Craspase is a self-regulating protease that is sequence-specifically activated by viral RNA

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Bacteria encode on average five immune systems to protect themselves from viral infection. CRISPR-Cas immunity is often present and mounts an immune response upon sensing invading nucleic acids. We discovered a novel CRISPR-Cas system that is composed of a large ribonucleoprotein capable of sequence-specific recognition and cleavage of viral RNA. Uniquely, this protein forms a complex with a protease from the caspase family. We termed this complex Craspase (CRISPR-guided caspase) and, using biochemistry and cryo-electron microscopy approaches, demonstrate that the protease activates upon RNA binding and deactivates through cleavage of the bound RNA. Activated Craspase site-specifically proteolyzes a host-encoded protein, preventing virus propagation through host suicide.

Fundamental metabolic strategies of heterotrophic bacteria

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Through their metabolism, heterotrophic microbes drive carbon cycling in many environments. These microbes consume (and produce) hundreds to thousands of different metabolic substrates, begging the question to what degree their metabolic niches can be understood in terms of a small number of simplified functional categories: do we need to account for the detailed metabolic capabilities of each organism, or can these capabilities be understood in terms of a few well-conserved carbon utilization strategies that could be more easily interpreted and more robustly predicted? Based on the high-throughput phenotyping of a diverse collection of 186 marine bacteria on 140 carbon substrates, we show that the fundamental metabolic strategy of heterotrophic microbes can be summarized in terms of a single axis of variation, representing their preference for either glycolytic (sugars) or gluconeogenic (amino and organic acids) carbon sources. Moreover, an organism's position on this axis is imprinted in its genome, allowing us to successfully predict metabolic strategy across the bacterial tree of life. Our analysis also unveils a novel and general association between metabolic strategy and genomic GC content, which we hypothesize results from the difference in carbon:nitrogen supply associated with typical sugar and acid substrates. Thus, our work reveals a fundamental constraint on microbial evolution that structures bacterial genomes and communities and can be leveraged to understand diversity in functional terms, beyond catalogs of genes and taxa.

Endogenous viral elements in shrew genomes provide insights into Flaviviridae ancient history

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Introduction: As viral genomic imprints in host genomes, endogenous viral elements (EVEs) shed light on the deep evolutionary history of viruses, ancestral host ranges, and ancient viral-host interactions. EVEs are thus genomic fossils to compensate for the absence of physical fossil traces of viruses. Flaviviridae are an important family of viruses, including well-known human pathogens, such as Zika, dengue, or hepatitis C viruses. Most EVEs derived from Flaviviridae have been identified in arthropods, but none, to date, in the genome of mammals, even though the family encompasses numerous mammal-infecting members.
Methods: We conducted a comprehensive in silico screening of a 689 publicly available mammalian genomes. Positive hits were validated in biological samples of the species of interest and related species to confirm presence of the EVE.
Results: Our study identified two novel Flaviviridae-like EVEs in the reference genome of the Indochinese shrew (Crocidura indochinensis), a first in mammals. Homologs of these novel EVEs were subsequently detected in an additional 27 shrew species, including 26 species representing a wide distribution within the Crocidurinae subfamily and one in the Soricinae subfamily on different continents.
Conclusion: Based on this wide distribution, we estimate that the integration event occurred before the last common ancestor of the subfamily, about 10.8 million years ago, attesting to an ancient origin of pestiviruses and Flaviviridae as a whole.

Formation of structurally-defined biofilm aggregates in methanogenic cocultures is associated with the increased expression of signal transduction and chemotaxis genes

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Introduction:
In anaerobic environments, microorganisms are often found in organized biofilm assemblages, essential for efficient exchange of biochemical molecules. However, no knowledge exists on the biofilm formation in the cocultures of fatty-acid oxidizing acetogenic bacteria (like Syntrophobacter fumaroxidans) and hydrogenotrophic methanogenic archaea. Evidence and mechanistic insights into the aggregation abilities of these bi-cultures are required to understand ecological relationships between these anaerobic microorganisms, widespread from human gut to wastewater treating bioreactors. Here we report pioneering insights into morphological and biochemical changes occurring during the aggregation of methane-producing bi-cultures into mm-scale biofilm clusters.
Methods:
Bi-cultures of Syntrophobacter fumaroxidans and methanogens (Methanospirillum hungatei or Methanobacterium formicicum), 10% v/v of each, were grown at 37°C in a bicarbonate-buffered mineral salt medium containing propionate (20 mM) and 1.5 bar N2/CO2 (80/20 (v/v)). Morphological changes in the cocultures were monitored with fluorescent and scanning electron microscopy. Changes in the substrate/product turn-over rates were monitored with high-performance liquid/gas chromatography. Changes in the gene expression in dispersed and aggregated cocultures were investigated through RNA sequencing and differential expression analysis (DEseq).
Results:
We observed formation of aggregates in both bi-cultures within 5 months of cultivation. Localization of S. fumaroxidans within aggregates differed depending on the methanogen added. Biofilm aggregates had 2x faster substrate/product turnover rates, compared to the dispersed cocultures. DEseq results point to statistically significantly higher expressed genes for signal transduction, polysaccharide secretion, iron transporters and chemotaxis in the aggregated cocultures, compared to the dispersed ones.
Conclusion:
This report on biofilm-forming ability of the cocultures of syntrophic fatty-acid oxidizing bacteria and methanogenic archaea provides a deeper fundamental understanding of the physiology of these anaerobic microorganisms, which are essential for the global biogeochemical carbon cycling. Future studies will investigate cocultures' time-resolved genes expression profiles and production of signaling molecules to fully understand the mechanisms of biofilm formation.

Salmonella O-antigen Protects Against Kupffer Cell-Mediated Clearance During Bloodstream Infections

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The liver will capture and eradicate the vast majority of Gram-positive and Gram-negative bacteria that enter into the bloodstream, via the largest population of resident macrophages, the Kupffer cells. Unlike in any other organ, these macrophages reside directly within the liver capillary network (sinusoids), where they perform the almost inconceivable task of selectively catching bacteria out of the circulation, while ignoring all other blood components. The capture of bacteria by Kupffer cells out of the circulation is mechanistically distinct from bacterial phagocytosis in a test tube. Under high-flow conditions high shear forces need to be overcome and distinct receptors are utilized for this process. For instance, the capture of Gram-positive bacteria depends on the recognition of cell-wall associated lipoteichoic acid through the Kupffer cell specific receptor CRIg.
Using 'state-of-the- art' intravital microscopy in living mice we found that some Salmonella species can evade this important clearance mechanism. To unravel the molecular underpinnings, we utilized transposon mutagenesis followed by next generation sequencing and we identified several mutants in the O-antigen (LPS) synthesis pathway. Rough Salmonella strains (O-antigen deficient) become fully susceptible to clearance by KC's, whereas E. coli strains expressing the salmonella O-antigen become more resistant against Kupffer cell mediated clearance. In addition, we found that vaccination against the Salmonella O-antigen induces immunoglobulin and Kupffer cell dependent clearance. This study identifies a crucial step in salmonella pathogenesis and might provide new vaccine targets or treatment options for salmonella infections.

IgM GlcNAc-targeting antibodies are more functionally cross-reactive than IgG

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Antibody therapy is a promising alternative strategy to treat or prevent antimicrobial resistant bacterial infections. After binding their antigen, antibodies can induce a broad range of effector functions, ranging from neutralising the bacteria to activating immune cells and the complement system. For the development of effective antibody therapies, it is crucial to understand how different antibody isotypes induce effector functions for a specific antigen. Here, we compared the IgG and IgM variants of three different monoclonal antibodies (mAbs) that were characterised against specific N-acetylglucosamine (GlcNAc)-modifications on Staphylococcus aureus in their ability to cross-react with different GlcNAc-modifications. Using flow cytometry, we show that IgM mAbs recognise more GlcNAc-modifications on S. aureus than their IgG counterparts, and that these IgM interactions are effective in inducing phagocytosis and complement activation. By using wall teichoic acid beads that contain specific GlcNAc-modifications, we show that the IgM mAbs indeed recognised different GlcNAc-modifications than their IgG counterparts were originally characterised for. Furthermore, we show that GlcNAc-targeting IgM's, of which the IgG variant is specific for S. aureus, also bound to a broad range of bacterial species expressing a surface GlcNAc, including Gram-positive and Gram-negative bacteria, and activated complement on Escherichia coli. Using a mutant library, we pinpointed a GlcNAc in the lipopolysaccharide as the target on E. coli for two out of the three IgM mAbs. In conclusion, we show that GlcNAc-targeting mAbs are more intra- and interspecies cross-reactive when expressed as IgM than as IgG.

Genomic DNA transfer in Mycobacterium marinum

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Introduction

Conjugation is one of the most efficient routes for horizontal gene transfer and genome variation in bacteria, but this process is highly variable between different species. We identified a novel conjugative plasmid in Mycobacterium marinum that seems to be unique for these bacteria. The conjugation machinery of this plasmid requires the action of both a type IV–like and a type VII secretion system. In this study we set out to identify the origin of transfer and the possibility of chromosomal transfer through this system.

Results

First, we confirmed the essentiality of the relaxase for conjugation and identified the origin of transfer (OriT), which was situated close to the relaxase gene. By introducing OriT on a standard mycobacterial shuttle plasmid and providing the conjugative functions in trans we were able to efficiently introduce recombinant plasmids by conjugation into other mycobacteria, including mycobacterial species with limited genetic accessibility, such as Mycobacterium kansasii.

Next, we introduced the OriT on the chromosome and selected for transfer events. We obtained colonies and genome sequencing indicated that large fragments of genomic DNA of up to 400 kb had been transferred to other M. marinum strains. Surprisingly, genomic DNA transfer could also be observed in a control strain without OriT, indicating that this process is independent of OriT on the donor chromosome. Genomic DNA transfer was only achieved when the conjugative plasmid pRAW was present in the donor strain and can also be used to complement mutations in the recipient.

Conclusion

These experiments show that M. marinum is capable of genomic DNA transfer, a process that is driven by the donor strain that has a conjugative plasmid and does not depend on an OriT on the chromosome. Together, these results show that the mycobacterial conjugation machinery provides new tools to expand the genetic toolbox for mycobacteria.

Candida albicans and Staphylococcus aureus reciprocally promote virulence

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Candida albicans is a prevalent oral commensal which can become pathogenic during immune deficiencies. C. albicans pathogenesis involves upregulation of various virulence factors (e.g. biofilm formation, switching yeast to hyphal growth, invasion, and secretion of lytic enzymes and peptides) usually leading to localized and superficial infections. However, occasionally lethal bloodstream infections (BSI) may develop (candidemia). Interestingly, at least 20% of candidemia cases are known to coincide with bacteraemia. Staphylococcus aureus, the third most co-isolated bacteria during candidemia, is a leading cause of primary BSIs which occur without a known portal of entry. Accordingly, recent studies have suggested that C. albicans is able to facilitate S. aureus invasion and dissemination. However, the direct influence of co-culturing on virulence has not yet been studied. In this study we aimed to examine the direct effects of S. aureus on both C. albicans hyphal formation and invasive properties through agar invasion assays and (timelapse) microscopy. Moreover, using qPCR and proteomics we further investigated the effects of co-culturing on growth and secreted virulence factors. Results show that co-culturing significantly increases hyphal formation and invasion while facilitating increased S. aureus integration into the biofilm in a more adherend and complex structure. qPCR results indicate that this increase in S. aureus is likely promoted by C. albicans pH control. Finally, the exoproteome of these biofilms showed significant increases in secreted candidal and staphylococcal virulence factors during co-culturing compared to the respective mono-cultures. Altogether, these results show that C. albicans and S. aureus reciprocally promote virulence during co-culture.

Molecular epidemiology of an outbreak of Parainfluenza Type 3 virus among patients on a haematology unit.

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Introduction
Parainfluenza virus type 3 (PIV3) is an important cause of respiratory infection, morbidity and mortality in haematology patients. When implementing infection prevention measures to control an outbreak, it is important to know whether new infections are due to nosocomial transmission or are being introduced from the community. We describe an outbreak of PIV3 on the adult haematology ward of a tertiary referral hospital in the Netherlands. PIV3 sequence analysis was performed retrospectively and phylogenetic analysis was used to assess if the main route of transmission was among patients or whether PIV3 viruses were being introduced from the community.

Methods
All haematology patients with respiratory symptoms were screened for relevant respiratory viruses by real-time-PCR. PIV3 positive samples with a Ct value of <31 were further characterized by sequence analysis of the haemagglutinin-neuraminidase gene.

Results
Between July and September 2022, 31 haematology patients tested positive for PIV3 three or more days after being admitted, suggestive for nosocomial infection. Infection control measures were put in place including; isolation of positive patients, creation of patient cohorts, screening of all patients for PIV3 by real-time-PCR and wearing face masks by patients, staff and visitors at all times. In total, 29 PIV3 strains were sequenced. Phylogenetic analysis showed that one PIV3 strain was responsible for the outbreak. After intensifying infection prevention measures including the avoidance of all contact between patients, limiting the number of visitors and screening all newly admitted patients by PCR, the outbreak was brought under control.

Conclusions
In a suspected outbreak setting, the molecular characterization of PIV3 strains can help to determine if there is transmission of one viral strain or multiple introductions of different viral strains. This information can be used to confirm if it is an outbreak and guide the implementation of temporary strict and effective infection control measures.

Chronic bottom trawling in the North Sea partially explains taxonomic and functional shifts in benthic microbial communities

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Bottom trawling is a prevalent industrial fishing practice with which heavy equipment is dragged over extensive areas of the seafloor. This severely impacts the local environment and the resident organisms. Direct effects of bottom trawling include the resuspension of large amounts of sediment, oxygenation of anaerobic layers, the release of sulfides to the water column and high mortality rates among benthic organisms. There is little known on how bottom trawling affects microbial communities in the sediment and the metabolic processes they facilitate.
Here, we evaluated if bottom trawling intensity in the North Sea explains taxonomic and functional changes in sediment microbiota. We characterized microbiota in 340 samples of the upper sediment layer at a regional scale with 16S rDNA amplicon sequencing and conducted a spatial analysis using generalized additive mixed models (GAMMs). Bottom trawling intensity partially explained variation in both taxonomic and functional composition of sediment microbiota and also changes in diversity could be linked to bottom trawling. Taxonomic diversity declined already at low trawling intensity, whereas functional diversity decreased only at high trawling intensities. Moreover, we found that predicted metabolic functions shifted in response to bottom trawling intensity. Predicted gene counts for aerobic respiration and nitrification increased and predicted gene counts for nitrate reduction and denitrification decreased.
This study provides a first look into the effects of bottom trawling on sediment microbiota at a regional scale. Besides the better understood impacts of trawling on macro-organisms, our results suggest that the ecosystem impact of bottom trawling reaches down to the microbial level. Importantly, the observed changes in functional diversity and metabolic functions imply that microbial processes at the basis of the ecosystem may be affected.

Valganciclovir for congenital cytomegalovirus infection in infants with isolated hearing loss (CONCERT): a nationwide non-randomized, controlled, phase 3 trial

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Introduction
There is no current consensus on treating infants with congenital cytomegalovirus infection (cCMV) and isolated hearing loss. We aimed to assess the efficacy of valganciclovir treatment to prevent deterioration of hearing loss in this population.

Methods
In a nationwide non-randomized controlled trial (ClinicalTrials.gov: NCT02005822), six weeks of valganciclovir was compared to no treatment in infants recruited through the Newborn Hearing Screening Program in the Netherlands. Eligible subjects were aged ≤12 weeks, born at term (≥37 weeks) with sensorineural hearing loss (>20 dB) and prospectively diagnosed with cCMV through dried blood spot testing, without prior clinical suspicion. The choice for treatment or inclusion in the refusal control group was left to the parents or guardians of subjects. Additional historical controls were sought by retrospective cytomegalovirus (CMV) diagnostics in infants with hearing loss. Primary endpoints were categorical and numerical change in best and total-ear hearing from baseline to follow-up at 18-22 months of age. Secondary endpoints were neurodevelopmental outcome, viral load and safety assessments.

Results
1,374 infants were tested between September 14, 2012 and December 1, 2016, 59 (4·3%) were positive. Thirty-seven participants were enrolled, with 25 in the treatment group and 12 in the combined control group. Hearing was more likely to deteriorate in the control group than in the treatment group (best-ear p=0·003, total-ear p=0·001). Mean functional hearing outcome deteriorated by 13·7 dB in the control group, compared to a slight improvement of 3·3 dB in the treatment group (difference 17 dB, 95% CI 2·6 – 31·4, p=0·02). Six (24%) adverse events occurred in the treatment group, three of which were reversible neutropenia, and one a severe adverse event unrelated to study drug.

Conclusions
In infants with cCMV and hearing loss, six weeks of valganciclovir prevents further deterioration of hearing loss at age 18-22 months.

The morphological plasticity of the plant endophyte Chitinophaga pinensis

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The increase of pesticide-resistant plant pathogens together with increased drought and temperature stress on plants due to climate change is a pressing threat to modern agriculture. Sustainable solutions to ensure crop health are necessary, and one approach is to make use of microbes that are part of the plants' natural microbiota. One promising candidate is Chitinophaga pinensis. This bacterium has been shown to have a disease-suppressive function and is further assumed to mitigate heat and drought stress for better plant survival. Using light microscopy and cryo-electron tomography, we studied the morphological plasticity of this organism. Under favorable conditions, the non-motile C. pinensis grows into ~40 µm long filaments. In this growth state, the bacterium produces large quantities of outer membrane vesicles and membrane protrusions. During continuous growth, cell size dramatically decreases to 600-700 nm round cells. Previous reports have classified these small cells as spores, but our results demonstrate that they neither resemble morphological nor metabolomic characteristics of a spore. However, the small cells of C. pinensis do share the capability of hitchhiking with some spores. Hitchhiking is an energy-saving way of movement of a non-motile microorganism by attaching to a motile one. This behavior increases the chance of finding a suitable environment. The unique characteristics of this bacterium likely contribute to their plant growth promoting attributes as well as allowing them to thrive both inside and outside of plants. We now aim to understand the function of morphological change, hitchhiking behavior, and outer membrane vesicle production play inside plant tissue.

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

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Methanol serves as energy and carbon source for microorganisms in various environments. It is produced during degradation of pectin and lignin from dead plant biomass. Additionally, it is produced geochemically from CO2 and H2 in deep subsurface environments. Anaerobic methanogens and acetogens convert methanol using a pathway involving a vitamin B12-dependent methyltransferase (MT). The MT-system was thought to be prevalent in anoxic environments for methanol conversion, whereas in oxic environments methanol is generally oxidized by alcohol dehydrogenases (ADH). The simultaneous occurrence of both pathways was recently described in the sulfate reducer Desulfofundulus kuznetsovii strain 17T.

D. kuznetsovii strain TPOSR was isolated at our laboratory. In contrast to D. kuznetsovii strain 17T, the genome of strain TPOSR lacks essential genes of the MT pathway and therefore relies on a vitamin B12-independent ADH for methanol metabolism. The characterization of the ADHs of both strains helps to understand the advantage of either pathway, for example in competition with other microbial groups.

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

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

Alcaligenes faecalis accumulates hydroxylamine as a central intermediate during heterotrophic nitrification

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Despite numerous studies conducted on heterotrophic nitrifiers, our understanding of the underlying mechanism remains limited. Whilst strictly depending on organic carbon respiration, heterotrophic nitrifiers can convert ammonium via hydroxylamine to nitrite or nitrate and have been reported to form nitric oxide, nitrous oxide, and dinitrogen gas.
Here, we investigated the heterotrophic nitrifier Alcaligenes faecalis to elucidate the mechanism, genetic basis, and physiological benefit of nitrification during carbon respiration. A. faecalis was cultivated in batch cultures fed with isotopically labelled substrates and in continuous reactor cultures at different carbon-to-nitrogen ratios. For all culture conditions, we determined growth yield, soluble inorganic nitrogen, and gas production kinetics, as well as gene expression and metabolite production.
We showed that A. faecalis produces hydroxylamine, nitrite, nitrous oxide, and dinitrogen gas under a wide range of carbon-to-nitrogen ratios. In contrast to previous reports, the growth yield of A. faecalis did not increase during nitrification. Nitrous oxide and dinitrogen gas production could not be explained by canonical denitrification. Instead, the production of nitrogen gases was likely due to chemical reactions between hydroxylamine, nitrite, and nitric oxide. Recently, genes responsible for nitrogen gas formation from hydroxylamine were described for Alcaligenes ammonioxydans. However, despite the upregulation of this gene cluster, hydroxylamine accumulated in reactor cultures. Furthermore, increasing ammonium concentrations induced an upregulation of salt-stress response genes.
These findings add to the discussion of energy conservation through heterotrophic nitrification and challenge the view of combined nitrification and denitrification in A. faecalis. Furthermore, the copious production of hydroxylamine raises questions about the benefit of this physiology. Our transcriptomics results rather suggest that A. faecalis may be undergoing ammonium-induced salt stress. Hydroxylamine could therefore result from a detoxification mechanism induced by high intracellular ammonium concentrations, indicating that heterotrophic nitrification in general might be a stress response rather than an energy conserving metabolism.

From a Case-Control Survey to selecting a Diagnostic Viral Gastroenteritis Panel for testing of General Practioners' patients

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Introduction:
Previously, a case-control study was conducted to elucidate on the interpretation and relevance of positive results when molecular methods are used for the detection of pathogens putatively involved in gastroenteritis. Following our analyses on bacterial and protozoal agents we here report on the viruses: The clinical relevance of a broad range of 11 possible gastroenteritis viruses was investigated in order to establish the most appropriate diagnostic panel for our general practitioner (GP) patients.

Methods:
Internally controlled multiplex real-time PCRs for a broad range of possible pathogenic gastroenteritis viruses, performed in 1340 cases and 1100 controls.

Results:
The prevalence of any virus in symptomatic cases and asymptomatic controls was 16.6% (223/1340) and 10.2% (112/1100), respectively. Prevalence of astrovirus (adjusted odds ratio (aOR) 10.37; 95% confidence interval (CI) 1.34-80.06) and norovirus II (aOR 3.10; CI 1.62-5.92) was significantly higher in cases versus controls. Rotavirus was encountered only in cases. We did not find torovirus and there was no statistically significant relationship with cases for salivirus (aOR 1,67; (CI) 0.43-6.54)), adenovirus non-group F (aOR 1.20; CI 0.75-1.91), bocavirus (aOR 0.85; CI 0.05-13.64), enterovirus (aOR 0.83; CI 0.50-1.37), human parechovirus (aOR 1.61; CI 0.54-4.77) and sapovirus (aOR 1.15; CI 0.67-1.98). The viral load (Ct value) did not discriminate between carriage and disease in PCR-positive subjects. Our own multiplex diagnostic gastroenteritis panel based upon the present case-control study in the GP population demonstrated to suit better than the available commercial panels.

Conclusions:
In our population a diagnostic gastroenteritis panel should contain adenovirus group F, astrovirus, noroviruses, and rotavirus. Case-control studies as ours are lacking and should also be carried out in populations from other epidemiological backgrounds.

Population size directs alternative trajectories of antibiotic resistance evolution

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To predict adaptive mutation trajectories, mutations with high rates and mutations with large fitness benefits are key, as they are expected to drive most evolution. However, their contribution is expected to depend on population size, as competition between alternative mutants (i.e. clonal interference) in large populations will benefit large-effect mutations even when they occur at low rates. We show that replicate small and 100-fold larger bacterial populations adapt to a novel antibiotic using similar numbers, but different types of mutations. Small populations repeatedly substitute similar high-rate structural variants, including the deletion of a non-functional □-lactamase, and evolve modest resistance levels. Large populations more frequently use the same low-rate, large-benefit point mutations, including those activating the □-lactamase, and reach 50-fold higher resistance levels. Our results demonstrate the impact of mutation bias also under strong clonal interference and highlight the fundamental role of population size in mediating the contribution of high-rate and large-benefit mutations, facilitated by a trade-off between rates and fitness effects of different mutation classes.

Meta-omics analyses reveal insights into the ecology and physiology of Thorarchaeia in anoxic Aarhus Bay sediments

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

Double-blinded randomized controlled trial assessing the effect of three consecutive faecal microbiota transplantations on hepatic steatosis in patients with non-alcoholic fatty liver disease

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Introduction:
Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disease, in which the role of the gut microbiome is increasingly recognised. Besides lifestyle changes, there is currently no treatment available for NAFLD. This double-blinded randomized controlled trial assesses the effect of three consecutive faecal microbiota transplantations (FMT) on hepatic steatosis as a potential treatment strategy for NAFLD.

Methods:
Patients with NAFLD, diagnosed by ultrasound or VCTE FibroScan were recruited from hepatology outpatient clinics of the Leiden University Medical Center and affiliated hospitals. Participants were randomized 1:1 to three times (t=0; t=3; t=6 weeks) autologous or allogeneic FMT, infused directly into the duodenum during gastroscopy. FMT material was derived from two different donors (1:1) containing a stable, highly diverse and butyrate-rich microbiome. We assessed changes in hepatic steatosis (MRI-PDFF), liver biochemistry, and gut microbiome composition over a 12-week period.

Results:
In total, 20 patients participated (10:10). We found no significant change in MRI-PDFF in patients receiving allogeneic (18.6% (SD 9.1%) to 17.7% (SD 9.8%) (p=0.37)) or autologous FMT (15.7% (SD 8.4%) to 15.4% (SD 7.4%) (p=0.59)) (between-group difference: -0.54%, p=0.63) after 12 weeks. Triglycerides decreased over time after allogeneic FMT (coeff: -0.46 (95% CI: -0.90;-0.017), p=0.042) compared to autologous FMT, whilst no difference in effect was observed in ALAT, ASAT, AF and gGT. We observed no difference in gut-microbial alpha diversity after allogeneic versus autologous FMT (baseline vs week 12, mean difference allogeneic: 1.9% (SD 5.1%) vs autologous: 2.2% (SD 5.0%), p=0.90, 95%CI[-5.5, 6.2]).

Conclusion:
Triple allogeneic FMT significantly decreased plasma triglycerides in NAFLD patients over the course of 12 weeks, but did not affect hepatic steatosis. No effect on ALAT, ASAT, AF, gGT, or gut-microbial alpha diversity could be observed. Additional clinical and metagenomic analyses are needed to further elucidate the role of the gut microbiome in NAFLD.

Respiratory epithelial cytokine responses are pathogen specific and differ per anatomical region of the upper respiratory tract.

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The respiratory epithelium is the first cellular layer in the human body that encounters airborne pathogens. Multiple microorganism inhabit specific regions of the upper respiratory tract, where they can either reside as commensals or cause infection. Hence, when studying host-pathogen interactions it is important to use a model system that resembles the epithelia of the respiratory tract to investigate their properties, and response to viral and bacterial infections. For this purpose, we obtained primary epithelial cells from the nose (n=7), nasopharynx (n=3), and bronchiae (n=4) from different donors. We cultured these primary epithelial cells on an air-liquid interface and validated their differentiation status using immunofluorescence, and haematoxylin-and-eosin staining. After 3 to 6 weeks of culture, fully differentiated epithelial layers could be observed containing ciliated cells, goblet cells, and secretory cells. To our knowledge we are the first to generate a highly differentiated nasopharyngeal in vitro model where nasopharyngeal cells propagate and differentiate up to 5 weeks. Next, these nasal, bronchial, and nasopharyngeal epithelial models were stimulated with IFNγ, Streptococcus pneumoniae, Neisseria meningitidis, or respiratory syncytial virus (RSV) for 48 hours. Nasopharyngeal cells showed the highest responses for CCL2, CCL3, IFN-λ2,3, and IL-6 after meningococcal infection compared to the other tissue types and stimuli. RSV infection induced IFN-λ1, and IFN-λ2,3, production in nasal and bronchial cells, but not in nasopharyngeal cells. Pneumococcal infection induced generally low cytokine responses in all tissue types, except for CXCL5 in bronchial cells. In short, our data show that indeed both the pathogen and the origin of the epithelial cells in the upper respiratory tract determine epithelial cytokine responses. This stresses the importance of the usage of a suitable cell model system when investigating respiratory host-pathogen interactions, as these region-specific epithelial models could provide novel insights into interactions between host-tissues and specific microorganisms during disease.

The establishment of a controlled human pneumococcal infection model in the Netherlands.

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Introduction
Streptococcus pneumoniae frequently colonises the human upper respiratory tract without causing symptoms, but it is also an important cause of morbidity and mortality in risk groups. Controlled human infection studies (CHIS) that involve the deliberate exposure of research participants to infectious agents under controlled conditions can help provide a better understanding of host-pathogen interactions during colonisation, which can be used to develop more effective colonisation-reducing vaccines. Here, we describe preliminary microbiological results from the first Dutch pneumococcal CHIS, based on the model that was previously established in the United Kingdom (NCT05361499).

Methods:
Nineteen healthy adults (aged 18-49 years) were enrolled between May and December 2022 and intranasally inoculated with S. pneumoniae (strain BHN418; serotype 6B; 160,000 colony forming units). Safety and colonisation were monitored over 4 weeks with onsite visits at days 3, 7, 14 and 28 post inoculation. Antibiotic treatment was given after the last visit if colonisation was present. Pneumococcal colonisation was assessed by culture of nasal wash samples.

Results:
The rate of pneumococcal colonisation, defined as having at least one culture-positive nasal wash after challenge, was 42% (8/19). Colonisation was mainly detected during the first week after challenge and cleared naturally in 5/8 participants, with 3 participants receiving antibiotic treatment at day 28. None of the participants developed pneumococcal disease. Upper respiratory tract symptoms were reported by 95% (18/19) of the participants and were generally mild. Incidence and severity of symptoms were not different between colonized and not colonized individuals.

Conclusion:
We successfully performed a pneumococcal CHIS in the Netherlands in which participants were intranasally inoculated with a S. pneumoniae 6B strain, with a similar colonization rate and safety profile as observed in the UK. This paves the way for future pneumococcal CHIS in the Netherlands. Further microbiological and immunological analysis of collected samples is ongoing.

The immunological effects of COVID-19 vaccination after past infection in lung transplant recipients

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Introduction
Vaccination has reduced the mortality of COVID-19 significantly. Lung transplant recipients (LTRs) are particularly at risk of severe COVID-19, and have a poor response to vaccination, as the majority were shown to be non-responders. To investigate the immunological responses to vaccination after a natural infection, we studied a group of 19 LTRs who had already had a SARS-CoV-2 infection.
Methods:
Lung transplant recipients who were more than 40 days after SARS-CoV-2 infection , were vaccinated twice, at a 28 day interval. Blood for humoral and cellular analysis was taken at baseline, at 28 days before the second vaccination dose, 28 days after the second vaccination dose, and 6 months after the second vaccination dose. Antibodies to S and N proteins were measured as well as neutralizing antibodies. T-cell activity and functionality was measured using Interferon Gamma Release Assay (IGRA), ELIspot, and Flow cytometry.
Results:
LTRs included in the study, were a median of 200 days after natural infection. Both S and N-antibody levels were higher in LTRs who had experienced SARS-CoV-2 more recently. Following the first vaccination, S-antibodies titers increased significantly, and a further increase was observed after the second vaccination. N-antibodies decreased during the study period.
In the ELispot assay, an increase in IFN producing T-cells was observed following the first vaccination. Following the second vaccination the number of IFN producing spots decreased significantly in 10 LTRs. Flow cytometry showed increased expression of exhaustion markers.
Six months later, median antibody titers were significantly higher than before vaccination, at 3209 (IQR 673-4260) versus 138 (IQR 38-529) BAU/ml ($p<10e-4$).
Conclusion
Vaccination of LTRs with past SARS-CoV-2 infection leads to increased and durable humoral and cellular responses. The relevance of the decreasing number of reactive T-cells, and the increase in exhaustion markers after the second vaccination dose, is not clear.

Staphylococcus aureus : colonization of burn wounds and the innate defense

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Patients with extensive burns are susceptible to opportunistic pathogens due to their compromised immune system. Despite high standards of care, colonization with micro-organisms, Staphylococcus aureus in particular, is a common feature of larger area burn wounds. We have been using ex vivo human burn wound models successfully to test antibacterial therapies, but results with S. aureus were highly variable. Our aim is to gain insight into the factors that cause differences in survival and colonization of S. aureus in this skin model.
The colonization potential of 10 S. aureus strains with different genetic background was determined in burn wound models with skin of different donors. Bacterial genomic sequences were determined by MinION platform. A cytokine panel and presence of antimicrobial peptides (AMPs) were analyzed by Legendplex and Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR), respectively. Cytokines were measured in plasma samples from 18 burn patients positive for S. aureus, burn patients negative for S. aureus (n=11) served as controls.
Sequencing confirmed the different genetic backgrounds of the used strains. Colonization ability was strain dependent, although small differences in survival were noted between skin donors. Presence of bacteria in burn wound models resulted in a reduced level of IL6 in the medium, other cytokines were not affected. mRNA expression of AMPs HBD2, HBD3 and IL8 was slightly increased while expression of AMP LL37 was reduced in the presence of a poor colonizing strain in burn wound models. A good colonizing strain only induced expression of AMP RNase7. A relation between AMP expression and bacterial survival was however not found. Strikingly, plasma IL10 levels highly correlated with (susceptibility for) colonization of burn wounds by S. aureus.
Together, our data indicate that the ability of S. aureus strains to direct the host response to conditions more beneficial for survival and colonization differs between strains.

Imvanex vaccination induces low levels of monkeypox virus-neutralizing antibodies but strong virus-specific T-cell responses

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The global outbreak of mpox (formerly monkeypox) outside previously endemic areas was declared a public health emergency of international concern by the World Health Organization in July 2022. While smallpox vaccination efforts were stopped in the 1970s following the eradication of variola virus, modified vaccinia virus Ankara-Bavarian Nordic (MVA-BN, also known as Imvanex, JYNNEOS, or Imvamune), a third-generation smallpox vaccine, is available, authorized, and in use as a vaccine against mpox. However, data demonstrating cross-reactive immune responses against monkeypox virus (MPXV) induced by vaccination were lacking. Here, we show that a primary vaccination series with two doses of MVA-BN in immunologically-naive individuals yielded only relatively low levels of MPXV-neutralizing antibodies, while strong cross-reactive and MPXV-specific T-cell responses were induced. Our data furthermore indicate that dose-sparing of an MVA-based influenza vaccine led to lower overall MPXV-neutralizing antibody levels, whereas a third vaccination with the same MVA-based vaccine significantly boosted the antibody response. Additionally, MPXV-neutralizing antibodies were detected in individuals after historic smallpox vaccination, in some cases more than 70 years later, and after MPXV infection. Using a combination of different assays, serological differentiation between MVA-BN-vaccinated and MPXV-infected individuals was possible. As the role of both MPXV-neutralizing antibodies and MPXV-specific T-cells as correlates of protection against disease and transmissibility are currently unclear, we conclude that cohort studies following vaccinated individuals are necessary to assess vaccine efficacy in at-risk populations to prepare for future outbreak scenarios.

Fusobacterium nucleatum secretes metabolites that induce autophagy and cell death in colorectal cancer cells

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Colorectal cancer is the third most prevalent cancer in the world. The most established bacterial contributor to colorectal cancer is Fusobacterium nucleatum. Until now, research has mainly focused on the role of the F. nucleatum surface adhesins in tumor progression. However, the impact of the F. nucleatum secreted metabolome has been understudied thus far.
To address this, we assessed the effect of F. nucleatum along with different bacteria isolated from inflammatory bowel disease patients on the organoid viability of colorectal cancer organoids. In contrast to the other bacterial isolates tested, sterile conditioned culture supernatant of F. nucleatum clearly reduced organoid viability as measured by intracellular ATP levels using CellTiter-Glo. Further characterization showed that the active factor in the culture supernatant is smaller than 3kDa and heat stable. An Annexin V/PI staining did not show induction of apoptosis in HK1 cells or HT-29 colorectal cancer cells as result of F. nucleatum culture supernatant. Using an HT-29 autophagy LC3 reporter system, we showed that Fusobacterium nucleatum and several other Fusobacterium species were able to induce autophagy. The induction of autophagy in HT-29 cells was confirmed by an LC3 western blot, showing increased conversion of LC3-I to LC3-II after stimulation with Fusobacterium culture supernatants. Induction of autophagy by Fusobacterium spp. is independent of most of the common TLRs and NLRs, as autophagy was still induced in the HeLa-57a cell line, which is unresponsive to these ligands.
Altogether, we show that F. nucleatum secretes a small molecule that reduces organoid viability, in part through the induction of autophagy. The dysregulated induction of autophagy by F. nucleatum could be an important aspect in the regulation of cancer cell viability and overall cancer development. Identification of factor(s) responsible for the induction of autophagy may lead to novel anti-cancer therapeutic strategies.

New Anti-mycobacterial Compound with a novel Target shows Cooperativity and Synergy with approved Antibiotics

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Tuberculosis, caused by Mycobacterium tuberculosis, remains the deadliest bacterial infectious disease worldwide. Therefore, there is an urgent need to develop new antibiotics and treatment strategies. However, tuberculosis drug development is challenging due to several aspects. First, mycobacteria have a unique cell envelope consisting of an inner membrane and a highly impermeable outer membrane, which hampers antibiotics from reaching their intracellular target. Second, newly developed antibiotics should ideally have a novel mechanism of action and work synergistically with the current treatment regimens. This study aimed to identify compounds that would permeabilize the mycobacterial outer membrane and therefore render bacteria more susceptible to antibiotics. We screened a small benzothiazole scaffold compound library using an ethidium bromide uptake assay, which is often used to evaluate membrane permeability. In this assay, M.marinum was used as a surrogate for M.tuberculosis, and we identified the compound V2.6, which increased ethidium bromide uptake by 5-fold. Further testing revealed that the compound does not affect bacterial growth in culture, but it reduced infection in the M.tuberculosis-macrophage infection model and the zebrafish embryo infection model of tuberculosis. Due to its effect on the membrane, we hypothesized that the compound might show cooperativity together with other antibiotics. Using the M.marinum-zebrafish model, we showed that the combination of rifampicin and V2.6 results in 100-fold higher activity compared to the single-drug treatment. Moreover, V2.6 showed synergistic and additive effects in vitro with several well-established antibiotics. By generating spontaneous resistant mutants and further genetic studies, we identified and confirmed that TpvA is the target of V2.6. To conclude, our study identified a new anti-mycobacterial compound that targets the novel drug target TpvA. Due to the compound's effect on mycobacterial outer membrane permeability, V2.6 has excellent potential to become the backbone of a synergistic drug regimen.

What drives the diversity of electrogenic cable bacteria?

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Salinity gradients are characteristic of coastal ecosystems and are considered one of the main drivers of microbial diversity. Electricity-producing cable bacteria inhabiting coastal sediments are currently classified into two main genera: freshwater genus Candidatus Electronema and marine genus Candidatus Electrothrix. As the overall diversity of cable bacteria remains uncertain and likely underestimated, our objective was to expand and synthesize the current database of 16S rRNA gene sequences assigned to cable bacteria.

Firstly, incubations of sediments from a broad range of salinities (0-33) and geographical locations (Belgium, the Netherlands, Sicily, Spain) were set up to enrich for cable bacteria. Microsensor profiling (O_2, H_2S, pH and electric potential) of sediments was conducted to scan for the unique geochemical fingerprint of electrogenic sulfide oxidation by cable bacteria. Single cable bacteria filaments were then extracted from the enrichment incubations to sequence the full-length 16S rRNA gene. Additional sequences were mined from public databases to include a wider variety of environments. In total, more than 130 sequences from around the globe were compiled to construct an updated phylogenetic tree of cable bacteria.

Our results indicate that the current diversity of cable bacteria is largely underestimated as 4 potential new genus-level and 32 new species-level clades were identified. 16S rRNA gene sequences from our brackish samples from Spain and Sicily could not be assigned to any of the described cable bacteria species and thus possibly represent new species.

Unlike typical cable bacteria environments, the sediments from Sicily and Belgium are low in free sulfide. Therefore we hypothesize that, next to salinity, the availability of reduced sulfur species may play an important role as a driver of cable bacteria diversity. Overall, our data supports challenging the previous classification into a strictly marine and a strictly freshwater genus as several phylogenetic clades appear to tolerate wide salinity ranges.

“Candidatus Siderophilus nitratireducens”: a low-temperature, Nap-dependent nitrate-driven iron oxidizer within the new order Siderophiliales

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Globally, about one third of the nitrogen applied to agricultural soils is oxidized to nitrate and leaks into groundwater. While nitrate is not harmful, its reduction products are a serious threat to both humans and animals. Yet, in general current drinking water production processes do not eliminate nitrate. Its removal is challenging due to the lack of organic matter in groundwater. On the contrary, inorganic electron donors such as iron are present in high concentrations. Nitrate-dependent iron-oxidizers (NDFOs) couple the reduction of nitrate to the oxidation of iron, and have been extensively studied in pure cultures. However, their relevance and ability to thrive in natural and engineering ecosystems is poorly understood. Here, we propose a new drinking water treatment scheme using nitrate-dependent iron-oxidizing bacteria.

A pilot-scale RSF was continuously fed with iron- and nitrate-rich anaerobic groundwater. The rapidly formed biofilm fully removed nitrate, and iron was consumed at a stoichiometric molar ratio of 0.15 ± 0.04 Fe2+:NO3-. We combined short and long read sequencing to resolve the taxonomic and functional composition of the microbial community, seemingly divided in two distinct niches. Autotrophic iron oxidizers performed the initial denitrification, while organoheterotrophic organisms present in lower abundances completed the reduction of the intermediates to dinitrogen gas. Phylogenetic analysis revealed that the main member of the community (19.3% relative abundance) belonged to a new order-level within Gammaproteobacteria.

We propose to name the new species “Candidatus Siderophilus nitratireducens” gen.nov., sp.nov., a member of the new order Siderophiliales. Unexpectedly, non-electrogenic nitrate reductase Nap is the only denitrifying reductase present in “Ca. Siderophilus nitratireducens”, delimiting further the already narrow ecological niche of iron oxidizers. In conclusion, not only we document the labour-division in oligotrophic denitrifying communities between iron-dependent autotrophs and organoheterotrophs, but also expand the taxonomic and thermodynamic breadth of NDFO with the new order Siderpholiales.

Monoclonal antibodies effectively potentiate complement activation and phagocytosis of Staphylococcus epidermidis in neonatal human plasma

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Introduction
Staphylococcus epidermidis is the most common pathogen found in neonatal intensive care units (NICU). Since hypogammaglobulinemia is an important risk factor in preterm neonates, clinical studies have thus far focused on antibody supplementation with pooled intravenous immunoglobulins from healthy donors, but with little success. Monoclonal antibodies (mAbs) are successful in preventing infectious diseases such as Respiratory Syncytial Virus. Potentially, mAbs could also provide protection against neonatal sepsis.

Methods
Nine different IgG1 mAbs that recognize Staphylococcal surface components were tested to determine binding to S. epidermidis strain ATCC14990 and 20 clinical strains obtained from the NICU. We also investigated the effect of Fc-mutations that improve IgG hexamerization on complement activation and phagocytosis of S. epidermidis. To do so, bacteria were opsonized with mAbs in the presence or absence of either adult serum or r-Hirudin neonatal cord blood plasma as a complement source, and their immune activating potential was studied by flow cytometry. Finally, we studied the phagocytic capacity of neonatal neutrophils in whole blood, for which we used preterm and term neonatal cord blood samples.

Results
mAbs rF1, CR5133 and CR6453 showed the strongest binding to S. epidermidis ATCC14990, and CR5133 and CR6453 bound the majority of clinical isolates (19/20). We observed that activation of the complement system is essential to induce efficient phagocytosis of S. epidermidis, and that it could be enhanced by hexamer-enhancing mutations. We found that classical pathway complement activity in neonatal cord blood plasma is comparable to adult levels, and that mAbs could greatly enhance phagocytosis of S. epidermidis in the presence of neonatal plasma. Furthermore, our whole blood assays suggest that phagocytic capacity of neutrophils from term neonatal blood is similar to adult neutrophils.

Conclusion
Our findings provide insights that are crucial for optimizing anti-S. epidermidis mAbs as prophylactic agents for neonatal bloodstream infections.

Rapid diagnostics of joint infections using Molecular Culture

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Introduction: Diagnosis of joint infections by culture often requires several days as bacteria often exist in a dormant state in biofilms. This long diagnosis time severely hampers rapid and tailored antibiotic treatment. Molecular assays have the potential to drastically reduce time to diagnosis. We developed the Molecular Culture test, a PCR based assay that combines length polymorphisms of the 16S-23S interspace rDNA region with phylum-specific fluorescently labelled primers to identify bacteria to species level. This assay takes approximately four hours, thus offering a much faster turn-around time than culture. In this study, we evaluated the diagnostic accuracy of this test procedure on joint aspirate samples.
Methods: A total of 293 native and 298 prosthetic joint samples (residual material from routine diagnostics) were subjected to DNA isolation optimized for joint aspirates followed by Molecular Culture. Outcome of Molecular Culture was compared to routine culture results.
Results: 192 out of 591 (32.4%) samples were positive in routine culture whereas Molecular Culture detected 223 (37.7%) positive samples. For the 4 most prevalent detected species, the numbers detected by routine culture, Molecular Culture and concordant detections were respectively: Staphylococcus aureus 59-73-56; Staphylococcus epidermidis 35-34-29; Streptococcus dysgalactiae 14-20-14, Escherichia coli 11-17-10. The 10 discordant detections for these species where Molecular Culture was negative mostly involved low-load routine culture outcomes. Twelve from the 35 extra detections by Molecular Culture were supported by identical routine culture results from other samples from the same patient. The semi-quantitative output of Molecular Culture corresponded well with the loads reported by routine culture.
Conclusions: Molecular Culture showed favourable performance for diagnosing joint infections. Combined with its fast turnaround time, Molecular Culture may provide a much-needed option for faster diagnosis and treatment of patients suffering from native- and prosthetic joint infections.

Impact of COVID-19 on the Euregio Meuse-Rhine: remarkable seroprevalence differences between Belgium, the Netherlands, and Germany.

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Introduction

A prospective longitudinal study was designed to determine the impact of COVID-19 on inhabitants of the Euregio Meuse-Rhine (EMR) region, covering the border area between Belgium, the Netherlands, and Germany. Seroprevalence of anti-S (spike) and anti-N (nucleocapsid) antibodies were examined to give insight into acquired infections and vaccination status when vaccination campaigns were enrolled.

Methods

A sample of 3500 inhabitants was selected per country. Questionnaires and self-finger-prick blood samples were collected in Spring and Autumn of 2021. Samples were tested for anti-S and anti-N antibodies. Weighted seroprevalence was calculated.

Results

Response rates for Belgium, the Netherlands, and Germany were 7.4%, 15.1%, and 15.4%. In Spring, the seroprevalence of anti-S antibodies was 65.9% for Belgium, 64.8% for the Netherlands, and 69.8% for Germany. Seroprevalence of anti-N antibodies in anti-S-positive participants was 3.2%, 12.3%, and 3.2%. In Autumn, the seroprevalence of anti-S antibodies increased to 94.5%, 91.8%, and 94.5%. Seroprevalence for anti-N antibodies increased for Belgium to 3.5%, and was 4.4%, and 1.7% for the Netherlands and Germany.

Conclusions

A remarkable high anti-N antibody response was seen in Spring in the Netherlands. The anti-S antibody responses reflect the progress in vaccination in the populations. A large number of seroreversions for N-directed antibodies were observed within 6-8 months, while S-directed antibodies remained positive, making anti-N-directed antibodies suitable for detection of relative recent SARS-CoV-2 infections.

The Salmonella adhesin Rck mediates entry through the EGF receptor in a MUC13-dependent manner

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The intestinal mucus layer protects epithelial cells from commensals and pathogens such as the enteropathogen Salmonella enterica. In addition to soluble mucins secreted by goblet cells, epithelial cells express various transmembrane mucins on their surface. Transmembrane mucins have highly O-glycosylated extracellular domain, transmembrane domain, and cytoplasmic tail. While classically regarded as barrier against infection, our lab showed the enteropathogen Salmonella enterica expresses the giant adhesin SiiE that utilizes the transmembrane mucin MUC1 for apical invasion into enterocytes. Salmonella can also invade cells via a zipper mechanism, when its outer membrane protein Rck binds to the epidermal growth factor receptor (EGFR), and initiates receptor-mediated endocytosis. Interestingly, MUC1 and MUC13, have EGF-like domains and/or have been shown to interact with signaling receptors of the EGFR family. The importance of Rck-EGFR invasion pathway and the contribution of transmembrane mucins remain to be determined.

To study Rck-mediated bacterial invasion, we expressed either full-length Rck, or truncated Rck (Rck) that cannot mediate invasion in Escherichia coli. As infection model, we selected the human colon cell lines HT29-MTX and HRT18 that express high levels of both MUC1 and MUC13 and generated MUC1 and MUC13 knockout lines using CRISPR-Cas9. Rck-mediated bacterial invasion was similar in MUC1 KO cells compared to WT cells. Surprisingly, bacterial invasion was dramatically decreased in MUC13 KO HT29-MTX and HRT18 cells. To determine the specificity of the Rck-mediated invasion, we pretreated the intestinal cells with the EGFR inhibitor gefitinib prior to the bacterial invasion assay. In both types of intestinal cells, EGFR inhibition dramatically decreased bacterial invasion. Immunofluorescent confocal microscopy showed that bacterial adherence was not affected in the MUC1 or MUC13 knockout cells. Together, these data demonstrate that invasion mediated by the Salmonella adhesin Rck is dependent on EGFR activity and that the abundant intestinal transmembrane mucin MUC13 promotes this invasion pathway.

Reduction of waste after patient discharge from the intensive care unit using results of admission screening for highly resistant microorganisms

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Introduction
There is an urgency to make healthcare more sustainable, without affecting patient safety. To prevent pathogen transmission via the environment at the intensive care unit (ICU), patient rooms are cleaned after patient discharge and unused products are disposed. This generates 3-9 kg waste per patient per room. We hypothesized that the results of ICU admission cultures for highly resistant microorganisms (HRMO), already part of our surveillance program, could be used to decide on the indication of product disposal. We aimed to assess whether these targeted infection prevention and control measures at the ICU could reduce unnecessary waste.
Methods
This retrospective study, at the Erasmus University Medical Center, included anonymous patient ICU admission and isolation data between 01-01-2019/30-09-2022. Patients are screened (throat and rectum) for HRMO on admission, e.g. carbapenem-resistant Gram-negatives; negative cultures are known within 72 hours. We retrospectively categorized admissions into products to be disposed or not, whereby products should be disposed after admissions with isolation precautions and after admissions <72 hours. Products could not have been disposed after admissions of >72 hours without isolation precautions during the full admission.
Results
We included 3923 ICU patients, with 4423 admissions per patient per room. Isolation precautions were applied in 1012 (22.9%) admissions. Admission duration was >72 hours for 2505 (56.6%) admissions, of which 1750 (69.9%) admissions did not require isolation precautions. Therefore, in 2673 (60.4%) of admissions, products were to be disposed, while for 1750 (39.6%) this would not have been necessary.
Conclusion
The use of negative ICU screenings cultures to decide whether or not to dispose patient room products could reduce on average 40% waste (e.g. 10,500 kg waste reduction) produced after patient discharge from the ICU. The new waste policy was implemented based on these results and will be further evaluated from environmental, financial, and microbiological perspective.

Extracellular polymeric substance from seawater-adapted aerobic granular sludge: enrichment of polyanionic fraction and exploration into its potential application for sepsis treatment

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Sepsis is a severe infectious disease, where extracellular histones play a major role in the death of sepsis patients. Anionic polymers, like heparins, have shown the potential to treat sepsis by neutralizing the cationic histones and thus blocking cytotoxicity. Currently, the raw material for heparin is from animal livestock, which comes with great risks (e.g. shortages due to diseases). Sulfated polymers, similar to heparin, have been detected in the extracellular polymeric substances (EPS) of aerobic granular sludge (AGS). As sulfated polymers are mainly reported to be produced by marine bacteria, it was hypothesized whether the EPS from seawater-adapted AGS could be used as raw material for therapeutical treatments. This study aims to enrich the anionic fraction of the EPS and explore its potential application for sepsis treatment. AGS was grown in a lab-scale bubble column reactor fed with acetate and under seawater conditions (35 g/L). The EPS was extracted under alkaline and heat (80°C) condition and fractionated by molecular weight through size exclusion chromatography. The histone binding was tested by native gel electrophoresis. AGS was found to be dominant with “Candidatus Accumulibacter” and stable granulation was observed. The two highest molecular weight fractions (>5,000 - 438 kDa) of seawater-adapted EPS were dominant in carbohydrates and were enriched with sulfated glycoconjugates up to 2-fold. Unfractionated EPS and the fractions of seawater-adapted EPS successfully neutralized the positive charge of the two histones involved in sepsis cytotoxicity. These results indicate that anionic enriched EPS from seawater-adapted AGS has the potential to be developed as a sepsis treatment based on the binding by histones.

Salt Stress: discovering osmoregulation in freshwater anaerobic methane-oxidizing archaea

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Coastal freshwater wetlands are natural hotspots for methane production, with increased susceptibility to anthropogenic and climate change-derived impacts. Methane-oxidizing microorganisms can alleviate this methane production using a diverse range of available electron acceptors. In this study, we characterized the physiological response of sea-level rise-derived salinization in an enrichment of nitrate-reducing and typically freshwater anaerobic methane-oxidizing Ca. Methanoperedens archaea (ANME-2d). We hypothesized that Ca. Methanoperedens reduces its methane oxidation potential while preserving its dominance in the community. We aimed at elucidating a yet uncharacterized ANME-osmoregulation strategy in these archaea.

Salt concentration was gradually increased from freshwater to brackish (1.5%) in a granular-growing Ca. Methanoperedens (enriched for ~60-80%) anoxic bioreactor. Activity of the culture was followed by measuring ammonium, nitrate and nitrite. Methane oxidation potential was determined by whole bioreactor methane-labelled (13C-CH4) activity assays using Gas Chromatography-Mass Spectrophotometry (GC/MS) at freshwater, brackish and marine (3.5%) salinities. Osmolyte production during the stepwise salt increase was explored by untargeted metabolomics employing a Liquid Chromatography (LC)/quadruple - time of flight (Q-TOF) system. The Ca. Methanoperedens osmoregulation potential and abundance in the community was monitored via metagenomics and 16S rRNA gene amplicon sequencing. Changes in metabolism were analysed via metatranscriptomics and metaproteomics.

Ca. Methanoperedens remained active and the most abundant community member, with over 60% of the mapped reads. Methane oxidation potential decreased from freshwater to brackish, but remained stable when salt was increased from brackish to marine concentrations. We were able to identify N6-Acetyl-Beta-L-Lysine as the possible metabolite regulating the osmolarity in the cell and confirm that the complete production pathway was exclusive to the Ca. Methanoperendes genome.

Congruent with our hypothesis, we showed that the freshwater Ca. Methanoperedens remained active although with some reduced methane oxidation potential. Our investigation is the first one to link osmolyte N6-Acetyl-Beta-L-Lysine production to an ANME enrichment culture.

Endocytosis-like DNA uptake by cell wall-deficient bacteria

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Bacteria have developed sophisticated macromolecular machines to take up DNA from the environment, which facilitate DNA transport across the cell wall. Over the past years, it has become clear that bacteria may also thrive without their cell wall. These cell wall-deficient cells are formed when they are exposed to environmental stresses such as antibiotics or a high osmotic pressure. We here show how such cells take up DNA independent of the canonical machinery involved in natural transformation. Instead, these cells use a eukaryotic-like endocytosis mechanism, whereby membrane invagination leads to the encapsulation of extracellular material. The uptake process is energy-dependent and can be stalled by canonical endocytosis inhibitors. We show that wall-deficient cells can take up a variety of molecules, including plasmid DNA, polysaccharides and 150-nm lipid nanoparticles. Using state-of-the-art cryo-electron microscopy, we discovered that these cells contain complexes of vesicles and protrusions, indicating the cell membrane is highly dynamic. Given that cell wall-deficient bacteria are considered a model for early life forms, our work provides a possible mechanism for primordial cells to acquire new genetic material or food before invention of the bacterial cell wall. This work has recently been published in Nature Communications (1).

Reference

1) Kapteijn, R., Shitut, S., Aschmann, D. et al. Endocytosis-like DNA uptake by cell wall-deficient bacteria. Nat Commun 13, 5524 (2022). https://doi.org/10.1038/s41467-022-33054-w

Gene gain facilitated endosymbiotic evolution of Chlamydiae

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Chlamydiae is a bacterial phylum composed of obligate animal and protist endosymbionts. Together with its next relatives, Chlamydiae forms the Planctomycetes–Verrucomicrobia–Chlamydiae (PVC) superphylum, however most other members are primarily free living. This makes the PVC superphylum an interesting group for studying the transition to and long term evolution of endosymbionts. How Chlamydiae transitioned to an endosymbiotic lifestyle and later diversified over billion-year timescales is still largely unresolved. Here, we reconstructed Planctomycetes–Verrucomicrobia–Chlamydiae species relationships and modeled superphylum genome evolution. Obligate endosymbionts are generally expected to undergo genome reduction and metabolic streamlining. We expected chlamydiae to have evolved from a large ancestor with complete metabolic potential, subsequently leading to smaller or similar genome sizes found in extant chlamydiae. Gene content reconstruction from 11,996 gene families suggests a motile and facultatively anaerobic last common Chlamydiae ancestor that had already gained characteristic endosymbiont genes. Counter to expectations for genome streamlining in strict endosymbionts, we detected substantial gene gain within Chlamydiae. We found that divergence in energy metabolism and aerobiosis observed in extant lineages emerged later during chlamydial evolution. In particular, metabolic and aerobic genes characteristic of the more metabolically versatile protist-infecting chlamydiae were gained, such as respiratory chain complexes. Our results show that metabolic complexity can increase during endosymbiont evolution, adding an additional perspective for understanding symbiont evolutionary trajectories across the tree of life.

Diversity of cfr-carrying plasmids in livestock-associated methicillin-resistant Staphylococcus aureus from humans in the Netherlands

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In the Netherlands, livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA, clonal complex CC398) represents 25% of isolates submitted to the national MRSA surveillance. To study transmission of LA-MRSA in hospitals, isolates from nine hospitals were analyzed which revealed the presence of the cfr gene. This gene is often located on plasmids and encodes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (termed PhLOPSA phenotype). The objective of this study was to analyze cfr-carrying plasmids and compare them to cfr-plasmids found previously in the Netherlands.

A total of 555 LA-MRSA isolates collected in 2017 to 2019 from unique patients in nine hospitals participating in the Dutch MRSA surveillance were sequenced using Illumina short-read sequencing. Sequence data was used for resistance gene identification (AMRFinder), and whole genome multilocus sequence typing (wgMLST). cfr-positive isolates were subjected to long-read sequencing to reconstruct plasmids. Antibiotic susceptibility testing was performed using broth microdilution.

The cfr gene was identified in four out of 555 (0.7%). These isolates were submitted by three hospitals in 2017 and 2018, were collected for screening purposes, and expressed the PhLOPSA phenotype. All persons from whom the LA-MRSA were isolated reported contact with livestock. Based on wgMLST, the four cfr-positive LA-MRSA isolates were unrelated since the allelic distance was >94 alleles. The cfr gene was localized on plasmids of variable sizes ranging from 36-98-kb, which displayed low similarity of 5-61%. Comparison of these four cfr-carrying plasmids with five previously reported cfr-carrying plasmids of 14-35-kb (Schouls et al., 2022) from unrelated LA-MRSA also revealed low similarity (5-87%) and variable %G+C (29.5-32.8%).

The proportion of cfr-positive LA-MRSA isolated from humans was low. The human cfr-carrying plasmid population in the Netherlands is highly diverse, suggesting multiple introductions and because of its association with multiresistance its presence should be closely monitored in humans as well as in livestock.

Unraveling the ability of Aeromonas sp. to degrade and utilize the biopolymer chitin

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Introduction: Aeromonas are ubiquitous inhabitants of man-made and natural aquatic ecosystems. Occasionally, Aeromonas also grows in drinking water distribution systems, which is undesired due to the pathogenicity of some members of this genus. In The Netherlands, Aeromonas sp. is considered an indicator strain for biological stability of the drinking water. However, the growth of Aeromonas in highly oligotrophic environments is currently only poorly understood. Possible nutrient sources are represented by biopolymers such as chitin, which is the structural component of the exoskeleton of insects, some invertebrates and the cell walls of fungi.

Methods: Aeromonas bestiarum, Aeromonas media and Aeromonas rivuli were grown on M9 minimum salt medium amended with either glucose or amorphous chitin. Large-scale quantitative proteomics was performed using a QE plus Orbitrap mass spectrometer (Thermo Fisher Scientific, Germany). The shotgun raw data were searched against the proteome databases of each Aeromonas species, obtained from UniprotKB and NCBI, using PEAKS Studio X. The enzymatic chitin degradation pathway analyzed by Vanquish UPLC and a QE focus Orbitrap mass spectrometer (Thermo Fisher Scientific, Germany).

Results: In this study we first demonstrated that Aeromonas species can efficiently grow on chitin as sole carbon and nitrogen source. Quantitative cellular proteomics furthermore validated fundamental reorganization of the Aeromonas proteome when switching to chitin as sole nutrient source. Finally, the analysis of the secretome revealed the hydrolytic enzymes that allow efficient degradation and utilization of this biopolymer. Interestingly, albeit the studied Aeromonas species could efficiently grow on chitin, their degradation and uptake system slightly differ.

Conclusion: We demonstrate that Aeromonas species can efficiently grow and thrive on chitin as sole carbon and nitrogen source. This supports the hypothesis that Aeromonas may use the exoskeleton of invertebrates present in the drinking water distribution system as nutrient source.

A one year pilot surveillance of hypervirulent Klebsiella pneumoniae in Dutch patients

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Introduction:
Hypervirulent Klebsiella pneumoniae (hvKp) is associated with invasive infections in previously healthy individuals and development of multiple abscesses. Hypervirulence appears associated with virulence genes involved in iron acquisition and genes associated with hypermucoviscosity, located on a plasmid. In contrast to Asia, hvKp is considered rare in Europe. This prospective pilot surveillance aimed to determine occurrence and clinical, epidemiological and genomic characteristics of hvKp in the Netherlands.

Methods:
In 2022, all Dutch medical microbiology laboratories were requested to submit suspected hvKp isolates (based on clinical criteria). Suspected isolates submitted ad hoc in 2020/2021 were also included. Clinical and epidemiological patient data were collected by a web-based questionnaire. Next-generation sequencing (NGS) and whole-genome multi-locus sequence typing (wgMLST) were performed, enabling assessment of antimicrobial resistance genes (ResFinder), virulence factors (Kleborate), and genetic relatedness.

Results:
Fifty-three suspected hvKp isolates were received (46 in 2022 and seven in 2020/2021). Of 41 patients with completed questionnaires, 12 (29%) were female and the median age was 69 years (interquartile range 57-74). Twenty-nine (71%) had an abscess, mainly in the liver, and 25 patients (61%) had bactaeremia; 13 patients (32%) had both. Twelve patients were born in the Netherlands, 11 abroad (Asia: 8; Africa: 2; USA: 1), and for 18 it was unknown. Of 12 patients with known recent travel history, one had travelled outside Europe (Korea). Only one isolate was resistant to multiple antibiotics, though not to carbapenems. Nineteen of 48 sequenced isolates had a maximum virulence score based on the presence of genes encoding for yersiniabactin, colibactin and aerobactin. Of these, 13 were from MLST sequence type 23. WgMLST of 39 isolates showed low genetic relatedness.

Conclusion: The number of hvKp infections in the Netherlands appears low and the hvKp population is genetically diverse. There were no indications of transmission of hvKp.

Seroprevalence and risk modeling of future outbreaks for mpox among men who have sex with men in the Netherlands

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Background
The mpox outbreak among mainly men-who-have-sex-with-men (MSM) in Europe reached a peak in July 2022, followed by a steady decline. Currently the level of viral circulation in Europe is very low. In the Netherlands, risk groups were invited for vaccination with Imvanex (modified vaccinia virus Ankara [MVA]). To assess how immunity contributed to the containment of the outbreak we performed an immunological survey among MSM in Rotterdam. These data, in combination with literature data, were subsequently used to develop a mathematical model to predict how the current seroprevalence level would impact future outbreaks.

Methods
Sera of 315 MSM who visited the Centers for Sexual Health in Rotterdam in September 2022 were tested for the presence of poxvirus-specific IgG antibodies, and MPXV and MVA neutralizing antibodies (nAbs). A stochastic model was developed, based on the assumption that the outbreak was started by a superspreading event. We calibrated the model to the exponential phase of the 2022 mpox outbreak in the Netherlands and the final total number of individuals (1,200-1,800), that were infected 120-180 days after the outbreak started. A new outbreak was modelled at different seroprevalence levels.

Results
Poxvirus-specific IgG antibodies were detected in 143/315 sera (45%). From 70 positive sera, 10% showed a nAb pattern compatible with infection and 37% showed a nAb pattern compatible with Imvanex vaccination. Using our model, we demonstrated that with increased seroprevalence of poxvirus-specific antibodies, the size and duration of a new outbreak would be strongly decreased. For the current seroprevalence level, the outbreak size was modelled to be 108 cases (interquartile range, IQR, 60-147) with a peak after an average of 52 days (IQR 33–68).

Conclusions
Our seroprevalence and modeling data emphasize the importance of risk group vaccination to prevent future mpox outbreaks and provide a framework for public health policy-making.