highly variable between as well as within strains, and additionally isolated WTA is unstable. We have therefore developed a model using synthesized RboP hexamers that are enzymatically modified by recombinant Tar glycosyltransferases to create the different WTA glycoforms. This model enabled highly sensitive and specific detection of human antibodies interacting with specific WTA glycotypes, without the need for isolating unstable aureusWTA or affinity purifying WTA-reactive antibodies from serum. IgG of all four subclasses deposited on the various WTA glycotypes at different levels and both IgG and IgA induced efficient neutrophil phagocytosis of WTA-coated beads. In addition, we observed that mucosal secretory IgA has a distinct WTA glycotype target distribution from serum IgG or IgA, suggesting that the mucosal and systemic compartments of the adaptive immune system encounter different predominant WTA glycotypes. These synthetic WTA molecules thereby provided a new and invaluable tool to dissect the interaction between . aureusWTA and host receptors at the molecular and structural level. In addition, these synthetic WTA analogues can be further explored as . aureusWTA vaccine antigens and used as a platform for the design and optimization of therapeutic antibodies targeting aureusWTA.

O016

Mucosal immune response to controlled human Bordetella pertussis challenge

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Introduction
Pertussis, or whooping cough, is caused by the Gram-negative bacterium Bordetella pertussis. This highly contagious respiratory disease affects children and adults of all ages and was responsible for high infant mortality and morbidity before the widespread implementation of a whole cell pertussis vaccine (wP) in 1954. Due to safety concerns, the wP vaccine was replaced by an acellular pertussis vaccine (aP) in 2005, consisting of a limited number of purified pertussis protein antigens. Although aP vaccination remains highly effective against severe morbidity and death in infants, studies have suggested that aP-induced immunity may be less effective against infection. At present, an estimated 9% of the Dutch population >9y shows serological evidence of recent exposure to B. pertussis. Although the majority of these infections are presumably asymptomatic, the high circulation rate poses a threat to newborns too young to be protected by vaccination. At present, the correlates of protection against (subclinical) infection remain largely unknown. The establishment of a controlled human infection model for B. pertussiswill facilitate the development and evaluation of novel pertussis vaccines that not only target prevention of disease but also infection. In this study, we evaluated the mucosal immune response to intranasal challenge with B. pertussisin human volunteers.
Methods
Healthy adult volunteers (n=15) were challenged with 10^5 colony forming units (CFU) of B. pertussisstrain B1917 and followed up for clinical signs of pertussis disease for 14 days while being kept in isolation to avoid contagion. After 14 days, azithromycin was given once a day for three days to eradicate possible B. pertussiscolonization. Volunteers were discharged at day 16. Bacterial load was determined at various timepoints. Mucosal lining fluid was obtained at different time points using nasosorption devices and analyzed for B. pertussis-specific antibodies and inflammatory mediators.
Results
Of the 15 volunteers challenged with B. pertussis,12 became culture-positive. In the three culture-negative volunteers, we observed two ‘waves’ of B. pertussis-specific mucosal IgA and IgG at five and 14-15 days after challenge. Of note, volunteers with high bacterial densities showed virtually no mucosal antibody response. Our findings suggest that an early IgA/IgG response may be correlated with control of colonizing B. pertussis. Analysis of the inflammatory responses is ongoing.
Conclusion
A local early increase in pertussis-specific mucosal IgA and IgG may be protective against colonization with Bordetella pertussis. Correlation of the data with serum antibody levels and systemic responses will help us to understand the mechanisms of protection against colonization.

O017
Competition for glycans at the mucosal interface
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Introduction: The human gastrointestinal tract is colonised with a diverse microbial community, that plays crucial role in human’s health. In the gut, host-derived glycan structures form a protective mucus layer attached to the epithelial cells that separates the bacteria from the host. These mucus-derived glycans are utilised by bacteria that have adapted to this niche of the gastrointestinal tract. The mucosa-associated bacteria form a microbial network participating in nutrient exchange, communication with the host, development of the immune system, and resistance against invading pathogens. Two distinct gut microbiota members that are known to use mucus glycans are Akkermansia muciniphilaand Bacteroides thetaiotaomicron.The aim of this study is to evaluate the interaction of these two bacteria to explore if they interact with each other and the host.
Methods: Four groups of mice (n=20) were used at the gnotobiotic facility at University of Götenburg, in

Sweden. The first group of mice was kept germ free, hosting no bacteria at all and thus serving as a negative control. The second group was inoculated with Bacteroides thetaiotaomicronand the third group was inoculated with A. muciniphilaserving as positive controls. The last group was inoculated with both bacteria. Samples were taken from two different parts of the gut: the cecum (Ce) and the distal colon (DC). In vitro fermentations were used to study more closely the interaction between these species and to compare with the in vivoexperiment. Microbial presence of the different parts of mice gut and in the bioreactors was determined by quantitative PCR (qPCR) while SCFAs' production was analysed by HPLC. The differential gene expression between double and mono-cultures in the different parts of the gut was characterised by transcriptomics.
Results: qPCR showed similar amount of bacteria in mono and double associated mice in the different parts of the mice gut and in the in vitroexperiment. Moreover, SCFAs production is enhanced in the double culture in the bioreactors compared to mono-culture. Next to that, transcriptome analysis of the microbiota did exhibit significant differences in the expression of the genes between the different conditions. As such, B. thetaiotaomicronrevealed upregulation of different Polysaccharides Utilisation Loci (PULs) between mono- and double culture in both cecum and distal colon. Interestingly, A. muciniphilaupregulates only in the presence of B. thetaiotaomicrona gene cluster encoding for glycosyl transferases and capsular polysaccharide biosynthesis proteins.
Conclusion: The results showed that both B. thetaiotaomicronand A. muciniphilaare not affected in abundance when grown together but adapt their transcriptional responses as a result of co-existence. The next step in our analysis is to look at the host response.

O018
The identification of a novel Erysipelotrichaceae species, an immunogenic, IBD-associated mucin degrader
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The human gut microbiota plays a central role in intestinal health and disease. However, many of its bacterial constituents are functionally still largely unexplored. A crucial prerequisite for bacterial survival and proliferation is the creation and/or exploitation of an own niche. For many bacterial species that are linked to human disease, the inner mucus layer was found to be an important niche. To explore how such commensals are able to effectively colonize this dense mucus layer, we screened the genomes of mucus-colonizing commensals for mucolytic enzymes that may contribute to this process. In a previously uncharacterized, IBD-associated bacterium, from the family of Erysipelotrichaceae, we identified multiple secreted Carbohydrate Active Enzymes (CAZymes).These CAZymes are able to degrade mucin O-glycans after which some of the liberated monosaccharides can be utilized for bacterial growth. Furthermore, the enzymatic degradation of the mucin glycans sensitizes the mucus layer for further degradation. Therefore these enzymes can likely aid in the colonization of the intestinal mucus layer and be a crucial factor for competing with other bacteria. Moreover, mice colonized with this strain show decreased inner mucus layer thickness and are more prone to developing colitis in both IL10-/- and RAG1-/- backgrounds. Therefore this novel bacterium may provide new insights into the mucosal niche and the development of colitis.

O019
Calcifying bacteria in sponges belong to different classes of Proteobacteria
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Introduction
Endosymbiotic calcifying bacteria, named calcibacteria due to the calcareous spherules they produce, were first identified in the Altlanto-Mediterranean sponge Hemimycale columella(Uriz et al. 2012). Later, seven calcibacteria OTUs belonging to the Alphaproteobacteria were identified in sponges, corals and seawater (Garate et al. 2017), which allowed to hypothesized on the role of this bacteria in calcification of early Metazoa. Moreover, similar calcareous spherules were observed in other sponges from different latitudes. The aim of this study was to analyze and compare the microbiota of the sponges with calcareous spherules to obtain more insight in the phylogeny of calcifying bacteria in sponges.
Methods
The studied sponge species were: Hemimycale columella, H. mediterranea,and Cliona viridisfrom western Mediterranean Sea, H. mediterraneafrom Adriatic Sea, H. arabicaand Crella cyathophorafrom

Red Sea, C. cyathophorafrom Indo-Pacific Ocean, and Cinachyrella allocladaand Cynachyrella sp. from Caribbean Sea. Tag-pyrosequencing analyses of 16S rRNA gene were performed with 454 Roche platform, and data were analyzed using QIIME 1.4.0 pipeline (Caporaso et al. 2010).
Results
The studied sponges had species-specific microbiomes with Proteobacteria as the most abundant phylum (> 70% relative abundance) in all the species reported as LMA sponges, except the two from the Caribbean Sea, which belong to the HMA sponges. All of the species except Cinachyrellasp. harbored large numbers of calcareous spherules. The known calcibacteria OTUs were the most abundant sequences in the microbiota of the spherules-harboring species, except in C. allocladaand H. mediterranea.Surprisingly, the microbiota of the latter species, morphologically cryptic with H. columella, lacked the known Alphaproteobacteria calcibacteria but were dominated by a Betaproteobacterium OTU. Hybridization experiments on this species using a specific Betaproteobacteria probe were positive, indicating that taxonomically distant bacteria were involved in sponge calcification.
Conclusions
Calcibacteria-sponge symbioses are widespread in temperate and warm seas, and calcibacteria are the most abundant bacteria in almost all the sponges with calcareous micro-spherules. Taxonomically distant bacteria are involved in sponge calcification. Given the phylogenetic closeness of these OTUs to those present in corals, the hypothesis of the role of intracellular bacteria in calcification processes of Early Metazoa gains support.

O020

An altered gut microbiota establishment in early life is linked with the development of atopic dermatitis

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Introduction
Gut microbiota maturation in early life is a dynamic process that is still incompletely understood. It has been hypothesized that the first year of life represents a critical time frame for the maturation of the gut microbiota. Inadequate microbiota maturation can cause immune deregulations linked to manifestation of non-communicable diseases, such as atopic dermatitis, later in life.
Within the context of the PAPS cohort, we investigated the establishment and maturation of the gut microbiota of children from birth up to school-age in association to the subsequent development of atopic dermatitis, using a novel approach.
Methods
Within the context of the PAPS cohort, fecal samples from 311 children were collected at ages 5, 13, 21, 31 weeks and 6-12 years and profiled by amplicon sequencing of the 16S rRNA V4 hypervariable gene region. Information on lifestyle, diet and medication was retrieved through interviews and development of atopic dermatitis was monitored through regular clinical examination by a pediatrician. In order to describe the establishment of the gut microbiota, correlation analysis as well as network analysis were performed in R. To investigate the establishment of the gut microbiota in relation to the subsequent development of atopic dermatitis we applied for the first time on a microbiota study, a joint modeling to our longitudinal and survival data.
Results
Our findings show that the complexity of the microbiota gradually increases over time and this increase is mostly driven by the duration of breast feeding (p=0.001) as well as the age of introduction of solid food (p=0.003) as showed by Envfitanalysis. Moreover, the microbial interaction network changes over time with different bacteria dominating these age-dependent networks. These results suggest that infant diet, rather than other environmental or genetic factors, is crucial for maturation of the infant gut microbiota.
Subsequently, to investigate the potential link between alterations in gut microbiota maturation and the development of atopic dermatitis, we introduced the application of a joint model. This novel approach enables the use of repeatedly collected infant fecal samples (longitudinal analysis) and link its variations with the probability to develop atopic dermatitis at a given age (survival analysis). The results show that a decrease in microbial richness of one unit (Shannon index p=0.0001) is associated with a 4.8-fold increased risk to develop atopic dermatitis at a given age.
Conclusion
Altogether our findings suggest that adequate gut microbiota maturation during the first year of life is crucial to reduce the risk of atopic dermatitis later in life.

O021

The role of microbiome and soil composition on eelgrass seed germination and outgrowth

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Resilient coastal ecosystems that are governed by mangroves, salt marches or seagrasses are of fundamental importance in maintaining marine biodiversity. In the Netherlands, efforts are made to re-introduce eelgrass (Zostera marina) in both the Zeeuwse delta as well as the Waddenzee. Progress is made in methods of seed treatment and planting, but re-introduction success is still unpredictable. Therefore, more insight is needed in factors influencing Z. marinagrowth. In terrestrial plants it is known that microorganisms present in the soil are crucial for plant development, and we hypothesise the same is true for seagrasses. Therefore, we study the effect of sediment type and microbiome composition on the germination and outgrowth of Zostera marinaseeds.
As a starting point, we designed laboratory experiments allowing us to test two types of sediment, both either non-treated or gamma-sterilized. We used mud from Uithuizen mudflats and North Sea beach sand. We followed seed germination and outgrowth on these sediments, and simultaneously identified the microbiome of both the bulk sediment and the rhizosphere. Each sediment type had a different microbiome content, and the rhizosphere microbiome differed from the bulk soil, already with relatively small seedlings. We observed improved germination on pre-sterilized soils, indicating the presence of pathogenic activity in the non-sterilized samples. Mud resulted in stronger growth compared to beach sand. Pre-sterilized mud gave rise to rather uniform seedlings, whereas untreated mud resulted in a large spread in seedling development, with some seedlings growing up to twice the size of the seedlings in pre-sterilized mud.
Pre-treatment by gamma-sterilization of the sediments effected both microbe abundance (as measured by total DNA concentrations) and diversity. Whereas the bacterial population seems to partly recover after the pre-sterilization, the archaea did not. The experimental setup was such that we did not completely isolate the individual growing cylinders, so re-colonization of the pre-sterilized sediment through aerosols was possible.
These initial results of our controlled setup are encouraging, and allow for the planning of experiments where we can specifically manipulate parts of the microbiome in this setup. This will allow testing of direct effects of bacterial species and consortia, providing empirical insights in the factors influencing eelgrass seedling success.

O022

Vegetative Cell and Spore Proteomes of Peptoclostridium difficile show finite differences and reveal potential biomarkers

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Vegetative Cell and Spore Proteomes of Peptoclostridium difficileshow finite differences and reveal potential biomarkers

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Introduction: Clostridium difficile-associated infection (CDI) is a health-care-associated infection mainly transmitted via highly resistant endospores from one person to the other. In vivo, the spores need to germinate in to cells prior to establishing an infection. Bile acids and glycine, both available in sufficient amounts inside the human host intestinal tract, serve as efficient germinants for the spores. It is therefore, for better understanding of Peptoclostridium difficilevirulence, crucial to study both the cell and spore state with respect to their genetic, metabolic and proteomic composition.
Methods: In the present study, mass spectrometric relative protein quantification, based on the $^{14}N/^{15}N$ peptide isotopic ratios, along with the one-pot sample processing method, has been achieved. For this purpose a special ^{15}N-yeastolate medium has been used for the growth and labeling of the vegetative cells. The ^{14}N spores have been prepared in CloSpore medium.
Results: The analysis has led to quantification of over 700 proteins from combined spore and cell samples and revealed that the proteome turnover between a vegetative cell and a spore for this

organism is moderate. Additionally, specific cell and spore surface proteins, vegetative cell proteins CD1228, CD3301 and spore proteins CD2487, CD2434 and CD0684 are identified as potential biomarkers for P. difficileinfection.
Conclusion:
1) The one-pot sample processing method along with ^{15}N metabolic labelling using ^{15}N-yeastolate has, for the first time, enabled a reproducible, combined cell and spore quantitative proteome analysis of the anaerobic pathogen P. difficile630. The method is extendable to other spore-forming as well as other pathogenic or non-pathogenic microbes with complex media requirements.
2) The analysis outlines a relatively modest proteomic adaptation of this evolutionarily and clinically important anaerobic pathogen, when as a survival strategy, it completes spore formation.
3) The analysis has revealed specific cell and spore surface proteins, vegetative cell-specific proteins and spore-specific proteins as potential candidate biomarkers.

O023
Inhibition of bacterial Type-5 secretion by small molecules
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Introduction. Promising targets for the development of novel antibiotics are bacterial virulence factors, which are required to establish and maintain an infection within the host and to spread to a new host. Most virulence factors are secreted across the bacterial cell-envelope to exert their function in the host, for instance as adhesins or toxins. Hence, interference with bacterial secretion systems would be a valid strategy to interfere with bacterial infections. In Gram-negative bacteria most secreted virulence factors are secreted via the autotransporter (AT) pathway, also known as type V secretion. AT secretion is semi-autonomous and only requires the assistance of two membrane-based protein complexes: Sec and Bam. Importantly, ATs play a crucial role in the establishment of severe infections, like meningitis, whooping cough and peritonitis.
Methods. To screen for AT inhibitors we developed a simple fluorescence-based high-throughput assay. The assay reports on the stress induced by the accumulation of the model AT hemoglobin protease (Hbp), expressed from a plasmid in Escherichia coli (E. coli), in the periplasm when secretion is inhibited at the level of the outer membrane. Here, we present data demonstrating successful application of the assay to identify compounds that inhibit secretion of Hbp, but do not inhibit bacterial growth.
Results. We first validated the stress assay by expressing the Hbp secretion incompetent mutant Hbp-110C/348C, known to accumulate in the periplasmic space and to trigger the cell-envelope stress response. After extensive optimization we found a robust increase in fluorescent intensity upon expression of Hbp-110C/348C compared to wildtype Hbp, showing that E. colicells that experience cell-envelope stress can be identified. After validation we screened 1600 compounds from the VUF fragment library and found one fragment-based compound that leads to cell-envelope stress upon Hbp expression. With a secondary assay we confirmed that this compound indeed inhibits Hbp secretion in E. coliin a dose-dependent manner.
Upon further characterization of the compound we observed that the inhibitory effect on AT secretion is generic. However, the compound does not interfere with other secretion systems present in pathogenic Gram-negative bacteria, such as Type-III secretion. Interestingly, the compound also affects the insertion of outer membrane proteins (OMPs), a process that, like AT secretion requires a functional Bam complex in the outer membrane. In addition, compound treated cells do not show general “heat shock” stress and only show growth inhibition at very high concentrations of compound. Finally, the compound induces the release of vesicles that appear to assemble in short chains

Conclusion. (1) We developed an assay that can be used to screen for compounds that interfere with AT secretion. (2) In an initial screen we identified an AT inhibitor that might target the Bam-complex.

O024
How are A. fumigatus conidia protected against peroxides?
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Introduction: Fungi protect themselves against ROS (Reactive Oxygen Species) components using superoxide dismutates, catalases and melanin. DHN-melanin in the cell wall of conidia of A. fumigatus and catalases were previously shown to be involved in conidial-peroxide protection. Here we investigated the role of DHN-melanin and catalases in more detail to elucidate the mechanisms of

conidia of A. fumigatusto protect themselves against peroxide.
Methods: The sensitivity of the hyphae was measured using a peroxide plate assay. Peroxide was added to a hole in the middle of the plate and after 16 hours of incubation the inhibition zone was measured. Sensitivity of conidia to peroxide was measured by adding conidia to a peroxide solution and plating serial dilutions at different time points, after overnight incubation the CFU's were counted. Expression of the catalase genes was monitored by RNA isolation and a qPCR analysis.
Results: Peroxide-plate assays showed increased peroxide sensitivity of a pksP, lacking DHN-melanin, (UV mutant) as compared to its WT background (ATCC46645) and, remarkably, the pksPcomplemented strain was not rescued. Interestingly, in this assay no difference in peroxide sensitivity was observed between a pksPdeletion mutant and its WT (CEA10). However, when dormant conidia of these WT and pksPmutants were directly exposed to peroxide in solution no difference in sensitivity was observed. Analysis of gene expression of different catalase genes in dormant conidia using qPCR indicate that the expression of the catA, cat1 and cat2 genes was strongly increased in CEA10 ΔpksPbut actually reduced in the ATCC ΔpksPmutant.
Conclusion:
These results suggest that absence or presence of DHN-melanin and expression of catalases in dormant conidia is not correlated with conidial-peroxide sensitivity since no difference was observed in direct peroxide exposure in solution.
Differences in catalase gene expression might explain the differences in peroxide sensitivity between the two different pksPmutants in the plate assay.

O025

Gene co-expression analysis identifies gene clusters associated with isotropic and polarized growth in Aspergillus fumigatus conidia

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Background
Aspergillus fumigatusis a saprophytic fungus that extensively produces conidia. These microscopic asexually reproductive structures are small enough to reach the lungs. Germination of conidia followed by hyphal growth inside human lungs is a key step in the establishment of infection in immunocompromised patients. Until now, transcriptome and proteome studies in A. fumigatuswere only performed on breaking of dormancy and early germination. In this study, we used RNA-Seq and focused on the later stages of germination in A. fumigatus. These stages are characterized by two distinct morphological phases as observed in A. niger.The first morphological change is swelling of the cell, referred to as isotropic growth. The second, polarized growth is characterized by the formation of a germ tube. These morphological changes are probably induced by a selective set of genes. Therefore, RNA-Seq was used to analyze the transcriptome of dormant and germinating A. fumigatusconidia.
Methods
A.fumigatusstrain AfIR974 and strain AfIR964 were used in duplicate for transcriptome analysis. RNA was extracted on five different time points in germination and the mRNA library was constructed using TruSeq® RNA Sample Preparation v2 Guide (Illumina®, Inc., San Diego, USA) according to manufacturer's instructions. We constructed a co-expression network, identifying genes with similar expression patterns. These expression patterns may be associated to the distinctive morphological phases seen in conidial germination. Additionally, the constructed co-expression network was used to detect highly connected genes in the module.
Results
Construction of a gene co-expression network revealed four gene clusters (modules) correlated with a growth phase (dormant, isotropic growth, polarized growth). Transcripts levels of genes encoding for secondary metabolites were high in dormant conidia. During isotropic growth, transcript levels of genes involved in cell wall modifications increased. Two modules encoding for growth, cell cycle and DNA processing were associated with polarized growth. In addition, the co-expression network was used to identify highly connected intermodular hub genes. These genes may have a pivotal role in the respective module and could therefore be compelling therapeutic targets.
Conclusion
Generally, cell wall remodeling is an important process during isotropic and polarized growth, characterized by an increase of transcripts coding for hyphal growth, cell cycle and DNA processing when polarized growth is initiated.

O026

The impact of active HRMO surveillance in patients with prolonged hospitalization

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Introduction
In healthcare facilities, active surveillance of patients who are at risk for carriage of highly resistant microorganisms (HRMO) is one of the key pillars to prevent cross-contamination, allowing early detection. Nevertheless, a substantial proportion of HRMO is detected unexpectedly in patients without known risk factors. Increased length of in-hospital stay, among other factors, has been associated with a higher risk of HRMO-carriage. We assessed the impact of a new policy comprising active surveillance for HRMO in patients hospitalized ≥14 days.

Methods
Retrospective data were collected from patients hospitalized ≥14 days at the Rijnstate Hospital, Arnhem between December 2016 and March 2018. Patients were tested for methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococcus (VRE), and carbapenem-resistant Enterobacteriaceae (CPE) by culture and molecular testing performed on rectal, throat and nasal swab samples. Source and contact-tracing investigations were performed by the infection control team. Main outcome variables were the number of contact tracings indicating nosocomial transmission and the number of MRSA, VRE and CPE-carriers identified using active surveillance in patients hospitalized ≥14 days.

Results
A total of 1899 screening sets from 1765 patients were analysed. In 24 patients culture results were positive for HRMO: 13 isolates were VRE positive and 11 were MRSA positive, accountingfor 40.7% (11/27) of newly identified hospitalized MRSA carriers.On two occasions, contact-tracing investigations revealed nosocomial spread after which further transmission was prevented by reinforcing infection control measures. No CPE-carriers were identified.

Conclusion
Identification of patients with prolonged hospitalizations carrying MRSA and VRE has led to transmission-based precautions and contract tracings to reduce the risk of transmission to other patients and healthcare workers. Our findings suggest that active surveillance might be extended from traditional risk groups to patients with prolonged hospitalizations.

O027

The investigation of a nosocomial outbreak with vancomycin resistant enterococci; a comparison of amplified fragment length polymorphism and whole genome sequencing

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Objectives.Recognition of nosocomial outbreaks with antimicrobial resistant (AMR) pathogens and appropriate infection prevention measures are essential to limit the consequences of AMR pathogens to patients in hospitals. Because unrelated, but genetically similar AMR pathogens may circulate simultaneously, rapid high-resolution molecular typing methods are needed for outbreak management. We compared whole genome sequencing (WGS) and amplified fragment length polymorphism (AFLP) during a nosocomial outbreak of vancomycin-resistant Enterococcus faecium(VRE) that spanned 5 months.
Methods.Hierarchical clustering of AFLP profiles was performed using unweighted pair-grouping and similarity coefficients were calculated with Pearson correlation. For WGS-analysis, core single nucleotide polymorphisms (SNPs) were used to calculate the pairwise distance between isolates, construct a maximum likelihood phylogeny and establish a cut-off for relatedness of epidemiologically linked VRE isolates. SNP-variations in the vanBgene cluster were compared to increase the comparative resolution. Technical duplicates of 2 isolates were sequenced to determine the number of core-SNPs derived from random sequencing errors.
Results.Of the 721 patients screened for VRE carriage, AFLP assigned isolates of 22 patients to the outbreak cluster. According to WGS, all 22 isolates belonged to ST117 but only 21 grouped in a tight phylogenetic cluster and carried vanBresistance gene clusters. Sequencing of technical duplicates

showed that 4-5 SNPs were derived by random sequencing errors. The cut-off for relatedness of epidemiologically linked VRE isolates was established at <10 core-SNPs. The discrepant isolate was separated from the index isolate by 61 core-SNPs and the vanBgene cluster was absent. In AFLP analysis this discrepant isolate was indistinguishable from the other outbreak isolates, forming a cluster with 92% similarity (cut-off for identical isolates ≥90%). The inclusion of the discrepant isolate in the outbreak resulted in the screening of 250 patients and quarantining of an entire ward. Conclusion.Compared to AFLP, WGS provided higher resolution with implications for outbreak management.

O028

Mechanisms of colistin resistance in nosocomial Escherichia isolates.

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Introduction: The increasing rate of multidrug resistance in nosocomial infections with Gram-negative bacteria has led 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. In this study, we examined the mechanisms by which clinical E. colistrains have evolved resistance to colistin.
Methods: Ten colistin-resistant E. colistrains from bloodstream infections were obtained from the diagnostic laboratory of our hospital. Whole genome sequencing was used to identify SNPs and indels that differentiated the genomes of colistin resistant E. colistrains from the most closely related strain from a set of 210 publicly available Escherichiachromosomal sequences. The effects of the identified mutations in basRSwere determined by expression of alleles of basRSclinical strains, and those in which mutations had been reversed through inverse site-directed PCR mutagenesis, in a basRSdeletion mutant in E. coliBW27848. Lipid A modifications were determined by MALDI-TOF.
Results: A total of 1140 Escherichiastrains from bloodstream infections isolated in the period 2006-2015 were available, of which 10 were colistin resistant (0.87%). Phylogenetic analysis showed that the colistin-resistant E. colistrains were not clonally related, and that one E. albertiistrain had been mistyped as E. coliin standard clinical procedures. Whole genome sequencing showed that colistin resistance was associated with mutations in genes encoding the BasRS (PmrAB) two-component system in nine strains, while one strain carried the colistin resistance gene mcr-1. Expression of the mutated basRSalleles showed that five mutated alleles caused colistin resistance in E. coliBW27848. The reversal of the identified mutations in these alleles, caused the strains to become susceptible to colistin again. Lipid A modification by phosphoethanolamine was observed in all clinical resistant strains, with additional palmitoylation in three strains.
Conclusion: In our collection of invasive Escherichiastrains, the mechanisms that lead to colistin resistance are limited to mutations in the gene encoding the BasRS two-component system and the acquisition of mcr-1. Colistin resistance in invasive E. coliappears to be rare, presumably due to the limited number of viable evolutionary trajectories that lead to resistance.

O029

Antimicrobial evaluation of a 3D printed dual antibiotic-loaded PLGA microparticles-GelMa hydrogel

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Introduction: taphylococcus aureus(. aureus) is one of the most common pathogen in orthopaedic-associated infections. Total hip arthroplasties show infection rates of 0.5 to 1% and total knee arthoplasties of 1-4%. Fracture fixation devices have infection rates ranging from 1 to 2% for closed fractures and even higher rates up to 30% for open fractures. These infections are not easy to diagnose and extremely difficult to treat due to the ability of the pathogens in biofilm to resist the immune response and antibiotic treatment. Clinically, implant infections are prevented by antibiotic prophylaxis, the placement of antibiotic laden bone cements, and the use of minimally invasive surgical procedures. However, conventional biomaterials such as bone cement are compatible with a limited number of

antibiotics and they show poor antibiotic release profiles. It is therefore necessary to develop novel antimicrobial delivery strategies incorporated in the implant or as a coating to prevent infection.
Methods: In order to meet this requirement, a 3D printed dual gentamicin and rifampicin delivery system based on gelatin methacrylate (GelMa) hydrogels was developed as a biomaterial coating. GelMa hydrogels have been widely investigated as tissue regeneration scaffolds and for generation of bioartifical implants. The antimicrobial release activity was evaluated in vitroagainst . aureusRN4220, and against derived strains resistant to either gentamicin or rifampicin, or against a combination of both antibiotic resistant strains.
Results: The GelMA scaffold co-delivering gentamicin and rifampicin was able to eradicate the mixture of . aureusstrains resistant to gentamicin and to rifampicin. In addition, confocal and electron microscopy confirmed the efficacy of the scaffold.
Conclusion: In conclusion, 3D printing GelMa scaffolds show promising in vitroresults as an antimicrobial drug delivery system to prevent orthopedic implant infections. Moreover, 3D printing enables the design of a gradual and controlled antibiotic release system that can be tailored to different types of implants and geometries.

O030

Methicillin-resistant Staphylococcus argenteus from surveillance specimen misidentified as methicillin-resistant Staphylococcus aureus

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Introduction
MRSA is an important pathogen that has been detected as cause of a variety of infections and has been responsible for major outbreaks in clinical settings. In the Netherlands, the so-called 'search and destroy' policy, which includes screening for MRSA in patients suspected for MRSA carriage, has been of great importance in preventing the spread of MRSA. For the sake of both the patient and the infection control within a clinical setting, correct identification of MRSA is of great importance. We present a case of a falsely identified MRSA, obtained from a throat swab as part of the MRSA surveillance.
Methods
MRSA diagnostics was performed according to the laboratories routine practice: swabs were inserted in the enrichment broth (MHB + 6.5% NaCl) and incubated overnight. Real-time PCR was performed targeting mecA/mecC, SA442 gene (Martineau) and PhHV (internal control). Culture was performed when mecA/mecC and SA442 genes were detected. . aureusisolates were identified by MaldiTOF and methicillin resistance by testing for susceptibility using both a cefoxitin disk and cefoxitin screen (Vitek). Confirmation was done by testing the isolate in the initial real-time PCR.
Every first MRSA isolate was sent to the national reference center (RIVM) for confirmation and MLVA typing. In addition, NGS was performed using the Illumina HiSeq 2500. The resulting data was used for MLST, cgMLST and ResFinder resistome analysis.
Results
The enrichment broth tested positive for mecA and SA442, of which subsequently a cefoxitin resistant . aureusisolate was cultured. Confirmation of the isolate was positive for mecA (Ct-value 21.7) and SA442 (Ct-value 41.3). Because of this unusual large difference in Ct-values, the isolate was also tested in the GeneXpert MRSA assay in which it was identified as MRSA. Other conventional, phenotypical methods all pointed towards the finding of MRSA.
At the reference center, the mecA PCR tested positive and the isolate was identified as . aureusby MaldiTOF. MLVA typing resulted in a non-typable isolate since only 2 of 7 tandem repeats could be identified. Using NGS data, MLST resulted in ST2250, a type not seen before in the Dutch MRSA surveillance. CgMLST failed with only 704 of the 1,861 available . aureusaccessory genes identified. Resistome analysis showed that the isolate carried the beta-lactamase genes mecA and blaZ, as well as the tet(K) and dfrG genes, conferring resistance to respectively tetracycline and trimethoprim. BLAST analysis of the five largest contigs eventually identified this isolate as taphylococcus argenteus(formally known as . aureusCC75).
Conclusion
Here, we present a methicillin resistant taphylococcus argenteus(MRSArg) that has been misidentified as MRSA by both the regular molecular methods and the conventional methods.
To the best of our knowledge, this species has not earlier been described in the Netherlands. Review of the literature shows that this species is endemic in South-East Asia and Australia, contains the same

virulence factors as and is able to cause comparable infections as . aureus.Especially for epidemiological reasons, correct identification and discrimination between . aureusand . argenteusseems to be obligatory.

O031

Cross-border occurrence of an OXA-48 plasmid identified in three different Enterobacteriaceae species.

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Background
Antimicrobial resistance is a worldwide problem. A “One Health” approach towards detection of resistance is important as resistant bacteria are found in all domains, from humans and animals to the environment. The i-4-1-Health project aims to elucidate the prevalence and transmission of antibiotic resistance in humans and animals in the Dutch Belgian cross-border region. To assess transmission within and among healthcare institutions in this project, collected resistant bacteria are characterized by whole genome sequencing to detect the presence of resistance genes and identify clonal relatedness of these isolates. Two perianal swabs, taken from a single patient, contained different bacterial species harboring an OXA-48 gene. This plasmid-associated gene encodes resistance to carbapenems, a class of last-resort antibiotics. As short-read sequencing is unable to determine whether plasmid transmission has occurred, a combination approach of short and long-read sequencing was applied to answer this question.
Methods
During ward-based prevalence surveys in six Dutch and three Belgian hospitals, 1.719 patients were screened for rectal carriage of antimicrobial resistant bacteria. Pre-enriched perianal swabs (FecalSwab, Copan Diagnostics, Italy) were selectively cultured for the presence of ESBL- and carbapenemase-producing Enterobacteriaceae and vancomycin-resistant enterococci using selective chromogenic agar plates (ChromID, bioMérieux). So far, 300 Dutch isolates were whole genome sequenced on the MiSeq platform (Illumina CA, USA). Assembled genomes were screened for the presence of acquired-resistance genes and typed using cgMLST (Bionumerics software package, Applied Maths). Long-read sequencing by Nanopore sequencing was performed for fiveOXA-48-positive (OXA-48+) Klebsiella pneumoniaeisolates, one OXA-48+ Escherichia coliisolate and one OXA-48 negative (OXA-48-)K. pneumoniae isolate with an identical cgMLST as the OXA-48+isolates, obtained from two perianal samples from one Dutch patient; two OXA-48+isolates (K. aerogenes and K. michiganensis) from two Belgian patients; and a 2011 Maasstad OXA-48+outbreak K. pneumoniae isolate. Sequencing data were assembled using Unicycler. Plasmid data analysis was performed with Bandage, and plasmid sequences were subsequently aligned using blastn.
Results
All 5 OXA-48+K. pneumoniae were clonally related with, on average, 1 locus difference between isolates on cgMLST. Interestingly, one susceptible K. pneumoniae from the same patient was also clonally related to this cluster, but was OXA-48-, indicating loss or gain of OXA-48 by this isolate or cluster. Analysis of the hybrid assemblies showed that all OXA-48 genes were located on a 64kb IncL/M plasmid. Multiple alignment of these plasmids revealed that these were identical, indicating horizontal gene transfer (HGT) by plasmid transfer between K. pneumoniae and E. coli. Remarkably, this plasmid was virtually identical (1 bp insert) to a plasmid found in an OXA-48+K. aerogenesfound in a Belgian hospital. Comparison to the NCBI nucleotide database showed that this plasmid was not previously identified.
Conclusions
1. Long read sequencing revealed the transfer of a novel OXA-48 carrying plasmid between pneumonia and E. coliobtained from the same patient.
2. A virtually identical plasmid was identified in a aerogenesisolated in a Belgian hospital, indicative for cross-border occurrence. Additional analysis of all OXA48+ isolates might shed light on the route of possible transmission

O032

Results from the IBESS study: comparison of infection risks, severity of disease and human-to-human transmission of infections with Shigella spp. and EIEC

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Introduction: higella spp.and entero-invasive Escherichia coli(EIEC) are bacteria causing the same disease, and are difficult to distinguish in Medical Microbiological Laboratories (MMLs). This hampers application of infectious disease control regulations, as physicians and MMLs are obligated to report shigellosis cases towards health authorities, while EIEC infections are not under such regulations. In 2016 and 2017, the cross-sectional Invasive Bacteria E. coli/ShigellaStudy (IBESS) was conducted to get more insights into risk factors for infection with, disease outcomes and human-to-human transmission of EIEC and to compare them to infections with higella spp.
Methods: Patients were included if they had intestinal complaints and higella spp./EIEC was detected in their fecal sample, submitted to one of the 15 participating MMLs for regular diagnostics. Patients were then requested to participate in IBESS and answered, with their consent, multiple questions about their demographics, known risk factors for shigellosis, experienced symptoms, and their knowledge of related patients. From the symptoms, two different severity scores were calculated, both were modifications of the Vesikari Score (de Wit, Kortbeek et al. 2001, Freedman, Eltorky et al. 2010). Differences in infections with higella spp.compared to infections with EIEC were calculated using multivariate regression models with the following confounders: sex, age and MSM contact of patients, co-infections, effect of underlying diseases, medication use, and bacterial load. All analyses were performed using SAS 9.4 and R 3.4.3., significance being defined as $p < 0.05$.
Results: Patients with EIEC infections are more likely to have a co-infection with another enteric pathogen (9%) in comparison to patients with shigellosis (2%). 53% of EIEC cases reported ingestion of contaminated food and 3% reported MSM contact as sources of infection, against 26% and 22% of patients suffering from shigellosis ($p = 0.02$). EIEC cases reported longer duration of diarrhea (median (IQR) = 14 (7-21 days) and more excrements when vomiting (median (IQR) = 3 (1.5-9 excrements/24H) than cases with Shigellosis, with 10 (6-14) days of diarrhea and 2 (1-4) excrements per day, with $p < 0.0001$ and $p = 0.001$, respectively. When calculating severity scores, one of the severity scores showed a significant difference ($p = 0.045$) between shigellosis (mean ± SD: 7.5 ± 2.7 points) and EIEC infections (6.4 ± 2.6 points), the other score showed no significant difference ($p = 0.943$) between shigellosis (7.3 ± 2.8 points) and EIEC infections (7.4 ± 3.3 points). No significant differences were detected in the presence of human-to-human transmission or the number of related patients in EIEC infections compared to shigellosis.
Conclusion: The distribution of infection sources of EIEC infections is significantly different from shigellosis cases. Although some individual symptoms indicate a more severe course for EIEC cases, this is not reflected in calculated severity scores, which indicate a more severe course or equal severity for shigellosis. The minimal differences in infection risks, severity of disease and human-to-human transmission, do not support the current difference in approach of infectious disease control regulation for infections with EIEC compared to Shigellosis.

O033

Culture-free genotyping of Neisseria gonorrhoeae revealed distinct strains at different anatomical sites in a quarter of patients

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Background: Resistance of Neisseria gonorrhoeae(NG) is increasing for a wide range of antibiotics. Therefore, surveillance of circulating strains is important. A widely used technique for surveillance is N. gonorrhoeaemulti-antigen sequence typing (NG-MAST) genotyping that provides data on transmission of NG. NG-MAST relies, however, on culture and culture success rates are low, particularly in extra-genital samples. Our aim was to develop a culture-free genotyping method that is compatible with NG-MAST database, which facilitates genotyping of NG detected at separate anatomical sites in individual patients. Furthermore, we aimed to gain insight in the frequency of patients infected with distinct NG strains at separate anatomical sites.
Methods: Novel NG specific primers for both NG-MAST PCR targets porBand tbpBwere designed and

technically validated by assessing the analytical sensitivity, cross-reactivity with 32 non-gonoccocal Neisseriaspecies, and concordance with NG-MAST. Amplified porBand tbpBtargets were characterized using Sanger sequencing with NG-MAST primers. Clinical application was assessed on selected paired clinical samples (n = 205 samples) from concurrent NG infections at different anatomical sites of 98 patients (81 men who have sex with men and 17 women) visiting our sexually transmitted infections clinic.
Results: Typing could be consistently performed on samples with a PCR quantification cycle (Cq) value <35. Furthermore, none of the 32 non-gonoccocal Neisseriaspecies were PCR-positive for either porBor tbpBand our method was concordant with NG-MAST. Routine culture was performed for 95 of the 205 clinical samples and 59 (62.1%) were culture-positive. Culture-free NG-MAST improved the typing rate from 62% (59/95) for cultured samples to 94% (89/95) compared to culture-dependent NG-MAST. In total, 90.2% (185/205) of the selected paired clinical samples were successfully genotyped with our method. Paired samples of 80 of 98 patients were genotyped, revealing distinct NG strains in separate anatomical sites in 25% (20/80) of the patients. In three samples, the notorious genogroup G1407, linked with ceftriaxone resistance in NG, was observed of which only one (ST2212) was culture-positive and susceptible for ceftriaxone.
Conclusions: This NG-specific genotyping method can improve NG surveillance as it facilitates genotyping of non-culturable and extra-genital samples. Furthermore, a quarter of patients were infected with multiple NG strains, which is missed in current culture-dependent surveillance. Inclusion of data on non-culturable and concurrent NG strains will improve NG surveillance and might provide more insight in transmission networks.

O034

Import of Brucella canis positive dogs into the Netherlands - implications for animal and human health

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Brucellosis is a zoonosis caused by bacteria from the genus Brucella. In the Netherlands brucellosis is notifiable in humans and all mammal species. Brucellaspp. had never been isolated from dogs in the Netherlands till November 2016 when the first canine case was detected.
This first case was a 1 year old, castrated, female mixed breed dog which was imported from a rescue centre in Romania in February 2016. The dog showed clinical signs of back pain and impaired range of motion. A CT-scan showed a multifocal discospondylitis. Bacteriological culture from the affected intervertebral discs was found positive for Brucella canis. The dog was serologically positive (SAT = 400 IE/ml), urine culture was negative. As the dog already had clinical signs upon arrival in the Netherlands, this case was considered an import case from Romania.
To raise awareness among veterinarians, information was disseminated by signalling services, websites and newsletters. This resulted in more than 30 notifications at the Netherlands Food and Consumer Product Safety Authority in 2017 and 2018. So far, 19 dogs were found serologically positive for B. canis. In 11 of these serological positive dogs, B. caniswas cultured from urine (n=5), blood (n=3), intervertebral disc (n=1), synovial fluid (n=1) and post mortal from spleen and lymph nodes (n=1). All serological positive dogs were imported from Eastern Europe: Romania (n=8), Bulgaria (n=10) and Croatia (n=1). The most prominent clinical signs in the dogs were back pain and impaired range of motion; in 8 dogs a CT-scan showed a multifocal discospondylitis.
A risk assessment was performed to evaluate potential hazards for animal and public health. All Dutch B. caniscases were considered import cases. Import of dogs from Eastern Europe occurs frequently and poses a potential threat of importing B. canisinto the Netherlands. Infected dogs might show reproductive problems (e.g. orchitis and abortion), but in castrated dogs, lameness and spinal pain are most prominent. Transmission of B. canisamong dogs occurs by direct contact; in addition to discharge from reproductive organs, B. caniscan be shed in urine, saliva and nasal secretions. Antimicrobial treatment of dogs with brucellosis might lead to clinical improvement, but the bacterium will not be eliminated. Therefore, clinically recovered dogs might still pose a threat of spreading B. canis.To prevent further spread of the infection castration of intact dogs or euthanasia might be justifiable measures.
Transmission of B. canisfrom infected dogs to humans is possible through direct contact with infected dogs or their excreta. Laboratory workers, veterinarians and animal care takers might be at increased risk. In general B. canisseems to cause less severe infections in humans compared to other Brucellasp., but a proper risk assessment can only be performed when more information is available. The prevalence

of human B. canisinfections is probably underestimated as the diagnosis might be missed due to nonspecific clinical signs and absence of accurate serological tests for B. canisantibodies in humans.

O035

Nontuberculous mycobacterial pulmonary disease and Aspergillus co-infection: Bonnie and Clyde?

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Introduction: Patients with chronic obstructive pulmonary disease, cystic fibrosis or bronchiectasis are prone to nontuberculous mycobacterial pulmonary disease (NTM-PD) and other opportunistic infections including infections by Aspergillus fumigatus. Co-infections are difficult to identify as diagnostic criteria for NTM-PD and chronic pulmonary aspergillosis (CPA) overlap. Aspergillusserology is part of NTM-PD diagnostic work up in our reference center. In a retrospective cohort study, we assessed the frequency of AspergillusIgG seropositivity and its relation to disease outcome in patients with NTM-PD. Furthermore, we studied symbiosis of Mycobacterium aviumand M. abscessuswith A. fumigatus, in vitro.
Methods: We selected all patients in our center who met British Thoracic Society (BTS) diagnostic criteria for NTM-PD between January 2015-January 2018 and had AspergillusIgG serology results. Patients with Cystic Fibrosis were excluded from the analysis. For all included patients, we registered clinical, microbiological and radiographic features. We applied NTM-NET definitions for NTM-PD treatment outcomes and European Respiratory Society diagnostic criteria for CPA.
In vitrosymbiosis of A. fumigatuswith M. aviumATCC700898 and M. abscessusCIP104536 was assessed using CAMH medium supplemented with supernatant of the other genus. Cultures of A. fumigatuswere supplemented with mid-log phase and stationary phase supernatants of M. abscessusCIP104536 and M. aviumATCC700898 liquid cultures. NTM growth was measured by colony forming unit (CFU) counting of liquid cultures at defined time intervals. Growth of liquid A. fumigatuscultures was evaluated by determination of optical density over time.
Results: Forty-seven patients met the inclusion criteria (53% female, mean age 64.4±9.7 years). Thirty (63.8%) patients showed positive AspergillusIgG, with a mean level of 67.2±56.1 mg/L. Thirty-seven patients had sputum fungal cultures performed yielding Aspergillusspecies in 19 patients (51.4%; 18 A. fumigatus, 1 A. niger). Fourteen of all 47 patients (37.8%) had positive cultures with Aspergillusand elevated AspergillusIgG levels and thus met CPA diagnostic criteria. Thirty-three patients received >6 months of treatment for NTM-PD. Microbiological cure rates at the end of NTM-PD treatment were lower for AspergillusIgG-positive patients (27%) than for IgG-negative patients (50%; p=0.036)
M. abscessusand M. aviumgrowth rate was not influenced by A. fumigatusculture supernatants. A. fumigatusshowed a strongly decreased growth rate in medium supplemented with M. abscessussupernatant (optica density [OD] after 70 hours 0.43 vs 0.67). On the contrary, M. aviumsupernatant increased the A. fumigatusgrowth rate, compared to non-supplemented growth controls (OD after 70 hours 1.15 vs 0.67).
Conclusions: 1) In this NTM-PD cohort, co-infection with Aspergillus fumigatuswas common as 64% percent of the patients had positive AspergillusIgG. 2) Fourteen NTM-PD patients (37.8%) met diagnostic criteria for CPA, with positive cultures and AspergillusIgG positivity. 3) Treatment outcomes of NTM-PD are worse in AspergillusIgG-positive patients, compared to AspergillusIgG-negative patients. 4) M. aviummay aggravate Aspergillusinfection by direct interaction, promoting Aspergillusgrowth. 5) All patients with NTM-PD need to be screened for Aspergillusco-infection.

O036

Diagnostic blood PCR and metagenomics in community acquired pneumonia.

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Introduction In severe acute infections like pneumonia, rapid information on species, virulence factors, and antibiotic resistance markers can impact treatment decisions. In addition, bacterial genotyping is essential for surveillance purposes. Metagenomics applied directly on patient specimens could overcome delay from blood culture, subsequent analysis and the limitation on number of genetic targets tested. We studied the feasibility of metagenome sequencing on serum samples from pneumonia patients.
Methods Serum samples were collected from 200 adults presenting to the emergency department at the St. Antonius Hospital in Nieuwegein with community acquired pneumonia between 2004-2006 and stored at -20^{o}C for >10 years. All patients underwent extensive systematic microbiological work-up

including serotype-specific serologic testing for . pneumoniae. Patients were stratified according to microbiology results, DNA was isolated from 200µl serum, and pneumococcal DNA-load was determined by qPCR. Selected specimens underwent metagenome sequencing (Illumina NextSeq, 3Gb per sample) and reads were compared to the genome of the corresponding blood culture isolate.
Results Currently we report on 50 patients, representing groups with particular microbiology results. Pneumococcal qPCR was positive on 200µl stored serum from 10 out of 17 patients with a pneumococcal bacteraemia. In non-bacteraemic pneumonia patients with a positive pneumococcal urinary antigen test, pneumococcal DNA was identified in 3 out of 10 tested samples. Furthermore, pneumococcal origin of pneumonia was demonstrated in 2 out of 11 patients who had been receiving antibiotics, without any micro-organism identified by extensive routine microbiology testing. No serum was positive among 10 patients with solely pneumococcal seroconversion and 2 patients with confirmed Legionella pneumophilainfection. Metagenome sequencing on sera with a pneumococcal DNA load of 10^4 genome copies/ml resulted in 2/3 capture of the entire pneumococcal genome, with accurate identification of the prophage virulence factor pblBpredictive of 30-day mortality in pneumococcal disease.
Conclusion The presence of pneumococcal DNA in stored serum samples from pneumonia patients was identified by qPCR. In the current study we demonstrated the feasibility of subsequent metagenome sequencing on sera, providing genotypic information on species and virulence factors. Ongoing follow-up studies aim to assess the clinical value of serum metagenomics at varying bacterial loads for the entire cohort.

O037

Parainfluenzavirus infections in lung transplant recipients; from bench to bedside

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Introduction: Community Acquired Respiratory Virus (CARV) infections are an important source of morbidity after Lung transplantation. CARV infections are associated with new onset of Chronic Lung Allograft Dysfunction. Among these CARV infections, human parainfluenza virus (HPIV) infections have been recognized as a significant cause of morbidity in the form of Bronchiolitis Obliterans Syndrome (BOS) in lung transplant recipients (LTR). Ribavirin has been proposed as potential treatment for paramyxoviruses, but there is no consensus over the effectiveness of this drug, especially for HPIV.
Methods: We report a bedside to bench and back strategy by using an in vitromodel of human airway epithelium cells of bronchial origin (HuAEC) in an air-liquid-interface to study the efficacy of experimental HPIV-3 inhibitors with subsequent retrospective assessment of the effectiveness of ribavirin treatment in these patients.
Results: 12 LTR were identified with an HPIV infection with median FEV1 decline of 8.3% (IQR 21%); 9 had BOS grade < 3, of whom 6 developed new or progressive BOS.
We then studied the replication of HPIV-3 on HuAEC and the antiviral effect of nucleoside analogues. Apical infection with a clinical isolate of HPIV-3 showed significant viral replication. Cells were exposed, at the basal site, to nucleosides (100 µM ribavirin or 100 µM favipiravir) from days -2 until +4 post-infection. A clear inhibition (>4 log HPIV-RNA reduction) of HPIV-3 replication in the presence of ribavirin was observed while there was no effect of favipiravir. When ribavirin treatment was stopped, HPIV-3 replication increased in 48 h. to the level of untreated cultures.
Lastly we compared outcomes of 21 ribavirin treated vs. the 12 untreated LTRs. Overall incidence of new or progressive BOS at 6 mo. post-infection was 10/28 (< BOS 3; 36%), median FEV1 decline was 4.9% compared to pre-infection (p<0.01). At baseline no differences in BOS-grade pre-infection, FEV1 decline at presentation, underlying disease, time since transplantation, coinfections or HPIV-subtype between the groups were seen. Ribavirin treated LTR showed significantly lower median FEV1 decline 6 mo. post-infection vs.pre-infection compared to ribavirin untreated LTR (2.9% [IQR 4.7%] vs.8.3% [IQR 21%] p=0.04). Incidence of new or progressive BOS was also significantly lower in the ribavirin group compared to ribavirin untreated (4/19 [21%] vs.6/9 [67%] p=0.04).
Conclusion: In vitroresults of ribavirin are confirmed by observational data on HPIV infections in LTR. Preservation of long-term FEV1 in LTR seems a potential benefit of ribavirin but needs to be studied in a larger cohort.

O038

Benzene degradation under anoxic conditions by a nitrate-reducing bacterium that lacks

anaerobic hydrocarbon degradation pathways

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Benzene is an important aromatic component of petroleum that can be readily degraded aerobically, whereas anaerobic degradation is challenging due to the high stability of benzene under anoxic conditions. We recently reported concurrent transcription of genes involved in aerobic and anaerobic benzene degradation pathways in a benzene-degrading denitrifying continuous culture. The transcription of genes encoding enzymes involved in oxygenase-mediated aerobic benzene degradation suggested the presence of oxygen, possibly formed via a nitric oxide dismutase. However, oxygen production from denitrification for benzene degradation could not be unequivocally observed using the denitrifying microbial consortium. Here, we report the isolation of Pseudomonas stutzeristrain BN from this consortium. Strain BN showed indications of oxygen production from denitrification during anaerobic benzene degradation. Interestingly, the genome of strain BN neither encodes any of the known anaerobic hydrocarbon degradation pathways nor nitric oxide dismutase, but in contrast, encodes several oxygenase-dependent aerobic pathways for aromatic compound degradation. These findings imply that strain BN uses novel aromatic degradation pathways in which oxygen or a nitrogen compound formed during denitrification can be used for benzene activation. Further experiments are underway to define the underlying genes and pathways using a combination of transcriptomic and proteomic analysis, genome editing using CRISPR-Cas, stable isotope labeling, and compound-specific isotope analysis.

O039

Complexome analysis of the nitrite-dependent methanotroph Methylomirabilis lanthanidiphila

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The anaerobic bacterium Methylomirabilis lanthanidiphilacouples the oxidation of methane to CO_2 to the reduction of nitrite to N_2 in the absence of oxygen. To activate methane, M. lanthanidiphilais proposed to employ an intra-aerobic pathway. Nitrite is first reduced to NO by cytochrome cd_1nitrite reductase. Two molecules of NO are subsequently dismutated into O_2 and N_2 by a putative NO dismutase. This intracellularly produced O_2 can then be used to activate methane by particulate methane monooxygenase. Genomic analysis identified two genes that encode putative candidate proteins, which could catalyze NO dismutation. In addition, there are a number of apparent redundancies in the genome of M. lanthanidiphila, including a quinol-dependent NO reductase, two additional divergent NO reductases, one aa_3oxidase, two Rieske-cytochrome bcomplexes and two membrane-bound and one periplasmic nitrate reductase. In this study, the complexome of M. lanthanidiphilawas determined to investigate if all protein complexes proposed to be involved in its metabolism were functionally expressed and if the apparent genomic redundancy was also present on protein level. Protein complexes were isolated from M. lanthanidiphilaand separated by Blue Native PAGE. Gel lanes were cut into 60 slices and after tryptic digestion peptides were analyzed using LC-MS/MS. This allowed hierarchical clustering of protein migration patterns and identification of the subunit composition of the detected complexes. The metabolic model of M. lanthanidiphilawas validated by identification of all major protein complexes. Moreover, the apparent genomic redundancy was corroborated on a protein level, especially for the proteins involved in nitrogen cycling. These proteins might play an important role in balancing the redox state and (toxic) substrate concentrations in the cell.

O040

Enrichment and physiological characterization of a novel comammox Nitrospira

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Introduction: Nitrification, the oxidation of ammonia to nitrate via nitrite, was considered to be catalyzed by two distinct microbial guilds. However, the recent discovery of the first complete ammonia oxidizers (comammox), belonging to the genus Nitrospira, overturned this paradigm. The widespread occurrence of comammox bacteria in engineered and natural ecosystems indicates their important role within the biogeochemical nitrogen cycle as well as their potential implications in many biotechnological processes.

In order to study the physiology and biotechnological relevance of these enigmatic microorganisms, highly enriched cultures are required. Here, we describe the first detailed physiological characterization of a novel comammox Nitrospiraspecies enriched from a recirculating aquaculture system.
Materials and methods:
Comammox Nitrospirabacteria were enriched using a continuous membrane bioreactor, supplied with limiting concentrations of ammonium. Metagenomic analysis and quantitative fluorescence in situhybridization (FISH) were employed in order to verify the enrichment of comammox bacteria in the enrichment culture. Moreover, high-throughput combined with long-read sequencing was used in order to obtain the closed genome of the highly enriched in the system comammox Nitrospira. The nitrification kinetics of the enrichment culture were inferred from substrate-dependent oxygen consumption rates determined by microrespirometry.
Results:
Continuous cultivation in a membrane bioreactor system under substrate limitation yielded a high enrichment of comammox Nitrospira. Comammox bacteria were found to be the only ammonia-oxidizing organisms present in the system while they constituted approximately 80% of the overall microbial community. By combining 2nd and 3rd generation sequencing we obtained the closed genomes of a novel comammox and canonical Nitrospiraspecies. Physiological characterization of the enrichment culture demonstrated high affinities for both ammonium and oxygen. The calculated ammonia affinity was in the range of non-marine ammonia-oxidizing archaea and similar to that of N. inopinata. Unexpectedly, a strong substrate inhibition was observed at extremely low ammonium concentrations.
Conclusions:
Our data indicate the adaptation of the enriched novel comammox Nitrospirato highly oligotrophic environments, as was predicted to be the main environmental driver selecting for comammox bacteria. This physiological adaptation suggests that comammox bacteria could outnumber canonical ammonia oxidizing prokaryotes in engineered ecosystems with low ammonium loading rates. In conclusion, this study presents novel physiological aspects for the first comammox enriched from an engineered habitat and further expands our understanding of the ecological niche of these organisms and their potential role in many biotechnological applications.

O041

Nitrogen removal pathways in enhanced simultaneous nitrification and denitrification

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Nitrogen removal is essential in wastewater treatment because nitrogen compounds are the main cause of eutrophication and can be detrimental to receiving natural water bodies. To remove nitrogen, wastewater treatment plants often combine nitrification and denitrification to convert toxic ammonia into harmless dinitrogen gas. The microorganisms that perform these two processes are often spatially separated as they operate under different conditions. However, simultaneous nitrification and denitrification (SND) can be achieved, as was observed during saline wastewater treatment (Wang et al., 2017). In SND, microbial communities performing nitrification and denitrification are simultaneously active in one reactor. This reduces treatment costs by reducing aeration requirements and the need to add organic carbon. Additionally, excess sludge removal would be greatly reduced. However, the mechanisms of SND are still not fully understood. In order to investigate the possible nitrogen removal pathways involved, a SND performing culture was obtained by inoculating a sequencing batch reactor with sludge from the aeration tank of a municipal wastewater treatment plant. The biomass was grown on polypropylene fibers and fed with synthetic wastewater containing a low carbon to nitrogen ratio. After 45 days of enrichment, the ammonia removal efficiency of the culture was 72%. The effluent nitrite and nitrate concentrations were lower than 0.01 mg/L, indicating that more than 97% of the ammonia removed was converted into mainly nitrogen gas. Metagenomic sequencing was performed to explore the microbial composition of the culture, which revealed Zoogloeaand Nitrospira-like organisms to constitute the most dominant microorganisms. Additionally, we investigated the different nitrogen conversion processes occurring in the enrichment culture using batch activity and inhibition assays. Surprisingly, these experiments indicated that processes not blocked by the common nitrification inhibitors contributed 55% of the total ammonia removal and produced 51% of the emitted greenhouse gas nitrous oxide. These results demonstrate the feasibility of SND to treat wastewater with low C:N ratios. A better understanding of the nitrogen conversion processes occurring in this culture will increase our knowledge about nitrogen removal mechanisms and help to improve sustainable wastewater treatment.
Reference

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O042

Bio-methanol production by the methanotrophic extremophile Methylacidiphilum fumariolicum SolV

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Methanol is an important chemical feedstock, used as fuel and added to the denitrification process in wastewater treatment plants. The annual production of methanol is estimated to be 138 billiard liters. The current methanol production process uses natural gas and produces methanol via a multistep process operated at high temperature (>1000 °C) and high pressure (100 bar). Only natural gas can be used for the chemical production methods, since more sustainable methane sources, such as biogas, contain too many impurities and too low methane concentration. Therefore there is an increasing demand to produce methanol from more sustainable resources at more ambient conditions. Methanotrophs are bacteria that generate energy by the oxidation of methane into carbon dioxide. The first step of the oxidation pathway consists of methane to methanol conversion catalyzed by the enzyme methane monooxygenase. In the second step, methanol is oxidized into formaldehyde by methanol dehydrogenase (MDH). After this, formaldehyde is converted into formate and in the final step, formate is oxidized into CO_2. In order to convert a methanotroph into a methanol producer, the MDH activity needs to be inhibited.
Methylacidphilum fumariolicumSolV is a thermoacidophilic methantroph growing at 55 °C and pH 3. This extremophile contains a Xox-type MDH which requires a lanthanide for its activity. The MDH is inhibited by removing the cofactor from the cultivation medium. In this study, methanol production is investigated in non-growing cell suspensions, in batch incubation and in continuous cultures. Our data showed that there was no methanol production observed in non-growing cells. In batch experiments, methanol production was only observed during the cultivation in absence of lanthanides. The effect of growth rate on methanol production rates and yields was investigated in chemostat culture. The culture was supplied with limited amount of lanthanides and hydrogen gas to supply sufficient energy for growth. This study shows that methanol production only occurs in growing cells, methanol production rate is growth rate dependent and the conversion efficiency increases with growth rate.

O043

Degradation of methoxylated aromatic compounds by a methanogenic archaeon: Unravelling central metabolism of Methermicoccus shengliensis

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The main source of the greenhouse gas methane are methanogenic archaea. This emphasizes 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.) shengliensisis 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 a methoxylated compound. The encoded enzymes resemble an enzyme system of acetogenic bacteria used for degradation of methoxylated aromatics and are most likely essential for methoxydotrophic methanogenesis. Four genes were chosen for heterologous expression in E. coliand subsequent purification by affinity chromatography. Next to this demethoxylation system, genomic and transcriptomic analysis also revealed two interesting soluble heterodisulfide reductase (Hdr) complexes to be present in Methermicoccus, associated with proteins resembling the F420 hydrogenase subunit beta (FrhB) and the formate dehydrogenase subunit alpha (FdhA). With bioinformatics and enzymological assays we aim to get further insight into the role of those complexes.
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O044

A human organoid model to study S. suis intestinal translocation

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Introduction: treptococcus suisis a zoonotic pathogen that can cause septic shock and meningitis in pigs and humans. Human infections are mainly caused by . suisserotype 2, while many other serotypes can be found in pigs. In humans, the consumption of undercooked or raw pig products contaminated with . suiscan lead to systemic infections, implying that the gastrointestinal tract is a potential entry point for the pathogen. We previously used Caco-2 cell monolayer as a model for the human gut epithelium and found an increased adherence of . suisserotype 2 to the Caco-2 cells, compared to serotypes 1 and 9. In contrast, the ability to translocate through the Caco-2 cell monolayer was greatly dependent on the . suisgenotype and especially . suisclonal complex 1 demonstrated high translocation. To further advance our research and gain novel insights in the host-pathogen interaction, such as cell type specific interactions by which . suiscrosses the human intestinal epithelium, we developed a two-dimensional human organoid derived intestinal epithelium model.
Materials/methods: Fetal derived organoids were cultured in matrigel domes and, after propagation, harvested and seeded on Transwell membranes. Monolayer formation was followed in time by measuring the trans-epithelial electric resistance. Cell differentiation markers and tight junctions were stained using specific antibodies. After 14 days of Transwell culture, (fluorescently labeled) . suiswere added to the apical side of the monolayer. Translocated . suiswas quantified and monolayer integrity and bacterial adherence are imaged by immunofluorescence staining.
Results: In the intestinal epithelial monolayer enterocytes, paneth cells, goblet cells, enteroendocrine cells and stem cells were identified and the presence of tight junctions and adherence junctions was confirmed. Additionally, cells showed an apical-basolateral differentiation. An initial translocation experiment showed that at a multiplicity of infection of 1, the monolayer prevents . suistranslocation. However, at a multiplicity of infection of 50, . suisserotype 2 was able to translocate and showed localization at the cell-cell borders.
Conclusions: 1) The generated model includes almost all cell types found in the human small intestine and enables research on host-pathogen interactions at the intestinal epithelial surface. 2) . suisserotype 2 is able to translocate through the intestinal monolayer. 3) Localization of . suisat the cell-cell border could indicate that . suistranslocates paracellular.

O045

The highly dynamic phospholipidome of C.jejuni

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In response to changes in their environment bacteria need to change their protein repertoire, but also the lipid composition in the membrane. The main components of bacterial membranes are phospholipids that are composed of two fatty acids, a glycerol moiety, a phosphate group and a variable head group. Bacteria can modify or replace existing phospholipids to adjust their membrane viscosity to match environmental requirements.
By using high performance liquid chromatography (LC-MS/MS) we identified and followed the changes that took place in the phospholipidome of the human pathogen C.jejuniby growing it for 4 days under different oxygen and carbon conditions. By RNA-seq we studied the transcriptional change of the genes involved in this process. The phospholipidome of C. jejunicompromises more than two hundred different phospholipids and it displays a high variation dependent on the oxygen availability or age of the Campylobacterculture. The ratio of the main phospholipidhead groups PE and PG changed rapidly by age of culture as well as the number of cyclopropane bond containing fatty acids. Hugh amounts of the phospholipids of C. jejuniare lysophospholipids (30-45%), which are produced by phospholipase PldA and influence the molitity of this bacterium at low oxygen conditions. As in several pathogenic bacteria, accumulation of lysolipids is crucial to cause disease or to survive after phagocytosis, the role of the C. jejuniphospholipids in Campylobacteriosis might be an underestimated factor.

O046

Bacteroides fragilis is more prevalent in Crohn's disease exacerbations while strengthening the intestinal epithelial barrier in a strain-dependent manner

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Background: Crohn's disease (CD) is a chronic relapsing inflammatory gastro-intestinal disease with a high disease burden. Until today, the pathophysiology is not completely understood. Next to host genetics and environmental factors, impaired intestinal barrier function and microbiota seem to play a role in the onset and course of CD. Among others, Bacteroides fragilishas frequently been associated with CD. In addition, recombinant B. fragilistoxin (Bft) was found to disrupt the intestinal epithelial barrier in vitroby cleaving the adherens junction protein E-cadherin. Furthermore, Ubiquitin was found as a potential virulence factor, acting on host immune response. This study therefore aims to investigate the role of B. fragilisin the pathophysiology of CD, focusing on its interaction with the intestinal epithelial barrier.
Methods: To investigate the presence of B. fragilis, B. fragilistoxin (Bft) and Ubiquitin genes, we selected 183 CD patients with active or remissive state from our extensive population-based IBD South Limburg cohort. Disease activity was determined by faecal calprotectine levels (<100 µg/g = remission; ≥250µg/g = exacerbation) and faecal DNA was investigated by qPCR. Data were analyzed using Chi-square test. To examine the impact of B. fragilison the intestinal epithelial barrier, we subsequently cultured and isolated six B. fragilisstrains with various genetic profiles of bftand ubiquitinfrom two healthy subjects, three CD patients and one ATCC strain (25285). Next, bacteria-free culture supernatant as well as outer membrane vesicles (OMVs) were isolated and luminally applied to colonic adenocarcinoma-derived Caco-2 cell monolayers. After 24 h incubation, the difference in transepithelial electrical resistance (TEER) was determined and compared to the vehicle control.
Results: B. fragilisprevalence was 15 % higher ($p<0.023$) in active CD patients compared to remission. Bftand ubiquitinprevalence was comparable in both groups. Interestingly, TEER results demonstrate that concentrated culture supernatant of bftpositive B. fragilisstrains increased the TEER ($p<0.001$) compared to bftnegative strains or vehicle control, suggesting an improved epithelial integrity. However, isolated OMVs of bftpositive or bftnegative strains did not show any alterations in TEER.
Conclusion: This study confirms in a large well-defined patient cohort that B. fragilis, but not bftor ubiquitinpositive strains specifically, is more prevalent in active CD, suggesting that it might play a role in exacerbations. Surprisingly, B. fragiliscomponents did not impair the epithelial barrier and components of bftpositive strains even improved intestinal barrier function, which warrants further investigation. This unexpected finding stresses the relevance of extending current research on the functional role of relevant microorganisms.

O047

Aroma formation by lactic acid bacteria at near-zero growth rates

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Lactic acid bacteria (LAB) encounter long periods of nutrient limitation during food fermentation processes like cheese ripening. This leads to severe reduction of the growth rate. Particular LAB survive these periods of extremely slow growth while still contributing to flavour formation in the fermented product.
In this study a dairy-isolate of Lactococcus lactisis grown in retentostat cultures on a chemically-defined medium to study its physiology and aroma formation capacity at near-zero growth rates. During the cultivations, the growth rate decreased from 0.025 h^{-1} to less than 0.001 h^{-1}. The biomass increased about 8 times and the viability of the cells remained above 80% as monitored by live/dead staining. However, plate counts did not show a significant increase suggesting that a part of the population went into a viable but non-culturable state. Interestingly, the maintenance requirement of this dairy strain decreased 7 times at near-zero growth rates compared to high growth rates (from 2.55±0.36 to 0.36 mmol $ATP.gDW^{-1}.h^{-1}$). Possibly, the cells minimize their energy expenditure for protein turnover by switching its protein management from proteolysis of damaged proteins followed by re-synthesis to repair of damaged proteins
In the retentostat cultivations, more than 60 different volatile organic compounds were identified by HS SPME GC-MS. Similar to aroma formation during cheese ripening, some products of amino acid degradation increased at near-zero growth rates. Moreover, acetoin formation decreased at near-zero growth rates while 2-butanone increased. Analysing samples that still contained cells showed that

particular lipophilic compounds, mainly long-chain alcohols, aldehydes and esters, accumulated in the cells and/or cell membranes.
In conclusion, retentostat cultivation offers a unique tool to study aroma formation by lactic acid bacteria under industrially relevant growth conditions.

O048

Bacterial spore germination; molecular mechanisms and germination inhibitors.

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Introduction
Bacterial spores are ubiquitous in nature, can survive food preservation processes and subsequently may cause, upon outgrowth, food spoilage as well as safety risks [1]. The risk is exacerbated by the heterogeneous germination and outgrowth behavior of isogenic spore populations [2]. Our studies focus on the characterization of the mechanisms involved in spore development and germination and identification of antimicrobial strategies.
Methods
For B. subtiliswe used defined media in which the sole nitrogen source $^{14}NH_4Cl$ was replaced with $^{15}NH_4Cl$. For metabolic labelling of pathogenic P. difficilea rich medium was used in which a yeast hydrolysate (yeastolate) was the sole nitrogen source. The yeastolate was made from accharomyces cerevisiaeCEN. PK1137D grown at 37°C in a defined CBS medium [3] modified with ^{15} NH_4Cl, replacing (NH4)2SO4 as the sole nitrogen source. Yeast cells were harvested by centrifugation (5000 ×g, 30 min) and washed with water. The protocol to generate yeastolate was adapted from previous studies [4,5]. The final yeastolate medium contained 2% ^{15}N-yeastolate, 2% glucose, and 0.2% NaCl. For proteomics analysis the recently published ‘one-pot method’ was used [6]. Live-imaging and fluorescence microscopy [7] allow us to analyze the presence of the ‘germinosome’ in B. subtilisat single spore level, the spore germination process and outgrowth mechanisms.
Results and Conclusions
The proteomics analyses showed characteristic sets of proteins necessary for ‘kick-starting’ life upon spore germination. 16 out of 18 glycolytic enzymes were identified in B. subtilisas well as 71 out of 104 proteins involved in amino acid biosynthesis. For P. difficile80% of the quantified proteins are common to vegetative cells and spores, indicating a modest proteomic changeover for the synthesis of its survival structures. Proteins slightly more abundant in spores than cells were putative amino acid permeases that might be crucial to Stickland fermentation upon spore germination. Interestingly, shared between spores and cells are proteins involved in the Wood-Ljungdahl pathway of acetogenesis reinforcing the key role of the pathway in P. difficile(Abhyankar et al., Plos Pathogens submitted).
We used Structured Illumination Microscopy (SIM) and GFP reporter proteins to visualize clusters of germination receptors (Germinosomes) in B. subtilis. Our data show that B. subtilisspores contain 2 to 3 Germinosomes per spore (Wen et al., JoVE under review). Its impact on models of spore germination is being assessed. The presence of germinosomes in other spore formers (the pathogens P. difficileand B. cereus) is currently being probed. Finally, we analyzed human thrombocidin derived TC19 and TC84 antimicrobial peptides. We show that a perturbed membrane fluidity is central to their mode of action.
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O049

Proteomes of cells and spores of an IPTG-inducible kinA strain of Bacillus subtilis versus wild-type.

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Introduction
Bacillus subtiliscan form metabolically dormant spores that are able to survive harsh environmental

conditions. A phosphorelay controls the initiation of sporulation, starting with expression and auto-phosphorylation of the kinase KinA. KinA transfers phosphoryl groups to Spo0A, through Spo0F and Spo0B. Accumulation of Spo0A~P_i above a threshold triggers sporulation. Both our own current studies as well as previous research has shown that inducing kinAexpression in Bacillus subtilisbearing kinAunder control of an IPTG responsive hyperspankpromoter ($P_{hy\text{-}spank}$) triggers sporulation independently of the culture medium being nutrient poor or nutrient rich. With this strain a time resolved detailed proteomic study of spore formation becomes possible as sporulation can be induced in all cells simultaneously by the addition of IPTG. In order to be able to compare the proteome of $P_{hy\text{-}spank}$ kinAwith wild-type (WT) B. subtilisthe base-line proteomes of both their cells and spores must be established. Here we report on such studies.

Methods

A derivative of Bacillus subtilisPY79 bearing a $P_{hy\text{-}spank}$ kinAwas grown in a defined, MOPS-buffered medium which contained 40 mM glucose. IPTG was added to a final concentration of 100 µM when the culture reached OD600 of 0.7 and growth was continued for another 90 minutes. Next, the culture was diluted 1 in 5 with medium devoid of glucose. Cells and spores were harvested at the moment of glucose dilution and 1 day after that. Cells and spores of B. subtilisPY79 were harvested in the same way. MOPS medium was used to metabolically label all proteins with ^{15}N by replacing the sole nitrogen source $^{14}NH_4Cl$ with $^{15}NH_4Cl$. Identical amount of cells and spores were mixed to prepare peptides samples. A one pot proteomics method was used for analysis [1]. Electrospray Ionization(ESI) -FTICR Mass spectrometry was used to quantitatively analyze tryptic protein digests. Wet heat resistance and germination behaviour of spores were tested according to established protocols[2,3].

Results

IPTG induced and not induced spores and spores of the wild-type strain showed equivalent resistance to wet heat. IPTG-induced spores were obtained at high yield (70%) but were delayed in phase-darkening (germination ensu strictu) compared to not induced spore from $P_{hy\text{-}spank}$ kinAas well as wild-type spores upon AGFK induced germination. Comparing $P_{hy\text{-}spank}$ kinAand wild-type cells showed that a group of sigma A controlled genes were downregulated in the KinA strain. Also 16 out of 20 germination related genes were downregulated in the KinA strain which could explain the observed delay in spore germination.

Conclusions

The $P_{hy\text{-}spank}$ kinAstrain, suitable for the homogeneous induction of sporulation, displays some characteristic differences in protein expression in vegetative cells as well as spores. Currently we use this data as a baseline for comparisons in time resolved detailed proteome analyses of the deposition of spore proteins upon IPTG induced spore formation in the $P_{hy\text{-}spank}$ kinAstrain.

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O050

Essential? Maybe not. FtsZ-less cell division in Planctomycete bacteria.

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Planctomycetes are an enigmatic phylum of bacteria that share a number of unique characteristics, such as a complex endomembrane structure. Planctomycetes have been hypothesised to be a rich source of secondary metbolites and anammox Planctomycetes have found widespread application in waste water treatment. Planctomycetes perform cell division either by budding or by a process that resembles binary fission, both of which are currently not understood as Planctomycetes lack most of the known and essential cell division genes such as ftsZ. To figure out how cell division is performed in these organisms, fluorecent fusions and knockouts where created in the budding type-strain Planctopirus limnophilaand the phenotype was characterized using time-lapse phase contrast, confocal and fluorecent microscopy.

O051

Localization of bacterioferritin and encapsulin nanoparticles in the compartmentalized anammox bacterium Kuenenia stuttgartiensis

L. Claret Fernández, S.H. Peeters, R. Mesman, M.S.M. Jetten, S. Lindhoud, L.A.M.P. Van Niftrik
Radboud University, Microbiology, Nijmegen

Preferred session:
Microbes up close: subcellular microbiology and single-molecule techniques

Laura Claret Fernández, Stijn Peeters, Rob Mesman, Mike Jetten, Simon Lindhoud, Laura van Niftrik
Microbiology, Institute for Water & Wetland Research, Faculty of Science, Radboud University, The Netherlands
Anaerobic ammonium-oxidizing (anammox) bacteria convert ammonium and nitrite to dinitrogen gas with nitric oxide and hydrazine as intermediates. They are important players in the global nitrogen cycle and are applied in wastewater treatment for the removal of nitrogen compounds. Anammox bacteria have an extraordinary cell plan with a major “prokaryotic organelle” called the anammoxosome. The anammox reaction takes place in the anammoxosome and is proposed to be coupled to energy conservation by establishing a proton motive force over the anammoxosome membrane which can subsequently be used for ATP synthesis. However, parts of the molecular mechanism of this extraordinary metabolism and its link to the unusual cell plan remain unknown. Especially the role of the numerous (>10) hydroxylamine oxidoreductase (HAO)-like octaheme proteins is unclear. Interestingly, the anammoxosome contains iron-rich nanoparticles that could be bacterioferritins, encapsulins or an as of yet unknown nanoparticle. It is hypothesized that the function of both the encapsulins and bacterioferritins is linked to the anammox HAOs. Bacterioferritins are iron storage protein particles. Encapsulins are nanocompartments known to carry cargo proteins or enzymes – in the anammox case an HAO. Here we investigated the location and function of the K. stuttgartiensisbacterioferritins and encapsulins. We purified and characterized the bacterioferritins directly from Kuenenia stuttgartiensiscells. The K. stuttgartiensisencapsulins were heterologously expressed in and purified from Escherichia coli.Currenlty, immunogold localization of both nanoparticles is being performed on Kuenenia stuttgartiensiscells to determine their location in the cell.

O052

Molecular insights into the killing of E. coli by Membrane Attack Complex pores

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Introduction
The complement system is an important effector of the innate immune system in clearing bacteria. On Gram-negative bacteria, complement activation results in direct killing via a large pore-forming protein complex called the Membrane Attack Complex (MAC). MAC formation is initiated when bacteria are recognized by complement and labelled with surface-bound convertase enzymes. These convertases can cleave complement component C5 into C5b, which together with C6, C7, C8 and multiple copies of C9 assemble the MAC. Due to the complex composition of the Gram-negative cell wall compared to a single-membrane eukaryotic cell, the precise mechanism by which the MAC kills Gram-negative bacteria remains unclear. Recently, we have established that local assembly of the MAC by a convertase is essential to form pores that are capable permeating both the outer and the inner membrane to subsequently kill Gram-negative bacteria. This study aimed to gain more molecular insight in why local assembly of pores by a convertase is essential for bacterial killing.
Methods
E. coliwere labelled with convertase enzymes to study the early stages of MAC formation with recombinantly expressed MAC components in the absence of other serum components. Flow cytometry was used to assess binding of complement components to the surface and lysis of the outer and inner membrane. Confocal microscopy and atomic force microscopy were used to visualize the distribution of MAC on the bacterial surface.
Results
We show that the MAC precursor C5b6 is transiently associated to the surface by interacting with the convertase. Moreover, we show that immediate interaction of C5b6 with downstream MAC component C7 is not required for outer membrane lysis, but is required to destabilize the inner membrane and subsequently kill E. coli. Trypsin shaving of MAC precursors reveals that bactericidal MAC precursors are more properly inserted into the bacterial cell wall and can still form a bactericidal pore after trypsin treatment. Interestingly, some E. colistrains that are not killed by the MAC do show extensive MAC deposition and outer membrane lysis. These pores are readily cleaved by trypsin, suggesting that these bacteria survive because proper insertion of the MAC into the bacterial cell wall is prevented.
Conclusion

Altogether, these findings highlight that MAC assembly is a target-specific process that requires immediate insertion of the MAC precursor into the bacterial cell wall to form a pore that can kill E. coli. Moreover, these data suggest that some E. colistrains can prevent cell death not by preventing MAC formation, but by preventing this proper insertion of the pore into the cell wall. These findings open up avenues to better understand how cell wall lysis in Gram-negative bacteria leads to cell death and to use this knowledge in therapeutic development.

O053
The archaellum: how archaea swim
Sonja Verena Albers[1]
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The archaeal motility structure, the archaellum, is a unique structure, which is evolutionary not related to bacterial flagella, but shows structural homology to type IV pili. The archaellum is a rotary motility structure which achieves forward propulsion of the cells by ATP hydrolysis. We have analysed the subunit interaction of archaellum components from different archaeal species and performed detailed studies on archaellum assembly in the crenarchaeon *Sulfolobus acidocaldarius* and euryarchaeon *Haloferax volcanii*. In the presentation our current understanding of archaellum assembly and how rotation of the structure is achieved, will be discussed.

O054
Micro-Scale Warfare with Macro-Scale Implications: Mechnisms and Consequences of Interbacterial Antagonism
Joseph Mougous

O055
A surprising souvenir from India…
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, Nederland

A 37 year old male travelled for business to India and presented within a week after return with diarrhea and rash at the ER. How to act?

O056
Why you should't rush: implications of a rash decision during a grand round.
M.R.A. Welkers
Amsterdam UMC lokatie AMC, AMSTERDAM, Nederland

A 26-year old homosexual man travelling back from a festival in Poland falls ill with a rash and a cough. Is it a primary HIV-1 infection or maybe something else? Intuitively, every clinician would say: let's go have a look. But what happens when it is something else?

O057
Epidemiology of measles; the risk of measles among healthcare workers in the Netherlands
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Measles virus is one the most contagious pathogens. The number of reported cases declined rapidly in the Netherlands after implementation of routine measles vaccination in 1976. However, outbreaks still occur in socio-geographically clustered populations refusing vaccination for religious reasons. Outside outbreaks occasional patients who often acquire measles abroad, and small clusters related to these imported infections are reported. The patient characteristics between outbreak and non-outbreak periods differ. In Europe, the number of measles infections has increased recently with around 27.000 cases reported in 2017 and 2018 from EU countries. European countries where suboptimal vaccination coverage resulted in measles outbreaks include Romania, Italy, France and Greece.
In the last measles outbreak in the Bible Belt in 2013/2014, 2700 cases were reported. The median age of the patients was 10 years, 94% was unvaccinated and 7% was hospitalized. In the non-outbreak years 2015-2018, in total 53 measles cases were reported in the Netherlands. The median age of the

patients was 28 years, 73% was unvaccinated and around half were hospitalized. Two-thirds of these patients were infected abroad or were import-related secondary infections.
It is important to ensure health care workers have protective immunity against measles. Firstly, for individual protection, since they are at risk of exposure to measles patients, and secondly, to avoid onward transmission to vulnerable patients. During the 2013/2014 outbreak 19 health care workers (HCW) got infected. Most were working in a general practice and three in a hospital. In 2015-2018 four HCW got measles infection, of whom three in a hospital. 61% of the 23 HCW were unvaccinated. Onward transmission from these 23 infected health care workers was not observed, but did occur in a separate hospital outbreak later in 2014.
The risk of measles among healthcare workers in the Netherlands is low, but as the epidemiology of measles is changing, measles among health care workers may become more important. Relevant factors are suboptimal protected birth cohorts around the introduction of vaccination, an increased number of people with only vaccine-induced immunity, waning of vaccine-induced immunity and less boosting due to decreased circulation of measles virus. In 2018, Portugal reported an outbreak in a tertiary hospital which affected nearly 90 health care workers of whom 80% were completely vaccinated. In a Dutch hospital outbreak in 2014, 6/8 cases (75%) were completely vaccinated. However, the clinical picture and infectiousness of measles in vaccinated persons differs from a primary infection. The relevance of measles in vaccinated persons for transmission will be discussed.
During the 2013/2014 measles outbreak advice on MMR-vaccination of HCW was issued. However, a study among hospitals in 2014 indicated that adherence to this advice was suboptimal as less than half had implemented the minimum set of measures considered necessary to adequately prevent measles in HCWs. The national recommendations on measles pre-exposure measures, and the policy in case of exposure to measles virus in the hospital regarding serological screening of contacts (both patients and health care workers), the implications of different assays for this, and control measures will be discussed.

O058
Correlates of protection from measles: (what) should we test?
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Virus neutralizing antibodies as measured by plaque reduction neutralization (PRN) assays are a widely accepted correlate of protection for measles. However, the assay is labour-intensive and time-consuming. There are many other antibody detection methods available, but not all of them measure functional or protective antibodies. VN antibodies are mostly directed to conformational epitopes on the hemagglutinin, whereas many of the commercially-manufactured ELISA kits detect non-protective antibodies to internal viral proteins. These kits are often less sensitive than PRN tests at low antibody levels, which are found in a (small) proportion of vaccinated individuals. Moreover, although detection of protective PRN antibodies infers protection, their absence does not automatically mean that someone is not protected. Measles has a long incubation time, and secondary immune responses mediated by vaccination-induced memory lymphocytes can reduce both the clinical impact and risk of virus transmission. Hospitals should identify health care workers at increased risk of contracting measles as preventive measure.

See LCI website: https://lci.rivm.nl/richtlijnen/mazelen (bijlage 8, juli 2013)

O059
Panel discussion: how do we protect health care workers and their patients from measles after occupational exposure
No abstract

O060
Gut bacteria in the spotlight: discovery of a toxin breakdown pathway in the cabbage root fly
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Pest insects lead to excessive agricultural and therefore economic losses on crops worldwide (1). In recent years, research on insect-microbe symbioses has recognized that microbial symbionts may play a role in protecting against toxic molecules from plant defenses, leading to a form of defensive symbiosis between the pest insect and microorganisms termed detoxifying symbiosis. We investigated the

detoxifying symbiosis of cabbage root flies (*Delia radicum* L. (Diptera: Anthomyiidae)) which are challenged by toxic isothiocyanates from their host plants (2,3). Using biochemical, genomic and environmental microbiology techniques we found that microorganisms residing in the gut of cabbage root fly larvae degraded isothiocyanates by enzymes belonging to a new hydrolase enzyme family (4). Further investigation into this novel enzyme family revealed interesting properties with potential consequences to the ecology of the insect as well as phytopathogenic bacteria of the genus *Pectobacterium*.

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O061

Deep impact: sponge-associated microbes and bioactivity change with depth

Detmer Sipkema

Marine sponges are leading organisms for discovery of novel bioactive compounds from nature. Yet, factors involved in shaping their microbial community and chemical potential are poorly understood. The large majority of studies are from shallow water, while the microbiota and bioactivity of deeper sponges are largely *mare incognita*. Here we studied the impact of depth on the prokaryotic communties and metabolomes in marine sponges. For all sponge species studied, we find that microbiome and metabolite composition vary with depth and up to 29% of the variation in a microbiome and up to 17% of the variation in a metabolome were attributed to depth.

O062

The benefit of plant-Streptomyces interactions

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Streptomyces are filamentous bacteria that produce versatile natural products (NPs) relevant for agriculture, biotechnology and medicine, including the majority of the antibiotics we use in the clinic. Genome sequencing has unveiled that many antibiotic biosynthetic gene clusters (BGCs) are poorly expressed under routine laboratory conditions. This is a potential reservoir of untapped antibiotics that could be used to fight antimicrobial resistance (AMR). To identify them and elucidate their bioactivity, we need to uncover the triggers that elicit their production. Importantly, many *Streptomyces* live in symbiosis with eukaryotes, and in particular with plants. These interactions have likely played a key role in the evolution of the high chemical diversity and regulation of NPs.The STW Perspective program BacktoRoots aims to enhance plant growth and productivity by exploring and ultimately exploiting microbial communities[2]. The 'cry for help' hypothesis entails that plants suffering from biotic stress produce signals that activate the production of protective molecules by plant-associated microbes like *Streptomyces*. We aim to uncover these signals by studying plant-*Streptomyces* interactions and harness them for the screening of new antibiotics. To study plant-*Streptomyces* interactions we made use of several imaging techniques, including electron microcsopy. We were able to image the endophytic life-style of *Streptomyces*, as well as colonization and attachment of the spores to plant seeds[3]. In addition, genes responding to plant hormones were found by RNA sequencing and molecular cloning. We show that the plant-defense hormone jasmonic acid elicits the production of antibiotics, affects *Streptomyces* development and even may result in new phenotypes.&#13&#13&#13[1] van der Meij, A., Worsley, S., Hutchings, M., van Wezel, G.P. (2017). Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiol Rev.&#13&#13
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impact of plant hormones on their antimicrobial activity. Antonie van Leeuwenhoek.

O063
Virally induced mortality of Antarctic algae
C.U.M. Eich, S.B.E.H. Pont, R. Middag, C.P.D. Brussaard
NIOZ, 'T HORNTJE (TEXEL), Nederland

Marine phytoplankton, forming the base of most pelagic food webs, are not only grazed by zooplankton but also lysed by viruses. Recent studies have shown that the share of viral lysis is comparable to grazing. However, hardly any data are available for the Antarctic Ocean. Viral infection by lytic viruses results in the lysis of the host cell upon release of the progeny viruses. As such, viral lysis shunts particulate organic matter away from the higher trophic levels towards regeneration within the microbial loop. It is also hypothesized that viral lysis positively contributes to the bioavailability of iron, a micronutrient often limiting the growth of Antarctic phytoplankton. Having joined a cruise to the Amundsen Sea (Southern Ocean), I will present the mortality rates (viral lysis compared to grazing) of the different phytoplankton populations and discuss these in relation to their abundances, growth rate and community composition. I will show that also the cold water Antarctic phytoplankton are sensitive to viral infection and lysis rates are of ecological importance.

O064
Role of the gut microbiota in patients with inflammatory bowel diseases
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Our intestine harbours a complex community of microbes, collectively called the microbiota, which plays a crucial role in our health. The past decades have been characterised by an explosive increase in studies focusing on the intestinal microbiota in health and disease. This increase has been largely facilitated by fast developments and the application of high-throughput culture-independent technologies to study the microbiota in relation to health and disease. Notably, approaches using 16S ribosomal RNA or its encoding gene as a marker for bacterial identification have been widely applied. These studies have not only demonstrated that each individual has a unique microbiota composition but also that aberrations of the microbiota have been associated with several diseases, including inflammatory bowel disease (IBD). Despite the fact that several microbes or microbial groups have frequently been correlated to IBD, such as lower *Faecalibacterium prausnitzii* and higher Proteobacteria abundances, specific microbial associations are often inconsistent between different studies. Hence, there is a current need to explore what the causal links are between the microbiota and our health than only identifying associations. Fecal Microbiota Transplantation (FMT) is a radical way to interfere with the microbiota in IBD patients by replacing one's own microbiota with that of a healthy donor, that offers possibilities to identify causal associations between microbes and IBD. Observations from the TURN trial demonstrated that only a fraction of ulcerative colitis (UC) patients showed remission after FMT, both in patients receiving donor- or their own microbiota. In depth comparative microbiota composition analyses identified signature microbes for donor and recipient microbiota that are predictive for success or failure, and that the success of FMT relies on a perfect match between donor and recipient. The next step is to identify the specific role of these microbes in the intestine and how this is related to the success or failure for remission in IBD. This seminar will present and discuss the current status of insights into the microbiota in relation to health and IBD, and discuss what the current research challenges are and how these can be approached using examples from own research.

O065
Natural variations of microbiota in large populations
Sasha Zhernakova

Gut microbiota plays an important role in predisposition to various diseases, including immune, metabolic, psychiatric conditions, and response to treatment. The composition of the gut microbiota is highly variable across individuals. In this presentation, I will discuss the variations of gut microbiota in

population cohorts, and factors that influence gut microbiota composition, including technical covariates, host and environmental factors, and host genetics.

O066

How changes of the microbiota provoke colonization and disease by the intestinal pathogen Clostridioides difficile

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How changes of the microbiota provoke colonization and disease by the intestinal pathogen *Clostridioides difficile*

Background: *Clostridioides difficile* infection (CDI) usually develops in a disturbed gut microbiota associated with previous antibiotic use, but the gut microbiota composition in patients asymptomatically colonized with *C. difficile* is not well investigated. Knowledge on this topic could aid in designing gut microbiota-based treatments to prevent colonization and development of CDI.
Materials/methods: During a case-control study in two large Dutch hospitals, 1527 faecal samples collected from patients at admission were screened for *C. difficile* colonization. Gut microbiota composition from a subset of patients with *C. difficile* colonization (CDC, n=41) and without *C. difficile* colonization (controls, n=43) was investigated through 16S rRNA gene amplicon sequencing and compared to patients with clinically and microbiologically diagnosed CDI (n=41).
Results: The three patient groups were comparable in mean age and gender distribution (p=0.76 and p=0.47, respectively). Antibiotic use in the last three months was present in 97.6% of CDI patients, 73.2% of CDC patients and 59.5% of controls (p<0.001). Controls, CDC and CDI patients could be significantly separated from each other based on overall microbiota composition (p=0.001). Only in controls, the presence or absence of prior antibiotic use could identify two subgroups based on overall microbiota composition (p=0.035). Bacterial diversity was significantly decreased in CDC and CDI patients (p<0.01). *Clostridioides* abundance (most likely representing *C. difficile*) increased in a step-wise manner from controls (0.2 0.9%) to colonized patients (0.7 2.2%) and CDI patients (2.5 2.9%). As controls could be subdivided in antibiotic and non-antibiotic users, this group was split in controls with (C+AB) and controls without (C-AB) antibiotic use. Gut microbiota of CDI patients was characterised by significantly increased abundance of *Bacteroides* and *Veillonella* compared to CDC patients and both control groups. In contrast, both control groups had a significantly higher abundance of *Eubacterium hallii* than colonized patients. Association networks further indicated that in CDI patients *Clostridiodoides* was negatively associated with *Fusicatenibacter*, and that in CDC patients *Clostridioides* was positively associated with *Veillonella*. As *Fusicatenibacter* was also decreased in CDC patients as compared to C-AB, this may indicate a potential beneficial role for *Fusicatenibacter* against *Clostridioides*.
Conclusions: 16S rRNA gene amplicon sequencing well presented the obvious difference in *Clostridioides* abundance between the different patient groups. *C. difficile* colonized patients were characterised by a disturbed microbiota, independent of prior antibiotic use. *Eubacterium halii* may be protective against *C. difficile* colonization, *Fusicatenibacter* against *C. difficile* colonization and/or infection, while *Veillonella* was associated with both *C. difficile* colonization and infection. Future mechanistic studies are necessary to investigate whether these genera affect *C. difficile* colonization and infection in a causal manner.

O067

Guideline for probiotics to prevent and treat antibiotic-associated diarrhea in The Netherlands

Ger Rijkers

O068

A novel sulfur oxidation system revealed in dimethyl sulfide-degrading Hyphomicrobium denitrificans

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While many sulfur-oxidizing bacteria operate the well-established Dsr (dissimilatory sulfite reductase)

pathway, other bacterial and archaeal sulfur oxidizers lack this pathway. Bioinformatic analyses have indicated an alternative metabolic route involving a heterodisulfide reductase (Hdr)-like protein complex [reviewed in 1] but direct genetic evidence for this suggestion has not been available. &#13We addressed this major knowledge gap using *Hyphomicrobium denitrificans* (DSM 1869^T). This Alphaproteobacterium typically grows on C1-compounds like dimethylamine but also uses the volatile organic sulfur compound dimethyl sulfide (DMS) as sole carbon and energy source during aerobic respiration. DMS is degraded to methanethiol from which formaldehyde and sulfide are released [2]. The latter is then fully oxidized to sulfate by wildtype *H. denitrificans*. In contrast, a knockout strain lacking a viable Hdr-like system was completely incapable of growth on DMS. Complementation with a plasmid containing the complete *hdr*-like genes under a constitutive promoter rescued the phenotype [3]. A novel lipoate-binding protein (LbpA) resembling GvcH, a component of the glycine cleavage system, was identified as an important player in the Hdr-like sulfur oxidation pathway [4]. Comparative proteomic as well as immunological data showed that the hyphomicrobial Hdr-like proteins as well as LbpA are specifically induced by DMS, thus further strengthening the notion that this complex catalyzes a key step in sulfur oxidation not only in *H. denitrificans* but also in a wide range of other environmentally important sulfur oxidizers [3]. In analogy to known reaction cycles involving lipoate-binding proteins, LbpA may serve as a substrate-binding entity presenting the sulfur substrate to catalytic sites of the Hdr complex. At the same time, the intramolecular disulfide bond of lipoate may cycle between oxidized and reduced states ($E^{0'}$ = -0.29 V) and the electrons released upon reoxidation of dihydroliponamide could be directly transferred onto NAD^+. If part of the electrons arising from sulfane sulfur oxidation could thus be directly transferred to NAD^+, this would significantly decrease the need for energy-requiring reverse electron flow and provide reducing equivalents at a redox potential negative enough for direct reduction of carbon dioxide during lithoautotrophic growth on reduced sulfur compounds. Further to these considerations, evidence is provided that the assembly of lipoylated LbpA requires a specific pathway encoded by genes associated with prokaryotic *hdr-lbpA* gene clusters [4]. &#13&#13[1] Venceslau et al 2014 BBA 1837, 1148&#13[2] Eyice et al 2017 ISME J 12, 145-160&#13[3] Koch et al. 2018 ISME J 12, 2479-2492&#13[4] Cao et al 2018 eLife 7, e37439

O069

What happens in the membrane, stays in the membrane; the role of quinones during microbial respiratory adaptation

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Respiratory adaptation is a key process for bacteria upon changing environmental condition. Proton-pumping enzymes are expressed under aerobic conditions while non proton-pumping enzymes under anerobic conditions. However, the role of quinones in these processes, and the reasons behind switching between ubiquinone and menaquinone is poorly understood. This is because quinone-quinol interconversions in a lipid membrane environment by respiratory enzymes is a technically challenging area of study. Here, four quinone-utilizing proteins cymA, cytochromes bo_3 and Bd, and NADH Dehydrogenase 2, have been explored from the model microbes *Shewanella oneidensis, Escherichia coli* and *Caldalkalibacillus thermarum* TA2.A1 respectively. The purpose of the switch between the use of MQ and UQ upon anaerobic/aerobic conditions is explored alongside the influence of cardiolipin. Co-reconstitution of cbo_3 with the *E. coli* F_1F_o ATP synthase revealed that if the quinone regulation is lost, cytochrome bo_3 is capable of pumping protons faster than the F_1F_o ATP synthase is synthesizing ATP. This indicates a loss of regulation would lead to cell death *via* alkalization. However, this type of regulation is not possible in mammals, the terminal oxidase does not directly interact with quinones. Interestingly, the method of prevention of proton overloading may be Ca^{2+} driven, which has come to light as a key regulator of mitochondrial mito-flashes – giving insight into the reasons for evolutionary changes in terminal oxidase vs quinone utilization.
Lastly, we present a theory on the structural of quinones alongside the influence of a membrane environment on membrane protein activity with a view to combat pathogenic microbes.

O070

Thermostable [NiFe] hydrogenase isolated from an acidothermophilic methanotroph with high affinity for hydrogen gas at ambient oxygen

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For decades it has been known that soil systems can consume atmospheric hydrogen concentrations

(0.530 ppmv or 0.4 nM). However, only ten years ago the first microbes able to do so were isolated. These microorganisms encode for the novel group 1h [NiFe] hydrogenases, which are presumed to be responsible for atmospheric hydrogen oxidation. These enzymes were hypothesized to have a high affinity for hydrogen, be thermostable and insensitive to oxygen. So far, biochemical data is scarce and studies have not yet led to the confirmation that it is indeed a highly active enzyme with an affinity constant in nanomolar range. Since group 1h [NiFe] hydrogenases are thought to have a thermophilic origin, a thermophilic member of the Verrucomicrobia phylum was investigated.&#13 &#13The methanotroph *Methylacidiphilum fumariolicum* SolV was isolated from a hot and extremely acidic volcanic ecosystem. It is able to grow below pH 1 and up to 65, and is dependent on rare-earth elements for growth. This extremophile encodes for two different hydrogenases, one of which belongs to the novel group 1h [NiFe] hydrogenases. The ability to consume hydrogen as additional energy source gives a major advantage for methanotrophs in ecosystems where methane concentrations fluctuate. *M. fumariolicum* SolV can grow on hydrogen gas without methane or oxidize both compounds simultaneously where hydrogen oxidation provides extra reducing power for methane oxidation. In fact, this mixotrophic lifestyle has contributed largely to the success of these methanotrophs in volcanic ecosystems. &#13 &#13This study is the first to describe the purification of a hydrogenase from a methanotroph. Isolation of this enzyme from native biomass indeed shows the hypothesized group 1h [NiFe] hydrogenase characteristics: a thermostable, highly active, high-affinity enzyme that is active at ambient oxygen concentrations. Kinetic studies using a membrane-inlet mass spectrometer (MIMS) revealed for the first time an isolated hydrogenase with an affinity in the nanomolar range. This supports the hypothesis that group 1h [NiFe] hydrogenases have a high affinity for hydrogen and are responsible for the consumption of atmospheric hydrogen. The enzyme is thermostable with a half-life of 30 min at 80 C and functions optimally at pH 8 and 80 C. Although genetic analysis predicted the protein to be residing in the cytoplasm, activity of the enzyme is primarily localized in the membrane. By transport of electrons yielded from hydrogen oxidation to the membrane, the enzyme can provide reducing power for methane consumption. These findings show that indeed group 1h [NiFe] hydrogenases have an unprecedentedly high affinity for hydrogen and could aid in consuming environmental methane. This competence could therefore mitigate global warming, since methane is an important greenhouse gas 25 times more potent than CO_2.

O071
Computational methods for microbiome analysis
Marcus Claesson

O072
Garbage in, Garbage out: Impact of raw data quality and data analysis in downstream genomic analysis
João André Nogueira Custódio Carriço

O073
Great interest of students creates challenges in higher professional education
E Hilhorst
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In the Domain Applied Sciences, the national collaboration between higher professional educational institutions in the applied sciences, we have a great challenge to manage our increase in students in the study 'Biology and Medical Laboratory Research'.
Nine Universities of Applied Sciences (UAS) at eleven different locations offer this program, a very popular study among students. In the period 2010-2014 the new influx of students increased from around 1100 to almost 1700. After a decrease in 2015, we are back at the level of 2014 this study year. The large number of students creates problems in offering these programs, for example insufficient laboratory facilities, a shortage of qualified teachers and insufficient internships. Due to these consequences it is difficult to guarantee the quality of the education. Therefore, the Numerus Fixus (NF) was introduced by several Universities of Applied Sciences (UAS) in 2015. The NF resulted in commotion in the politics and the media and therefore part of the UAS decided to abolish the NF after a few years.
By consulting our professional field, the problem became clear. The job activities in mainly the diagnostic laboratories are changing quickly due to automation. Therefore, the number of staff needed in diagnostic labs decreased and therefore there is less time for them to supervise an internship. On the other hand,

the number of internships at universities are also decreasing because research groups at universities are stimulated by internal financial incentives to offer their own bachelor students internships instead of our students.
The UAS have to take action by looking for other possibilities for internships and for possibilities in changing the content of the program to meet the need of our professional field. We would like to discuss the possible solutions we see with the audience, and we hope for more suggestions!

O074
Getting BSc students off to a flying start via a Student Centered Approach
Nina Scheres
For the BSC Biomedical Sciences studies at the University of Amsterdam, a new interactive course was developed for first year students. The goal was to create a student-centered learning environment that sends first year students off to a flying start, by not only giving them basic knowledge of cell biology, microbiology and immunology, but also introducing them to academic learning. Using a flipped classroom set-up, the course consisted of lecture-days centered on a clear topic. Students started with self-study and teacher-guided group work, then took an online knowledge quiz, and after this could attend an interactive lecture. The content of the lectures was based on student questions and their results in the knowledge quiz of that day (hence: Just-in-time-teaching). This led to high attendance (70-90%), active participation, motivated students, and a high passing rate (80%). Furthermore, student evaluations showed that students were very enthusiastic. They appreciated the structure and clarity, and the pleasant atmosphere and good learning environment. Although this course was a demanding task for teachers and developers, in which strong collaboration between a team of teaching professionals and a research group was essential, we can conclude that it was highly successful and definitely worth the effort.

O075
Large scale teaching technology
Robbert van Veen

O076
To honour or not to honour? Exploring the benefits of honoursprograms in applied sciences
Willem van Leeuwen

The benefits of honours programs are often topic of discussion. What is the added value for both student and institute? Which students would start such a program? In our experience highly motivated students in the field of applied science tend to favour an honours program focussed on acquiring knowledge and experience in their chosen field of study. To facilitate these students, we have developed an honours program to further challenge themselves as young professionals in an extracurricular setting. In the program each student participates in studies proposed by a lector or university professor for at least one year. They work in the professional research laboratory setting to further acquire new skills. Furthermore, the students have a personal coach appointed, in order to evaluate the progress of these students for both professional and (inter)personal skills. In this session we, the lector and students, would like to share their experience with this honours program specifically focussed on students in the field of applied sciences. What have we learned so far? What are the benefits for both students and lector? What are the possible bumps in the road? Why have these students applied for this program and why are they selected?

O077
***StudentTalentCafe*, a workshop creating a personal mission**
Aart van den Dool, lecturer & Noud Verstappen, student Biomedical researc. Avans University of Applied Sciences, Academy Technology for Health & Environment, Breda &

Introduction
Personal development of students – leadership in new forms of education; build trust, explore resilience, raise awareness of personal talents that help experience personal Identity.
Methods
Peer-group workshops conducted by teachers, coaches & student-coaches
Results
We share with the audience how Students get insights in personal owned questions in their

development. We share anonymous student results.

Who am I? What makes me happy? How do I stay motivated? Am I doing what I am good at?

What topic do I want to do for my Internship/Graduation? How can I find the inspiration that I need? How can I step in the world and allow essential experiences, as start of my personal curiosity – my mission journey?

Conclusion
Students achieve a better understanding in vulnerability, self-belief and gain ownership in owned choices. Avans University of Applied Sciences offers a personal approach, guiding students to develop their personal compass in different phases of the education path. A train-de-trainer model for student- and study coaches supports the development towards personalized education. We show how we connect generations in education, building power in worldwide themes.

O078
Exploiting Virus Biology to Develop Cancer Therapeutics
John Bell

O079
Clinical translation of the oncolytic adenovirus Delta24-RGD for treatment of gliolastoma
Martine Lamfers

The oncolytic virus (OV) Delta24-RGD, also known as DNX-2401, is currently under clinical investigation for the malignant brain tumor glioblastoma (GBM). This serotype 5 adenovirus was modified with a 24 base pair deletion in the E1A region of the virus, restricting its replication to cells with dysfunctional retinoblastoma pathway, ie cancer cells. In addition, the Arg-Gly-Asp (RGD) peptide sequence was inserted into the fiber knob of the virus to enhance infectability of GBM cells, which are low in Coxsackievirus-adenovirus receptor but high in integrin expression. An overview will be provided of the preclinical studies that led to the recently finalized clinical phase I/II trial testing local infusion of Delta24-RGD in recurrent GBM patients. Trial-associated studies performed to assess local and systemic immune responses will be discussed, as well as ongoing research directed toward enhancing Delta24-RGD efficacy by combination therapy with immune checkpoint inhibitors and the development of assays that may predict therapeutic outcome to OV therapy.

O080
Evolution of reovirus-based oncolytic viruses
Iris J.C. Dautzenberg, S.T.F. Bots, V. Kemp, D.J.M. van den Wollenberg, R.C. Hoeben
Oncolytic viruses are able to selectively infect and kill cancer cells. Their dual mode of action, direct tumour-cell killing and induction of increased anti-tumour immunity, make them highly promising agents for cancer therapy. Reovirus type 3 Dearing is a potent representative of this new type of treatment and has been shown safe in a multitude of clinical trials. Our research group focusses on improving the anti-tumour efficacy of oncolytic reovirus by means of forward and reverse genetics strategies. We generated a panel of reovirus mutants with an expanded tumour-cell tropism and increased tumour-killing abilities.

O081
The use of Newcastle Disease virus in oncolytic viro-immuno therapy
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Erasmus MC, ROTTERDAM, Netherlands

In the last few decades, the application of oncolytic viruses (OVs) in cancer patients has shown to be a promising treatment strategy with encouraging results for a variety of tumors. OVs selectively infect and damage tumours either by directly killing tumour cells or by promoting an anti-tumour response towards all tumour cells. Nowadays, several oncolytic viruses are tested in clinical trials with promising results, including the Newcastle Disease Virus (NDV).
NDV is a replication competent oncolytic virus belonging to the family *Paramyxoviridae* with a natural avian host range. The strains are categorized in three groups based on disease severity in birds: avirulent (lentogenic), intermediately virulent (mesogenic) and highly virulent (velogenic). We have shown that a mesogenic strain containing a multibasic cleavage site in the fusion protein has significant higher antitumor effect *in vitro* as well as *in vivo* tumor models compared to the lentogenic strain.

However, mesogenic strains cause outbreaks of severe disease in poultry. To avoid the risk of an outbreak, we generated a virus with similar anti-tumour efficacy as the mesogenic strain, but with less virulence for avian species.
To this purpose, we deleted the V protein, an avian specific IFN antagonist, in a mesogenic NDV strain. We used different strategies to delete this protein, because the open reading frame of the V protein is overlapping with the open reading frame of the essential phosphoprotein P. As a results, the V protein is only transcribed when there is frameshift caused by stuttering of the polymerase protein. The strategies to delete the V protein included complete deletion of the V open reading frame or mutations at the frame shift position. Subsequently, four viruses with a deletion of V were obtained and their avian specificity and oncolytic effects were determined.
Viruses with a deletion of the V protein were found to be attenuated in avian cell lines as well as in embryonated duck and chicken eggs compared to the control virus. In addition, deletion of the V protein had minimal effect on the oncolytic efficacy of these mutant viruses compared to control viruses. Infection experiments in chicken will ultimately proof the environmental safety of these mutant viruses. In conclusion, the results so far, demonstrate that the virulence of mesogenic NDV (containing a multibasic cleavage site) can be reduced by deleting the V protein and infection experiments in chicken will determine whether these viruses are safe to be used in oncolytic viro therapy studies.

O082
European approach to diagnose and treat prosthetic joints infections
Andre Trampuz

O083
New antibiotic-free approaches to cure orthopaedic infections
Harrie Weinans

O084
Radiological diagnosis of orthopaedic infections
I.J.E. Kouijzer
Radboudumc, NIJMEGEN, Netherlands

Prosthetic joint infection is a severe infection which significantly affects the patient's quality of life. Often prolonged antibiotic treatment and repeated surgical approaches are needed, especially when the diagnosis of prosthetic joint infection is delayed. To ensure an earlier and successful treatment for the patient with preserving joint functionality, a prompt diagnosis of this infection is needed. This lecture will provide an overview of radiological and molecular imaging in diagnosing prosthetic joint infection.

O085
Induction heat to eradicate microganisms from orthopaedic implants
B.G. Pijls, I.M.J.G. Sanders, E.J. Kuijper, R.G.H.H. Nelissen
LUMC, LEIDEN, Nederland

Prosthetic joint infection (PJI) is a devastating complication following total joint arthroplasty. Presently, patients with infected implants often require multiple surgical procedures and prolonged antibiotic therapy. This treatment is invasive and may therefore be impossible in patients with high comorbidity factors. Furthermore, increasing antibiotic resistance of bacteria and allergies of the patient to antibiotics can limit the choice of antibiotics. It is therefore vital that new treatments for the prevention and treatment of PJI are developed.
Non-contact induction heating of metal implants is a new and promising future treatment for PJI. Induction heating uses pulsed electromagnetic fields (PEMF) to induce eddy currents within metal objects in a non-invasive manner. These eddy currents are electrical currents within the metal object that oppose the change in PEMF as derived from Faraday's law of electromagnetic induction, and consequently cause heating of the metal implant. In theory, every metal implant is suitable for induction heating, depending on its anatomical situation. The major advantage of induction heating of metal implants is that only the metal implant is actively heated and induction heating has no direct heating effect on the surrounding tissue. This results in the heat generated being directed onto the bacterial biofilm that is on the metal implant.
This talk explores the possibilities, challenges and in-vitro results of non-invasively treating PJI with induction heating.

O086

Next generation sequencing: first diagnostic one-stop shop in clinical microbiology

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Current molecular diagnostics of human pathogens provide limited information that is often not sufficient for outbreak and transmission investigation. In addition, when used for detection of pathogens often a biased approach is used. In this presentation an overview of the use of next generation sequencing in clinical microbiology and infection prevention will be given. Next generation sequencing (NGS) determines the DNA sequence of a complete microbial, viral or fungal genome in a single sequence run, and from these data, information on resistance and virulence, as well as information for typing is obtained, useful for outbreak investigation. The obtained genome data can be further used for the development of an outbreak-specific screening test. Like every new technology adopted in microbiology, the integration of NGS into clinical and routine workflows must be carefully managed. As the microbiology laboratories have to adhere to various national and international regulations and criteria for their accreditation, quality control issues for using WGS in microbiology, including the importance of proficiency testing, are presented. In addition, applications of NGS in the clinical setting are discussed, such as outbreak management, molecular case finding, characterization and surveillance of pathogens, rapid identification of bacteria using the 16S-23S rRNA region, taxonomy, and metagenomics approaches on clinical samples. Finally, we share our vision on the use of NGS in personalised microbiology in the near future, pointing out specific requirements.

O087

How Will Digital PCR advance clinical microbiology?

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Digital PCR (dPCR) counts DNA molecules, separated by limiting dilution, in a defined volume providing high accuracy estimation of copy number. The method offers high reproducibility, improved sensitivity when measuring rare genetic variants and greater confidence for low level, trace analysis. This talk will discuss how these factors can enable new measurements to be performed when investigating viral and bacterial infections while also highlighting how the method could revolutionise routine molecular analysis, either directly or in support of established methods.

O088

Implementation of a Rapid Influenza A/B and RSV Molecular Assay at the Emergency Department of a Teaching Hospital

M. Hermans[1], J. Lankelma[1], K. Geraats[1], M. Sterks[1], M. Janssen[1], J. Kroon[1], E. Hazenberg[1], R. Van Gageldonk-Lafeber[2], J. Jaspers[1], S. Lutgens[1]
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Background
The seasonal influenza epidemic poses a significant burden on hospitals, both in terms of capacity and costs. Structural cuts in healthcare budgets have reduced the number of hospital beds to the extent that during influenza waves many hospitals need to temporarily close their wards and cancel elective operations. In the 2017/2018 influenza season we explored whether rapid influenza testing at the Emergency Department (ED), as part of a hospital wide approach to cope with the influenza epidemic, would improve hospital patient flow.

Methods
A PCR-based influenza A/B/RSV Point of Care Test (PoCT; Cobas Liat, Roche; 20 minutes turnaround time, CLIA-waived) was implemented in accordance with ISO22870:2016 at the ED and at the reception desk of the Microbiology Laboratory of the Jeroen Bosch Hospital. The Cobas Liat systems were unidirectionally connected to the laboratory information system. All ED nursing staff and microbiology reception personnel received 1,5 hours of training to operate the system. Confirmatory testing on the Panther-Fusion was carried out for 121 patients.
In parallel, a temporary 'influenza-ward' equipped with 15 beds for influenza-positive patients was

established to increase efficiency of infection control by enabling cohort nursing.

Results
All patients presenting at the ED with symptoms of acute respiratory infection (ARI) and body temperature =38C were tested with the influenza PoCT. The =38C criterion was maintained only at the beginning of the season since many patients had influenza without a fever. Test results were, without authorization, directly available in the Electronic Medical Records. Molecular trained key-user laboratory technicians supported all aspects of the PoC testing.
Of 1546 patients presenting with ARI at the ED of the Jeroen Bosch Hospital, 624 (40%) tested positive for influenza. Performance of the PoCT was good. After the initial influenza A/B/RSV-test 5,1% was unresolved, after retesting these unresolved specimens, 0,8% remained unresolved. Confirmatory testing revealed no discrepancies, in addition no contamination was detected.
The implementation of the PoCT resulted in strongly decreased time from ED presentation to sample collection (194 to 47 min) and time from sample collection to result (1094 to 62 min) compared to the previous influenza season. In general, hospital patient flow improved with a decreased percentage of admitted influenza patients (91% vs 73%) and shorter length of subsequent stay (median 5.86 vs 4.61 days) compared to the previous influenza epidemic.
The costs of the implementation of the PoCT were estimated at 75.000 euro, of the other hospital preparations 25.000 euro. Savings were roughly estimated to be in the order of 500.000 euro.

Conclusion
The hospital-wide approach to cope with the influenza epidemic, including the implementation of the PoCT at the ED and microbiology reception, was successful. It did allow us to handle significantly more ARI patients and to keep wards almost continuously open, while keeping additional costs within limits. We recommend hospitals to explore possibilities for improving patient flow during the influenza epidemic.

O089
Extreme PCR, extreme possibilities?
Petra Wolffs

O090
Organs-on-Chips
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Organs-on-chips are advanced in vitro models that exhibit organ-level functionality and which are engineered by culturing human cells and tissues in microfluidic devices with fluid flow, mechanical actuation, soluble gradients and well-controlled three-dimensional structures. Organs-on-chips have already been successfully applied as models in biomedical science and for studies of toxicity and efficacy of drug candidates.&#13Successful engineering of organs-on-chips relies on advanced microfabrication, human cell culture and stem cell technology, sensor technology and imaging, and measurement of functional endpoints that can be integrated with data from other assays.&#13Based on literature, as well as the presenter's own work on the integration of blood vessels in organs-on-chips, the state-of-the-art in organ-on-chip engineering will be summarized. Moreover, key advantages and limitations of organ-on-chip technology will be described, illustrated by examples from the presenter's own work.&#13Organs-on-chips can be powerful as in vitro models of human organs and diseases. The future impact that they will have in disease modeling and treatment development will be strongly dependent on further technological development, as well as on multidisciplinary collaboration between engineers and biomedical, pharmaceutical and regulatory stakeholders.

O091
Models for studying arbovirus pathogenesis
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In the past two decades, the world has experienced a remarkable increase in the frequency and size of arbovirus epidemics, the Zika epidemic being the most recent example. Despite innovations in diagnostics and vaccine development, it remains extremely challenging to stop an arbovirus outbreak in

its tracks. One way of improving our preparedness for novel arbovirus outbreaks is to increase our knowledge about pathogenic arboviruses that circulate in exotic areas. This knowledge includes the competence of indigenous vectors to transmit these arboviruses and the susceptibility of indigenous target species, including livestock, wildlife and humans. This presentation will demonstrate how novel *in vivo* and *in vitro* models can be used to study arbovirus transmission and pathogenesis and how this knowledge can facilitate preparedness.

O092

In vitro models of virus induced arthritis

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Virus-induced arthralgia has previously been reported following infection with the alphaviruses particularly due to Ross River virus (RRV) and chikungunya virus (CHIKV). Many viruses other than alphaviruses, such as hepatitis C virus (HCV), measles virus (MV) and human immunodeficiency virus (HIV), are also known to affect bone functions which subsequently induces osteoarticular complications. These viruses target osteoblasts (OBs) and alter their function. OBs are mainly bone forming cells and are responsible for the deposition of bone matrix as well as mineralization of the bone matrix. In case of Zika virus (ZIKV) infection, arthralgia is reported in over 70% of symptomatic ZIKV cases including persistent or recurrent arthralgia for more than 30 days. ZIKV has also been detected in synovial fluid of a patient with arthralgia. In a recent case of miscarriage associated with ZIKV infection, ZIKV displayed tropism for fetal mesenchymal stem cells (MSCs, osteoblast precursors) in the perichondrium. However, it is not known if MSC- derived osteoblasts are susceptible to ZIKV infection, and whether infection of osteoblasts affects their function thereby contributing to ZIKV pathogenesis and ZIKV-associated osteoarticular complications. Therefore, in the current study, we investigated the role of osteoblasts in ZIKV infection and bone-related pathology. The effects of ZIKV infection on osteoblast differentiation, and function were monitored by quantifying activity and gene expression of key biomarkers, using human bone marrow-derived mesenchymal stromal cells (MSCs, osteoblast precursors). MSCs were induced to differentiate into osteoblasts and we found that osteoblasts were highly susceptible to ZIKV infection. While infection did not cause a cytopathic effect, a significant reduction of key osteogenic markers such as *ALP*, *RUNX2*, calcium contents and increased expression of *IL6* in ZIKV-infected MSCs implicated a delay in osteoblast development and maturation, as compared to uninfected controls. Moreover, in order to identify key pathways affected due to ZIKV infection, we performed host transcriptomic response analysis using next-generation RNA sequencing. These data show that ZIKV infected osteoblasts mount a robust interferon response as expected, but these cells also exhibit modifications in cell cycle and lipid metabolism related pathways. In conclusion, we have developed and characterized a new *in vitro* model to study the role of bone development in ZIKV pathogenesis, which will help to identify new targets for developing therapeutic and preventive measures.
Keywords: Zika virus, arthralgia, mesenchymal stem cells, osteoblast.

O093

Tissue microarrays for studying the host range of viruses

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Viral receptor expression is a major determinant for tropism and pathogenicity. Tissue and host specific receptor expression is, however, for many viruses poorly understood. Here we set out to increase our knowledge on the host range of viruses using tissue microarrays. To this end, healthy tissues of various hosts were collected and after formalin-fixation stored in paraffin. In particular, tissues of various species were collected after necropsy at the Faculty of Veterinary Medicine, and microscopically evaluated by a certified veterinary pathologist. When no or very limited microscopic lesions were detected, tissue cores were selected and combined into array format. Such tissue usually consist between 10 and 60 tissue cores, but can be tailored to one's needs, for example as multi-species single organ system or single-species multiorgan arrays. Using our avian tissue microarray comprised of tissues of *Anseriformes* and *Galliformes,* we revealed that binding of recombinant viral attachment proteins of avian respiratory coronaviruses and influenza viruses matched to those cells that are infected *in vivo*, demonstrating the

strength of such approach to predict tissue tropism. The ability to compare tissues from various species, including human, therefore allows prediction of the zoonotic ability of a virus of interest. When combined with other techniques like glycan arrays, tissue microarrays can be used to elucidate receptors, like the novel glycan receptor we recently indeed identified for avian enteric coronaviruses. Furthermore, tissue microarrays have the potential to determine detailed receptor-binding properties of viruses and their differences between species as observed for influenza A viruses. Finally, the contribution of specific viral attachment protein mutations to transmissibility or species-specific binding can be elucidated. Several examples highlighting the requirements, benefits and drawbacks of this method will be discussed. Taken together, tissue arrays comprise the complexity of all biologically relevant viral attachment factors and can be used to gain crucial information for enhancing our understanding of virus tropism.

O094
Molecular insight into antibody-dependent complement activation enhances bacterial clearance
Suzan Rooijakkers

O095
The prospects of phage therapy
Stan Brouns

O096
Making NRPS the ribosomal way
F. Ruijne, O.P. Kuipers
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Natural products, like the last-resort antibiotic daptomycin, are produced by nonribosomal peptide synthetases&#13(NRPSs) and comprise interesting structural elements for the development of novel antimicrobials, with high&#13efficacy and relatively low probability of inducing resistance. These large multimodular enzymes produce complex&#13cyclic lipopeptide antimicrobials in an assembly line-like fashion. Creating analogues of these complex products&#13to improve and expand the therapeutic applications and antibacterial spectrum by altering the enzymatic&#13modules is however challenging and laborious. Therefore, we sought to synthesize these peptides&#13in a ribosomal way, making it possible to create analogues by simple mutagenesis of the genetically encoded&#13peptide. In order to ribosomally synthesize these complex peptides, several post-translational modification&#13enzymes need to be orchestrated in time and space *in vivo* to recognize the same precursor peptide to act upon.&#13Recognition of the precursor peptide will be ensured by a hybrid leader peptide, which guides the enzymes to the&#13core peptide for modification. Post-translational modifications include macrocyclisation and epimerisation,&#13followed by an *in vitro* lipidation step. The resulting plug-and-play system of modification enzymes will allow for&#13the facile biosynthesis of a whole variety of new-to-nature peptides, hereby aimed on the production of novel&#13antimicrobials. Furthermore, this research will provide valuable knowledge on the mechanisms of these posttranslational modification enzymes to further develop the production of bioactive peptides. In this presentation, progress and strategies on the development of these new-to-nature NRPS peptide analogues will be discussed.

O097
Autotrophy at the thermodynamic limit of life: what are the metabolic tricks of methanogenic archaea?
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Methanogenic archaea generate about 1 giga ton of methane per year, a greenhouse gas that could be used as a biofuel [1]. The methanogenesis pathway constitutes the primordial source of energy of these strict anaerobes, yielding approximately ATP per formed methane. Such low energy yield obliges them to thrive at the thermodynamic limit of life: they must save ATP through genius strategies if they want to grow and survive. For instance, contrary to acetogenic bacteria, methanogens cannot offer any ATP consumption for CO_2-assimilation; they have to use an unconventional way extremely efficient catalytically and energetically to support their fast growth. The presentation will explain at the molecular

level how the chemical tricks of the heterodisulfide reductase:hydrogenase and formylmethanofuran dehydrogenase complexes team up to realize an ATP-independent CO_2-fixation. These highly elaborated enzymes (post-translational modifications, O_2-sensitive metallo-cofactors) have been purified and crystallized from methanogens under anaerobic conditions.
The 1.9crystal structure of the formyl-methanofuran dehydrogenase (FwdABCDFG), presents an astonishing new mechanism. CO_2 is firstly reduced by low-potential electrons to formic acid, which then diffuses through a 43-long tunnel to a second active site, where it condenses with methanofuran to formyl-methanofuran. The first active site, formed by the subunits FwdBD related to tungsten-containing formate dehydrogenases, is accessible only via a 40-long narrow tunnel allowing specifically CO_2 to reach the catalytic center. The second active site is harbored by FwdA (related to binuclear metal center amidohydrolases) to which methanofuran is bound. The unique electron-supplying core of the tetrameric $Fwd(ABCDFG)_4$ supercomplex contains an astounding network of 46 electronically coupled [4Fe-4S] clusters, which might serve as an electron-storage device or mediate communication [2][3].
FwdABCDFG is fuelled by low-potential electrons generated by the heterodisulfide reductase:hydrogenase (HdrABC:MvhADG). The [NiFe]-hydrogenase (MvhAG) initiates the reaction by transferring electrons from H2 to the flavin center of HdrA. At this stage, the enzyme uses a Flavin-based electron bifurcation mechanism: The reduced Flavin is able to split two electrons of same potentials in one electron of low potential and one electron of high potential. In this case, electrons of high potential reduce the heterodisulfide that is produced by the last step of methanogenesis and electrons of low potential will reduce ferredoxin carrier(s) to fuel FwdABCDFG (CO2-fixation). Our structure at 2.35 describes at the molecular level the different stages of the reaction and highlights a new type of catalytic iron-sulfur (non-cubane) involved in the heterodisulfide reductase reaction [4].
We will conclude our talk by our last research illustrating other examples of energy-coupling machineries involved in methanogenic archaea metabolism and physiology [5].
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O098

An in depth look into the methanol metabolism of the acetogen Moorella thermoacetica strain AMP

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Microbial interactions need to be studied to get a better understanding of biogeochemical cycles in nature. In microbial communities, microorganisms establish distinct relationships, from competition to mutualism. Syntrophy is a type of mutualism in which two microorganisms concert their metabolic activity to make the conversion of a certain compound thermodynamically (more) favorable. This type of syntrophic relationship is common in anaerobic environments, where many of the conversions rely on methanogens, acting as H2 and/or formate scavengers. Obligate syntrophic relationships have been studied in fatty acid-oxidizing microbes, but facultative syntrophy, between acetogens and methanogens has received little attention. The metabolism of the acetogen Moorella thermoacetica was shown to be influenced by the presence of methanogens (Dolfing et al. 2008). In this work we aim to get insight into the mechanisms underlying syntrophic behavior in co-cultures of Moorella thermoacetica strains and methanogens growing on methanol by combining physiological data and omics analyses. After an initial screening of several M. thermoacetica strains, we discovered that Moorella thermoacetica VPI 12954T grows on methanol only when the methanogen Methanothermobacter thermoautotrophicus strain ΔH is present (obligate syntrophy), while other M. thermoacetica strains (including strain AMP previously isolated in our laboratory) (Jiang et al. 2008) could grow on methanol both alone or with the methanogenic partner (facultative syntrophy). Currently, we investigate the physiological and genetic differences between these obligate and

facultative syntrophic co-cultures. We observed that M. thermoacetica strain AMP growing in mono-culture and in co-culture with M. thermoautotrophicus did not exhibit significant differences in lag phase and rates of methanol consumption and acetate production. In contrast, methanogenic co-cultures of M. thermoacetica strain VPI 12954T had a significantly longer lag phase than equivalent methanogenic co-cultures of M. thermoacetica strain AMP (i.e. 20.06 ± 0.1 vs 4.0 ± 0.1 days, $p<0.001$. In addition, the genomic and transcriptomic and analyses will shed new light on the interactions that govern mutualisticsyntrophic growth in these cultures.

O099

Characterization of a novel Verrucomicrobial methanotroph from Pantelleria island

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The island of Pantelleria is a volcanic environment with high greenhouse gas emissions of methane and CO_2, at high temperature and low pH. These conditions allow the growth of extremely adapted methanotrophs that are able to consume methane as energy source. For a long time methanotrophs were considered to be only present in the phylum Proteobacteria, but in 2007 a novel species from a volcanic mud pot in Italy was isolated and discovered to be part of the new phylum Verrucomicrobia. The present study expands our knowledge on the verrucomicrobial methanotrophs by describing a new bacterial species from the *Methylacidimicrobium* genus. This isolate, named *Methylacidimicrobium thermophilum* A8, contains a genome of 2.3 Mb that includes all the genes involved in methane metabolism; in particular, two copies of the lanthanide dependent *xoxF* methanol dehydrogenase were found, which is an enzyme that is known to convert methanol to formate or formaldehyde. Furthermore, this bacterium has an optimum temperature of 50C and a broad pH optimum ranging from pH 3 to pH 5. Additionally, the doubling time was determined and appeared to be 13h.

O100

Geomicrobiological factors influencing metal attenuation in extremely acidic and metal-rich mine pit lakes in Huelva, Spain

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Mining operations produce large amounts of waste, which, upon oxidation, can result in the generation of extremely acidic wastewaters with high concentrations of metals, termed acid mine drainage (AMD) waters. AMD forms when metal sulphide ores are exposed to water and an oxidizing agent such as oxygen or Fe(III), which catalyse their conversion to metal ions and sulphuric acid. A notable example occurs when abandoned open-cut mining sites are no longer actively pumped dry. They then fill up with ground-, surface and rainwater to the phreatic level, resulting in the formation of acidic, metalliferous artificial lakes-a widespread phenomenon at mining sites around the world. Physicochemical characteristics such as dissolved metal species and sulfate concentration profiles vary between pit lakes, as they are determined by local geology and are influenced by chemical and biological factors. The presence of high concentrations of sulphate, combined with anoxic conditions, makes these lakes habitats for extremely acidophilic sulphate reducers. As sulphate reduction results in the production of sulphide, sulphate-reducing activity further influences lake chemistry by precipitation of the dissolved metals as sulphide minerals (e.g., CuS, As_2S_3, PbS, ZnS, FeS_2).
We aim to understand how microbial activity might influence the physicochemical characteristics of pit lakes. With this purpose, we sampled three different pit lakes in Spain for chemical and microbiological analysis. Two of the lakes were located in the Iberian Pyrite Belt mining district, Huelva, and the other lake was situated in Minas de la Unin, Murcia. Sampling depths for microbial community analysis were based on pH, redox potential, conductivity and oxygen gradients measured on site at the time of sampling. Microbial diversity was analysed by 16S rRNA gene amplicon sequencing. Lipids were also analysed in an attempt to correlate microbial species with lipid markers. SEM microscopy was used on suspended particulate matter retained in membrane filters to detect microbe-mineral interactions. Results showed strong variation of the metal concentration profiles in the three lakes. Preliminary SEM analyses showed cell-like morphologies attached to sulphide minerals. Microbial community analysis is now in progress in order to assess the diversity of microorganisms present, as well as the presence of potential novel extreme acidophiles and their impact on water chemistry.

O101

Methane oxidation in anoxic lake waters mediated by novel facultative anaerobe methanotrophs

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Methanotrophic bacteria play a key role in limiting methane emissions from stratified lakes. It is generally assumed that methanotrophic bacteria are active in the oxic-anoxic transition zone, where they make use of the available oxygen to oxidize methane. However, our research on a eutrophic, seasonally stratified lake shows that a formerly known aerobic methanotroph of the genera *Methylobacter* is performing methane oxidation in the anoxic water column. Likely, this *Methylobacter* couples nitrate reduction to methane oxidation, as genes encoding for nitrate reductases were detected in its genome. This is supported by the experimental observation that the addition of nitrate to incubation experiments strongly enhanced the methane oxidation rate. Methane oxidation by *Methylobacter* was observed in both oxic and anoxic incubations, with anoxic rates being 3 times higher than oxic methane oxidation rates, accompanied by an increased relative abundance of *Methylobacter* from 7 % in oxic incubations to 35.4 % in anoxic incubations. PmoA protein analysis showed that this type of *Methylobacter* is most closely related to *Methylobacter tundripaludum*, and likely represents a novel species.

O102

In vivo imaging of Aspergillus fumigatus infections

Greetje Vande Velde

Invasive lung infection by Aspergillus fumigatus is an important life-threatening complication for already vulnerable patients. Nevertheless, our knowledge on etiology, resistance development and effective treatment thereof still contains important gaps. To tackle these challenges, preclinical investigators typically rely on end-stage procedures that are useful, but provide only a snapshot of disease processes that are essentially dynamic in time and space. Moreover, evaluation of fungal burden and therapy effectiveness often relies on survival analysis or enumeration of pathogens from isolated tissue homogenates that inherently suffers from large variation, resulting in high numbers of animals that need to be sacrificed in order to reach statistical significance. Our mission is to develop non-invasive multimodal imaging technology that allows evaluating repeatedly host response, infection progression and therapy effectiveness in live experimental models of invasive aspergillosis. The combination of several different imaging modalities (MRI, micro-CT, optical imaging such as photonic imaging and intravital fibered confocal fluorescence microscopy) overcomes the limitations of using individual methods, and provides thereby complementary information and novel insights into the dynamics of infection and host response in space and in time. This approach provides us with novel longitudinal readouts and quantitative imaging-derived biomarkers of infection and host response that have demonstrated to increase the accuracy and power of animal experiments. This approach immediately reduces manifold the amount of animals needed for aspergillosis and antifungals research, and will ultimately improve patient care.

O103

Aspergillus taxonomy, diversity and resistance

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Aspergilli cause a wide spectrum of infections including cutaneous manifestations, otomycosis and invasive infections such as pulmonary aspergillosis and endocarditis. *Aspergillus fumigatus* is the predominant agent of invasive pulmonary aspergilosis (IPA), followed by *A. flavus* and *A. terreus*, depending on the medical centre; however, also IPA due to other rare aspergilli (e.g. *A. calidoustus*, *A. lentulus*, *A. udagawae*) are reported. In the last decade, new insights have resulted that certain well-known *Aspergillus* species appear to be species complexes. This might lead (initially) to confusion; however, a correct identification has a function. Clinically, identification of unknown *Aspergillus* isolates to species may be important given that different species have variable susceptibilities to multiple antifungal drugs. Thus, knowledge of the species identity may influence the choice of appropriate antifungal therapy. Ideally, identification should be unequivocal, accurate, simple and immutable. In this paper, an overview of taxonomic changes and developments in *Aspergillus* is given. Furthermore, data is presented on intrinsic (and acquired) antifungal resistance in Aspergilli.

O104

Successful rescue treatment with aztreonam and ceftazidim/avibactam of a Dutch kidney transplant patient with sepsis due to a pandrug-resistant, New Delhi metallo-β-lactamase-producing *Klebsiella pneumoniae*

Key words: bacterial drug resistance, carbapenemase-producing Enterobacteriaceae, *Klebsiella pneumoniae*

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Introduction: Combining aztreonam and ceftazidim/avibactam is a potential treatment in multidrug-resistant Enterobacteriaceae that produce New Delhi metallo-β-lactamase (NDM). However, evidence of clinical efficacy and safety is limited and aztreonam for intravenous use is currently not available in the Netherlands. We describe the first Dutch patient with sepsis due to a pandrug-resistant, NDM-producing *K. pneumonia* successfully treated with ceftazidim/avibactam and intravenous administration of aztreonam powder for nebulizer solution.

Materials/methods: We tested antimicrobial susceptibility of the strain using Vitek-2 and MIC gradient tests. For colistin susceptibility we used broth microdilution. Carbapenemase genes were tested by an in-house PCR. To test for potential synergy between different antibiotics, we combined gradient tests and compared MICs with MICs of single gradient tests.

Results: In December 2018, urinary culture of an asymptomatic kidney transplant patient showed a NDM-producing *K. pneumoniae* resistant to all antimicrobials tested, including carbapenems, aztreonam, beta-lactam/beta-lactamase inhibitors, colistin and tigecyclin. Nine days later, she was admitted for suspected kidney transplant pyelonephritis. We regarded aztreonam combined with ceftazidim/avibactam the most promising treatment option, but aztreonam intravenous solution was not available in the Netherlands. As the patient's condition deteriorated, we decided to administer aztreonam designated for inhalation therapy intravenously. The subsequent day her blood culture became positive with the pandrug-resistant *K. pneumoniae*. Immunosuppressive therapy was reduced and the patient received supportive care for sepsis. Approximately 20 hours after start of the combination therapy, she started to improve. She recovered completely with 14 days of therapy without signs of adverse events. In vitro synergy testing indicated clinically relevant synergy between aztreonam and ceftazidim/avibactam based on combination gradient testing. No other clinically relevant synergy was found in vitro.

Conclusions: We report successful rescue treatment of a Dutch patient with sepsis due to a pandrug-resistant, NDM-producing *K. pneumoniae* using aztreonam inhalation formula as intravenous therapy in combination with ceftazidim/avibactam and reducing immunosuppressive therapy. Aztreonam for intravenous use should become available in the Netherlands. Future studies need to define efficacy and safety of this promising treatment combination in patients with serious infections due to pandrug-resistant, NDM-producing *K. pneumoniae* and other Enterobacteriaceae.

O105

CRISPR/Cas9 and gene editing for the elimination of persistent viral infections

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Keywords: virus, latency, reservoirs, CRISPR/Cas9, meganuclease

Persistent viruses, such as herpes simplex (HSV), hepatitis B (HBV), and Human immunodeficiency virus (HIV) remain serious causes of human suffering and death. After initial infection, these viruses establish latency or stable persistence in long-lived cells. Current antivirals can suppress viral replication, but they do not eliminate the persistent viral forms, which serve as the source for viral reactivation once treatment is stopped. To address the need for novel therapeutic approaches to cure these infections, we have evaluated the use of gene-editing endonucleases to directly target persistent virus in vivo. For HSV, we have demonstrated that AAV can efficiently deliver anti-HSV meganucleases to the trigeminal (TG) and superior cervical (SCG) ganglia of mice with latent infection, resulting in a reduction in ganglionic HSV of up to 90%. For HBV, we have observed that AAV-delivered anti-HBV CRISPR/Cas9 leads to dramatic improvement in human hepatocyte survival in a humanized mouse model. For HIV, the use of gene editing in vivo remains challenging due to the distributed nature of the reservoir, and thus new and innovative delivery approaches are key. Taken together, our results support continued efforts toward gene editing as an antiviral strategy, emphasize the importance of efficient delivery for in vivo gene editing applications, and should encourage workers in the field to appreciate the full diversity of available gene-editing nucleases.

O106

How to tackle the most successful pathogen on earth

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Tuberculosis remains a major health threat and better control measures are needed. These include better diagnostics, drugs and vaccines. More recent technical achievements have added biomarkers to this list. Recent findings revealed that host biomarkers based on transcriptomic or metabolomic signatures can predict onset of active tuberculosis in household contacts of newly diagnosed index cases. More than a dozen vaccine candidates against tuberculosis are undergoing clinical testing and one candidate has provided a protective signal in a phase IIb trial. The attenuated live vaccine VPM1002 is based on a genetically modified BCG in which the urease C gene had been replaced by the listeriolysin gene. VPM1002 has successfully completed phase I and II clinical trial testing and has entered phase III clinical efficacy trials. First, it is being tested whether VPM1002 can prevent recurrence of tuberculosis in individuals who had been cured from active disease by drug treatment. Second, prevention of infection and disease by VPM1002 will be tested in HIV exposed and unexposed neonates. Third, prevention of disease in household contacts of newly diagnosed tuberculosis index cases will be assessed. This presentation will describe current progress in the development of biomarkers and vaccines as intervention measures against tuberculosis.

O107

Antimicrobial resistance in humans: prevalence and spread

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The i-4-1-health-project is a crossborder one health project in which hospitals nursing homes, public health agencies, universities, veterianry medicine and companies on both sides of the Flemish-Dutch border work together intensively. The project uses objective and reproducible methods to measure the quality of infection control, antbiotic use and antimicrobial resistance in several domains. &#13The overarching principle of the project is: 'you cannot manage what you cannot measure'. &#13The prevalence of various highly resistant micro-organisms (HRMO) was measured in hospitalized patients in 9 hospitals.&#13A total of 1304 patients were included. The prevalence of resistance was in general higher in Belgium compared to The Netherlands. The only exception were carbapenemase producing Enterobacteriaceae which were rarely observed in both countries.&#13Also the prevalence of antimicrobial usee was measured. The proportion of patients that received antibiotics was comparable in both countries.However, the type of agents was different. In Belgium Piperacillin-tazobactam and vancomycin were most often used whereas in The Netherlands Amoxicillin-clavulanic acid and cefuroxim had the highest frequency. &#13The cleanliness of hospitals was measured using ATP. On average the Belgian hospitals had lower ATP values than the Dutch hospitals.&#13&#13In conclusion: the i-4-1-health project implemented a standardized method to measure antimicrobial resistance, antimicrobial use and the quality of infection control. There were substantial differences between the two countries which may offer targets for improvement. Repeated measurements can objectify the effect of these interventions. &#13&#13&#13&#13

O108

Reducing antimicrobial use and its effect on AMR in livestock in the Dutch-Belgian cross-border region: the i-4-1-Health project

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Keywords: livestock, antimicrobial resistance, antimicrobial use, Belgium and the Netherlands

Background:
Public and private policy in livestock farming aim to reduce total use and increase prudency of antimicrobial use (AMU). Cultural as well as policy driven differences in human and veterinary healthcare exist between countries with respect to AMU. Especially in border regions cross border travel of patients, animals as well as healthcare and livestock workers can be expected with its associated AMR genes. The aim of the cross-border project i-4-1-health (www.i41Health.eu) is to reduce AMU on 30 poultry and 30 pig farms with above average AMU and investigate its association with changes in prevalence of ESBL- (ESBL-PE) and carbapenem (CRE) and ciprofloxacin-resistant Enterobacteriaceae (ciproR-E).

Methods:
Sixty farms were visited four times in ~1.5 yr. First, farm biosecurity (using BioCheck.Ugent™), AMU and infection risks of animals and humans were evaluated. Subsequently farmers were guided towards reduction of AMU using a newly developed coaching method. At three visits, fresh fecal droppings were sampled (Fecal Swab, Copan Diagnostics) in a stratified-random design. The presence of ESBL-PE, ciproR-E and CRE was investigated employing non-selective broth enrichment (TSB, Copan Diagnostics) and selective media (bioMérieux, in-house), followed by antimicrobial susceptibility testing (disk diffusion or Vitek2, bioMérieux) and phenotypic ESBL confirmation (combination disk diffusion, Rosco).

Results:
First of all, no CRE were found. Prevalence of resistant bacteria varied across farms and differed between countries as well as between species. Higher levels of ESBL-PE and ciproR-E were found in Belgian samples compared to Dutch samples, but levels of ciproR-E were high in samples from both countries.

Conclusions:
The i-4-1-health project provides opportunities to compare levels of antibiotic resistance in border regions, create awareness and study the origin and relevance of these differences.

O109
The IRIS-app
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The Infection Risk Scan (IRIS) is a tool to measure the quality of infection control and antimicrobial use in hospitals and nursing homes (NH)[1,2]. However, manual registration, analyses and reporting is time consuming and error-prone. An Interreg Vlaanderen-Nederland grant made it possible to create an IRIS-app as part of the i-4-1-Health project. The IRIS-app consists of a user-friendly module for the registration of 1) the use of indwelling medical devices, 2) use of antimicrobial therapy, 3) environmental contamination, 4) hand hygiene performance, 5) personal hygiene of healthcare workers, 6) presence of infection control preconditions and 7) carriage and clonal relatedness of highly-resistant microorganisms. Analysis of the data is fully automated, resulting in an easy-to-read report, including a risk profile and improvement plot. The IRIS-app enables uniformity and standardization in infection control and is easy to use through its simple design.
https://www.ncbi.nlm.nih.gov/pubmed/29541449Willemsen I, Kluytmans J. Antimicrob Resist Infect Control. 2018 Mar 9;7:38
https://www.ncbi.nlm.nih.gov/pubmed/27900923Willemsen I, Kluytmans JA. Ned Tijdschr Geneeskd. 2016;160:D518. Dutch.

O110
Whole Genome Sequencing (WGS) webtool for track and trace of antimicrobial resistance
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Introduction
Antimicrobial resistance is a worldwide problem. A 'One Health' approach towards identification of

transmission routes of antibiotic resistant microorganisms across sectors is important to prevent spread of resistance. The i-4-1-Health project aims to elucidate the prevalence and transmission of antibiotic resistant microorganisms in humans and animals in the Dutch Belgian cross-border region. To assess transmission within and among healthcare institutions and farms, resistant bacteria are collected and genotypically characterized by whole genome sequencing to detect the presence of resistance genes and identify clonal relatedness of these isolates. For this purpose, a webtool was built with the aim to quickly confirm and manage outbreaks of the most relevant hospital acquired infection (HAI) bacterial species through NGS-based epidemiological and antimicrobial resistance profile analysis.

Methods and Results

The webtool was built to manage both raw sequencing data from Illumina sequencing and preassembled genomes. This webtool contains functionalities for (i) assembly of raw sequencing data, (ii) genomic characterisation of bacterial isolates (iii) wgMLST based epidemiological analysis of clonal relatedness, (iv) screening of isolates against resistance and plasmid databases. Geographical analysis of results will be implemented. Intended users comprise both hospital laboratory mangers, data researchers and infection prevention specialists, with access and output specific for each role.

The backbone of the webtool consists of validated analysis algorithms, most of which already found their way to the scientific community. In general, validation was performed by comparison of results obtained through the pipeline with results obtained through classical, widely accepted, analysis techniques (e.g. wgSNP, maldi-TOF, Kirby-Bauer method for antibiotics resistance).

The webtool provides a one click solution for analysis of sequencing data. After uploading of sequencing- and metadata, the webtool automatically detects the species from the data and takes cares of all the necessary analyses and computations. Yet, it also provides the user with the necessary quality parameters, such as number of contigs, N50, number of retrieved loci, to allow a quality assessment at all times. The results can be analysed at different levels of complexity: summary reports provide a quick overview of the most relevant results, while detailed reports allow deeper analysis and to investigate why, for instance, an isolate was reported resistant to certain antibiotics. Finally, it also allows analysis of the bigger picture' as it can graphically show relations between isolates, either from different institutes or with previously analysed isolates.

Conclusion

This webtool provides rapid feedback of NGS-based epidemiological data on HAI bacterial isolates to confirm and manage hospital acquired infection outbreaks to different levels of professional users. The standard reporting and easy access will facilitate better turnaround time of epidemiological data and the option to analyse institute data in wider geographical context.

O111

Where do eukaryotes go in the tree of life?

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The origin of eukaryotic cells was a major event in the history of life. Our understanding of eukaryogenesis has made enormous progress in recent years, thanks in part to new techniques for sequencing environmental microbes and better methods for inferring phylogenetic trees. In my talk, I will present new phylogenomic analyses that bear on several of the open questions about the origin and early evolution of eukaryotes. These include (i) the nature of the relationship between Asgard archaea and eukaryotes and (ii) the earliest divergences among the major lineages of eukaryotes following their origin. Working further back in time, I will also present some work on rooting the tree of life. While most analyses assume evolution 'from prokaryotes to eukaryotes', different roots have major implications for our understanding of early cell evolution. I will re-evaluate recent evidence for a root between prokaryotes and eukaryotes, which --- if true --- implies that both the classical 'three domains' and the more recent 'two domains' trees are profoundly wrong and misleading.

O112

Assigning new sulfate reduction and alcohol cycling roles across the microbial tree of life

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The Prairie Pothole Region of North America is the tenth largest wetland ecosystem in the world. Wetlands in this region sustain extremely high methane emissions and the highest sulfate reduction rates reported to date, estimated to account for a significant fraction of organic carbon conversion into carbon dioxide gas. Alcohols have been hypothesized to substantially support such high microbial

activity given that millimolar concentrations of ethanol and isopropanol were measured in sediment pore waters, becoming depleted in periods of highest activity. Despite the importance of these wetlands for regional carbon and sulfur cycling and greenhouse gas emissions, microorganisms responsible for these processes are poorly characterized.
Here, we present a metagenomics-based investigation of microorganisms responsible for sulfate reduction and alcohol production in Prairie Pothole wetland sediments. Eighteen sediment samples were collected from two wetlands, representing three seasons (winter, spring and summer) and three depths (surface, middle and deep sediments). DNA was extracted and sequenced on the Illumina HiSeq 2500 platform. Following read assembly and binning, unbinned contigs and draft genomes were scanned for marker genes for sulfate reduction and alcohol fermentation.
We recovered 162 *dsrA* and 206 *dsrD* sequences, as well as reconstructed 24 candidate sulfate reducer genomes assigned to seven phyla. These genomes encoded the potential for utilizing a wide variety of electron donors (methanol and other alcohols, methylamines, and glycine betaine, lactate, acetate, glucose) and acceptors (not only sulfate, but also oxygen and nitrate). Additionally, we recovered 62 genomes of candidate alcohol-cycling microorganisms spanning 16 phyla. These genomes encoded a variety of novel alcohol dehydrogenases - including 19 novel putative isopropanol dehydrogenases affiliated to phyla for the first time suggested to metabolize isopropanol. The most frequently encoded pathway potentially accounting for alcohol production was a *Pyrococcus furiosus*-like fermentation which can involve a pyruvate:ferredoxin oxidoreductase. Mixed acid fermentation and heterofermentative lactate fermentation were also frequently encoded.
In conclusion, phylogenetic and metabolic diversity may be key to high sulfate reduction and alcohol production in Prairie Pothole wetlands. Our results support the hypothesis that alcohols sustain high microbial activity and carbon cycling, with implications for greenhouse gas emissions from these wetlands, and advance our understanding of alcohol fermentation in wetlands. Finally, the novel microorganisms and alcohol dehydrogenases reported here have potential for future biotechnological applications in commercially valuable alcohol production.

O113

Ultra-small archaea - a diverse and deep-branching archaeal lineage?

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The diversity and deep evolutionary origins of archaea is of fundamental importance for our understanding of the evolution of life on Earth. Recently, cultivation-independent approaches have allowed the reconstruction of genomes of uncultivated microorganisms and revealed that archaea are much more diverse and widely distributed in the biosphere than assumed previously. For example, archaea were shown to include a large diversity of organisms with extremely small cell and genome sizes and limited metabolic capabilities. Currently, these organisms belong to at least 10 different lineages, which seemingly comprise a deep-branching monophyletic superphylum referred to as DPANN. However, the extent of the diversity of DPANN archaea and their phylogenetic placement remain a matter of debate. Here, we describe 13 genomes of the thus far uncharacterized, deep-branching archaeal UAP2 lineage, which seems to represent a new phylum within the DPANN archaea. Similar to other DPANN archaea, UAP2 have small genomes and a limited metabolic gene set. For instance, members of this group lack key genes for amino acid and vitamin biosynthesis and have a low number of transporters. However, UAP2 genomes encode all subunits of a V-type ATP synthase, partial gluconeogenesis and a non-oxidative pentose phosphate pathway, a ribulose-bisphosphate carboxylase, ADP-forming acetyl coenzyme A synthetase and several key lipid biosynthesis genes. These findings suggest that UAP2 archaea could ferment simple carbohydrates to generate energy, however, they also point towards some dependency on symbiotic interactions with other organism groups. Results from initial phylogenetic analyses suggest that UAP2 branches deeply with regard to DPANN archaea and sharing features with both free-living and symbiotic archaea indicates that UAP2 occupies a key position to address DPANN monophyly. Thus, subsequent in-depth genomic and phylogenetic analyses as well as ancestral reconstructions will likely help to provide important insights into the evolution of symbiosis in archaea.

O114

Unravelling the ecophysiological dynamics of core human intestinal bacteria in a defined consortium

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One of the major challenges in understanding the ecological processes and metabolic interactions in the human microbiome is the high level of complexity. The trophic interactions in the intestinal tract facilitate co-existence of complementary species that share resources. However, competition is often more prevalent than complementarity in the human intestinal microbiome. In order to understand how competing species co-exists, it is important to understand their metabolic lifestyle in presence of other species in systems that are sufficiently well characterized. However, studying metabolic interactions as well as identifying emergent biosynthetic pathways resulting from multiple interacting species is challenging due to the complexity of natural microbiota. Defined microbial communities with known composition offer a unique opportunity to understand metabolic interactions in the human microbiome. We have designed an ecophysiology guided minimal bacterial community to incorporate four features that are relevant for the functioning of the colonic intestinal microbiome: a) being representative of the prevalent and abundant core bacteria; b) having a higher proportion of competitive than complementarity interactions; c) having functional redundancies at each trophic level; d) having trophic interactions via breakdown of complex dietary carbohydrates and fermentation by-products. The first results from our in-vitro experiments that demonstrate both the compositional and functional features of the diet based minimal microbiome in presence of different dietary carbohydrates will be discussed.

O115

Transforming metagenomics through long-read sequencing

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Obtaining the genome sequence of a microorganism constitutes an important first part in the design of many studies. The genome enables the exploration of the metabolic potential of an organism, permits comparison to other strains and species and provides the foundation for transcriptomics and proteomics studies. The advent of next-generation sequencing (NGS) technologies (e.g. Illumina sequencing by synthesis, PGM IonTorrent semiconductor sequencing, 454 Pyrosequencing) has enabled many labs to routinely sequence isolate cultures at low cost. However, the vast majority of microorganisms known today cannot be cultivated using conventional techniques, challenging the recovery of genomes for these species. Continuous cultivation techniques, including bioreactor systems, can be utilized to obtain cultures that are enriched in the target organism(s), potentially reducing the complexity of obtaining a genome through *in silico* analysis.

Metagenomic methods offer opportunities to study elusive, difficult to culture microbes. In a metagenomics approach, microbial communities are sampled directly from the environment, circumventing the necessity and complexity of cultivation and avoiding the potential biases that may arise from it. The composition and function of whole microbial communities can be studied in this manner. One of the central objectives of metagenomics is to correctly assign the assembled contigs forming the metagenome to genome bins, where a 'bin' is a set of contigs that are assumed to constitute a single genome. Several approaches are used to identify and exploit signals to distinguish and separate sequences into genome bins, including assessing sequence composition (e.g. GC content, codon usage, tetra-nucleotide frequencies), (differential) coverage binning (use of relative abundance data), use of linkage information (paired-end information, mate-pair data) and assignment of contigs through taxonomic classification and annotation.

In many metagenomics studies, 2nd generation sequencing platforms, generating short highly accurate reads, have been used to great success. However, various biases hinder the resolution of closed genomes. For instance, mandatory DNA amplification steps have difficulty amplifying GC rich regions, yielding uneven genome coverage. Furthermore, relatively short read lengths prevent the accurate reconstruction of repetitive sequences, palindromes and low complexity regions. These issues impose limits on the recovery of genomes from low abundant organisms and separation of similar strains. The use of long, multi-Kb reads has the potential to ameliorate and overcome many of these challenges. Recent developments of 3rd generation sequencing platforms, such as PacBio Single-Molecule Real-Time (SMRT) sequencing and Oxford Nanopore sequencing, have culminated in significant improvements in sequence accuracy and data throughput, making long-read technology an increasingly more suitable method for use in metagenomics approaches. Although long-read technology has great potential to advance metagenomics by reducing complexity of metagenome assembly and binning, new

methods and tools are required to harness the full power of the long-read data.
Based on case studies, I will show how long-read technology has enabled us to close genomes and extract highly similar strains from complex communities and enrichment cultures. Furthermore, I will discuss current developments and the future potential of long-read data in metagenomics.

O116
Organoid culture systems to study host-microbe interactions
Jerry Wells

O117
Th17 development in man and its relevance to Candida in Inflammatory Bowl Syndrome
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The identification of Interleukin-17 (IL-17)-producing CD4+ Thelper (Th17) cells as a T cell subset distinct from IFN--producing Th1 or IL-4-producing Th2 cells has had a tremendous impact on our understanding of the function of cytokines and T cells. Mouse model experiments have shown that bacteria and fungi are effectively challenged through amplification of neutrophil activity by IL-17A-producing effector CD4+ helper T cells. And indeed, also patients with inborn errors in IL-17 expression or IL-17R suffer from severe mucocutaneous candidiasis or infections with mostly gram negative extracellular bacteria e.g. K. pneumonia. On the other hand, Th17 cells have been associated with various autoimmune diseases including rheumatoid arthritis and inflammatory bowel disease (IBD).
The unchallenged paradigm is that development of effector T cells is directly driven by antigen-primed dendritic cells (DCs). In contrast to the mouse, human nave T cells do not develop into Th17 cells upon stimulation by activated DCs. However, recent data demonstrate that at inflammatory sites and secondary lymphoid structures, DCs are in close proximity and/or interact with innate immune cells, such as PMNs. Indeed, it has been demonstrated that basophils, the innate target of Th2 cells or NK cells, the target cell of Th1 cells, amplify the Th2 or Th1 cell development, respectively. Therefore, we hypothesized that neutrophils, the innate effector cell associated with Th17 cell responses may amplify or initiate the development of DC-driven Th17 cells from naive human $CD4^+$ T cells. Our data show that neutrophils are indispensable for dendritic cell (DC)-driven development of human Th17 cells from naive CD4+ T cells. We pinpointed the mechanism to elastase, which is released by activated neutrophils and processes DC-derived IL-8 of 77aa into a truncated form of 72aa driving Th17 cell development. Taken together, this newly discovered link between neutrophils and Th17 cell development supports the novel concept that innate cells are of paramount importance in polarizing adaptive immunity.
Treatment of patients suffering from the arthritic condition spondylo-arthritis, which is strongly associated with IL-17 and Th17 cells, with neutralizing antibodies against IL-17 is very succesful. In contrast, treatment of IBD (Crohn's Disease or Ulcerative Colitis) with anti-IL-17 was not beneficial for the patients whereas neutralizing IL-23, the DC-derived cytokine involved in Th17 cell development, is effective. It is speculated that the failure of anti-IL-17 to improve IBD may be the result of a shift in microbiome and candida presence in the gut.
During my presentation I will discuss these data.

O118
The role of the plasmids and the microbiome in antimicrobial resistance in Clostridioides difficile
Wiep Klaas Smits
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Clostridioides difficile (*Clostridium difficile*) is a gram-positive anaerobic enteropathogen. *C. difficile* infections (CDI) are a common complication of antimicrobial therapy, and recurrent infections are a clinical problem. A common first-line therapy for CDI is the 5-nitroimidazole prodrug metronidazole.

Despite a wealth of next generation sequencing data, the role of extrachromosomal elements in C. difficile virulence and pathogenesis is poorly understood. Only very few plasmids have been characterized and none of these have a clear phenotype associated with them. Our previous work on one family of plasmids suggests that they may be more common than currently assumed. We performed an in silico survey to identify putative extrachromosomal elements (likely plasmids) of *C. difficile*. We find that plasmids are abundant in *C. difficile* and that multiple plasmids can co-exist in the same strain. We identified a metronidazole resistant isolate in a patient suffering from recurrent CDI, that was initially diagnosed with a susceptible strain. Using whole genome sequencing we identified a plasmid, pCD-METRO, capable of conferring metronidazole resistance. The plasmid is identified in human and animal, and both toxigenic and non-toxigenic isolates, and is internationally disseminated. pCD-METRO is likely mobilizable, and does not carry any characterized metronidazole-resistance genes, suggesting a novel mechanism of resistance. Quantitative PCR suggests that the pCD-METRO replicon sustains a higher copy number than currently known *C. difficile* replicons. This is the first report of a clinically relevant phenotype associated with plasmid carriage in *C. difficile*.

O119

The human skin mycobiome and its major player Malassezia

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The skin is our largest organ and forms a physical barrier between the environment and the rest of our body. It is inhabited by complex communities of viruses, bacteria and fungi: the skin microbiome. This community of external microorganisms (and viruses) may serve as a protector by training our immune system and keeping pathogens at bay. If the skin barrier or the protective microbial skin community is disrupted, pathogens may cause skin disease or even systemic infections could be the result. When the immune system is down, even commensals may be able to cause health problems. In my talk I will discuss the fungal part of the skin microbiome with a strong focus on the genus *Malassezia*, the most abundant component of the healthy skin mycobiome. The genus *Malassezia* phylogenetically sits within the Ustilaginomycotina, which primarily are plant pathogens. During the course of evolution, the genus has adapted to human and warm-blooded animal skin through loss of genes coding for carbohydrate metabolism and gain of genes involved in breaking down external lipid sources. *Malassezia* maintains a complex relationship with its host and may take on different roles as commensal, pathogen, and there are even indications that it may serve as an active protector in specific situations. Much around the underlying mechanisms driving these relationships between *Malassezia* and host needs further clarification. And should the general rise of antifungal resistance cause concern here as well, considering the abundant presence of *Malassezia* on the skin and its two-faced 'behavior'?

O120

The European Regulation on In Vitro Diagnostic Medical Devices (IVDR) IGJ point of view

K. Maquelin
Health and Youth Care Inspection, UTRECHT, Nederland

The current legislation on in-vitro diagnostics (IVDs) will be replaced by the IVDR on May 26th, 2022. This regulation not only describes the requirements for manufacturers of IVDs, but also describes the criteria for using an IVD. The most significant change for medical microbiological laboratories are the limitations for laboratory developed tests (LDTs). In sharp contrast to today's situation, LDTs cannot be developed when an equivalent device is available on the market. Laboratories need to justify and document the development and use of LDTs. Additionally, a declaration of conformity to the requirements of the IVDR should be made publicly available. A final new aspect for LDTs is the requirement to review the experience gained with LDT and take all corrective actions needed in order to guarantee safe use in patient care.
Availability of all CE-marked IVDs after the IVDR is fully applicable is not 100% certain. Due to the new demands on pre- and post-market responsibilities, many manufacturers are reconsidering their product portfolio. In some cases a manufacturer will stop putting their IVD(s) to market because the profits will not outweigh the costs. In this situation a laboratory needs to have alternatives to continue their services. This presentation will focus on the changes the IVDR will bring for diagnostic laboratories. Some insight will be presented into the surveillance and enforcement responsibilities of the Health and Youth Care Inspectorate.

O121
UMC-Utrecht: Consequences of the IVDR for medical microbiologiccal laboratories
Edwin Boel

O122
Discussion

O123
The Dutch Labcode Set for AMR - what is it and how to implement it
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Objective
To study the feasibility of nationwide implementation of a standardized interface (HL7) using a subset (the Dutch Labcodeset) of internationally accepted terminologies (SNOMED CT, LOINC, UCUM) to communicate routine antimicrobial susceptibility testing results between the medical microbiology laboratory (MML) and the Dutch national antimicrobial resistance monitor (ISIS-AR).
Methods
In this pilot study six MML's mapped their internally used laboratory codes for micro-organisms, specimen types and antimicrobial susceptibility testing (AST) procedures and results to the codes available in the Dutch Labcodeset. Three laboratory information system vendors developed the standardized interface, using HL7 syntax, to report the AST results to the ISIS-AR database. Analysis of the data reported through this new interface compared to the traditional monthly reports was performed. For each of the five completed phases in this pilot study each MML filled out an evaluation form.
Results
Analysis of the data reported through the new interface compared to the traditional monthly reports showed high concordance. Based on the feedback gathered during the project and in the 30 evaluation forms, we developed a practice guide to facilitate the implementation steps. In conclusion, implementation of a standardized interface and codes (Dutch Labcodeset) is feasible.
Future perspectives
A new cohort of MMLs has started implementation of the Dutch Labcodeset for real-time AST reporting. The aim is to have full coverage of Dutch MMLs by the end of 2020.

O124
Surveillance, Lab2lab and research - application of the Dutch Labcode Set for AMR
R.A. Stegwee
National Institute for Public Health and the Environment (RIVM), BILTHOVEN, Nederland

Current Surveillance
The Dutch system for surveillance of antimicrobial resistance is called ISIS-AR. A majority of the labs report their routine diagnostic tests for antimicrobial infections and their susceptibility to antibiotics. Currently these labs produce a monthly excerpt of the LIMS database that they send to the National Institute of Public Health and the Environment (RIVM). The ISIS-AR data managers then have to run scripts to translate the data into the format and coding of ISIS-AR, before they can upload it to the system. These scripts will identify unknown test codes or unknown codes for the results (typically for microbes). These translation failures need to be checked and verified or corrected by the originating lab. This whole process can easily take 3-6 months to complete.
Near real-time Surveillance
With the implementation of the Dutch Labcode Set for AMR, the RIVM only has to maintain one translation: from the standard codes to the ISIS-AR code. The labs, however, will have to maintain their own translations or mappings from their internal LIMS codes to the standards codes in the Dutch Labcode Set. So how is this different? The fact that the translation takes place in the LIMS means that the originating lab will spot translation errors directly and can correct them before the data is sent to the RIVM. In addition, we provide means for direct reporting. This is how we achieve near real-time AMR surveillance.
Exchange between labs
However, near real-time surveillance is just one of the reasons for introducing the Dutch Labcode Set for

AMR. The second application that we are introducing right now is electronic ordering of genetic typing of specific types of resistant microbes (MRSA and CPE at this point in time). The genetic typing order and the prior results that indicate the reason for genetic typing are taken directly from the LIMS and sent to the Type-Ned application using the Dutch Labcode Set. After the RIVM lab has completed the genetic typing, the results are sent back electronically and the LIMS can incorporate them directly in their workflow. This Lab2lab interface conforms to the international standard for electronic exchange of lab orders that a lab outsources to an external lab. Thus, a lab that has implemented Lab2lab for genetic typing of isolates by the RIVM, can use the same standard for routine outsourcing of lab work to external labs. This will greatly reduce the amount of work that is currently involved with outsourcing.

Sharing research data

An increasing number of scientific studies rely on sharing data from routine diagnostics or from scientific research created in multiple labs across the country (or even the world). The FAIR principles for research data only make sense when the terminology used in coding lab tests and results is compatible across the data sets. The Dutch Labcode Set for AMR provides an international standard terminology for AMR data.

O125

Discussion on the Dutch Labcodeset for AMR

R.A. Stegwee
National Institute for Public Health and the Environment (RIVM), BILTHOVEN, Nederland

Based on the two contributions on the introduction of the Dutch Labcode Set for AMR in this session, we would like to hear your opinion on the potential future use of the standards that we introduce in routine AMR reporting and Lab2lab communication. The following questions may guide this discussion:

- How would your work benefit from near real-time surveillance?
- Are you able to capitalize on the ability for standardized Lab2lab communication?
- Have you experienced incompatibility of AMR data in your work?
- Will the Dutch Labcode Set address all such incompatibilities, or will some remain unsolved?
- Would you be able to use standardized data on the characteristics of the specimen?
- Which area of microbiology would you like to see tackled next, beyond AMR?

All participants are invited to contribute to this discussion, even to send in their ideas in advance to the moderator (robert.stegwee@rivm.nl).

O126

CELLULAR IMMUNE RESPONSES INDUCED BY VACCINIA VIRUS-BASED VECTOR VACCINES

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Introduction

Recombinant viral vaccine vectors allow delivery of any antigen of choice, resulting in the induction of antigen-specific B and T cell responses *in vivo*. Vaccinia virus (VACV) and its attenuated derivative modified vaccinia virus Ankara (MVA) are of special interest for vector vaccine development because large sequences of foreign DNA can readily be inserted into the viral genome. Vaccination with a vaccinia-based vaccine induces immune responses against the antigen of interest, but also against the vector itself. The latter is potentially beneficial as formation of an immune stimulatory environment could enhance the immunogenicity of the vaccine. However, a potential drawback of using vaccinia-based vaccines that pre-existing immunity against the vector - induced by smallpox vaccination or previous vector-based vaccination - and immunomodulatory proteins produced by the backbone might interfere with the induction of desired immune responses against the antigen of interest. Therefore, we addressed the effect of pre-existing vaccinia-specific immunity on the immunogenicity of MVA-based influenza vaccines in a mouse model. Additionally, to obtain a thorough understanding of the mechanisms by which VACV induces cellular immune responses, we investigated temporal changes in the expression of host and viral proteins on the surface of VACV-infected cells.

Methods

The effect of pre-existing vaccinia-specific immunity on vaccination was systematically addressed in mice with and without vaccinia-specific (pre-existing) immunity induced by priming. These mice were vaccinated with different recombinant (r)MVA influenza vaccines, followed by a lethal challenge with an

avian influenza virus. Clinical parameters, and serological and T cell assays were used to determine the effect of pre-existing immunity on the protective efficacy of the MVA-based vaccines. Quantification of the plasma membrane proteome at 6, 12, 18 and 24h after VACV infection of HFF cells was performed by biotinylating the cell surface proteome followed by affinity purification of biotinylated proteins, digestion, labelling with tandem-mass-tag reagents and mass spectrometry.

Results & conclusions

Serological assays showed that the effect of pre-existing vaccinia-specific immunity on induction of antibody responses by rMVA in the mouse model was limited. In contrast, pre-existing vaccinia-specific immunity completely prevented the induction of antigen-specific T cell responses by rMVA vaccination. Therefore, it is crucial to take vaccinia immune status of vaccine recipients into account when using MVA-based vaccines that aim at the induction of virus-specific T cells in humans. Furthermore, these results highlight the importance of acquiring a fundamental understanding of the immune response induced by the vector. The plasma membrane profiling experiment shows selective manipulation of several classes of natural killer (NK) cell ligands on VACV-infected cells. Establishing the molecular mechanism of action of this viral manipulation and the effect on activation of cellular immune responses is essential for intelligent vector vaccine design.

O127

An alphavirus replicon-based cancer vaccine; from bench to bedside

Toos Daemen

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Immunotherapeutic strategies targeting human papillomavirus (HPV) are critical for eliciting effective immunity in patients with (pre)malignant cervical lesions. Alphavirus-based strategies are attractive platforms due to the high-level of transgene expression as well as activating both humoral and cellular immunity. Our group pioneered a recombinant viral vector system based on Semliki Forest virus, an alphavirus, encoding for HPV E6 and E7 (rSFVeE6,7). rSFVeE6,7 demonstrated robust E6/E7-specific immunity with complete regression of HPV-specific tumors in mice. With these promising findings, we now performed a phase 1 trial.

In this presentation, preclinical studies, the GMP production process of a clinical batch of the rSFVeE6,7 vaccine and set up and results of the clinical trial will be presented.

O128

Flavivirus versus alphavirus replicon vaccines

Gorben Pijlman

O129

Successes and challenges of DNA vaccination of fish

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Perhaps not everybody knows, but intramuscular DNA vaccination of fish can be extremely effective. Our group recently reported the development of an intramuscular DNA vaccine against the fish rhabdovirus Spring Viremia of Carp Virus (SVCV). This vaccine conferred up to 100% protection to carp even at a single dose of 0.1 µg DNA/g of fish of a plasmid encoding the SVCV glycoprotein. Characterization of the adaptive immune responses, revealed the presence of serum neutralizing antibodies and of memory T cells in vaccine-protected animals. Unfortunately, the same vaccine did not confer protection when administered orally.

Currently we are investigating the potential of commensal probiotic bacteria as vehicles to deliver the DNA vaccine orally or by immersion. To this end we engineered *Lactococcus lactic* probiotic bacteria to carry the DNA vaccine and the listeriolysine (LLO) gene. The latter, when expressed in the bacteria, will favour escape from the phagolysosome, thereby increasing the chances of DNA delivery to host cells. Our preliminary data show that modified *L. lactic* bacteria are very effective in delivering DNA plasmid to fish cells *in vitro*, and that listeriolysin is required for this process, supporting their potential

use as vaccine vehicles also *in vivo*.

O130
Viral assembly and mechanics scrutinized at the single particle level
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With the advent of single particle approaches, the study of molecular mechanisms in biology has taken a new turn. For instance, using Atomic Force Microscopy (AFM) we are now able to zoom in on (supra)-molecular processes [1-7]. In this way, interactions and dynamics of proteins, lipids and nucleic acids can be studied. After a brief introduction of the background and potential of several single-particle approaches, I will start by discussing the mechanics and material properties of viruses and cellular protein nanocages; In particular by revealing the existence of pre-stress in nanoshells, by scrutinizing the interactions between capsids and viral RNA or DNA and by showing how viral infectivity is in essence a mechanical process. Next, using dual-trap optical tweezers, I will discuss a single-particle approach to study self-assembly of viruses. This starts by performing real time binding experiments of capsid proteins on dsDNA. By optimising assembly conditions, a significant shortening of the DNA molecule is observed in the optical tweezers set-up, indicative of successful capsid assembly and genome packaging by the virus. The experiments indicate that in this way stable structures are formed and the assembly pathway is discussed. All in all these studies have revealed that our optical tweezers and atomic force microscopy results provide a window into the complex kinetics of viral capsid formation as well as into the fascinating mechanical structure of viral particles.
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O131
Mitochondrial DNA maintenance in trypanosomes: a complex story
Torsten Ochsenreiter

O132
An 'unexpected' souvenir
Femme Harinck

O133
Her headache gives me a headache
Mirjam Dautzenberg

O134
Mixed infections
Alieke van der Hoeven
Multiple infections in a single patient: a clinical case presentation.

O135
Itch and eosinophilia, just bad luck?
Myrte Tielemans