P01

Seroprevalence of human Ascaris, Toxocara, and Toxoplasma in the Netherlands: results from the PIENTER-3 study

Dr. Rens Zonneveld[1,2], MD PhD K Stanoeva[1,3], Msc O van den Berg[4], Ing D Hoek-van Deursen[1], PhD F van der Klis[5], PhD E Franz[4], PhD I Friesema[4], MD T Kortbeek[1]
[1]RIVM, [2]Amsterdam UMC, [3]ECDC, [4]RIVM, [5]RIVM

Introduction
The helminths Ascaris and Toxocara and protozoan Toxoplasma are ubiquitous parasites with zoonotic potential from their definitive hosts, which are domestic pigs, cats and dogs, and cats, respectively. In data from two previous cross-sectional serosurveys amongst the Dutch population with a 10 years interval, PIENTER-1 (1996-1997) and PIENTER-2 (2006-2007), a significant decrease in Toxocara (10.7% to 8.0%) and Toxoplasma (40.5% to 26.0%) seroprevalence, yet significant increase in Ascaris (30.4% to 41.6%) seroprevalence was observed. Presence of antibodies was higher with increasing age. Here, we present preliminary data from the third Dutch cross-sectional serosurvey, PIENTER-3, collected in 2016-2017.

Methods
Study design of the PIENTER-3 serosurvey was similar to the two earlier serosurveys and described elsewhere. In short, questionnaire and serum sampling were performed among different age categories and in nested municipalities representing all regions in the Netherlands. Samples were tested using in-house ELISAs for presence of IgG antibodies against antigens derived from Ascaris suum, Toxocara canis and Toxoplasma gondii, respectively. Samples were tested in duplicate and a ratio ≥1.0 between the optical density (OD) of the samples and the cut-off was considered positive. Discrepant results between duplicates were excluded. Statistical analysis was performed with R software. Comparison of seroprevalence rates was performed using a chi-square test. A two-sided p-value of ≤ 0.05 was considered statistically significant.

Results
A total of 6,229, 6,360, and 6,326 samples were included in the final analysis and seroprevalence was 45.4% (95% CI 44.1-46.6%), 9.1% (95% CI 8.4-9.8%), and 29.7% (95 CI 28.6-30.9%), for Ascaris, Toxocara, and Toxoplasma, respectively. Seroprevalence in 2016 was significantly higher when compared to 2006 for Ascaris ($p<0.0003$), and Toxoplasma ($p<0.0001$). Seroprevalence for Toxocara was higher in 2016, but not statistically significant ($p=0.08$). For all parasites, seroprevalence increased with age up to 64.6% (95% CI 59.0-69.9) for Ascaris, 17.2% (95% CI 13.3-22.0%) for Toxocara, and 76.4% (95% CI 71.2-80.9%) for Toxoplasma in individuals aged 76 to 90 years.

Conclusion
The results from this preliminary analysis show that overall seroprevalence for Ascaris has increased and Toxocara remained similar in the Dutch population between 2006 and 2016. Interestingly, the stark decrease for Toxoplasma seroprevalence between 1996 and 2006 did not continue into 2016. The data also confirmed the higher presence of antibodies with increasing age found in the previous two serosurveys. Further analysis of the PIENTER-3 data is needed to establish trends in risk factors for infection and relevance of environmental or food-borne transmission routes for infection for which public health interventions may apply.

P02

Microbiological insights into the haloalkaline biodesulfurization process

Suyash Gupta[1,2], M Sc. R de Rink[3,4], dr.ir JBM Klok[1,4], dr CM Plugge[1,5], prof.dr G Muyzer[2]
[1]Westsus, European Centre of Excellence for Sustainable Water Technology, [2]University of Amsterdam, [3]Wageningen University & Research, [4]Paqell B.V., [5]Wageningen University & Research

Microbiological insights into the haloalkaline biodesulfurization process

Biodesulfurization is an economical and eco-friendly process to remove H2S from gas streams by using haloalkaliphilic sulfur-oxidizing bacteria (SOB). These bacteria oxidize dissolved sulfide to elemental sulfur that can be further used as fertilizer or fungicide. Recent technological advancements in the biodesulfurization process have increased the sulfur selectivity from 90% to 96.6%[1]. However, less is known about the microbiology involved in the updated process. Hence, the aim of this study was to analyze the diversity and ecophysiology of the microbial community present in the biodesulfurization process. For this, biomass was obtained from different runs of a pilot-scale biodesulfurization plant in which each operational run corresponded to a different selection pressure for the SOB in the system. Both DNA- and RNA-based 16S amplicon sequencing was performed to determine the microbial diversity in the different operational runs and to discriminate between active and non-active community members. The results showed that non-active bacterial populations varied with the different operational runs, but there was no change in the active bacterial populations. However, the relative abundance of the active bacteria varied with each run. The microbial community also changed with the time of operation for each run. However, there was no difference in microbial diversity in the different sections of the system. In all cases of comparison, we found that there was a difference between active and non-active bacterial populations. Some dominantly present and active SOB were members of the genera Alkalilimnicola and Thioalkalivibrio. Our results indicate that it is essential to determine the active microbial community members as they might be the key players in the haloalkaline biodesulfurization process at the moment of sampling.

Reference:

1. de Rink R, Klok JBM, van Heeringen GJ et. al (2019) Increasing the Selectivity for sulfur formation in Biological gas desulfurization. Environ Sci Technol 53:4519–4527. doi: 10.1021/acs.est.8b06749

P03

Culturing novel nitrifiers through cell sorting

Msc Pieter Blom[1], dr. M van Kessel[1], prof. dr. ir. MSM Jetten[1], dr. S Lücker[1]
[1]*Radboud University*

Nitrification was long regarded as a two-step process that is performed by separate guilds of ammonia and nitrite oxidizing microorganisms, respectively, but the recent discovery of complete ammonia oxidizing (comammox) bacteria resulted in a shift of this paradigm. The to date only available comammox Nitrospira pure culture yielded interesting physiological insights, but more isolates are needed to test whether the observed traits are common to all comammox bacteria. The characterization of an additional highly enriched comammox Nitrospira already indicated fascinating differences in their physiology, as this species surprisingly was inhibited by low concentrations of its substrate ammonium. This exemplifies the urgent need to obtain pure cultures of novel and distinct nitrifying microorganisms in order to study their phenotype under defined conditions and to understand their contribution to nitrogen cycling in natural and engineered ecosystems. However, classical cultivation methods have for a long time overlooked the existence of comammox bacteria, which not only illustrates the recalcitrance of these fastidious bacteria to cultivation, but also our still incomplete knowledge about nitrifying microorganisms. Here, we present a workflow for the targeted enrichment and isolation of novel ammonia oxidizing microorganisms, including comammox bacteria, from complex environmental samples. Specific in vivo fluorescent labelling of ammonia monooxygenase, the key enzyme required for ammonia oxidation, was combined with fluorescence-activated cell sorting (FACS) into 96-well plates containing mineral medium amended with ammonia and nitrite Determination of nitrate production in these wells allowed to distinguish complete nitrifiers from canonical ammonia oxidizers, and wells containing both types of nitrifier were selected for further cultivation and physiological characterization. Applying this workflow to complex enrichment cultures derived from activated sludge and biofilm in recirculating aquaculture system biofilters, we managed to obtain several highly enriched cultures containing comammox Nitrospira and two apparently pure Nitrosomonas-related ammonia oxidizing bacteria. In conclusion, we demonstrate that our approach is well-suited to isolate novel ammonia-oxidizers from complex environmental samples, circumvents the challenges involved in classical cultivation techniques that hindered the isolation of many relevant nitrifying microorganisms, and thus will greatly advance our understanding of the environmental role and biotechnological potential of these intriguing microorganisms.

P04

Effect of acetic acid on growth, physiology and gene expression of Desulfobacillus acidiphilus

MSc Reinier Egas[1], Dr. Cornelia Welte[2], Prof. DZ Sousa[1], Dr. Irene Sánchez-Andrea[1]
[1]Wageningen University & Research, [2]Radboud University

Acid mine drainages (AMD) are streams characterized by their low pH and high concentrations of sulfate and heavy metals. If released to the environment, AMD have a detrimental impact on water quality and biodiversity. Sulfate-reducing bacteria (SRB) can aid in AMD bioremediation as the produced sulfide precipitates with metals (forming metal sulfides facilitating its separation). Bioremediation processes based on sulfate reduction are operational, but mostly based on neutrophilic SRB communities, requiring pH neutralization of the influent. This might be optimized by using acidophilic SRB (aSRB). The recently characterized aSRB, Desulfobacillus acidiphilus can completely oxidize acetic acid to CO2. This is advantageous as proton stress at low pH affects other stress factors like the fraction of undissociated organic acids. Insights in organic acid toxicity aids understanding aSRB adaptation to these extreme environments and their mechanisms for pH homeostasis. Knowledge gained might facilitate the application of aSRB in bioremediation processes, leverage optimization of existing techniques and increase cost-effectiveness by implementing alternative electron donor streams.

This study investigated the mechanisms employed by Db. acidiphilus to handle the effect of sub-lethal acetic acid toxicity. We hypothesize an increase in acetate oxidation and upregulation in proton stress resistance mechanisms, such as decarboxylation of amino acids, active proton export and cation pumps, to adjust the chemiosmotic gradient due to internal dissociation of acetic acid.

Comparative transcriptomics was performed on Db. acidiphilus grown under stress and non-stress conditions in pH-controlled bioreactors (pH 5.0). To induce stress at 70% of the maximum optical density (600 nm), acetic acid or an equal volume of water was injected in the spiked and control condition, respectively. First, the maximum sub-lethal limit of spiking with acetic acid was established in duplicate followed by comparing the maximum tolerated concentration of 7.5 mM acetic acid to a non-stress condition in triplicate. Samples for transcriptomics were taken of spiked and control conditions 2.5-3 hours after spiking (Sp1 and C1, respectively). Continuation of growth was confirmed based on optical density, and 26 hours after spiking another sample was taken of the stressed condition (Sp2) – after confirming Db. acidiphilus had recuperated with a significantly impaired growth rate from 0.0418 to 0.0033 h-1. In total 386, 148 and 245 differentially expressed genes (DEG) were identified for the comparisons Sp1-C1, Sp2-C2 and Sp1-Sp2, respectively. DEGs are identified by a log2 fold change > 2 and adjusted p-value < 0.01. The majority of DEGs are associated to the amino acid metabolism. Synthesis of the negatively charged amino acids glutamate and aspartate is downregulated in Sp1-C1 and largely recovered in Sp2. No DEGs are identified in proton stress response mechanisms such as F0F1-ATP synthase, cation transporters or decarboxylases despite the amino acid metabolism perturbations.

This study showed: (1) Db. acidiphilus can recover growth after spiking up to 7.5 mM of acetic acid in pH-controlled conditions. (2) Limited proton stress response to organic acid toxicity, no DEGs in active proton stress mechanisms. (3) DEGs after spiking mainly associated with the anion pool, indicating anion accumulation main stressor of organic acid toxicity.

P05

Differences in the methanol metabolism of the sulfate reducers Desulfofundulus kuznetsovii strains 17T and TPOSR

Lukas Friedeheim[1], prof. dr. D. Z. Sousa[1,2], prof. dr. I. Sanchez-Andrea[1], prof. dr. ir. A. J. M. Stams[1,2]
[1]Wageningen University, [2]University of Minho

In deep-subsurface environments methanol can be geochemically produced from CO2 and H2. There, it can act as an electron donor and carbon source for anaerobic microorganisms. Methanogens and acetogens generally convert methanol using a pathway initiated by a vitamin B12-dependent methyl transferase (MT). The MT system was thought to be prevalent in anoxic environments for methanol conversion, whereas in oxic environments methanol is oxidized by an alcohol dehydrogenase (ADH).
We previously described the simultaneous occurrence of both methanol conversion pathways (MT and ADH) in a single microorganism, the sulfate reducer Desulfofundulus kuznetsovii strain 17T (former Desulfotomaculum kuznetsovii). The genome of D. kuznetsovii strain 17T contains several ADH, and proteomics analyses abundance of one when the strain was grown in the absence of vitamin B12 and cobalt (precursor of vitamin B12). In a previous study, we have isolated another D. kuznetsovii strain (TPOSR), that is able to grow on methanol. When performing a genome comparison of the two strains we verified that, even though the two strains show a high degree of identity in their genomes, strain TPOSR lacks a number of genes from the MT operon that are vital for its function. In contrast to D. kuznetsovii strain 17T, strain TPOSR therefore relies on a vitamin B12-independent ADH for methanol metabolism. Genes encoding for ADHs in the two strains are highly similar. The alcohol dehydrogenase that has been reported responsible for methanol metabolism in D. kuznetsovii matches a homologue in the genome of strain TPOSR with 96% amino acid identity. When vitamin B12 and cobalt are omitted from the media, growth of D. kuznetsovii strain 17T is significantly impaired due to the lack of MT activity. Furthermore, higher residual methanol concentrations indicate activity of the ADH to a minimal methanol concentration of 5 mM. In the presence of vitamin B12 and cobalt, the active MT facilitates growth up to a concentration of 0.8 mM. Growth of strain TPOSR remains mostly unaffected by vitamin B12 and cobalt starvation. Interestingly, its ADH facilitates metabolism of methanol up to a minimal concentration of 2 mM, indicating a higher affinity in the absence of vitamin B12 and cobalt when compared to the type strain.
The results indicate that, despite the high degrees of genetic similarities between D. kuznetsovii strain 17T and strain TPOSR, their methanol metabolism shows differences. Strain TPOSR misses important parts of the MT operon, rendering it reliant on its dehydrogenase activity for methanol conversion. A deeper knowledge of the extent of these differences and their influence on methanol metabolism and kinetics could potentially help understanding the advantage of a more flexible methanol metabolism (like the one in strain 17T) for example in competition with other microbial groups.

P06

High methane production potential via various methanogenic pathways in the sediments of marine Lake Grevelingen after summer hypoxia

Anna Wallenius[1], MSc OM Zygadlowska[2], Dr. WK Lenstra[2], Dr. NAGM van Helmond[2], MSc GHL Nuijten[1], MSc J Venetz[1], Dr. P Dalcin Martins[1], Prof. dr. ir. CP Slomp[2], Prof. dr. ir. MSM Jetten[1]
[1]Radboud University, [2]Utrecht University

Introduction. Coastal ecosystems are a significant source of the potent greenhouse gas methane, contributing to up to 75% of marine methane emissions. Methane is typically produced in sediments by methanogenic archaea in the final step of organic matter degradation from fermentation end-products acetate (acetoclastic), carbon dioxide and hydrogen (hydrogenotrophic), and methylated compounds (methylotrophic). In marine sediments, over 90% of produced methane is filtered by anaerobic methane-oxidizing archaea (ANME) in the sulfate-methane transition zone (SMTZ) above the methanogenic layer. However, in coastal marine systems with a high organic matter input and prolonged periods of anoxic bottom waters due to water column stratification, conditions for methanogenesis near the sediment-water interface may become more favorable. This may reduce the methane-filtering efficiency in the SMTZ, enabling methane to escape into the water column and potentially to the atmosphere. Usually, methanogenesis occurs in deeper sediments as near the surface and in the SMTZ methanogens are outcompeted for the main substrates acetate and hydrogen. Methylotrophic methanogens can use non-competitive substrates such as methanol or methylamines and therefore can be found also in the upper sediment layers. However, their contribution to total methane emissions from coastal sediments is poorly understood. This study investigated potential methane production rates and pathways in the sediments of marine Lake Grevelingen (the Netherlands) after a period of bottom water anoxia in summer. We hypothesized that methane production rates would be highest below the SMTZ and would be enhanced by addition of different methanogenic substrates, indicating which pathways of methanogenesis might be favored in Lake Grevelingen sediments.
Methods. Sediment samples were taken in September 2020 with the RV Navicula, transported to the laboratory and sliced anoxically in 5 cm intervals. Incubation experiments and gas chromatography were used to measure the methane production potential in different sediment layers. Incubations were amended with various methanogenic substrates, i.e. acetate, hydrogen and carbon dioxide, methanol, and methanol and hydrogen.
Results. During the 2-month incubation period, methane production was observed in every sediment layer tested, from the top 5 cm to a depth of 40 cm, and the highest methanogenesis rate was recorded at the zone between 5-10 cm depth. Methanogenesis was enhanced in all incubations amended with methanogenic substrates, but acetate addition resulted in the highest measured methane production over time.
Conclusions. Together, these results indicate that: 1. Methanogenic archaea inhabit all layers of the sediment, 2. Methanogenesis co-occurs in the top layer together with sulfate reduction and organic matter mineralization, and 3. Methane is potentially produced via acetoclastic, hydrogenotrophic, as well as methylotrophic methanogenesis in Lake Grevelingen sediments. Follow-up studies including the analysis of the microbial community structure based on16S rRNA gene sequencing will help to further elucidate the identity of methanogenic microorganisms. Selected metagenome studies will provide insight into the potential methanogenic pathways responsible for methane production in these sediments.

P07

Diversity of key genes for the conversion of methanol in diverse anoxic marine sediments

Msc peter Fischer[1], dr L. Villanueva[2], prof. dr. D.Z. Sousa[1]
[1]Wageningen University & Research, [2]Royal Netherlands Institute for Sea Research

Methanol is a major driver in microbial metabolism. It is formed as an end product of fermentation, through geochemical formation and is distributed by atmospheric and marine deposits. In anoxic marine environments, methanol can be utilized in the metabolism of many microorganisms, for example sulfate reducing microorganisms or methanogens. The combination of relative ubiquity of this compound and the broad range of microorganisms capable of utilizing it makes it an interesting link in the global carbon cycle. However, there are still many unknowns about the distribution of methanol metabolism in marine anoxic ecosystems.
To gain further insights on methanol conversion in these environments, we have searched in 252 annotated anoxic marine metagenomes deposited at the Integrated Microbial Genomes & Microbiomes database for the presence of six key genes involved in methanol catabolism: three methanol methyltransferases, one methanol-derived formaldehyde dismutase, lanthanide-dependent methanol dehydrogenase and pectin esterase, which provides information in the utilization of methanol, its further incorporation in metabolism, as well as the putative origin of the compound in marine sediments. Metadata was utilized to distinguish between differences in spatial distribution of the samples, water column and sediment depth. Furthermore, on metagenomes from the same study on the same sample site with varying depth, we estimated the relative abundance of the investigated genes to infer the influence of water depth on the distribution of methanol cycling microorganisms.
We detected marked differences between samples of coastal sediments, where genes involved in methanol conversion appear to be ubiquitous, and deep sea sediments, where these genes are less abundant. Furthermore, within same-site samples, genes related to methanol metabolism appear to be most abundant in surface sediment than in deeper sediment. These observations will aid in the detection and isolation of novel microorganisms involved in methanol cycling, as well as expanding our knowledge on the fate of methanol in marine systems.

P08

Ruler of the seven seas – meta-analyses reveal the dominance of Nitrospinae in marine nitrite oxidation

Linnea Kop[1,2], Dr. H Koch[1], Prof. Dr. ir. M S M Jetten[1], Univ.-Prof. Dr. H Daims[2], Dr. S Lücker[1]
[1]Radboud University, [2]University of Vienna

Nitrite oxidation, the second step of nitrification, is catalyzed by nitrite-oxidizing bacteria (NOB) and has an important regulatory function in marine systems as it is the main source of nitrate in the ocean. The produced nitrate can be used as nitrogen source by many organisms and thus, especially in regions low in oxygen, nitrite oxidation counteracts nitrogen loss as gaseous N compounds. Additionally, marine NOB contribute to primary production by fixing large amounts of inorganic carbon, and thus have a significant impact on carbon cycling as well. Surprisingly, nitrite oxidation, which is assumed to rely on oxygen as terminal electron acceptor, has been detected in oxygen minimum zones (OMZs), areas in the ocean where oxygen concentrations are very low or below detection limit. To date, little is known about the identity and physiology of these marine nitrite oxidizers, and it remains enigmatic how nitrite is oxidized under apparently anoxic conditions. Studies on marine nitrifiers usually target a single oceanic region only, and thus a clear picture of the global distribution of the known marine nitrifiers is lacking. Here, we performed a read recruitment analysis against nitrite oxidoreductase (Nxr) gene sequences, encoding the key enzyme for nitrite oxidation, to determine the distribution of marine nitrite oxidizers in a large set of publicly available metagenomes. Throughout the ocean, the dominant nitrite oxidizers were affiliated with the phylum Nitrospinae, as was expected based on previous studies. While Nitrospinae dominated in most samples from oxygen minimum zones (OMZs), the community of nitrite oxidizers was more diverse in the Gulf of Mexico, where also Nitrospira nxrB sequences were present. The Gulf of Mexico is characterized by regions experiencing seasonal hypoxia and is heavily influenced by the extensive nutrient input from incoming rivers. This pattern of seasonally and spatially variable hypoxia differs from the stable anoxic water layers in open ocean OMZs, which could explain the differences in community composition of NOB. Intriguingly, nxrB sequences of cultivated Nitrospinae rarely recruit reads, while the majority of found Nitrospinae sequences mapped to those extracted from metagenomes from OMZs, showing that cultivated Nitrospinae are not representative of the majority of marine nitrite oxidizers. The observed distribution patterns of NOB suggest niche adaptation, but it still remains unclear how their life styles differ between habitats. For further insights, the metagenomic read recruitment analysis should be combined with pangenomic analyses to decipher the ecophysiology of the dominating NOB guild in the ocean.

P09

Removal of methane and ammonium during drinking water production using rapid sand filtration

Alje Boersma[1], S Haukelidsaeter[2], F Corbera Rubio[3], Dr. MAHJ van Kessel[1], Dr. PWJJ van der Wielen[4], Prof. Dr. MSM Jetten[1], Prof. Dr. CP Slomp[2], Prof. Dr. MCM van Loosdrecht[3], Dr. S Lücker[1]
[1]Radboud University, [2]Utrecht University , [3]Delft University of Technology, [4]KWR Water Research Insitute

Alje S. Boersma1, Signe Haukelidsaeter2, Francesc Corbera Rubio3, Maartje A.H.J. van Kessel1, Paul W.J.J. van der Wielen4, Mike S.M. Jetten1, Caroline P. Slomp2, Mark C.M. van Loosdrecht3, Sebastian Lücker1
1Department of Ecological Microbiology, IWWR, Radboud University, Nijmegen
2Department of Marine Biogeochemistry, Utrecht University
3Department of Environmental Biotechnology, Delft University of Technology
4KWR Water Research Institute, Nieuwegein, the Netherlands

Introduction. In The Netherlands, 58% of drinking water is produced from groundwater. The production of drinking water from groundwater is achieved through biofiltration using rapid sand filters (RSFs). RSFs contain complex microbial communities shaped by the chemical parameters of the inlet water and are used to remove undesirable compounds by a combination of microbiological and geochemical processes. In the case of groundwater, which has a relatively constant composition, this mostly concerns the removal of methane, iron, ammonium and manganese. In most RSFs, methane is removed by a preceded aeration step, which also oxygenates the water. Iron, ammonium and manganese are then removed in the filter bed. Here, we investigate the microbial and geochemical processes involved in drinking water treatment by sampling the RSFs of a treatment plant in the south part of the Netherlands.

Methods and results. Water and sand samples were taken from RSFs at the Breehei drinking water treatment plant, which offered the unique opportunity to study operationally diverse RSFs receiving the same groundwater. One system consists of aeration followed by RSF and is compared to a second system, that employs RSF without a preceding aeration step. We determined the chemical composition of the influent and effluent waters, while the sand samples were used for activity assays and metagenomic analysis to study the microbial communities. Metagenomics revealed a variety of methane and ammonia-oxidizing bacteria, and it became apparent that the removal efficiency of ammonium in these RSFs decreased under increasing methane concentrations. It was also found that key players of ammonium removal in these systems are complete ammonia oxidizers, commonly known as comammox Nitrospira. These bacteria are capable of ammonia oxidation to nitrite and subsequently to nitrate, a process that is mostly divided between ammonia-oxidizing organisms and nitrite-oxidizing bacteria.

Conclusion. The elucidation of the microbial communities of RSFs will contribute to our understanding of these ‘black box’ systems. This will make it possible to optimize the removal of methane, iron, ammonium and manganese, ultimately contributing to more reliable and sustainable production of drinking water.

P10

Microbial methane oxidation in the water column of Lake Grevelingen

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

[1]Radboud University, [2]Radboud University, [3]Utrecht University

Introduction: Coastal ecosystems cover only 16 % of the global ocean surface, however, it has been estimated that these estuarine- shelf ecosystems contribute up to 75 % of the marine methane flux to the atmosphere. Methane emissions from coastal zones are a result of the imbalance between anaerobic methane production and methane removal at the expense of various electron acceptors. In marine sediments, most methane is produced anaerobically below the sulfate-reduction zone and subsequently oxidized in the sulfate-methane transition zone by archaeal methanotrophs living in a syntrophic relationship with sulfate reducers. Although this so-called methane-oxidation filter removes up to 90 % of the methane produced in marine sediments, and therefore prevents most of the methane from reaching the overlying water column, it has been shown that its efficiency may vary over the seasons. This can lead to elevated methane concentrations in the water column e.g. during late summer hypoxia. Water column methane oxidation in coastal ecosystems is therefore an important process to mitigate methane emissions. However, the microbial dynamics of aerobic methane oxidation in the water column of marine ecosystems are not well explored. Here, we investigated the fate of methane diffusing from the sediment to the water column in marine Lake Grevelingen during summer stratification.

Methods: Water sampling in Lake Grevelingen was performed with the RV Navicula in September 2020. Oxygen profiles were measured by an oxygen-CTD probe, and methane profiles were determined by gas chromatography. Water samples from different depths were incubated at excess oxygen concentration and 13C-labelled methane was added to follow methane consumption by gas chromatography–mass spectrometry.

Results: The water column was stratified, with a narrow oxycline where oxygen concentrations rapidly decreased between 30 and 35 m. In the bottom water, high concentrations of methane (53 µM) were observed, as a result of high methane production but only partial methane removal in the sediments. The methane released from the sediments diffused upwards to the oxycline. Here, methane concentrations decreased with increasing oxygen concentrations, forming a methane-oxygen counter gradient. The subsequent, exponential decrease of methane concentrations at the lower part of the oxycline points to an active methanotrophic community along this methane-oxygen counter gradient. Interestingly, potential aerobic methane oxidation was observed at all measured depths (25, 30, 32 and 42 m), even below and above the methane-oxygen counter gradient. This might be explained by a high potential of the ambient methanotrophic community within the water column to rapidly adapt to high oxygen/low methane concentrations or to low oxygen/high methane concentrations.

Conclusion: From these results, we conclude that 1. Aerobic methane oxidation in the water column is an important pathway to mitigate methane emissions from Lake Grevelingen, and 2. The ability of the methanotrophic community to adapt to changing oxygen and methane concentrations might be crucial to maintain efficient methane removal in these systems. Future oxygen manipulation experiments together with 16SrRNA gene and metagenome sequencing will provide deeper insight into the microbial community structure and the flexibility of the methanotrophic community to adapt to rapidly changing oxygen conditions.

P11

Improved diagnosis of viral encephalitis in adult and pediatric hematological patients using viral metagenomics

Ellen Carbo[1], EP Buddingh[2], E Karelioti[3], IA Sidorov[1], MCW Feltkamp[1], PA von dem Borne[4], JJGM Verschuuren[5], ACM Kroes[1], ECJ Claas[1], JJC de Vries[1]

[1]Leiden University Medical Center, [2]Leiden University Medical Center, [3]GenomeScan B.V., [4]Leiden University Medical Center, [5]Leiden University Medical Center

Metagenomic sequencing is a powerful technique that enables detection of the full spectrum of pathogens present in any specimen in a single test. Hence, metagenomics is increasingly being applied for detection of viruses in clinical cases with suspected infections of unknown etiology and a large number of relevant potential causes. This is typically the case in patients presenting with encephalitis, in particular when immunity is impaired by underlying disorders.

In this study, viral metagenomics has been applied to a cohort of hematological patients with encephalitis of unknown origin.

Since viral loads in cerebrospinal fluid of patients with encephalitis are generally low, the technical performance of a metagenomic sequencing protocol was increased by using capture probes targeting all known vertebrate viral sequences, which significantly raised the sequence read count 100 – 10.000 fold.

Subsequently, the optimized viral metagenomics protocol was applied to a cohort of 41 hematological patients with encephalitis of unknown origin. In five out of 41 patients (12%), a virus was detected by viral metagenomics which had not been detected by current routine diagnostics. BK polyomavirus, hepatitis E virus, human herpes virus-6 and Epstein Barr virus were identified by this unbiased metagenomic approach.

This study demonstrated that hematological patients with encephalitis of unknown origin may benefit from early viral metagenomics testing as a single step approach.

DOI: 10.1016/j.jcv.2020.104566

P12

Lateral flow test for the rapid detection of Aspergillus fumigatus

Drs. Ben Ridwan[1], ing. E.D. de Vries[1], drs. B.U. Ridwan[1]
[1]*Comicro*

Introduction:
In 20-30% of COVID-19 patients in the intensive care unit Aspergillus species is present in the lungs (1). Aspergillosis in these patients can lead to a severe increase in mortality rates(2). Galactomannan testing is important to diagnose invasive pulmonary aspergillosis. However, Galactomannan ELISAs are resource demanding methods and are therefore not available to every microbiology laboratory or can only be performed in batches. A faster and simpler alternative is warranted. We evaluated the Olm diagnostics Aspergillus lateral-flow device (AspLFD) which can detect Aspergillus fumigatus in bronchoalveolar lavages (BAL) in about one hour. Our goal was to see if the AspLFD had a high enough sensitivity and specificity to be used as an alternative for galactomannan ELISAs.
Methods
10 BAL with a negative galactomannan ELISA and 10 BAL with a positive galactomannan ELISA were tested. All tests were performed according to manufacturer's instructions for contaminated BAL samples. Tests were marked negative when only the control line was visible and marked positive if both the control line and the test line were visible. Test were marked inconclusive if no control line was visible or if it was unclear whether a test line was visible. These evaluations were done by eye as well as by a digital CUBE lateral-flow reader. Lastly sensitivity and specificity were calculated.

Results:
Reading the tests by eye resulted in a sensitivity of 86% and a specificity of 100%. However, 6 of the tests gave an inconclusive result. By using the CUBE lateral-flow reader only 2 of the tests came back inconclusive. This resulted in a sensitivity of 75% and a specificity of 80%
Conclusion:
The AspLFD provides an easy to perform test that can rapidly identify Aspergillus fumigatus in BAL samples. Its sensitivity however is on the low side which can result in false positives. The test line of the AspLFD is also more difficult to interpret then the test lines of most other lateral flow tests because it often is very weak. A CUBE reader can help interpret these weak lines but with us this resulted in a lower sensitivity and specificity overall. Therefore, we recommend only using the CUBE reader on AspLFD test that are difficult to interpret by eye. Despite these shortcomings the AspLFD could still be a useful tool. This faster albeit slightly less accurate diagnosis could be used to start treatment while waiting for conformation of the test results by galactomannan ELISA.

1. Verweij, P. E., (2020). Diagnosing COVID-19-associated pulmonary aspergillosis. The Lancet Microbe, 1(2), e53–e55.
2. Koehler, P., (2020). Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. The Lancet Infectious Diseases, 0(0).

P13

Metagenomic analysis shows distinct Neisseria gonorrhoeae strains at separate anatomical sites occur more commonly than mixed strain infections: implications for surveillance

Brian Van Der Veer[1], Dr. PFG Wolffs[1], Prof. Dr. CJPA Hoebe[1,2], Dr. NHTM Dukers-Muijrers[1,2], Dr. LB Alphen[1]
[1]Department of Medical Microbiology, Care and Public Health Research Institute (CAPHRI), Maastricht University Medical Center, [2]Department of Sexual Health, Infectious Diseases and Environmental Health, South Limburg Public Health Service

Introduction: Mixed infections of Neisseria gonorrhoeae (NG) in one anatomical site could complicate surveillance as linked individuals might be missed with current genotyping methods. In our previous study, distinct NG strains at separate anatomical sites was observed in a quarter of patients. As only the dominant NG strain was characterized, additional strains could be present because of mixed infections. Therefore, we performed a follow-up study of samples using a metagenomic genotyping method. With this, we aimed to gain further insight in mixed infections and distinct NG strains at separate anatomical sites for surveillance.

Methods: NG specific primers were used to amplify NG-MAST PCR targets porB and tbpB in samples (n=33) of 16 men who have sex with men (MSM) with distinct NG strains at separate anatomical sites. Amplicons were sequenced using the Illumina MiSeq (250bp paired-end) and Nextera XT kit. Reads were split by NG-MAST PCR target using Burrow-Wheeler Aligner and alleles were called using split kmer analysis. Reads were stringently mapped to the dominant alleles to call low abundant alleles with the unmapped reads. To validate this pipeline, mixed strain datasets were generated in silico of all pairs of the 14 whole genome sequenced WHO strains (n=91). This dataset comprised relative abundances ranging from 0.01% to 50% of the second strain in benchmark mixed NG infection datasets. Mixed strains infections were defined as detection of at least two alleles with more than 1% dissimilarity in at least one target.Results: The analysis pipeline correctly detected all alleles in the mixed-strain in silico datasets with at least 1.5% abundance. Most samples (25/33) were well sequenced (at least 200,000 paired-end reads). The majority of the samples (26/33, 78.8%) were single NG infections. In seven samples from five patients a mixed NG infection was observed. In three patients, additional NG strains were detected, these are strains that were not detected in the previous study. The additional strains were ST11465 (5.7% abundance) for patient 1, ST1407 (30.8% abundance) and ST4431 (1.0% abundance) for patient 2, and a New ST (9.8% abundance) for patient 5.

Conclusion: We developed and validated a metagenomic genotyping method for NG. Despite the selected MSM with distinct NG isolates at different anatomical sites, most samples are single NG infection. Our study provides further evidence that mixed infections do occur but likely at low prevalence. However, resistant strains (e.g. ST1407) can be missed, potentially resulting in treatment failure and unnoticed transmission. This suggests that distinct NG strains at separate anatomical sites are more important for surveillance as it occurs more frequently than mixed infections.

P14

The effect of viral load and vaccination on the establishment of genital and anal human papillomavirus concordance in young women.

Phd Candidate Kahren Van Eer[1], MSc Student I Laâbi[1], Dr. RDM Steenbergen[2], Dr AJ King[1]
[1]National Institute For Public Health And The Environment (RIVM), [2]Amsterdam UMC, Vrije Universiteit Amsterdam

Introduction

Anal cancer (AC) incidence is rising in developed countries, particularly in women. A persistent high-risk human papillomavirus (HPV) infection highly increases AC risk. In 2009, young girls were offered a bivalent vaccine (against HPV16/18) which is expected to reduce AC incidence. Still, women with a history of HPV-associated genital lesions are more susceptible to AC development. Detection of identical HPV types in the genital and anal region of women is common, but occurs more in women who have (had) genital lesions as opposed to lesion-free women. Therefore, genital-anal HPV concordance may impose an increased risk of AC. Viral load possibly influences genital-anal HPV concordance, as it contributes to HPV concordance among heterosexual couples. Still, its role in intra-host genital-anal HPV concordance is unknown. Here, we examined genital and anal type-specific HPV concordant infections and the potential contribution of viral load. In addition, we analyzed the impact of the bivalent vaccine on concordant genital-anal HPV infection frequency.

Methods

A total of 1074 women from the PApillomavirus Surveillance among Sexually transmitted infection clinic YOungsters in the Netherlands (PASSYON) study provided a genital and anal swab. Total DNA isolation followed by HPV detection/genotyping was executed with the Magna Pure 96 and the SPF10-DEIA-LiPA25, respectively. Genital and/or anal HPV positivity was determined in 857 women. We performed type-specific qPCR assays for high-risk HPV16/18/33/35/39/45/51/52/56/58/59, potential high-risk HPV66, and low-risk HPV6/11. HPV copy number (copies/reaction) was normalized for cellular content with a β-actin qPCR assay, given as HPV viral load (copies/cell). The impact of the bivalent vaccine on concordant genital-anal HPV infection frequency was analyzed with eligible HPV-positive women with known vaccination status (n=416), either vaccinated (n=274) or unvaccinated (n=142).

Results

Concordant genital-anal infections with high-risk HPV were more frequent than with low-risk HPV. Furthermore, genital viral load of concordant genital-anal HPV infections was higher than in genital-only HPV infections. The anal viral load of concordant genital-anal HPV infections and anal-only HPV infections was similar. Anal-only HPV infections also occurred less or were absent for certain HPV types. The genital and anal cellular content was significantly different, therefore we compared HPV copy number between these regions. The copy number of virtually all HPV types in concordant genital-anal infections was higher in the genital region than the anal region. Single-genital and single-anal HPV infections showed similar copy numbers. Vaccination mostly impacted HPV infections with the bivalent vaccine types, with the greatest reduction in concordant genital-anal infections, followed by genital-only infections. No effect on anal-only infections was observed.

Conclusions

1. Concordant genital-anal infections with high-risk HPV were more frequent than with low-risk HPV.
2. Genital viral load, but not anal viral load, likely facilitates genital-anal HPV concordance.
3. Copy numbers of concordant genital-anal HPV infections are higher in the genital region than in the anal region. Overall, anal-only HPV infections occur less or are absent for certain HPV types. Therefore, HPV infections likely spread from the genital region to the anal region.
4. Vaccination mostly impacted concordant genital-anal HPV infections with the bivalent vaccine types.

P15

First identification of the multi-resistance gene cfr in livestock-associated methicillin resistant Staphylococcus aureus (LA-MRSA) in humans and in pig housing in the Netherlands

Dr Leo Schouls[1], Dr K. Veldman[2], Dr M. Brouwer[2], Dr C. Dierikx[1], BSc S. Witteveen[1], BSc. M. van Santen-Verheuvel[1], Dr A Hendrickx[1], BSc P. Hengeveld[1], Dr B. Wullings[3], BSc M. Rapallini[3], BSc B Wit[4], Dr E. van Duijkeren[1]

[1]National Institute for Public Health and the Environment (RIVM), [2]Wageningen Bioveterinary Research (WBVR), [3]Wageningen Food Safety Research, [4]Netherlands Food and Consumer Product Safety Authority (NVWA)

Introduction: In 2018 a project on surveillance of MRSA in man and animal in the Netherlands was started. MRSA isolates obtained from animals, dust from livestock farms and meat, were compared with isolates collected in the Dutch national MRSA surveillance to assess possible changes in the rate or nature of MRSA transmission between animals and humans.

Methods: Next-generation sequencing (NGS) data of MRSA isolates were generated. NGS data were used in whole-genome multi-locus sequence typing (wgMLST) to assess the genetic relationship between isolates. ResFinder software was used to determine the presence of acquired antibiotic resistance genes. To reconstruct the complete chromosomes and plasmids in a subset of isolates, both NGS and third-generation sequencing data, obtained by long read nanopore sequencing, were used in a unicycler hybrid assembly. Broth microdilution was performed to determine minimum inhibitory concentrations (MICs) of the MRSA isolates to a panel of 19 antimicrobial agents. Isolates obtained from humans, comprised 1,200 LA-MRSA isolates from 1,150 persons and 3,600 non-LA-MRSA isolates from 3,500 persons collected in 2008-2020, with half of the isolates originating from 2017-2019. In addition, NGS data of 327 MRSA isolates obtained from animals, meat or dust collected in livestock farms during 2008-2020 were used.

Results: We detected the presence of the multi-resistance gene cfr in five LA-MRSA obtained from humans in 2018 (1), 2019 (2) and 2020 (2) and in two isolates from dust samples obtained in one pig farm in 2019. Epidemiological data, only available from three patients, showed that two patients had been in contact with livestock and the other one claimed not to have had animal contact. One isolate was cultured from pus, one from urine and three were obtained from nasal swabs. The cfr gene methylates the 23S rRNA resulting in simultaneous resistance against five antibiotic classes: phenicols, lincosamides, oxazolidons, pleuromutilins and streptogramin A, known as the PhLOPSA phenotype. This phenotype was confirmed by MIC analyses of the isolates. The wgMLST showed that the cfr-carrying LA-MRSA isolates were genetically unrelated. In all seven isolates the cfr gene was located on plasmids. Remarkably, the plasmids differed considerably in size and composition from each other. These results show that there is no outbreak with a particular strain or spread of a cfr carrying plasmid with these exceptional resistance traits, but suggests multiple introductions of cfr in LA-MRSA in the Netherlands.

Conclusion: The finding of the multi-resistance gene cfr is worrisome and should be closely monitored. Linezolid is not routinely used to treat MRSA infections in the Netherlands, but is important to treat vancomycin-resistant enterococci infection and even classified as critically important antimicrobial by the WHO. In veterinary medicine linezolid is not used, but phenicols, pleuromutilins and lincosamides are and could select for LA-MRSA carrying the cfr gene. These findings show that it is important to combine and compare data obtained from MRSA surveillance in humans, animals and food from a One Health perspective.

P16

Coronavirus discovery by metagenomic sequencing: a tool for pandemic preparedness

Ellen Carbo[1], IA Sidorov[1], JC Zevenhoven-Dobbe[1], EJ Snijder[1], EC Claas[1], JFJ Laros[2,3], ACM Kroes[1], JCJ de Vries[1]
[1]Leiden University Medical Center, [2]Leiden University Medical Center, [3]National Institute for Public Health and the Environment

The SARS-CoV-2 pandemic is a prime example of the omnipresent threat of emerging viruses that can infect humans. A protocol for the identification of emerging novel coronaviruses by viral metagenomic sequencing in diagnostic laboratories is needed for pandemic preparedness. Therefore, we validated a protocol for metagenomic virus discovery to potentially prevent another large coronavirus outbreak.

The performance of a viral metagenomic protocol in a clinical setting for the identification of novel coronaviruses was tested using clinical samples containing SARS-CoV-2, SARS-CoV, and MERS-CoV, in combination with databases generated to contain only viruses of before the discovery dates of these coronaviruses, to mimic virus discovery.

Classification of NGS reads using Centrifuge, Genome Detective and Blast resulted in assignment of the reads to the closest relatives of the emerging coronaviruses. Low nucleotide and amino acid identity (81% and 84%, respectively, for SARS-CoV-2) in combination with up to 98% genome coverage were indicative for a related, novel coronavirus. Capture probes targeting vertebrate viruses, designed in 2015, enhanced both sequencing depth and coverage of the SARS-CoV-2 genome, the latter increasing from 71% to 98%.

The model used for simulation of virus discovery enabled validation of the metagenomic sequencing protocol. The metagenomic protocol with virus probes designed before the pandemic, can assist the detection and identification of novel coronaviruses directly in clinical samples. Implementation of virus discovery protocols in diagnostic laboratories may contribute to increased vigilance for emerging viruses and therefore aids in surveillance and pandemic preparedness.

DOI: 10.1016/j.jcv.2020.104594

P18

Swine flu in a hospitalised immunocompromised individual in the Netherlands during the COVID-19 pandemic without evidence of direct contact with pigs

Ph.d. Jozef Dingemans[1], Sharon van den Brink[5], Jeroen Cremer[5], Gabriel Goderski[5], Mariam Bagheri[5], Dr. Alvin Han[4], Elleke Leclercq[2], MD Ans Timmermans[2], Dr. Lieke van Alphen[1], MD Inge van Loo[1], MD Wil van der Zwet[1], Dr. Erhard van der Vries[3], Dr. Dirk Eggink[5], Dr. Harry Vennema[5], Prof. Dr. Christian Hoebe[1,2], Prof. Dr. Paul H. M. Savelkoul[1], Dr. Adam Meijer[5]

[1]MUMC+, [2]South Limburg Public Health Service, [3]Royal GD, [4]Amsterdam UMC, [5]National Institute for Public Health and the Environment (RIVM)

Influenza A virus infections usually follow a seasonal cycle, with rare cases being reported during the summer months. Moreover, due to extra hygiene measures during the COVID-19 pandemic, the amount of Influenza A virus infections has been extremely low even during peak season. Surprisingly, a case of influenza A infection was reported in a 31-year-old male with a severely impaired immune system, who was admitted to the hospital for stem cell transplantation by early September. The patient had tested negative for influenza A virus twice by RT-qPCR in routine screen for respiratory pathogens one week before transplantation. Seven days after transplantation, the patient started developing influenza-like symptoms including fever and headache. One day later the patient tested positive for influenza A virus in a throat flush sample and remained positive for 21 days while being symptomatic. During this period the patient was also treated with oseltamivir for a total of 5 days. At day 23 after the onset of symptoms, the patient had tested negative for the first time, recovered and eventually was discharged from the hospital by late November.

Remarkably, two of the patient's specimens with Cq values in the twenties as determined by an in-house PCR assay, yielded a negative result in a combined SARS-CoV-2 & Influenza A/B molecular-amplification-based point-of-care (mPOCT) assay, in contrast to an influenza A-positive sample with a similar Cq value from a different patient. Closer inspection of the influenza A signal in the amplification plot revealed a weak signal that remained below the threshold of the assay. Subtyping by PCR for the seasonal human influenza A viruses and for avian H5 at the National Influenza Centre location of the RIVM yielded negative results. Whole genome sequencing (WGS) characterized the virus as a Eurasian Swine influenza A(H1N1) virus clade 1C.2.1. This influenza A virus subtype and clade has been detected in several swine populations in Europe in recent years and shares no common genome segments with human influenza A(H1N1)pdm09 virus. In addition, WGS revealed the emergence of a variant with neuraminidase H275Y amino acid substitution after oseltamivir treatment. This amino acid substitution was absent before treatment started and present in about 80% of the viruses in the clinical specimen five days after completion of the oseltamivir course but almost completely lost eight days after completion of the course.

In an attempt to identify the source of infection, it was revealed that the patient had no direct contact with pigs or other animals during the incubation period. Nevertheless, a hemagglutination inhibition assay revealed consistent antibody titers against the isolated H1N1 virus in sera from the subject's parents, although cross-reactivity with A(H1N1) pdm09 was observed. Altogether, this serendipitous case report of swine flu highlights the importance of microbiological surveillance to identify potential candidates that could cause future pandemics.

P19

blaOXA-48-like genome architecture among carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in the Netherlands

Dr. Antoni Hendrickx[1], F Landman[1], A de Haan[1], S Witteveen[1], M van Santen-Verheuvel[1], L.M. Schouls[1], Dutch CPE surveillance Study Group[1]
[1]*National Institute for Public Health and the Environment (RIVM)*

Introduction The genes encoding the OXA-48-like group of carbapenem-hydrolyzing enzymes are found in high frequency among Enterobacterales isolates submitted to the Dutch national CPE surveillance. Therefore, we investigated the characteristics and diversity of the blaOXA-48-like carrying plasmids and chromosomes of Escherichia coli and Klebsiella pneumoniae isolates collected from 2014-2019 and compared these with genome sequences from 29 countries.
Methods Illumina next-generation genome sequencing was combined with Nanopore long-read sequencing to reconstruct blaOXA-48-like plasmids and chromosomes of E. coli and K. pneumoniae isolates. wgMLST, UPGMA clustering and automated resistome (ResFinder) and replicome (PlasmidFinder) analyses were performed. The Etest for meropenem was used to determine antimicrobial susceptibility.
Results In total, 47 and 132 complete blaOXA-48-like plasmids were reconstructed for E. coli and K. pneumoniae, respectively. Seven distinct plasmid groups designated as pOXA-48-1 to pOXA-48-5, pOXA-181, and pOXA-232 were identified. These plasmids were similar to internationally reported plasmids obtained from 29 different countries from North and South America, Europe, Asia and Oceania. The seven plasmid groups differed from each other in size, % G+C, presence of antibiotic resistance genes, replicon family and gene content. The plasmids within the pOXA-48-1 to pOXA-48-5 groups the containing IncL/M replicon were variable, and the pOXA-181 (ColKP3 and IncX3 replicons) and pOXA-232 (ColKP3 replicon) plasmids were conserved. The pOXA-48-1, pOXA-48-2, pOXA-48-3 and pOXA-48-5 groups contained a putative conjugation system, which was absent in the pOXA-48-4, pOXA-181 and pOXA-232 plasmid groups, indicating latter plasmids are non-conjugative. Furthermore, the pOXA-181 plasmids carried a virB2-virB3-virB9-virB10-virB11 type IV secretion system, and the pOXA-48 plasmids and pOXA-232 lacked this system. A group of non-related pOXA-48 plasmids from the Netherlands contained different resistance genes, non-IncL type replicons or carried no known replicons. wgMLST revealed that the blaOXA-48-like plasmids were found in a variety of genetic backgrounds. K. pneumoniae isolates carrying blaOXA-48 or blaOXA-232 were mostly resistant for meropenem (MIC >8 mg/L, according to EUCAST), whereas E. coli blaOXA-48, blaOXA-181 and chromosomal blaOXA-48 or blaOXA-244 isolates were mostly sensitive (<2 mg/L). Analysis of chromosomally localized blaOXA-48 and blaOXA-244 alleles revealed that these alleles were located on genetic elements of variable sizes and comprised a conserved region of pOXA-48 plasmids. The blaOXA-48-like genetic element was flanked by a direct repeat (DR) upstream of IS1R, and was found at multiple locations in the chromosomes of E. coli in limited genetic backgrounds.
Conclusions The overall composition of the blaOXA-48-like plasmid population in the Netherlands is conserved and similar to that for other countries, and is in line with global dissemination of blaOXA-48-like plasmids. Non-resistant E. coli harboring blaOXA-48-like plasmids possibly represent a reservoir from which resistance can spread to other Enterobacterales. A diverse blaOXA-48-like plasmid subgroup was present, and is likely the result of plasmid diversification in the Netherlands or of multiple introductions from abroad. In contrast to blaOXA-48-like plasmids, chromosomally encoded blaOXA-48-like alleles were found in a limited number of genetic backgrounds, but were localized at variable positions in chromosomes containing a DR and IS1R, suggesting multiple independent transposition events.

P20

Frequent detection of Treponema pallidum subspecies pallidum in different body locations and intra-patient homogeneity in patients with early syphilis

Helene Zondag[1], SA Nieuwenburg[1], SM Bruisten[1,2], MF Schim van der Loeff[1,2,3], HJC de Vries[1,2,5], AP van Dam[1,4]
[1]Department of Infectious Diseases, [2]Amsterdam Institute for Infection and Immunity, [3]Department of Internal Medicine, Division of Infectious Diseases, [4]Department of Medical Microbiology, [5]Department of Dermatology

Syphilis, caused by Treponema pallidum subspecies pallidum (TP), is a complex multi-stage infectious disease. Syphilis is thought to be most infectious in the primary, secondary and early latent stage of the disease. Systematic dissemination is known to occur within a few hours of transmission. In this study, we aimed to investigate the presence and molecular variation of TP at various body locations within patients in different syphilis stages.

We included 293 men who have sex with men clinically suspected for primary or secondary syphilis or serologically having latent syphilis at the sexually transmitted infections clinic in Amsterdam between December 2018 – December 2019. 70 (24%) had primary syphilis, 73 (25%) secondary syphilis, 86 (29%) early latent syphilis, 14 (5%) late latent syphilis and 50 (17%) did not have syphilis. Besides the routine samples taken during a routine STI screening visit, extra study samples were peripheral blood, a pharyngeal swab, an anal swab and an urine sample. TP-DNA was detected using a polA targeting qPCR. All positive TP samples were characterized using multi-locus sequence typing (TP-MLST) based on sequence analysis of three genetic regions (tp0136, tp0548, tp0705).

In the patients with primary syphilis, 63 ulcers and 46/280 (16%) study samples tested positive for TP-DNA, of which 2 (3%) peripheral blood samples, 7 (10%) pharyngeal swabs , 13 (19%) anal swabs and 24 (34%) urine samples. For the patients with secondary syphilis, we found TP-DNA in 10 ulcers and 125/292 (43%) of the study samples, of which 15 (21%) peripheral blood samples, 47 (64%) pharyngeal swabs , 37 (51%) anal swabs and 26 (36%) urine samples. In the early latent syphilis stage, 43/344 (13%) study samples showed TP-DNA presence, of which 5 (6%) peripheral blood samples, 21 (24%) pharyngeal swabs , 11 (13%) anal swabs and 6 (7%) urine samples. No TP-DNA was found in the late latent syphilis stage. Full TP-MLST types were obtained for the following TP-DNA positive samples: 1/22 (5%) peripheral blood, 35/75 (47%) pharynx, 10/61 (16%) anus, 23/56 (41%) urine and 50/73 (68%) ulcer. At least one TP-MLST full type was obtained from 48/70 (69%) patients in the primary, 35/73 (48%) in secondary and 10/86 (12%) in early latent stage. Among all 22 patients with 2 or more TP-MLST types, the TP-MLST type was identical at the different body locations. The most prevalent TP-MLST types were 1.3.1 and 1.1.1, detected in 39/93 (42%) and 17/93 (18%) patients.

TP-DNA was frequently detected in various body locations of MSM with primary and, even more, secondary syphilis. The intra-patient TP homogeneity suggests that the TP-DNA detected at the different body locations occurs from dissemination rather than from separate infections.

P21

Time-resolved analysis of Staphylococcus aureus invading the endothelial barrier

Elisa JM Raineri[1], H Yedavally[2], A Salvati[2], JM van Dijl[1]
[1]University Medical Center Groningen, University of Groningen, [2]Groningen Research Institute of Pharmacy, University of Groningen

Time-resolved analysis of Staphylococcus aureus
invading the endothelial barrier

Elisa J.M. Raineri*, Harita Yedavally*, Anna Salvati & Jan Maarten van Dijl

*These authors contributed equally to the work

Staphylococcus aureus is a leading cause of infections world-wide. Once this pathogen has reached the bloodstream, it can invade different parts of the human body by crossing the endothelial barrier. Infected endothelial cells may be lysed by bacterial products, but the bacteria may also persist intracellularly, where they are difficult to eradicate with antibiotics and cause relapses of infection. Our present study was aimed at investigating the fate of methicillin resistant S. aureus (MRSA) isolates of the USA300 lineage with different epidemiological origin inside endothelial cells. To this end, we established two in vitro infection models based on primary human umbilical vein endothelial cells (HUVEC), which mimic conditions of the endothelium when infection occurs. For comparison, the laboratory strain S. aureus HG001 was used. As shown by flow cytometry and fluorescence- or electron microscopy, differentiation of HUVEC into a cell barrier with cell-cell junctions sets limits to the rates of bacterial internalization, the numbers of internalized bacteria, the percentage of infected cells, and long-term intracellular bacterial survival. Clear strain-specific differences were observed with the HG001 strain infecting the highest numbers of HUVEC and displaying the longest intracellular persistence, whereas the MRSA strains reproduced faster intracellularly. Nonetheless, all internalized bacteria remained confined in membrane-enclosed LAMP-1-positive lysosomal or vacuolar compartments. Once internalized, the bacteria had a higher propensity to persist within the differentiated endothelial cell barrier, probably because internalization of lower numbers of bacteria was less toxic. Altogether, our findings imply that intact endothelial barriers are more likely to sustain persistent intracellular infection.

P22

The times they are a-changin': successful validation of an automated pipeline using WGS data to determine Salmonella serovars

Dhr. Kim Van Der Zwaluw[1], Alejandra Hernandez Segura[1], Anjo Verbruggen[1], Kirsten Mooijman[1], Eelco Franz[1], Thijs Bosch[1], Maaike van den Beld[1]
[1]*RIVM*

Introduction:
For more than 50 years, Salmonella isolates have been serotyped at the National Institute for Public Health and the Environment (RIVM) for diagnostic and surveillance purposes using slide agglutination with an extensive panel of antisera, supplemented with a screening method based on Luminex technology for the last six years. Salmonella serovars are based on the detection of O- and H-antigens. Over 2500 different serovars have been described, most of which belong to S. enterica subspecies enterica. The genetic code for the antigens of these serovars enables in silico detection. From this perspective, a pipeline for serotyping Salmonella using whole genome sequencing (WGS) data was developed and its use for diagnostics and surveillance in the Netherlands was validated in accordance to EN-ISO 15189 and EN-ISO 16140-6.
Methods:
A selection of 503 Salmonella isolates, comprising 181 different serovars from various origins and 100 non-Salmonella isolates were tested to assess the suitability of this approach against the criteria of EN-ISO 15189 and EN-ISO 16140-6: accuracy (inclusivity), analytical specificity (exclusivity), measurement trueness and measurement precision. Short read Illumina sequencing data were generated using the NextSeq platform, then processed with an in-house pipeline ("Juno") for de-novo assembly, including trimming and QC algorithms. An in silico Salmonella serotyper pipeline based on SeqSero 2 microassembly-mode was developed using Snakemake workflows and reproducible conda environments. The pipeline generates a multi-report containing the seroformula (e.g. 9:g,m:-) and a predicted serovar name (e.g. Enteritidis).
Results:
After identification, none of the 100 non-Salmonella isolates were designated as Salmonella, while 473/503 (94%) Salmonella isolates were correctly identified by the in silico serotyper. Retesting the 30 discrepant isolates with slide agglutination resolved 15 cases where the stored serovar differed from the serovar of the sequenced isolate. In nine of the remaining 15 discrepancies, an antigen was detected genetically, but was not expressed phenotypically. Because this outcome is not incorrect, these results were excluded from the accuracy-analysis. In four other cases, the seroformula found was correct, but a serovar name was not correctly assigned. This was corrected in the pipeline. In the remaining two cases, an O-antigen (O25) was phenotypically found but not detected in the sequence reads. These results accumulate to an accuracy (inclusivity) of 99% and a 100% score for analytical specificity (exclusivity) and measurement trueness. To determine the measurement precision, md5sum hashes of the multireports, generated multiple times under different conditions, were compared and 100% agreement was found. After retesting, all results were within the acceptability limits of both ISO standards.
Conclusions:
This validation in accordance with EN-ISO 15189 and EN-ISO 16140-6 shows that the in silico serotyper based on WGS data is a reliable method to determine the serovar of a Salmonella isolate. As a result, this approach has been implemented for isolates submitted to the RIVM starting January 2021. To prevent any incorrect results, isolates with results known to cause difficulties in this in silico method will still be confirmed using slide agglutination.

P23

Emergence of a cephalosporin reduced susceptible Neisseria gonorrhoeae clone between 2014-2019 in Amsterdam

Jolinda de Korne-Elenbaas[1,2], Dr S.M. Bruisten[1,3], Dr A.P. van Dam[1,2]
[1]Public Health Laboratory Amsterdam, [2]Amsterdam UMC, [3]Amsterdam UMC

Introduction
Emerging resistance against cephalosporins in Neisseria gonorrhoeae (Ng) is a major public health threat because those are antibiotics of last resort. Ceftriaxone resistance in Ng has often been associated with, among others, Multi-Locus Sequence Type (MLST) 1901 and carriage of a mosaic penicillin-binding protein-A (penA) gene. Recently, the emergence of a reduced susceptible strain belonging to MLST7827 was identified in Norway, which lacked the mosaic penA but carried mutations in both penA and porin-B (porB) genes. This strain has not been described as a reduced susceptible strain before, although its penA and porB mutations are already known ceftriaxone resistance determinants. Continuous surveillance is needed to monitor the spread and circulation of such reduced susceptible strains.

Methods
In this study, we analysed Ng isolates obtained from patients visiting the Sexually Transmitted Infections (STI) outpatient clinic between January 2014 and July 2019 in Amsterdam. Whole genome sequences (WGS) were obtained from 318 Ng isolates, of which 82 were reduced susceptible to ceftriaxone with a MIC ≥ 0.094 mg/L and the other 244 had MICs <0.094 mg/L. Typing was done using the MLST scheme and resistance mutations in penA and porB genes were identified. Genetic clustering among Dutch isolates was assessed by creating a phylogenetic tree. A phylogenetic tree including all publicly available MLST7827 isolates was created to assess genetic relatedness between Dutch- and global MLST7827 isolates.

Results
Seventeen main MLST clusters were identified among the Dutch isolates. Ceftriaxone reduced susceptibility was associated with MLST 1901, 7363 and 7827 clusters. MLST1901 and 7363 were reduced susceptible strains which predominated from 2014-2016 and MLST7827 emerged and became dominant during 2017-2019. Reduced susceptible MLST1901 isolates mainly carried a mosaic penA gene and porB G120K/A121N mutations and MLST7363 a non-mosaic penA gene with A501T mutation and porB G120K/A121D mutations. MLST7827 isolates mainly carried a non-mosaic penA gene with A501V mutation and porB G120K/A121D mutations, which were lacking in susceptible isolates of the same ST. The Dutch MLST7827 isolates were genetically strongly related to MLST7827 isolates from other European countries, including Norway. Among the publicly available MLST7827 isolates, 2 Chinese isolates were resistant to ceftriaxone with a MIC of 0.25 mg/L.

Conclusion
The ceftriaxone reduced susceptible MLST7827 strain emerged during recent years in Amsterdam. The co-carriage of penA A501V and porB G120K/A121D mutations was strongly associated with reduced susceptibility in this ST. Strong genetic clustering of Dutch, Norwegian and other European MLST7827 isolates indicate extensive circulation of this strain in Europe. The two Chinese MLST7827 isolates with MICs of 0.25 mg/L suggest that this strain is able to develop resistance and might lead to therapy failure in the future. Therefore, close monitoring of the spread and circulation of this strain with an alarming susceptibility profile is needed.

P24

Clinical evaluation of the Roche/SD Biosensor antigen rapid test with symptomatic and asymptomatic, non-hospitalized patients and its use for community testing.

Phd Zsofia Igloi[1], PhD JvB van Beek[1], PhD MV Vink[2], MD EF Fanoy[2], JV Velzing[1], GA Aron[1], PhD KB Benschop[3], WH Han[3], MD Boelsums[2], Professor MPGK Koopmans[1], MD CGvK Geurtsvankessel[1], RM Molenkamp[1]
[1]Erasmus MC, [2]Public Health Service Rotterdam-Rijnmond, [3]National Public Health Institute (RIVM)

Antigen rapid diagnostic tests (Ag RDTs) are simple-to-use tests usually in a lateral flow immunoassay (LFI) cassette format. Although Ag RDTs have lower sensitivity compared to RT-PCR they offer the possibility of rapid case detection at or near the point of care. Use of these tests allow faster detection and isolation of new cases which is essential in stopping the further spread of SARS-CoV-2.
First we have evaluated the Roche/SD Biosensor Ag RDT in a mild symptomatic population at a drive through public health service testing site. An additional nasopharyngeal swab was directly tested with the rapid test on site and results were compared to RT-PCR and virus culture. Date of onset and symptoms were analyzed using data from a clinical questionnaire. Following the satisfactory performance of the test, we have further validated it in a community testing project amongst people both with and without symptoms at mobile, walk in testing sites placed in the center of two districts in Rotterdam (Delfshaven and Feijenoord).
For the test evaluation we could include 970 persons with complete data. Overall sensitivity and specificity were 84.9% (95% Confidence Interval (CI): CI95% 79.1-89.4) and 99.5% (CI95% 98.7-99.8) which increased to 95.8% (CI95% 90.5-98.2) for people who presented within 7 days since symptom onset. For samples with a ct-value above 30, only 1/27 (4%) could be cultured but 8/27 (30%) were Ag RDT positive indicating that above a ct-value of 30 (E gene copy/ml 2.17E+05) the majority of samples are not infectious. Around 98% of all viable specimen with ct <30 were detected. For the community walk-in testing we included 1,736 (RT-PCR positive 7.7%) and 2,184 (RT-PCR positive 7.8%) respective of the two neighborhoods. Overall (data of both neighbourhoods combined) sensitivity and specificity of the Ag RDT was 88.0% (CI95% 83.8-91.5%) and 99.1% (CI95% 98.7-99.3%) which is very comparable with previous results. From all who tested positive by PCR, 15.7 % reported no symptoms. Ag RDT sensitivity was lower amongst asymptomatic 80.9% (CI95% 66.7-90.9%) however PCR ct-values were comparable between the two groups.
Antigen rapid tests can detect both asymptomatic and (mildly) symptomatic cases. Test sensitivity is lower for asymptomatic people which is expected due to the unknown course of the disease however viral load was not found to be significantly lower in this group. Symptomatic cases especially in the early phase of disease can be detected with high sensitivity thereby identifying the most infectious individuals. We recommend to use the Ag RDT in field settings to improve testing coverage. Immediate future directions include large scale risk driven testing approach utilizing rapid test. Furthermore, self-testing approaches need to be explored utilizing suitable sample type.

P25

Prevalence of SARS-CoV-2 RNA in conjunctival swabs of symptomatic COVID-19 patients

Msc Judith Veugen[1,2,3,5], Dr. M. Gijs[1,2], Dr. P.F.G. Wolffs[3,5], Prof. dr. P.H.M. Savelkoul[3], MSc J. Tas[2,4], Dr. B.C.T. van Bussel[4,5], Dr. M.D. de Kruif[6], Dr. R.M.A. Henry[7], Prof. dr. C.A.B. Webers[1,2], Dr. M.M. Dickman[1,2], Prof. Dr. R.M.M.A. Nuijts[1,2,8]

[1]Maastricht University Medical Center, [2]Maastricht University, [3]Maastricht University Medical Center, [4]Maastricht University Medical Center, [5]Maastricht University, [6]Zuyderland Medical Center, [7]Maastricht University Medical Center, [8]Zuyderland Medical Center

Introduction: Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has rapidly become a global health issue since it originated in Wuhan, China in December 2019. On March 11, 2020, the WHO declared COVID-19 as pandemic. The clinical presentation of a SARS-CoV-2 infection can range from asymptomatic infection to severe disease. The main clinical features of COVID-19 are fever, cough, fatigue, anorexia, dyspnea and myalgia. Conjunctivitis has been occasionally reported among COVID-19 symptoms. Human-to-human transmission occurs mainly through respiratory droplets, but other routes are under investigation because SARS-CoV-2 has been detected in several body fluids including blood, stool and saliva. In addition, SARS-CoV-2 has been found in tear fluid and conjunctival secretions of COVID-19 patients. The main purpose of this study is to evaluate the prevalence of RNA of SARS-CoV-2 in conjunctival swabs of a large cohort COVID-19 patients in the Netherlands.
Methods: A total of 243 symptomatic laboratory-confirmed COVID-19 patients were included in this observational multicenter study. Sample collection took place during the 'second wave' of the pandemic (from August to November 2020) in two medical centers in the Netherlands. From a subset of subjects (healthcare workers), consecutive conjunctival samples were available from follow-up visits.
Tear/conjunctival samples were obtained by gently sweeping the inferior conjunctival fornices of both eyes with a disposable swab. Samples were analyzed by in-house reverse transcription (RT) PCR for detection of SARS-CoV-2 RNA. Positive conjunctival samples were re-analysed for confirmation using the commercially available GeneXpert assay.
Results: Viral RNA was detected in conjunctival swabs of 17 out of 24 (7.0%) COVID-19 patients. The mean age of those 17 subjects was 46.7 ± 16.2 years (range 26 – 80) and the male-to-female ratio was 0.31 (4:13). One subject reported increased tearing as ocular symptom. Conjunctival samples were positive for viral RNA as long as 12 days after disease onset. Cycle threshold (Ct) values for conjunctival swabs (mean 34.5 ± 5.1, range 22.6 – 42.0) were significantly higher than of nasopharyngeal swabs (mean 16.7 ± 3.6, range 12.1 - 24.3) ($p < 0.0001$). No correlation ($r = -0.10$, $p = 0.70$) between Ct values of conjunctival and nasopharyngeal swabs was observed. The majority of positive conjunctival samples were detected during the first visit, while three subjects became positive during the second visit and one subject during the fourth visit. None of the subjects tested positive in the conjunctival sample more than once.
Conclusion: SARS-CoV-2 RNA was detected in conjunctival swabs of 7.0% of patients with laboratory-confirmed symptomatic COVID-19. Our results suggest a risk for ocular transmission of SARS-CoV-2 that requires vigilance of protecting the ocular surface by wearing protective equipment.

P26

Regulation of intestinal barrier functions by the transmembrane mucin MUC13

Celia Segui Perez[1], Dr. K. Strijbis[1], Prof. dr. J.P.M. Van Putten[1]
[1]*Utrecht University*

The transmembrane mucin MUC13 is highly expressed on the apical surface of enterocytes but also overexpressed by adenocarcinomas, such as colorectal cancer. Cell junctions, including tight junctions (TJs) and adherens junctions (AJs) play a crucial role in maintaining epithelial barrier integrity and prevention of bacterial translocation. The loss of cohesion between these structures can lead to invasion and thus metastasis of cancer cells. Using CRISPR/Cas9 technology, we knocked out the MUC13 gene in the intestinal cell line HRT18. These MUC13 knockout cells showed an elongated morphology, formed a more disorganized cell monolayer, and expressed altered cell junctions. Assays including transepithelial resistance (TEER) and paracellular permeability to FITC dextran particles demonstrated that MUC13 knockout cells built up a tighter barrier. Interestingly, the addition of the probiotic bacteria, Lactobacillus plantarum, led to a higher increase in the TEER of MUC13 knockout cells compared to wild type (WT) cells. Together, these data suggest that MUC13 negatively regulates epithelial barrier function. By performing subcellular fractionations, we observed an increased in barrier-forming claudins in the membrane fraction of MUC13 knockout cells, which is in line with the higher TEER. Moreover, we found differences in myosin light-chain kinase (MLCK), a regulator of tight junctions' permeability, and expression of target genes of the Wnt/β-catenin pathways. Our results indicate that MUC13 is an essential player in the regulation of cell junctions and that could be involved in opening of cellular junctions during intestinal inflammation. In adenocarcinomas, overexpression of MUC13 may contribute to a reduction of cell-cell interactions, and stimulation of cell migration.

P27

Growth conditions of Streptococcus pneumoniae affect its interaction with human primary respiratory epithelial cells.

Msc Esther Van Woudenbergh[1,2], MD N van Heerbeek[3], Dr M.I. de Jonge[2], Dr G den Hartog[1]
[1]National Institute for Public Health and the Environment, [2]Radboud Institute for Molecular Life Sciences , [3]Radboud University Medical Centre, Nijmegen

The respiratory epithelium is the first cellular layer in the human body that encounters airborne pathogens. When studying host-pathogen interactions it is important to closely mimic the natural conditions of the infection site. For this purpose, we have cultured primary human nasal and bronchial epithelial cells on an air-liquid interface that enabled the cells to form a highly differentiated epithelial cell layer, which we confirmed by immunofluorescence and haematoxylin and eosin staining. We infected these epithelial cells with Streptococcus pneumoniae that were either grown in chemically defined medium (CDM), which mimics the nutrient levels at the mucosal infection site, on blood plate, representing the invasive environment, or in regular rich growth medium, and the amount of epithelial bound bacteria was counted. Preliminary results show that there was a difference in bacterial binding levels to epithelial cells. S. pneumoniae grown in CDM showed significantly higher levels of adhesion than pneumococci grown on blood. These data could indicate that the bacterial growth conditions could impact the interaction of S. pneumoniae with the human respiratory epithelium. This knowledge can be implemented to adapt epithelial infection models to make them more physiological relevant.

P28

Interactions of bacterial pathogens with differentially glycosylated MUC1, relevance for Inflammatory Bowel Disease (IBD)

Msc Koen Giesbers[1], Dr. K Strijbis[1], Prof. dr. J.P.M. Van Putten[1]
[1]*Utrecht University*

Inflammatory Bowel Disease (IBD) effects over 6-8 million individuals worldwide. The intestine has the highest density of bacteria anywhere in the body, with 10^11-10^12 per cm3 colon. To prevent constant infection and/or penetration of bacteria, the intestine is lined with a layer of glycosylated proteins, called mucins. Mucins are mostly comprised of O-linked glycans, which are over 70% of their mass. The differences in glycosylation of mucins in relation to disease, in particular for the transmembrane mucin MUC1, has been extensively studied. Intra- and extra-cellular processes resulting in aberrant hypo-glycosylation of MUC1, mediated by high expression of sialyltransferases like ST6GalNac1 have been implicated in chronic inflammation and cancer metastasis. Under healthy conditions, the glycosylated MUC1 has an essential barrier function for pathogenic bacteria such as Helicobacter pylori and Campylobacter jejuni. However, our recent work demonstrated that Salmonella Enteritidis expresses a giant adhesin SiiE that specifically interacts with sialic acids on MUC1 and facilitates apical entry into enterocytes. We are currently investigating interactions of other pathogenic bacteria with MUC1 and want to determine the influence of alterations in MUC1 glycosylation. Since aberrant hypo-glycosylation of MUC1 has been implicated in IBD, we hypothesize that the difference in glycosylation may promote the binding of pathogenic bacteria such as Salmonella, and thereby predispose IBD patients to MUC1-targeted bacterial invasion. We will identify novel MUC1-binding bacteria from healthy individuals and IBD patients with a FACS-based screen. Furthermore, MUC1 glycosylation will be manipulated by overexpression of core O-glycosylation synthases and the generation of sialyltransferase knockouts in intestinal epithelial cells. With this work, we will gain insight into bacteria-mucin interactions in the healthy and inflamed intestine.

P29

microViz: enhanced microbiome data analysis and visualization with R.

David Barnett[1], Prof ICW Arts[1], Dr J Penders[2]
[1]Maastricht University, [2]Maastricht University Medical Center+

Introduction:

Modern microbiome research typically involves the use of next-generation-sequencing methods to profile the relative abundance of hundreds of microbial taxa across tens or hundreds of samples. Alongside increasing sample sizes, the amount of relevant metadata collected is growing, particularly in human cohort studies. These trends all increase the size and complexity of the resulting dataset, which makes its exploration and rigorous statistical analysis increasingly challenging.

Methods:

We have developed microViz, an R package for the statistical analysis and visualization of microbiota data. This software extends the functionality of popular microbial ecosystem data analysis R packages phyloseq, vegan and microbiome.

Results:

microViz offers several useful enhancements or additions to this toolbox including:
- a novel tree-based approach for compact and intuitive visualization of numerous microbe-metadata associations derived from (multivariable) statistical models;
- a novel approach for intuitive interactive exploration of the microbial composition of your samples using paired ordination and abundance bar chart visualizations in R Shiny;
- a novel approach pairing ordination and polar bar charts for comprehensive, intuitive and compact visualization of the similarity and composition of hundreds of microbial ecosystems;
- streamlined and user-friendly functionality for generating publication-ready ordination plots with ggplot2, accommodating constrained ordination, biplots and triplots, and automatic captioning designed to promote methodological transparency and reproducibility.

Beyond the main visualization functionality, microViz provides a suite of powerful tools for working with phyloseq objects including wrappers that bring functions from the popular dplyr package to phyloseq, to easily filter, select join, mutate and arrange sample data. All microViz functions are designed to work with R's pipe operator (%>%), to chain successive functions together and improve code readability. For user convenience, microViz documentation is hosted online via a pkgdown website on GitHub Pages, with extensive examples of code and output using example datasets.

Conclusion:

We anticipate that microViz will add a selection of powerful tools to the workflow of researchers already familiar with phyloseq and other ubiquitous microbiome R packages, as well as assisting researchers with less R programming experience to independently explore their data and generate publication-ready figures.

P30

Transmission after kidney transplantation of human polyomaviruses other than BK; an exploratory serological cohort study

Drs Sergio Kamminga[1,2], C.S. de Brouwer[1], A.L. van Rijn[1], J.I. Rotmans[3], H.L. Zaaijer[2], M.C.W. Feltkamp[1]
[1]Leids Universitair Medisch Centrum, [2]Sanquin Research, [3]Leids Universitair Medisch Centrum

Introduction
Human polyomaviruses (HPyVs) cause various diseases in immunocompromised patients, such as solid organ transplant recipients. BK polyomavirus (BKPyV) can persistently infect the kidneys and cause BK polyomavirus-associated nephropathy in kidney transplant recipients. BKPyV is transferred to kidney transplant recipients (KTR) through the allograft. Little is known about the route of transmission for other polyomaviruses. In this study, we aimed to provide serological evidence for transmission through the kidney allograft for the polyomaviruses JCPyV, MCPyV, TSPyV and HPyV9.

Materials and methods
Serum samples from KTR were gathered before and approximately 6 months after transplantation (n = 620), as well as paired blood donor serum samples as control (n = 174). These samples were assessed for HPyV-specific serology using a multiplex bead-based immunoassay. Results were compared between KTR and blood donors by applying linear mixed models.

Results
Increased seroresponses were observed in 14.8% of KTR for JCPyV, 7.1% for MCPyV, 10.6% for TSPyV, 8.1% for HPyV9 and 11.9% for BKPyV. Seroconversion was observed in 6.5% of KTR for JCPyV, 2.3% for MCPyV, 1.3% for TSPyV, 6.5% for HPyV9 and 0.6% for BKPyV. The linear mixed models showed a significant increase in seroresponse over time for JCPyV ($p < 0.001$), HPyV9 ($p < 0.001$) and BKPyV ($p < 0.001$), but not for MCPyV and TSPyV.

Conclusions
KTR are frequently exposed to JCPyV and HPyV9, next to BKPyV. The donor kidney is a probable origin of exposure and should be further studied by comparing donor and recipient seroresponses. The role of serologic JCPyV donor screening for KTR risk of JCPyV-related disease remains to be established.

P31

Importance of combined epidemiological and laboratory surveillance for Listeria monocytogenes and Shiga toxin-producing Escherichia coli (STEC) in a one health setting

dr. Maaike van den Beld[1], Kim van der Zwaluw[1], Menno van der Voort[2], Coen van der Weijden[3], Thijs Bosch[1], Eelco Franz[1], Ingrid Friesema[1]

[1]National Institute for Public Health and the Environment (RIVM), [2]Wageningen Food Safety Research (WFSR), [3]Netherlands Food and Consumer Product Safety Authority (NVWA)

Introduction: The National Institute for Public Health and the Environment (RIVM) employs a combination of epidemiological and laboratory surveillance for human infections with Listeria monocytogenes and Shiga toxin-producing Escherichia coli (STEC). Epidemiological surveillance consists of collection and analyses of mandatory notifications from public health services and physicians to the RIVM. For laboratory surveillance, medical laboratories submit their isolates for whole genome sequencing (WGS). In addition, Wageningen Food Safety Research (WFSR) analyses food and food production facility samples, collected by the Netherlands Food and Consumer Product Safety Authority (NVWA), for the presence of STEC and L. monocytogenes isolates and subsequently performs WGS. All WGS data is used to monitor pathogen circulation and to detect outbreaks using cluster analysis. Identification of a cluster involving human and food isolates often leads to targeted actions to end an ongoing foodborne outbreak. Here we evaluate the effect and potential improvements of this combined surveillance for STEC and L. monocytogenes for 2019 and 2020.

Methods: The presence and benefit of combined data for epidemiological and laboratory surveillance was determined. Cluster detection based on Illumina sequencing of received isolates was performed with cgMLST using Ridom SeqSphere+ 7.1.0. The clusters, detected outbreaks and actions resulting from STEC and L. monocytogenes surveillance for 2019 and 2020 were assessed.

Results: In 2019 and 2020, for 85% of L. monocytogenes notifications an isolate was received, and 95% of the Listeria infections of which an isolate was available were notified. A total of 181 Listeria patient isolates were received in 2019 and 2020. Using a cluster threshold of 7 alleles, 73 isolates (40%) presented as singletons and 108 were part of 38 clusters. In 24 (63%) of these clusters, isolates from food origin were also present. For 10 of the mixed human and food clusters, including a large outbreak associated to sliced cold meats, cluster identification led to ending an ongoing outbreak or improve food safety hygiene at food production facilities to reduce transmission of L. monocytogenes via food. For STEC, only for 27% of notifications an isolate was received, probably due to STEC protocols of laboratories, in which obtaining isolates is no longer standard. Additionally, only 44% of infections for which STEC isolates were available were reported to health authorities. In 2019 and 2020, 493 human STEC isolates were received. Using a cluster threshold of 7 alleles, 363 (74%) of these isolates were singletons, and 130 (26%) were distributed over 56 clusters. Three were of mixed human and food origin, consisting of one case and one food product with at least months between the isolates leading to no actionable results.

Conclusion: In 2019 and 2020, WGS-based surveillance and monitoring of L. monocytogenes from human and food origin resulted in actionable cluster-signals. Effective WGS-based STEC surveillance is hindered by the lack of available isolates, resulting in an incomplete picture of circulating STEC. The increasing trend of molecular diagnostics for STEC infections (and refraining from culturing) threatens effective WGS-based national surveillance with the purpose of detecting clusters and outbreaks.

P32

Heterologous expression of ethA and katG in Mycobacterium marinum enables the rapid identification of new prodrugs active against Mycobacterium tuberculosis

Mr Vien Ho[1]
[1]*Amsterdam University Medical Center*

Screening strategies for anti-tuberculosis compounds using Mycobacterium tuberculosis are time-consuming and require BSL-3 facilities, which makes the development of high-throughput assays difficult and expensive. Mycobacterium marinum, a close genetic relative of M. tuberculosis, possesses several advantages as a suitable model for tuberculosis drug screening. However, despite the high genetic similarity, there are some obvious differences in susceptibility to some tuberculosis drugs between these two species, especially for the pro-drugs ethionamide and isoniazid. In this study, we aimed to improve M. marinum as a model for anti-tuberculosis drugs identification by heterologous expression of two common drug activators, EthA and KatG. These two activators were overexpressed in M. marinum and the strains were tested against ethionamide, isoniazid and a library of established antimycobacterial compounds from TbAlliance to compare drug susceptibility. Both in vitro and in vivo using zebrafish larvae, these genetically-modified M. marinum strains showed significantly higher susceptibility against ethionamide and isoniazid, which require activation by EthA and KatG. More importantly, a strain overexpressing both ethA and katG was potentially more susceptible to approximately 20% of the anti-tuberculosis hit compounds from the TB Alliance library. Most of these compounds were activated by EthA in M. marinum. Four of these compounds were selected for further analysis and three of them showed obvious EthA-dependent activity against M. tuberculosis. Overall, our developed M. marinum strains are valuable tools for high-throughput discovery of potential novel anti-tuberculosis pro-drugs.

P33

CpnT toxin of Mycobacterium tuberculosis identified as a new ESX-5 substrate

Beatriz Izquierdo Lafuente[1], R Ummels[2], Dr. C. Kuijl[2], Prof. Dr. W. Bitter[1,2], Dr. A. Speer[2]
[1]AIMMS VU Universiteit, [2]Amsterdam UMC

Pathogenic bacteria such as Mycobacterium tuberculosis (Mtb) use secrete virulence factors to ensure the survival and dissemination when infecting host cells. The CpnT (Rv3903c) protein is thus far the only known toxin that is secreted by Mtb. This toxin acts as an NAD glycohydrolase and induces necrosis in eukaryotic cells. CpnT is composed of two domains where the C-terminal domain is responsible for enzymatic activity. CpnT is secreted across the bacterial cell envelope and released into the cytosol of the infected macrophages. However, its secretion system is still unknown. In this study, we investigated secretion of the CpnT toxin, using M. marinum as a model organism. CpnT does not have a clear secretion signal, but has some characteristics of type VII secretion (T7S) substrates, including a helix-turn-helix prediction followed by a secretion motif (YxxxD/E). A point mutation in this specific secretion motif , strongly reduced protein secretion, indicating that CpnT is as a substrate of the T7S machinery. The genome of mycobacteria encodes five different T7S systems, ESX-1 through ESX-5. We discovered that CpnT is specifically secreted in bacterial cultures by ESX-5, which is exclusively found in slow-growing mycobacteria. Additionally, we confirmed that secretion of CpnT depends on ESX-5 in vitro during macrophage infection studies. Surprisingly, while secretion of CpnT in bacterial culture relied solely on a functional ESX-5 system, secretion during macrophage infections was not only dependent on ESX-5 but also on ESX-1 and ESX-4. Since the ESX-1 system is required for phagosomal escape, these results suggest that the bacteria only release the CpnT toxin after reaching the cytosol of the macrophage. The role of the ESX-4 system is still unknown, but we confirmed that ESX-4 mutants, in contrast to ESX1 mutants, are not involved in phagosomal escape. To this point it is unclear what the function of ESX4 is during CpnT secretion in vivo. However, we observed that CpnT secretion in an ESX-4 mutant was recovered during co-infection with a wild type strain deficient in CpnT expression, suggesting that the defect of ESX-4 could be cross-complemented. This means that a secreted factor is somehow involved in this process. In summary, our data showed that three independent type VII secretion systems act in concert to facilitate secretion of the M. tuberculosis toxin CpnT during infection.

P34

An efficient molecular approach to distinguish chains of measles virus transmission in the elimination phase

Rogier Bodewes[1], L Reijnen[1], F. Zwagemaker[1], R.H.G. Kohl[1], J. Kerkhof[1], I.K. Veldhuijzen[1], R van Binnendijk[1]
[1]*National Institute for Public Health and the Environment (RIVM)*

Introduction
Measles viruses continue to spread globally, despite the availability of a safe and effective vaccine. Molecular surveillance of measles virus has become an essential tool to demonstrate whether cascades of infections in a certain region or country are the result of endemic spread or the repeatedly introduction of the virus in contained outbreaks. Currently, molecular surveillance of measles viruses worldwide is mainly based on 450 nucleotides of the C-terminal region of the nucleoprotein (N450).
However, as a result of the disappearance of particular measles virus clades over the past decades, this gene segment does not provide sufficient resolution anymore. This was recently experienced after a sudden increase of reported measles cases in 2018 and 2019 in the Netherlands. Whether this was the result of increased import of measles virus or spreading of the virus could not be discriminated, as the N450 sequences were identical.
The aim of the present study was to improve molecular surveillance of measles viruses by selection and subsequent Sanger sequencing of additional regions of the measles virus genome.

Methods
An alignment was made of recent complete genomes of measles genotype B3 and D8 viruses. Based on this alignment, three regions were selected with a relatively high sequence variation between strains, namely the partial non-coding region between the M and F gene (M-F NCRpartial; Sundell et al 2019), partial H gene (Hpartial) and partial L gene (Lpartial). Specific primers were designed to amplify and sequence (using Sanger sequencing) these regions of measles viruses detected in clinical materials in the Netherlands in 2018 and 2019.

Results
Results of this study and initial pilot experiments indicated that sequence data of the M-F NCRpartial, Hpartial and Lpartial could be obtained from >95% of clinical samples provided Ct-values were below 30. Analysis of obtained sequence data indicated that sequencing of these three regions resulted in an increase in molecular resolution for measles virus genotype B3 and D8 viruses, two of the four global genotypes currently predominant in the European region. Furthermore, this improved resolution was sufficient to support an epidemiology characterized by repeat introduction of measles virus rather than endemic virus spread in the Netherlands in 2018 and 2019.

Conclusion
Sequencing of the M-F NCRpartial, Hpartial and Lpartial regions of the measles virus is an efficient and useful approach for molecular surveillance of measles viruses.

P35

Complex microbial communities in long-term nitrifying bioreactors

Dr. Garrett Smith[1], Dr. M. van Kessel[1], Dr. S. Leucker[1]
[1]*Radboud Universiteit*

(Background)
One critical step of Wastewater Treatment (WWT) is nitrification, the oxidation of ammonia to nitrite by Ammonia Oxidizing Microorganisms (AOM), followed by the oxidation of nitrite to nitrate by Nitrite Oxidizing Bacteria (NOB). Bacteria capable of COMplete AMMonia OXidation (Comammox) are also found in these systems, which are expected to only be competitive under substrate-limited conditions. However, Comammox often co-occur with lower-affinity canonical AOM and NOB, and therefore the current understanding of nitrifiers insufficiently explains their observed ecology in WWT plants.
(Methods)
Two laboratory-scale bioreactors were inoculated with WWT biomass and incubated in tandem under substrate-limiting conditions. Kinetic measurements of oxygen, ammonia, and nitrite consumption were used to monitor the performance of the bioreactors. Metagenomic sequencing was used reconstruct genomic information of the dominant nitrifying microorganisms.
(Results)
Differential abundances implicate Comammox in nitrification under oxygen limitation, while nitrogen-limitation instead selected for conventional Nitrosmonas and Nitrospira were the predominant AOM and NOB, respectively, although all three types of nitrifiers were present in both reactors. Intriguingly, heterotrophs accounted for a majority of the communities and may recycle oxidized nitrogen to ammonia or nitrite, complicating predictions of nitrification performance of the reactors.
(Conclusions)
Despite relatively minimal nitrogen inputs and no exogenous organic carbon sources, (1) reactor communities remained diverse containing multiple nitrifying taxa with overlapping functionalities, and (2) rich heterotrophic community that are likely also involved in nitrogen cycling. Further analysis of these reactors will use targeted activity measurements to resolve the primary members responsible for nitrification and supporting the non-nitrifying members in these bioreactors.

P36

Predicting the structure and dynamics of membrane protein GerAB from Bacillus subtilis

BSc Sophie Blinker[1], dr. J. Vreede[2], prof. dr. P. Setlow[3], prof. dr. S. Brul[1]
[1]University Of Amsterdam, [2]University Of Amsterdam, [3]U Conn Health

Bacillus subtilis forms dormant spores upon nutrient depletion. Germinant receptors (GRs) in spore's inner membrane respond to ligands such as L-alanine, and trigger spore germination. In B. subtilis spores, GerA is the major GR, and has three subunits, GerAA, GerAB, and GerAC. L-Alanine activation of GerA requires all three subunits, but which binds L-alanine is unknown. To date, how GRs trigger germination is unknown, in particular due to lack of detailed structural information about B subunits. Using homology modelling with molecular dynamics (MD) simulations, we present structural predictions for the integral membrane protein GerAB. These predictions indicate that GerAB is an α-helical transmembrane protein containing a water channel. The MD simulations with free L-alanine show that alanine binds transiently to specific sites on GerAB. These results provide a starting point for unraveling the mechanism of L-alanine mediated signalling by GerAB, which may facilitate early events in spore germination.

P37

Generation, separation, and eradication of Bacillus subtilis persister cells

Shiqi Liu[1], Dr. S.A.J Zaat Zaat[2], Prof. Dr. S.B Brul[3]

[1]Swammerdam Institute for Life Sciences, University of Amsterdam, [2]Centre for Infection and Immunity Amsterdam (CINIMA), Academic Medical Centre, University of Amsterdam, [3]Swammerdam Institute for Life Sciences, University of Amsterdam

The antibiotic crisis, majorly caused by drug resistance and persistence, has led to severe public health problems. Compared to inheritable resistance mechanisms attributable to genetic alterations, persistence is a non-heritable phenotype that universally exists among almost all bacterial species. Persister cells are growth-arrested subpopulation that can survive possible fatal environments and revert to wild types after stress removal. Clinically, persistent pathogens play a key role in chronic, recurrent, and antibiotic-resilient infections. Persister cells can be formed during stress conditions such as exposure to energy limitation, immune cells, antibiotics, and various other antimicrobial compounds. Yet, instead of exhibiting a unified antibiotic tolerance profile, persisters show heterogenous abilities to withstand antimicrobials due to the different ways of obtaining persister-cells. Due to the (extremely) low metabolic activities of persister cells, traditional antimicrobials fail to kill them. As a novel alternative approach, antimicrobial peptides (AMPs) have been intensively investigated as one of the most promising strategies against these persisting bacterial forms. AMPs are based on short peptides generated as part of the defense system of most if not all living organisms and may be synthesized “ex-vivo”. With activities like membrane perturbation, AMPs are highly recommended as “next-generation antibiotics” with broad-spectrum antimicrobial activities. In general, molecular physiological research into persister formation and the mode of action of AMPs against persister cells are much desired (e.g. Liu et al. 2020 IJMS 21, 8967).

In this research, we aim at generating B. subtilis persisters via various means and characterizing resulting cells. B. subtilis persister cells are induced by different antimicrobial agents: 1) the uncoupling agent carbonyl cyanide m-chlorophenylhydrazone (CCCP), a proton-ionophore leading to a drop in cellular ATP level; 2) vancomycin, inhibiting the lipid II cycle of cell wall biosynthesis; 3) enrofloxacin, breaking double-stranded DNA; and 4) tetracycline, reversibly inhibiting protein synthesis. Current results indicated that B. subtilis persister cells can be induced by all tested stress conditions. However, the numbers of generated persister cells are different. Specifically, these stimuli generated persister cell numbers in the following order from high to low numbers: Vancomycin > Enrofloxacin ≥ CCCP > Tetracycline. This implies that the molecular mechanism relating to persister cells formation can be different as well. Upon exposure to these antimicrobial agents and staining with appropriate fluorescence dyes and/or labelling with fluorescent reporter proteins, it is expected that persisters can be separated by flow cytometry. Subsequently, pure persisting populations will be collected for further analyses, including transcriptomics to reveal the mechanism of persister cells formation under different stress conditions, microscopic observation for morphological analysis, and the efficiency and mode of action of AMPs against persisters. Preliminary studies show that AMP TC-19 and SAAP-148, derived from human platelet thrombocidin, and human cathelicidin LL-37, are highly effective against B. subtilis vegetative cells. Their activity against isolated persister cells will be investigated next.

With our research, we provide valuable information on the formation, separation, and eradication of persister cells, which is beneficial to control these special phenotypic variants and hence reduce their impact on public health.

P38

Despite excellent test characteristics of the cobas®4800 CT/NG assay, detection of oropharyngeal Chlamydia trachomatis and Neisseria gonorrhoeae remains challenging

Julius Van Niekerk[1], BMJW van der Veer[1], CJPA Hoebe[2], J van de Bovenkamp[3], C van Herk[3], IHM van Loo[1], LB van Alphen[1], PFG Wolffs[1]

[1]Maastricht University Medical Centre (MUMC+), [2]South Limburg Public Health Service, [3]Laboratory for Medical Microbiology (PAMM)

Objectives

Oropharyngeal Chlamydia trachomatis (CT) and especially Neisseria gonorrhoeae (NG) infections are common but few commercial nucleic acid amplification tests (NAATs) specify extragenital samples as intended use. The test characteristics of the cobas®4800 CT/NG assay are evaluated for oropharyngeal swabs.

Methods

The technical validation includes analysis of the specificity, sensitivity, dynamic range, linearity, efficiency and precision. The probability of detection curve combined with historical data enables estimation of potentially missed diagnoses. A clinical evaluation has been performed on a subset of 2798 clinical samples available from routine diagnostics. Results of the cobas®4800 were compared with in-house CT/NG polymerase chain reaction assays. Discrepant samples were tested with resolver assays and these results were considered decisive.

Results:

No cross-reactivity was seen in the analytical specificity analysis. High linearity (≥0.983 R^2), efficiency (89%-99%), and precision (0.1-0.9 cycle thresholds, i.e. Ct-values) were seen for both CT/NG. The limit of detection in oropharyngeal samples was $3.2x10^2$ inclusion-forming units/mL for CT and $6.7x10^2$ colony-forming units/mL for NG. Estimates on potentially missed diagnoses were up to 7.2% for CT and up to 24.7% for NG. Clinical sensitivity and specificity were evaluated with 25 CT, 86 NG positive and 264 negative samples, resulting in 100% and 99.6% for CT and 100% and 96.7% for NG respectively.

Conclusion:

The findings in this study demonstrate the utility of the cobas®4800 CT/NG assay for oropharyngeal samples. Despite being a highly accurate test, the range of reported Ct-values especially for NG suggest relatively low oropharyngeal loads. Hence, consistent detection over the full range of oropharyngeal loads could be impaired.

P40

Antibody-mediated natural killer cell activation and Fc-glycosylation in infants with severe respiratory syncytial virus infection

E.A. van Erp[1,2], AJ Lakerveld[1], E de Graaf[3], MD Larsen[3], RM Schepp[1], A Hipgrave Ederveen[4], IML Ahout[2], CAM de Haan[5], M Wuhrer[4], W Luytjes[1], G Ferwerda[2], G Vidarsson[3], Dr. Puck van Kasteren[1]
[1]RIVM, [2]RadboudUMC, [3]Sanquin, [4]LUMC, [5]UU

Introduction – Respiratory syncytial virus (RSV) is a major cause of severe lower respiratory tract infections in infants and there is no vaccine available. In early life, the most important contributors to protection against infectious diseases are the innate immune system and maternal antibodies. However, the mechanisms by which antibodies can protect against RSV disease are incompletely understood, as both antibody levels and neutralization capacity correlate poorly with protection. We therefore asked whether antibody-mediated natural killer (NK) cell activation correlates with RSV disease.

Methods – We performed an observational case-control study including infants hospitalized for RSV infection, hernia surgery, or RSV-negative viral respiratory tract infections. First, we determined RSV antigen-specific antibody levels in infant plasma using a multiplex immunoassay. Subsequently, we measured the capacity of these antibodies to activate NK cells. Finally, we assessed Fc-glycosylation of the RSV-specific antibodies by mass-spectrometry.

Results – We found that RSV-specific maternal antibodies potently activate NK cells in vitro. While the concentrations of RSV-specific antibodies did not differ between cases and controls, antibodies from infants hospitalized for severe lower respiratory tract infections (RSV and/or other) induced significantly less NK cell interferon gamma production than those from uninfected controls. Furthermore, NK cell activation correlated with Fc-fucosylation of RSV-specific antibodies, but their glycosylation status did not significantly differ between cases and controls.

Conclusion – Our results suggest that Fc-dependent antibody function and quality, exemplified by NK cell activation and glycosylation, contribute to protection against severe RSV disease and warrant further studies to evaluate the potential of harnessing these activities to develop an effective vaccine.

(van Erp et al., Clinical & Translational Immunology, 2020)

P42

Unravelling conserved generic Aspergillus spp germination pathways using cross-platform and cross-species transcriptomics

Tim Baltussen[1], J.P.M Coolen[1], P.E. Verweij[1], J. Dijksterhuis[2], W.J.G. Melchers[1]
[1]Radboudumc, [2]Westerdijk Fungal Biodiversity Institute

At least 600 Aspergillus species exist and inhabit all sorts of environmental niches worldwide. The main vehicles of distribution are single-celled survival structures, called conidia, that become airborne extremely easily. Inhaled conidia rarely cause an infection, however the combination of species able to grow at body temperatures and an immunocompromised person, an infection may develop. To establish an infection, germination of inhaled conidia is crucial. A more profound understanding of the transitions from dormant conidia via germ tube initiation to hyphal tip formation may be vital in the search for possible novel strategies to eradicate early infection.
To identify critical biological processes during germination, we performed a cross-platform cross-species comparative analysis of germinating A. fumigatus and A. niger conidia using transcriptional data from published RNA-Seq and Affymetrix studies. Firstly, to perform this cross-platform cross-species comparative analysis we used the following bioinformatic approach. i) a selection of 6,598 orthologues genes was necessary to integrate the two datasets which enclosed nearly 50% of the A. niger genes and over 50% of the A. fumigatus genes. ii) normalization of the intensities was done as both techniques have different expression values, i.e., RNA-Seq counts and microarray fluorescence intensities and iii) expression patterns during germination stages were compared.
A consensus co-expression network analysis identified four gene modules associated with germination. These modules showed numerous shared biological processes between A. niger and A. fumigatus during conidial germination. Specifically, the turquoise module was enriched with secondary metabolism, the black module was highly enriched with protein synthesis, the darkgreen module was enriched with protein fate, and the blue module was highly enriched with polarized growth. More specifically, enriched functional categories identified in the blue module were, vesicle formation, vesicular transport, tubulin dependent transport, actin dependent transport, exocytosis, and endocytosis. Vesicles transport cell wall modifying enzymes, substrates, and the cell membrane are required for expansion of the growing tip.
In this study, we demonstrated the possibility for comparative analysis between Aspergillus spp using two different transcriptional profiling platforms which introduces the opportunity to perform cost-effective insightful comparisons. 1) The cross-platform cross-species analysis confirmed the occurrence of conserved, generic, functionally important biological processes during germination, which are independent of a single technology. This is the more interesting as A. fumigatus and A. niger are taxonomically not very close within the taxon. 2) The consensus gene co-expression network detected modules associated with transcription and protein synthesis and polarized growth.

P43

Effect of faecal microbiota transplantation on procarcinogenic pks+ Escherichia coli in metagenomes of patients with multiple recurrent Clostridioides difficile infections

Sam Nooij[1,2,3], QR Ducarmon[1,3], JFJ Laros[3,4,5,6], RD Zwittink[1,3], JM Norman[7], B Roberts[7], HW Verspaget[8,9], JJ Keller[8,10], EM Terveer[1,2,3], EJ Kuijper[1,2,3,6]

[1]Leiden University Medical Center, [2]Netherlands Donor Feces Bank, [3]Leiden University Medical Center, [4]Leiden University Medical Center, [5]Leiden University Medical Center, [6]National Institute of Public Health and the Environment (RIVM), [7]Vedanta Biosciences Inc., [8]Leiden University Medical Center, [9]Leiden University Medical Center, [10]Haaglanden Medical Center

Introduction

Patients suffering from multiple recurrent Clostridioides difficile infections (rCDI) have a disturbed gut microbiota, which can be restored with faecal microbiota transplantation (FMT) from healthy donors. Despite extensive screening, these donors may carry bacteria in the intestinal tract that could pose long-term health risks, such as the procarcinogenic colibactin-producing (pks+) Escherichia coli. In this study, we screened deep-sequenced faecal metagenomes of faeces donors of the Netherlands Donor Feces Bank and adult FMT treated rCDI patients for E. coli and the pks gene island. Thereby, we determine the prevalence of pks among healthy donors and rCDI patients and evaluate the effect of FMT on pks-carriership.

Methods

In a cohort of 49 rCDI patients treated with FMT and matching donor samples, we retrospectively screened faecal metagenomes for pks+ E. coli and compared pks presence and absence, as well as quantities, between patient samples before and after treatment and compared with their respective donors. The pks island was quantified using a read-mapping approach to a reference sequence containing all 19 coding gene sequences, while E. coli was quantified using three single-copy marker genes. Metagenomes are classified as pks-containing (pks+) when one or more reads mapped to any of the 19 coding genes of the pks island and metagenomes with no reads mapping are pks-negative.

Results

Out of 38 donor samples included, derived from 8 donors, we detected pks in 11 samples (29%) from 3 different donors (37.5%). One donor was persistently colonised with pks+ E. coli at least half a year. Pks was present in 27 out of 49 patients (55%) before FMT treatment and 19/49 (39%) after FMT. Pks levels were generally higher in patients (pre-FMT median: 0.46 reads per kilobase per million; RPKM, post-FMT median: 1.35 RPKM) than in donors (median: 0.01 RPKM). Whether or not a patient was treated with a pks+ donor suspension affected the pks-status of the patient after FMT (Chi-squared, P=.02). The donor status especially affected persistence (8/9) and decolonisation (13/18) of pks in the group of patients that had pks prior to FMT (Fisher's exact test, P=.004).

Conclusion

1. Pks+ E. coli occurs in healthy donors and may persist at low abundances for more than six months.
2. Pks+ E. coli is prevalent and relatively abundant among rCDI patients, which indicates that these patients may be at increased risk of developing colorectal cancer.
3. FMT affects the pks-status of the patient. In particular, the pks-status of the donor influences pks-persistence in patients that were already colonised by pks+ E. coli.
4. FMT has a minor effect on pks in rCDI patients and the long-term health risks are not yet known. Besides, the rCDI patients in our cohort are mostly elderly patients with a number of comorbidities. Therefore, we think FMT is safe for rCDI patients and protocols need not be adapted for pks. However, in younger patients that are treated by FMT for other conditions it might be worth to screen for pks and assess its effects on the longer term.

P44

Lifestyle, Acid Suppression, and Carriage of Multidrug-Resistant Enterobacterales: A Population-Based Study in Dutch Adults

Drs Roel Willems[1], MD, PhD K van Dijk[1], PhD L Mollema[4], PhD CM Dierikx[4], PhD JWR Twisk[5], PhD FRM van der Klis[3,4], PhD SC de Greeff[2,4], MD, PhD CMJE Vandenbroucke-Grauls[1]

[1]Amsterdam Umc, [2]National Institute for Public Health and the Environment (RIVM), [3]National Institute for Public Health and the Environment (RIVM), [4]National Institute for Public Health and the Environment (RIVM), [5]Amsterdam UMC Vrije Universiteit Amsterdam

Introduction: Gastric acid-suppressing therapy has been linked to intestinal carriage of multidrug-resistant Enterobacterales (MRE); whether this association reflects confounding by lifestyle factors is unknown. We therefore aimed to examine the risk for intestinal carriage of multidrug-resistant Enterobacterales (MRE) associated with the use of acid suppressants, and to assess possible modifying factors in a population-based setting.

Methods: The study population comprised 2746 adult participants (≥18 years) who provided stool specimens within the framework of the PIENTER-3 survey, a Dutch nationwide population-based seroprevalence study. Data and specimens were collected between February 2016 and June 2017. All stool specimens were analyzed to detect Enterobacterales producing extended-spectrum β-lactamases (ESBLs) or carbapenemases; resistance enzymes were screened for by phenotypic assays and resistance genes were confirmed by polymerase chain reaction (PCR). Exposure and potential confounders (i.e. sociodemographic, lifestyle, medical) were measured via structured questionnaires. Multivariable mixed-effect logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs); control of selected covariates was guided by multiple causal directed acyclic graphs (DAGs).

Results: Data from 2746 Dutch adults were analyzed (1582 [57.6%] female; median [interquartile range] age, 50 [34-64] years); the crude and standardized prevalence of MRE carriage was 7.2% ([95% CI, 6.3%-8.2%]) and 7.5% (95% CI, 6.2%-8.9%), respectively. Approximately 11.5% of participants used acid-suppressants; current use of these agents was not associated with MRE carriage (adjusted odds ratio [aOR], 1.05; 95% CI, 0.63-1.73); lifestyle did not modify this association. Overweight or obesity (body mass index [BMI] ≥ 25 kg/m2) (aOR, 1.41 [95% CI, 1.02-1.96]), travel to the Eastern-Mediterranean, Western-Pacific or South-East Asia region (aOR, 3.11 [95% CI, 1.69-5.74]), non-Western ethnic origin (aOR, 1.96 [95% CI, 1.34-2.87]), and prior use of broad-spectrum antibiotics were associated with increased odds of MRE carriage (aOR, 1.72 [95% CI, 0.95-3.12]). Eighty-five percent (180/212) of the MRE isolates produced ESBLs of CTX-M groups; five participants carried carbapenemase-producing strains.

Conclusion: In this study, one in thirteen participants carried ≥1 MRE isolate in their stool. While the current use of acid suppressants was not associated with MRE carriage in the general population, overweight or obesity (BMI ≥25 kg/m2) yielded approximately 40% higher odds of carriage. Our findings suggest an as yet unrecognized role of obesity as a possible risk factor for colonization with resistant strains.

P45

Higher in vitro mucin degradation, but no increased paracellular permeability by faecal water from Crohn's disease patients

Heike Becker[1,2], N Kameli[1,3], A Rustichelli[2], B Heijnens[2], PhD F Stassen[1], PhD DMAE Jonkers[2], J Penders[1]
[1]Maastricht University, [2]Maastricht University, [3]Jazan University

Introduction: Crohn's disease (CD) is a chronic inflammatory gastro-intestinal condition with a variable disease course. Impaired intestinal integrity and microbial dysbiosis are associated with disease onset and exacerbations. We hypothesized that a perturbed microbial activity in CD patients may contribute to the impaired barrier function. Therefore, this study aimed to examine the impact of faecal bacterial products of active CD patients, CD patients in remission, and healthy controls (HC) on mucin degradation and intestinal epithelial barrier function in vitro.
Methods: Six HC and twelve CD patients were included. Disease activity was determined by endoscopy. Faecal water (FW) and bacterial membrane vesicles (MVs) from fresh faecal samples were applied on mucin agar to determine mucin degradation. Further, differentiated Caco-2 cell monolayers were exposed to FW and MVs to assess transepithelial electrical resistance (TEER) and paracellular junction stability using fluorescein isothiocyanate-labelled dextran of 4 kDa. Relative abundances of faecal bacterial genera were evaluated by 16S rRNA gene amplicon sequencing.
Results: FW-induced mucin degradation was higher in CD samples as compared to HC ($p<0.01$), but was not linked to specific bacterial relative abundances. FW resulted in 78-87% decrease of TEER in three of the remissive ($p<0.001$) but not in the active CD or HC samples. The decrease of TEER was not linked to increased paracellular permeability. MVs did not induce mucin degradation or epithelial barrier disruption.
Conclusion: The higher mucin degradation capacity of CD patient-derived FW might indicate contributions of microbial products to CD pathophysiology and warrants further investigation. Moreover, the altered epithelial resistance in some individuals is not due to paracellular alterations.

P46

Binding and internalization of RSV particles by platelets results in release of growth factors and chemokines

Anke Lakerveld[1], E A van Erp[1], P B van Kasteren[1]
[1]*National Institute Of Public Health And The Environment (RIVM)*

Respiratory syncytial virus (RSV) infection is a major cause of severe lower respiratory tract infections in risk groups such as infants and elderly. Severe RSV disease appears to result from a dysregulated immune response and is characterized by pronounced (neutrophilic) inflammation. Platelets are increasingly recognized as important players in the immune response against various pathogens, for example through the activation of neutrophils. Therefore, we hypothesized that RSV interacts with platelets and activates them.

To study platelet-RSV interaction, we isolated platelets from fresh blood of healthy adult volunteers and incubated these with sucrose-purified RSV-A2. Using flow cytometry, we could show binding and uptake of RSV particles by platelets. Furthermore, a multiplex immunoassay revealed increased concentrations of VEGF, PDGF-BB, CCL2, and CXCL5 in the supernatant of RSV-exposed platelets, all of which have been associated with RSV infection in humans. Incubation of platelets with recombinant RSV G protein suggests that VEGF secretion is induced by this protein.

To our knowledge, we are the first showing that platelets are able to bind and internalize RSV, subsequently inducing the secretion of several chemokines and growth factors, which may contribute to pulmonary inflammation during RSV infection.

P48

Prevalence of hepatitis delta virus among chronic hepatitis B carriers in a large tertiary center in the Netherlands

Boris Beudeker[1], dr, JC Voermans[1], dr. C GeurtsvanKessel[1], dr. RJ de Knegt[2], T Kuhlemann[1], dr. A Boonstra[2], dr. AA van der Eijk[1]

[1]Erasmus MC University Medical Center, [2]Erasmus MC University Medical Center

Background & Aims

Hepatitis D virus infection (HDV) is considered the most severe form of viral hepatitis with a poor prognosis due to high rates of liver cirrhosis, liver failure and liver cancer development. Considerable progress has been made in the discovery of novel HDV treatments and the prognosis of these patients is expected to improve. However, epidemiological data on HDV prevalence in Western countries is scarce and - importantly- the data is generally collected before the 2015 migration of refugees to Europe. Moreover, there is a dire need to implement validated surveillance strategies in at-risk patient populations. In our study, we now evaluate the prevalence of HDV infection between 2017-2019 in a tertiary center of a large, multi-ethnic city in the Netherlands, and we validate the reliability of a recently developed high throughput assay to detect antibodies against HDV.

Methods

All unique HBsAg-positive patients visiting the outpatient clinic between 2017 and 2019 were tested for HDV serology. Seropositive serum samples were further assessed by HDV RNA PCR and Sanger sequencing to identify the HDV genotype.

Results

This is the first report on HDV prevalence in the Netherlands. Out of 925 patients 3.7% tested seropositive for HDV, and HDV viremia was confirmed in 2.0%. The majority of patients (94%) had a migratory background and did not speak English or Dutch. We only detected HDV genotype 5 (N=3), and genotype 1 (N=15). Phylogenetic analysis demonstrated HDV1 clusters composed of sub-Saharan Africa isolates, central Asian, Turkish, Iranian and European isolates.

Conclusions

The prevalence of HDV infection in a tertiary center in the Netherlands was –with 2.0% among HBsAg-positive individuals – low and comparable to neighboring European countries. Patient are likely to have acquired HDV before migrating to Europe, as HDV genotypes matched with their country of origin. Screening risk-groups for HDV, with anti-HDV antibodies, was a reliable and fast screening tool that allows for improved patient care and prevention strategies.

P49

The effects of a virtual, real-time, antibiotic-team on antibiotic prescribing behaviour of elderly care physicians in eight Dutch nursing homes.

Md Mph Katja van Ewijk[1], MD MPH PHD C. Schneeberger[1,2], MD MSc PhD M.F. Schim van der Loeff[1,3], MD MSc F.M.H.P.A Koene[1,2]

[1]Public Health Service of Amsterdam (GGD Amsterdam), [2]Amsterdam University Medical Centre (AUMC), [3]Amsterdam University Medical Centre (AUMC)

Objectives: To assess the impact of a virtual, real-time, antibiotic-team (VAT) on the appropriateness of antibiotic prescribing behaviour of elderly care physicians, regarding urinary tract (UTI), respiratory tract (RTI), and skin and soft tissue infections (SSTI), in nursing homes residents. Design: Before-after trial. Intervention was the introduction of VAT consisting of a microbiologist, elderly care physician, and a pharmacist. Setting and participants: Eight nursing homes in Amsterdam, the Netherlands. Methods: The VAT was introduced to eight nursing homes on April 1, 2019 and continued for 11 months. Meetings were held via teleconferencing twice weekly. Patients were included when antibiotic treatment had been started. The VAT discussed and advised about treatment indication, antibiotic choice and necessary additional diagnostics to confirm diagnosis according to national guidelines. Data were retrospectively extracted from patient files on all infectious episodes for which antibiotics had been prescribed during the 12 months before (period I), and 11 months after the introduction of the VAT (period II). The appropriateness of antibiotic prescriptions was assessed using national guidelines and an algorithm developed for antimicrobial stewardship in nursing homes. Antibiotic prescription rates per 100 person-years (py) were estimated, and compared between periods I and II, expressed as an incidence rate ratio (IRR) with 95% confidence limits. The proportions of appropriately prescribed antibiotics were compared between the periods using the Chi-squared test, for all infectious periods combined and UTI, RTI and SSTI separately. Results: A total of 525 infectious episodes for which antibiotics were prescribed (between April 1, 2018 and March 1, 2020) were identified; 284 in period I and 240 in period II. Antibiotic prescription rates were 73 per 100 py for period I compared to 68 for period II. There was no significant decrease in overall prescription rates after introduction of the VAT compared to before (IRR 0.9; 0.95 CI% 0.8-1.1). Of all prescriptions during period I, 23.9% was assessed as appropriate, which increased to 40.3% after the introduction of the VAT ($p<0.001$).The percentage of appropriately prescribed antibiotics increased after introduction of the VAT regarding RTI (48.2% vs 12.5%; $p<0.001$) and SSTI (74.3% vs 47.5%; $p=0.02$). No significant difference in appropriately prescribed antibiotics was observed regarding UTI (28.6% vs 23.5%; $p=0.31$). After the introduction of the VAT, elderly care physicians performed bacterial cultures more often to support their antibiotic treatment (39.0% vs 27.5%; $p=0.005$) and consulted a clinical microbiologist (besides VAT meetings) more frequently during infectious episodes (24.0% vs 7.0%; $p<0.001$). Conclusion and implications: Implementation of VAT in nursing homes in Amsterdam, the Netherlands, was associated with significantly more appropriate antibiotic use overall, and for RTI and SSTI in particular. Improving prescribing behaviour of elderly care physicians regarding UTI might need extra strategies.

P51

Metagenomic profiles of Fecal microbiota derived vesicles in Crohn's disease patients reveal a potential role in pathogenesis

Nader Kameli[1,2], H.E.F Becker[1,3], Tessa Welbers[1], Professor D.M. Jonkers[3], Dr John Penders[1,4], Professor Paul Savelkoul[1,5], Frank Stassen[1]

[1]Maastricht University Medical Center+, [2]Faculty of Applied Medical Sciences, Jazan University, [3]Maastricht University Medical Center+, [4]VU University medical center, [5]Maastricht University

Background: Crohn's disease (CD) is a gastro-intestinal disorder characterized by a chronic inflammation with periods of exacerbation and remission. Gut microbiota dysbiosis is thought to play a crucial role in disease pathogenicity. In the current study, we aimed to investigate the role of membrane vesicles (MV), one of the main bacterial products in host-bacteria interaction, in CD pathogenesis by determine their microbiome composition in comparison to their corresponding bacterial origin in feces.
Methods: fecal samples from six healthy subjects (HC) and twelve CD patients (6 active disease, 6 in remission) were used in this study. Fecal bacteria membrane vesicles (fMV) were isolated by a combination of ultrafiltration and size exclusion chromatography. DNA was obtained from fMV fraction (MV-DNA), pellet of dissolved feces as bacterial-DNA (bDNA), and directly from feces as (fDNA). fMV were characterized by nanoparticles tracking analysis and cryo-electron microscope. Metagenomics profiling by sequencing 16s rDNA was conducted for assessing microbial co-diversity on DNA obtained from the three conditions.
Results: beta-diversity analysis revealed that microbiome compositions of MV-DNA are significantly different from those in fDNA or bDNA. While there are no differences between fDNA and bDNA. Microbial richness of MV-DNA showed a significant difference between HC and CD patients and also within CD patients. Profiling of fDNA and bDNA demonstrated that Firmicutes were the most dominant phyla, while in MV-DNA Bacteroidetes were most prominently present. Firmicutes and Proteobacteria belonging families and genera were significantly altered in the MV-DNA of CD patients. In particular, a reduction in Bifidobacteriaceae, Ruminococcaceae and Lactobacillaceae, and an increase in Enterobacteriaceae was noted in comparison to HC.
Conclusion: The microbial alterations of MVs in CD patients suggests a role for MVs in host-microbe interaction and CD pathogenesis. Future research is needed to unravel the exact role of MVs in disease initiation and/or progression.

P52

Bacterial agents in vulvovaginitis and vaginal discharge: a 10-year retrospective study in The Netherlands

Marjan J. Bruins[1], Dr C Oliveira dos Santos[1], Dr G.J.H.M. Ruijs[1]
[1]*Isala*

Introduction

Vulvovaginitis is a common problem in the GP's practice. A common cause is bacterial vaginosis (BV), others are Candida species infection and sexually transmitted infections (STIs). Usually GPs start treatment with metronidazole or antifungal drugs, based on physical and in speculo examination and possibly in-office diagnostic tests. Only if symptoms persist or recur, sometimes after several rounds of antimicrobials, a vaginal swab is sent in for culture and the detection of BV. Guidelines recommend culture only for identifying less susceptible Candida species. However, essential bacterial pathogens may escape diagnosis if culture is not or selectively performed. Until recently Nugent's scoring has been the gold standard for BV detection, but molecular methods have also been developed. These differ in targets and prices however, and are not available everywhere. We analysed retrospectively ten years of results of vaginal swabs processed at our laboratory, especially focussing on less frequently reported bacterial pathogens.

Methods

Microbiology results of vaginal samples sent in between January 2010 and December 2019 from GP patients with complaints of vulvovaginitis, comprising an abnormal non-bloody vaginal discharge, itch, irritation, redness or pain, were analysed. Repeat cultures from unique patients were included and considered to represent a new episode of vulvovaginitis if recurrent or persistent symptoms after treatment were reported or if the time between samples was at least one month. Patients with positive STI tests were excluded. Patients were divided into three groups according to age: paediatric/prepubescent (<12 years), premenopausal (12-51 years) and postmenopausal (>51 years). We calculated the proportions of isolated strains of Haemophilus influenzae, Streptococcus pneumoniae, beta-haemolytic streptococci groups A, C, F and G, Staphylococcus aureus and Candida species as well as of BV results.

Results

In total 16,381 cultures taken from 11,606 GP patients were processed, 14,508 discharge samples were tested for BV. The most frequently found agents of vulvovaginitis were Candida species and BV, with the highest prevalence in the group of premenopausal women. Haemophilus influenzae, streptococci and Staphylococcus aureus together were isolated in 5.5% of all cultures. A shift in pathogen proportions was seen between the age groups, with the highest percentages in children, a reduction in the age group of 12-51 years and an increase in postmenopausal women. Group A streptococci were frequently found in children (20.1%), much less in the age group of 12-51 years (1.0%) and in almost 10% of postmenopausal women.

Conclusion

Our findings show that if initial blind treatment of vulvovaginitis complaints fails, complete bacterial culture of vaginal discharge should be performed to detect all potentially pathogenic microorganisms. This way a faster and higher rate of successful treatment can be obtained and repeat visits to the GP and gynaecologist

can be avoided. For the detection of BV, molecular testing may seem attractive to process large numbers of tests and to obtain automated interpretations, but Nugent scoring still remains the fast and low-cost gold standard, which can be performed in any laboratory. We recommend incorporating the above insights into the next revision of guidelines on vaginal discharge.

P53

Selecting and using a minimal, defined microbiome to unravel functional role of gut microorganisms.

Pim Van Leeuwen[1], Dr MT Wortel[1], Drs. Ing. R. de Boer[1], Dr M. Lezzerini[1], Prof. Dr. S. Brul[1]
[1]*Universiteit Van Amsterdam*

The human gut microbiome houses a vast number of microorganism species. Every microorganism present contributes to the stability, metabolism, protection, and/or fermentation occurring throughout the lining of the human intestinal tract. Currently, big steps are being made in understanding the functional role of each member of the gut microbiome. Because of this large number of organisms, the models and systems quickly become too complex to analyze without the application of machine learning algorithms. To downscale the complexity of the problem, we propose working with a minimal gut microbiome comprised of specific organisms, chosen based on their known functional role within the human gut microbiome. By downscaling, we hope to eludicate the functional role of other microorganisms. From the available literature, different functional groups were first identified and then selected. To confirm the function of the microorganisms, a combination of in-vitro and in-silico approaches were used. First, the microorganisms were cultivated in mono-, co-, and tri-cultures. During growth, the metabolic products left in the medium were analyzed. Parallel to the in-vitro experiments, in-silico experiments using genome-scale models were used to design a minimal medium in which all microorganisms could grow. In order to cover as many functional niches as possible the microbiome model is upscaled to incorporate more species. The selected microbiome is then cultivated within different model situations, some of which containing human epithelial cell linings to mimic the conditions in the human gut. The cultures are followed using qPCR and selective plating. HPLC techniques, metabolomics, and proteomics can be used to track the metabolism of the minimal microbiome. The different interactions when species are added, subtracted or replaced can be studied and will yield us more information about the interactions found within the human gut, and ultimately help us to understand the functional role of these different microorganisms on a larger scale within the gut.

P54

Elevated mucosal antibody responses against SARS-CoV-2 are correlated with lower viral load and faster decrease in systemic COVID-19 symptoms

Janeri Fröberg[1,2], Joshua Gillard[1,3], R Philipsen[1,2,4], K Lanke[2], J Rust[1,2,4], D van Tuijl[1,2,4], Prof T Bousema[2], E Simonetti[1,2], C van der Gaast-de Jongh[1,2], M Bos[1,2], F.J. van Kuppeveld[5], B-J Bosch[5], N van der Geest-Blankert[6], C van Daal[6], prof. M Huynen[3], M de Jonge[1,2], Dr. D Diavatopoulos[1,2]

[1]Radboudumc institute for molecular life sciences, [2]Radboudumc, [3]Radboudumc, [4]Radboudumc, [5]Utrecht University, [6]Radboudumc

Introduction:
Transmission within households is an important contributor to the spread of SARS-CoV-2, as close contact within households facilitates early-onset pre-symptomatic transmission of the virus. Mucosal antibodies play a key role in protection against SARS-CoV-2 exposure, but their role during primary infection is not well understood.

Methods:
We assessed the timing, magnitude and complexity of mucosal antibody responses during primary infection with SARS-CoV-2 in 50 index cases and examined their relationship with viral load and clinical symptoms. Next to that, we looked at the infection rate in 137 household contacts and their number of symptoms, magnitude of viral load and production of SARS-CoV-2 antibodies.

Results & conclusion:
We observed the strongest increases in mucosal antibodies for IgM and IgG directed against S and RBD early after symptom onset, with elevated mucosal IgM levels associated with lower viral load. Increased RBD and S-specific mucosal antibodies correlated with decreases in systemic symptoms over the study period, while older age was associated with an increase in respiratory symptoms. Finally, we demonstrate that up to 42% of participating household contacts develop antibodies to SARS-CoV-2, including children, suggesting high transmission among household contacts. Child contacts were infected at a similar rate as adult contacts with similar viral load, but developed less symptoms compared to adults. Therefore the role of children in transmission might be underestimated.

P55

Local delivery of the antimicrobial peptide SAAP-148 from a 3D-printed degradable PLGA coating to prevent orthopaedic infections

Clara Guarch Pérez[1], D. Martínez-Pérez[2], Doctor M. Riool[1], Professor Doctor W. Święszkowski[2], Doctor S.A.J. Zaat[1]

[1]Amsterdam UMC, [2]Faculty of Materials Science and Engineering, Warsaw University of Technology

INTRODUCTION: Fracture fixation devices (FFD) have infection rates ranging from 5-10% for closed fractures and even higher rates up to 30% for open fractures1 causing devastating complications. Clinically, implant infections are prevented by systemic antibiotic prophylaxis, the placement of antibiotic laden bone cements, and the use of minimally invasive surgical procedures1. However, conventional biomaterials such as bone cement are compatible with a limited number of antibiotics and they show poor antibiotic release profiles which can lead to antimicrobial resistant development.
Cationic antimicrobial peptides (AMPs) have shown to successfully kill antimicrobial resistant bacteria. However, the application of AMPs in FFD is limited. Therefore, we studied the local incorporation of the AMP SAAP148 in poly-lactide-co-glycolic acid (PLGA) as a coating of titanium fixation plates made by powder-bed selective laser melting (SLM) technology. The coatings were applied by the droplet on demand (DOD) additive manufacturing technique. These devices will be tested in a in vivo femur fracture infection mouse model.
METHODS: The ink was prepared by dissolving PLGA (5kDa, 15kDa and 40kDa) and SAAP148 (10%w/w) in a mixture of organic solvents. Then, titanium coupons were coated (1, 2, 4 layers) by a DOD 3DPrinter (regenHU). The concentration of the peptide was measured by the µBCA assay and its antimicrobial activity was evaluated with the MIC/LC99.9% assays against Staphylococcus aureus JAR06.01.13. The cytotoxicity of the SAAP148 was also evaluated against the mouse cell lines RAW264.7 and pre-osteoblastic MC3T3-E1 with the WST-1 assay. On the other hand, the SLM printed plates were chemically polished and applied ex vivo in a mouse femur to confirm the dimensions.
RESULTS: The release assay showed a burst release of SAAP148 from the 15kDa and 40kDa PLGA containing coatings at the first 24h followed by a sustained release for the next 36days. 40kDa PLGA was selected for further development. Subsequently, one layer of coating and handmade showed the highest burst release of peptide within the first 24h and the antimicrobial activity of the eluates was confirmed by the LC99.9% assay. The MC3T3-E1 cell line did not have any reduction in cell proliferation after 48 and 72h. However, the RAW cells proliferation was reduced in the presence of the peptide after 48 and 72h.
DISCUSSION & CONCLUSIONS: SAAP148 has potent in vitro and in vivo bactericidal and antibiofilm forming activity2. The peptide was successfully incorporated in the 15kDa and 40kDa PLGA and in 40kDa by the DOD technique. The release kinetics of the peptide can be tailored based on the number of layers of coating. The antimicrobial activity of the constructs was confirmed. Further antimicrobial studies will be performed to evaluated in vitro and in vivo.
ACKNOWLEDGEMENTS: Research funded by PRINTAID (MARIE CURIE ETN No.722467). We thank prof. dr. P.S. Hiemstra, LUMC, Leiden, The Netherlands, for SAAP148.

REFERENCES:
1 Kavanagh, N., Ryan, E. J., Widaa, A., Sexton, G., Fennell, J., O'Rourke, S., .. & Kerrigan, S. W. (2018).
2 de Breij, A., Riool, M., Cordfunke, R. A., Malanovic, N., de Boer, L., Koning, R. I., ... & Kwakman, P. H. (2018).

P56

Implications of new EUCAST clinical breakpoints on clinical Aspergillus fumigatus isolates from the Netherlands

Jochem Buil[1,2], MC Tehupeiory-Kooreman[1,2], WJG Melchers[1,2], PE Verweij[1,2]
[1]Radboudumc, [2]Center of Expertise in Mycology Radboudumc/CWZ

Introduction: Azole resistance is associated with excess mortality in patients with invasive aspergillosis (IA) caused by Aspergillus fumigatus. Clinical breakpoints have been implemented by the EUCAST to guide antifungal therapy. Original classification involved resistant, susceptible and intermediate. The EUCAST decided to revise the intermediate category, introducing an "Area of Technical Uncertainty" (ATU). We set out to validate the revised azole ATU-breakpoints using a collection of clinical A. fumigatus isolates, that were sent to our mycology reference laboratory for susceptibility testing. This collection includes a large and diverse group of azole-resistant isolates and thus will help to determine if azole phenotypes are classified correctly.

Methods: Clinical A. fumigatus isolates received between January 2017 and December 2019. Screening for azole resistance was performed using a commercial agar-dilution method (VIPcheckTM, MediaProducts, Groningen, the Netherlands). In addition, a MIC test was performed using the EUCAST broth microdilution method for itraconazole (ITZ), voriconazole (VCZ), posaconazole (POS), and isavuconazole (ISA). For A. fumigatus isolates with a confirmed azole-resistant phenotype, the full Cyp51A gene was analyzed by PCR amplification and sequencing. All isolates were reclassified according to the revised breakpoints. Isolates with a POS MIC in the ATU were classified based on the ITZ susceptibility, while isolates with an ISA MIC in the ATU were classified based on the VCZ susceptibility. Furthermore, the dataset was analyzed using an additional rule for isolates with POS and ISA MICs in the ATU. In this analyses, the POS and ISA MICs in the ATU were classified based on both the VCZ and ITZ susceptibilities.

Results: A total of 2,274 clinical A. fumigatus isolates were included in the analysis. The highest resistance frequency was observed for ISA (701 isolates, 30.8%) and the lowest for VCZ (455 isolates, 20%). Overall, 396 (17.4%) susceptibility results of either ITZ, VCZ, POS or ISA resulted in MICs in the ATU, ranging between 216 (9.5%) isolates for VCZ and 49 (2.2%) isolates for ITZ. When the ATU rule was applied to isolates with POS MIC of 0.25 mg/l, 33 A. fumigatus isolates would be reported as POS S and 98 isolates as POS R. Among the 33 isolates classified as POS S, 16 showed a WT phenotype for the other azole drugs, while 17 (52%) did not. Of the 60 ISA ATU isolates 25 were classified as ISA S and 35 as ISA R. Among the ISA S isolates five (20%) showed non-WT azole phenotypes for other azoles. Reclassifying the isolates using the additional rule, 16 isolates would be reported as POS S and 115 isolates as POS R. Of the isolates classified as POS S, none showed non-WT azole phenotypes for the other azoles.

Conclusion: A large number of isolates in this dataset had a susceptibility result in the ATU. The classification of POS based on the ATU rules showed suboptimal performance as 52% of phenotypically non-wildtype isolates with POS MICs in the ATU were classified as POS S. The ATU rules could be improved by using additional rules to classify POS susceptibility.

P57

Do We Need to Change Catheter-Related Bloodstream Infection Surveillance in the Netherlands? - A Qualitative Study Among Infection Prevention Professionals

Msc. Janneke Verberk[1,2,3], PhD TII van der Kooi[2], MD, PhD LPG Derde[4], MD, PhD MJM Bonten[1,3], PhD SC de Greeff[2], MD, PhD MSM van Mourik[1]

[1]University Medical Centre Utrecht (UMCU), [2]National Institute for Public health and the Environment (RIVM), [3]University Medical Centre Utrecht (UMCU), [4]University Medical Centre Utrecht (UMCU)

Introduction

Catheter-related bloodstream infections (CRBSI) are a common healthcare-associated infection and therefore targeted by surveillance programs in many countries. Concerns, however, have been voiced regarding the reliability and construct validity of CRBSI surveillance in the Netherlands and the connection with the current diagnostic procedures. The aim of this study was to explore the experiences of Infection Control Practitioners (ICPs) and medical professionals with the current CRBSI surveillance and their suggestions for improvement.

Methods

In April - July 2019, focus group discussions (FGDs) and semi-structured interviews were conducted in order to gain insight in health professionals' experiences with the current Dutch CRBSI surveillance and their suggestions for improvements. There were two types of FGDs: 1) three FGDs involving ICPs from 19 different hospitals, discussing experiences with the CRBSI surveillance and their suggestions for improvement, and 2) one FGD with 9 medical professionals, concentrating on the infection entity, relevant patient groups and suggestions for future surveillance activities. FGDs were organized until no new themes were elicited. Thereafter, semi-structured interviews with ICPs from two other hospitals were held, where the interviewer investigated whether the points raised in the FGDs were recognized and confirmed by the interviewees. Data were audio recorded and transcribed verbatim. Analyses were performed using thematic analysis.

Results

Main themes derived from experiences with current surveillance were 1) ICPs' doubt regarding the yield of surveillance given the low incidence of CRBSI, the high workload and IT problems; 2) the experienced lack of leadership and responsibility for recording information needed for surveillance; and 3) difficulties with applying and interpreting the CRBSI definition. Suggestions were made to simplify the surveillance protocol, expand the follow-up and surveillance to homecare settings, simplify the definition and customize it for specific patient groups. Participants reported hoping for and counting on automatization solutions to support future surveillance.

Conclusions

This study reveals several problems with the feasibility and acceptance of the current CRBSI surveillance and proposes suggestions for improvement. This provides valuable input for future surveillance activities, thereby taking into account automation possibilities.

P58

An unusual outbreak of community-onset impetigo by Methicillin-resistant Staphylococcus aureus resistant to fusidic acid with increased virulence

Drs. Karuna Vendrik[1], EJ Kuijper[1], E. Denie[2], W. Silvis[3], M. Dimmendaal[4], A. de Boer[5], B. Postma[2], S. Bantjes[1], AF Schoffelen[1], H. Ruijs[1], F. Koene[6], M. Petrignani[6], LM Schouls[1], DW Notermans[1]
[1]National Institute for Public Health and the Environment, [2]Slingeland hospital, [3]Laboratorium Microbiology Twente/Achterhoek, [4]Municipal Health Service North and East Gelderland, [5]Praktijk voor huisartsenzorg Whemerhof, [6]Public Health Service of Amsterdam

Introduction
Methicillin-resistant Staphylococcus aureus (MRSA) is a well-known resistant pathogen in the hospital-setting. However, strains in the community are emerging, occasionally leading to outbreaks. The Netherlands has a low MRSA prevalence. Dutch medical microbiology laboratories send the majority of MRSA isolates from patients to the RIVM for the national MRSA surveillance. Isolates are then typed using multiple locus variable number of tandem repeat analysis (MLVA) and for a subset next-generation sequencing (NGS).

Methods
Between July and October 2019, an outbreak of impetigo with MRSA not responding to topical fusidic acid (first-line treatment) occurred, mainly localised in two towns from the eastern part of the Netherlands (outbreak region) with an epidemiological link to three cases from another region. NGS data was used for whole-genome multi-locus sequence typing (wgMLST) and to identify resistance genes and virulence factors. The rapid increase of cases led to collaboration between general practitioners, medical microbiologists, the Public Health Institute and municipal health services to stop the outbreak. General practitioners in the outbreak region were requested to submit samples for culture in case of no response to fusidic acid and treatment recommendations were altered. To obtain insight into the extent of the outbreak, three general practitioners from the outbreak region and eight from a nearby control region filled in a questionnaire. The number of prescriptions for mupirocin were requested from pharmacists from both regions.

Results
Typing of MRSA isolates send in for the national surveillance showed isolates with MLVA type MT4627 and the fusC gene, encoding fusidic acid resistance. This MLVA type was observed in only three earlier samples in the MRSA surveillance database. Furthermore, isolates were negative for Panton-Valentine leucocidin (PVL), but positive for exfoliative toxin genes eta and etb and virulence factor edinC. The national surveillance identified 55 cases with MLVA type MT4627, including 46 children. The median age was 6 and 44% was female. Among all cases, 84% had an infection with this MRSA, all skin lesions. All but one were community-onset. General practitioners from the outbreak and control region noticed an increase in impetigo unresponsive to fusidic acid, but the control region did not send samples for culture. Mupirocin prescriptions were increased. Besides fusidic acid, the isolates were resistant to beta-lactams, erythromycin, clindamycin and co-trimoxazole. The minimum spanning tree based on wgMLST results of 28 isolates showed clustering of 19 isolates from the outbreak region, including one from 2018, and three from another region. Six isolates from other regions differed slightly from the cluster. Two of these cases had recently visited Morocco and two others the Maldives. The combination of exfoliative toxins was not present in 3,800 sequenced isolates in our collection. Comparison to 460 genomes from an international database

showed a difference of 482 alleles between Dutch isolates and the nearest isolate in wgMLST. As of November 2019, only four new cases have occurred (n=2 from the outbreak region).

Conclusion

An outbreak with community-onset fusidic acid-resistant MRSA occurred with the uncommon MLVA-type MT4627 harbouring exfoliative toxin genes. Most cases were children with impetigo.

P59

The power and pitfalls of clustering: conserved developmental trajectories of the cecal microbiota in broiler chickens

Dr. Jannigje Kers[1,2], dr. FC Velkers[2], dr. EAJ Fischer[2], prof. JA Stegeman[2], prof. H Smidt[1]
[1]*Wageningen University and Research,* [2]*Utrecht University*

Introduction: There is a great interest in identifying gut microbiota developmental patterns to help identify strategies, for instance, to improve health and performance of broiler chickens. Cluster analyses are unsupervised learning methods that group data based on similarity. Clustering helps to simplify the complex and highly dynamic ecosystem of the intestinal microbiota. Previous literature, however, showed that outcomes of cluster analysis are highly dependent on the applied clustering algorithms. This study aimed to identify clusters with different clustering methods to increase insight into intestinal microbiota development of broilers.
Methods: A longitudinal observational study was performed. We followed ten well-performing Ross 308 broiler flocks on four different farms with a history of good production performance. From each flock, cecal content of nine broilers was collected across 10 different days between 0 and 35 days post-hatch. The cecal microbiota composition of 557 individual broilers was analyzed using 16S ribosomal RNA gene amplicon sequencing. Clustering was performed according to the partitioning around medoid (PAM)-based method using amplicon sequence variants and Jensen-Shannon divergence (PAM-JSD), Bray-Curtis dissimilarity (PAM-BC), unweighted UniFrac (PAM-UF), or weighted UniFrac (PAM-WUF). The optimal number of clusters was calculated using prediction strength, average silhouette index, Calinski–Harabasz index, and Laplace approximation. In addition, Dirichlet Multinomial Mixtures (DMM), a probabilistic model, was applied. All cluster analyses were also stratified by the age of the broiler, to identify potential clusters within an age group, across different flocks.
Results: All cluster algorithms showed support for two clusters, one of which was dominated by 7-day-old broilers, and one by 35-day-old broilers. Fourteen-day-old broilers were divided across both clusters. These two clusters can be defined as community types associated with different stages along the developmental trajectory of cecal microbiota. The predominant family in cluster 1 was Lachnospiraceae and in cluster 2 Ruminococcaceae. Despite the treatment of two flocks with antibiotics, these flocks did not show different clustering compared to untreated flocks. The stratified analyses of broilers of the same age showed that different clustering algorithms resulted in a variety of optimal cluster structures. This suggests a lack of age-specified clusters within 7-, 14- or 35-day-old broilers with the number of samples included in this study.
Conclusion: 1) This is the first study that showed conserved patterns of cecal microbiota development in different commercial broiler flocks. 2) In general, identifying clusters is sensitive to the applied algorithms, however, this might also be a matter of sample size. 3) Further investigation of mechanisms underlying microbiota development and function is required for improved management, diagnostics, and nutritional interventions.

P60

SARS-CoV-2 antigen testing in health care workers: a useful tool to prevent transmission in health care facilities

Dr. M.A. Fliss[1], Dr. X.W. Zhou[1], Dr. L. Rurenga-Gard[1], Prof.Dr. A.W. Friedrich[1], Prof.Dr. H.G.M. Niesters[1], Dr. C. Van Leer-Buter[1], Dr. Marjolein Knoester[1]
[1]*University Medical Center Groningen*

Introduction: Testing health care workers (HCW) with a risk factor of having a SARS-CoV-2 infection by antigen lateral flow assay before they start their workshift, may favour early detection of potentially contagious SARS-CoV-2 positive individuals. We present the sensitivity and specificity after ten weeks of antigen testing.

Methods: Since November 8, 2020, we have routinely tested HCW with symptoms compatible with a SARS-CoV-2 infection and HCW without symptoms but with a close COVID-19 contact. The Panbio COVID-19 Ag RAPID TEST DEVICE (Abbott) was used.
The symptomatic group was tested once, the asymptomatic group was tested repeatedly until ten days after last exposure. In each case, two swabs were taken, one for antigen testing at the test facility and one for polymerase chain reaction (PCR) in the Microbiology laboratory.

Results: 5599 antigen tests were taken between November 8, 2020 and January 14, 2020. Of those, 76 were positive in the antigentest, 5512 were negative and 11 were indeterminate. Of those 11 indeterminate results, 1 was found to be positive by PCR, 9 negative and 1 indeterminate. All 76 positive antigentest results were confirmed by PCR (specificity 100%). Of 5512 negative antigentest results, 5429 were confirmed to be negative by PCR, 47 were indeterminate and 36 were PCR positive. In other words, of 112 PCR positive results, 36 were found negative by the antigentest (sensitivity 68%). Looking deeper into the false negative results, 5 were missed with cycle threshold (Ct)-value <28 (sensitivity 94%), 11 were missed with Ct-value <30 (sensitivity 87%).
The majority of true-positive HCWs were symptomatic: 48/76 (63%); 23/76 (30%) underwent testing because of a close contact, but reported (very) mild complaints when they were tested positive; 5/76 (7%) were asymptomatic with a close contact. Of the false-negative test results, 15/36 were follow-up samples after a previously diagnosed infection. The 21 HCWs with a false-negative primary test were symptomatic in 5 (24%); 7 (33%) had (very) mild complaints when tested positive and 9 (43%) were asymptomatic with a close contact. From these data a sensitivity of 64% can be calculated for a-/oligosymptomatic close contacts (all Ct-values).

Conclusion: By using the combined test strategy of direct antigen testing followed by PCR among HCWs with a risk factor for SARS-CoV-2 infection, HCW could be prevented from starting their working shift while potentially being contagious. The PCR result at the end of the day provides a more sensitive result by which early stages of infection can be detected in asymptomatic HCWs with a close COVID-19 contact.

P61

A genetic cluster of multidrug-resistant Enterobacter cloacae complex ST78 harboring a plasmid containing blaVIM-1 and mcr-9 in the Netherlands

Dr. Antoni Hendrickx[1], Dr. Sylvia Debast[2], María Pérez-Vázquez[3], A. F. Schoffelen[1], D. W. Notermans[1], F. Landman[1], C. C.H. Wielders[1], J. E. Cañada Garcia[3], J. Flipse[2], A. de Haan[1], S. Witteveen[1], M. van Santen-Verheuvel[1], S. C. de Greeff[1], Ed Kuijper[1], L. M. Schouls[1], Dutch CPE surveillance Study Group[1]
[1]National Institute for Public Health and the Environment (RIVM), [2]Isala, [3]Centro Nacional de Microbiología, Instituto de Salud Carlos III

Introduction. Carbapenemases produced by Enterobacterales are often encoded by genes on transferable plasmids and represent a major health care problem, especially if the plasmids contain additional antibiotic resistance genes. As part of the Dutch national surveillance, medical microbiological laboratories submit Enterobacterales isolates suspected of carbapenemase production to the National Institute for Public Health and the Environment for characterization. In this study, the objective was to analyze the molecular characteristics of a genetic cluster of Enterobacter cloacae complex isolates collected in the period between 2015-2020.

Methods. Short-read and long-read sequencing enabled multi-locus sequence typing (MLST), pan-genome MLST (pgMLST) and chromosome and plasmid reconstructions. The pgMLST was based on an in-house E. cloacae complex scheme comprising 9,829 genes. Resistome and replicome analyses were performed using ResFinder and PlasmidFinder software. The Etest for meropenem and broth microdilution test for colistin were used to determine antimicrobial susceptibility. The carbapenem inactivation method was used to assess carbapenemase production.

Results. The pgMLST revealed that nine carbapenemase-producing E. cloacae complex isolates from three different hospitals differed less than 20 alleles and formed a genetic cluster termed EclCluster-013. Seven isolates were submitted by one hospital in the period of 2016-2020. In six patients cultures were taken for the purpose of screening upon hospital admission and three isolates were retrieved from patients with either a presumed respiratory tract infection or catheter-associated urinary tract infection. Two patients from two hospitals had been admitted to a hospital in Benidorm, Spain within two months prior to sampling. EclCluster-013 isolates belonged to MLST ST78, a globally disseminated lineage and harbored a 316 kb IncH12 plasmid which carried both the blaVIM-1 carbapenemase gene and the novel mcr-9 gene presumed to induce colistin resistance. The plasmid carried a mobile genetic element comprising qseB-qseC-wbuC-mcr-9 flanked by IS1R and IS5 insertion elements. The qseB-qseC genes encode a two-component system implicated in mcr-9 induction. The plasmid also contained genes encoding resistance other classes of antibiotics, including aminoglycosides, beta-lactams, fluoroquinolones, macrolides, sulphonamide, and streptogramin B. Automated antimicrobial susceptibility testing showed that EclCluster-013 isolates were multidrug-resistant, but susceptible for meropenem (<2 mg/L) and colistin (<2 mg/mL). To investigate a possible connection with Spain, two blaVIM-1/mcr-9 containing E. cloacae complex isolates retrieved from the autonomous community of Valencia were subjected to pgMLST, which demonstrated that they were unrelated to EclCluster-013. In addition, the size and composition of the blaVIM-1/mcr-9 plasmid in the isolates from Spain was different from the plasmid in the Dutch isolates.
Conclusion. The EclCluster-013 reported here represents a multidrug-resistant ST78 E. cloacae complex strain containing an IncH12 plasmid carrying both the blaVIM-1 carbapenemase and the mcr-9 colistin resistance gene in addition to other antibiotic resistance genes. Although two of the patients had been

admitted in a Spanish hospital, blaVIM-1/mcr-9 isolates from Spain and the Netherlands differed considerably, failing to confirm transmission. None of the isolates carrying a mcr-9 gene was resistant to colistin, suggesting that mcr-9 is not induced under laboratory conditions or that the modification of lipopolysaccharide by Mcr-9 does not lead to colistin resistance.

P62

Changes in demographics and AMR in the Dutch national antimicrobial resistance surveillance system (ISIS-AR) during the first wave of COVID-19

Md, Phd Annelot Schoffelen[1], PhD, Ir S.C. de Greeff[1], Prof, PhD, MD E.J. Kuijper[1], PhD, MD D.W. Notermans[1], PhD, MD A.F. Schoffelen[1], on behalf of the ISIS-AR study group
[1]*National Institute for Public Health and the Environment (RIVM)*

Introduction
The on-going coronavirus disease (COVID-19) pandemic, has resulted in profound changes in healthcare provided, with delay of many non-urgent hospital procedures and in the demographic characteristics of patients. These changes may affect the occurrence and the surveillance of antimicrobial resistance (AMR). In this study we investigated the AMR surveillance data in hospitalized patients during the first wave of the COVID-19 pandemic (1st wave, defined as 1 March to 1 July 2020), compared to the pre-COVID-19 period (defined as 1 October 2019 to 29 January 2020).

Methods
We used data from 21 medical microbiological laboratories (MMLs) with complete data during the total study period in the Dutch national surveillance system for AMR (ISIS-AR), which is based on data from routine antimicrobial susceptibility testing (AST) in MMLs. We compared the following demographic characteristics between time periods: the absolute number of patients with at least one isolate in the database, type of hospital department (ICU vs. non-ICU), patient's age and sex, and sample material. Additionally, we compared the proportion of 5 highly resistant microorganisms (HRMOs) between time periods; extended spectrum beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae, carbapenemase producing Enterobacterales (CPE), carbapenemase producing Pseudomonas aeruginosa (CPPA), multidrug-resistant P. aeruginosa, and methicilline resistant Staphylococcus aureus (MRSA).

Results
The monthly absolute number of patients with at least one isolate in the database was lower during the 1st wave, with a maximum reduction in April to 17,429, which was 81% of the mean monthly number of 21,494 pre-COVID-19. The proportion of isolates from ICUs increased from 8% (95%CI: 8-8%) pre-COVID-19 to 10% (10-10%) 1st wave. In ICUs, relatively more isolates were sampled from blood, (35% [33-37%] 1st wave versus 27% [25-29%] pre-COVID-19). Isolates from the ICUs were more often from male patients (66% [64-68%] versus 59% [57-61%]) and from the age categories 40-59 years (25% [23-27%] versus 20% [18-21%]) and 60-79 years (56% [54-58%] versus 51% [49-53%]).
The prevalence of all HRMOs in non-ICU departments was similar between both time periods. In the ICU, the proportion of ESBL in blood isolates increased, although not significant: from 15% (10-22%) pre-COVID-19 to 20% (12-32%) 1st wave. In data from all materials from the ICU, we found a non-significant rise in the proportion MRSA from 2% (1-4%) to 4% (2-7%). This increase did not evidently result from higher proportions of MRSA isolates in one or more specific regions in the Netherlands.

Conclusion
In ISIS-AR, the number of in-hospital patients with routine positive cultures with AST was lower during the 1st wave of the COVID-19 pandemic compared to the pre-COVID-19 period and we found demographic changes that were in line with the shifts in the patient population in Dutch hospitals. In the coming months we will further investigate the preliminary finding of the non-significant increase in proportion ESBL and MRSA in the ICU.

P63

Systematic screening for COVID-19 associated invasive aspergillosis in ICU patients; results and challenges

Drs Rebecca van Grootveld[1], Drs J van Paassen[2], Dr MGJ de Boer[3], Dr ECJ Claas[1], Prof Dr EJ Kuijper[1], Dr MT van der Beek[1]

[1]Leiden University Medical Center, [2]Leiden University Medical Center, [3]Leiden University Medical Center

Introduction

A high prevalence of COVID-19 associated pulmonary aspergillosis (CAPA) has been reported, though histopathological evidence is frequently lacking. To assess the clinical significance of Aspergillus species in respiratory samples of mechanically ventilated COVID-19 patients, routine screening for Aspergillus in tracheal aspirate (TA) was implemented.

Methods

From all adult COVID-19 patients admitted to the intensive care unit (ICU), TA samples were collected twice a week for Aspergillus screening by PCR and or culture. Bronchoalveolar lavage (BAL) sampling was performed in patients with a positive screening result if possible. Serum and BAL galactomannan (GM) were not performed routinely. Clinical information was obtained from the electronic patient record, and patients were categorised according to the recently published case definition for CAPA (Koehler et al. Lancet ID 2020). Non-bronchoscopic lavage was not performed so patients could not be classified as possible CAPA.

Results

Our study population consisted of 63 predominantly male patients, with a median age of 62 years and median ICU stay of 18 days. Aspergillus species were present in TA screening samples from 15 (24%) patients. Probable CAPA was diagnosed in 11 (17%) patients, and included four patients who were only GM positive in BAL and negative in screening. Serum GM was never positive and tracheobronchitis was not observed during bronchoscopy. Three of four patients with only a positive GM in BAL without other positive Aspergillus diagnostics were not treated as were two other patients; one positive in screening but only GM positive in BAL who died on the same day the GM result was available and one patient who was BAL PCR positive and who was improving clinically with no suspicion of invasive aspergillosis (IA). Six patients with probable CAPA were treated with antifungals and four died. Of the five patients who weren't treated with antifungals three died. One autopsy report was available from a patient with probable CAPA who was only BAL GM positive, but no histological evidence of IA was described. Concordance between TA and BAL for PCR and culture was 86%, and all TA culture-positives were confirmed in BAL. Treatment was withheld in three of fifteen patients with positive screening (20%) but negative BAL culture and PCR results.

Conclusion

Whether CAPA is a clinical entity that requires routine screening, invasive diagnostics and treatment is yet unknown. Positive culture, molecular detection and or antigen detection of Aspergillus species, do not equal infection and should not always prompt treatment with antifungals. However, mortality in patients with positive findings is high and establishing a causative role for Aspergillus is difficult. Our findings emphasize that histopathological studies are urgently needed as well as more information on the precise diagnostic performance of serum and BAL GM in this setting. Until the clinical relevance of Aspergillus species detected in respiratory samples of COVID-19 patients is better understood, minimal invasive screening by TA is a feasible method to monitor patients. Positive screening results should be an indication to perform a BAL to rule out upper airway colonization.

P64

Preclinical models of depression and the microbiome

Msc Tineke Banda[1,2,3,4], MD A. Lok[2,3], MSc P. van Leeuwen[1,3,4], BSc C. Maes[1,4], PhD M. Lezzerini[1,4], PhD A. Korosi[1,5], PhD S. Brul[1,2,4]

[1]Universiteit Van Amsterdam, [2]Urban Mental Health centre, [3]Amsterdam UMC, [4]Molecular Biology and Microbial Food Safety group, [5]Structural and Functional Plasticity of the Nervous System

The role of the gut microbiome in human health has received increasing attention recently. One major area focus is the reciprocal link between the microbiome and the brain, known as the Gut-Brain Axis (GBA). The GBA could potentially explain how environmental factors impact mental health. Additionally, it offers promising pathways for non-invasive, therapeutic intervention. Major Depressive Disorder (MDD) is the most common psychiatric disorder. Its heterogeneous presentation makes it difficult to unravel its disease mechanism and to treat MDD. Here, we try to elucidate the role of the microbiome in MDD. We use several preclinical models to do so, including a mouse model. We perform an exploratory analysis of faecal samples of mice who underwent the Early Life Stress (ELS) paradigm. Next, we use a minimal microbiome model to modulate the production of relevant metabolites. Through the use of this controlled model microbial consortia, the exact production of these metabolites can be charted. We look at the production of butyrate in particular, as this short-chain fatty acid (SCFA) has been shown to have multiple beneficial effects on health in general. Furthermore, butyrate is hypothesized to positively affect mental health. Therefore, these preclinical studies can both elucidate molecular mechanisms and yield clinically relevant therapeutic agents.

P65

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

Elias Vera[1], Ms Y Mora[1], Ms N. Monge[2], Ms A. Revtova[1], Mr JM van Dijl[1], Mr G Buist[1]
[1]Universitair Medish Centrum Groningen, [2]University of Bath

Key words

Bovine Mastitis, Staphylococcus aureus, Whole Genome Sequence, Bacterial Surface Proteins, Antigen

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

Methods
A combination of bioinformatics tools and immunological approaches was used to identify conserved immunogenic determinants of S. aureus mastitis isolates with special focus on cell surface-exposed proteins. Conserved potential immunogenic epitopes were selected using antigenicity plots in the CLC workbench. Further the B cell epitope prediction tool (Bepipre) was used to compare those conserved predicted epitopes with the epitopes that showed conserved antigenicity. Several selected proteins or their domains were purified and their antigenicity was tested with sera from infected cows to evaluate their immunogenic potential.

Results
By investigating the genome sequences of 63 S. aureus mastitis isolates, 24 conserved surface proteins were identified. Using the CLC pipeline and Bepipre, the respective sequences were aligned and, based on antigenicity plots and surface accessibility, conserved epitopes were identified in the selected surface proteins. Further, immunological assays using sera from twelve cows with mastitis revealed IgG responses against the cell surface located cell wall hydrolases Aly and Sle1 and their separately expressed subdomains.

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

P66

Performance of fungal diagnostics for the diagnosis of COVID-19 associated invasive pulmonary aspergillosis

Mehmet Ergün[1,2], R.J.M. Brüggemann[1,3], A. Alanio[4], S. Delliere[4], A. van Arkel[5], R.G. Bentvelsen[5,6], T. Rijpstra[7], S. van der Sar[8], K. Lagrou[9], N.A.F. Janssen[1,10], J.B. Buil[1,2], K. van Dijk[11], W.J.G. Melchers[1,2], M.H.E. Reijers[1,12], J.A. Schouten[13,14], J. Wauters[15], L.P. White[16], F.L. van de Veerdonk[1,10], P.E. Verweij[1,2]

[1]Radboud University Medical Centre, [2]Radboud University Medical Centre, [3]Radboud University Medical Centre, [4]Hôpital Saint-Louis, [5]Amphia Hospital, [6]Leiden University Medical Centre, [7]Amphia Hospital, [8]Amphia Hospital, [9]University Hospitals Leuven, [10]Radboud University Medical Centre, [11]Amsterdam University Medical Centre, [12]Radboud University Medical Centre, [13]Radboud University Medical Centre, [14]Radboud University Medical Centre, [15]University Hospitals Leuven, [16]University Hospital of Wales

Introduction

Invasive pulmonary aspergillosis (IPA) is increasing encountered as secondary infection in patients with severe viral pneumonia. Numerous cohort studies reported COVID-19 associated pulmonary aspergillosis (CAPA) in critically-ill COVID-19 patients (1-5). These studies have used various CAPA case definitions and diagnosing CAPA has been challenging due to low sensitivity of serum biomarkers and reluctance to perform bronchoscopy (6). We investigated the performance of Aspergillus diagnostic tests in various respiratory specimens obtained from patients with severe COVID-19, using the recently published 2020 ECMM/ISHAM CAPA case definition (7).

Methods

During the first corona wave in 2020, 219 critically ill patients with COVID-19 were prospectively included from the Netherlands, Belgium, France, and the United Kingdom. Patients were classified, and colonisation of the airway was considered when Aspergillus culture was only positive from respiratory tract specimens from the upper airways (e.g., sputum and bronchial aspirate). Diagnostic tests included Aspergillus culture, galactomannan (GM), (1,3)-beta-D-glucan (BDG) and Aspergillus polymerase chain reaction (PCR). For determination of performance characteristics of the diagnostic tests, proven and probable CAPA were considered true infection and possible CAPA, Aspergillus colonised, and no evidence of CAPA (including patients only positive for serum BDG) as not infected.

Results

Classification resulted in one proven CAPA case, 36 (16.4%) probable CAPA cases and 19 (8.7%) possible CAPA cases. Twenty-one (9.6%) patients were considered with Aspergillus, and in 142 (63.5%) patients there was no evidence of CAPA, including eight patients with positive serum BDG but no other evidence for Aspergillus.

The sensitivity of Aspergillus culture, GM, and Aspergillus PCR in BAL was 43.5%, 60.9%, and 47.6%, respectively, and in NBL the sensitivity was 50.0%, 51.5%, and 100%, respectively. For Aspergillus culture in bronchial aspirate and sputum, the sensitivity was 60.0% and 33.3%, respectively. In serum, the sensitivity of GM, BDG, and Aspergillus PCR was 14.6%, 35.7%, and 38.9%, respectively.

The 30-day mortality in 37 patients with proven or probable CAPA was 51.4% compared to 26.4% in 182 patients with possible CAPA, Aspergillus colonised, or no evidence of CAPA (odds ratio (OR) 2.870, p=0.003). Comparing proven and probable CAPA patients with and without positive serum biomarkers, the 30-day mortality in seven serum GM positive patients was 85.7% compared to 37.5% in 24 serum GM negative patients (OR 10.000, p=0.047), and in nine serum BDG positive patients the 30-day mortality was 88.9% compared to 36.8% in 19 serum BDG negative patients (OR 13.714, p=0.024).

Conclusion

BAL GM showed the best performance in critically ill COVID-19 patients suspected of CAPA. Interestingly, the performance of NBL GM was similar to that of BAL GM and may support the use of NBL for diagnosing CAPA. The low sensitivity of serum GM and BDG may indicate that angioinvasion is not common in critically ill patients with CAPA. However, 30-day mortality was significantly higher in CAPA patients with positive serum biomarkers GM and BDG, which indicates more severe invasive disease in these patients. Overall, histopathological studies are required to understand the pathophysiology of CAPA and to better validate our diagnostic approach.

P67

COVID-19-associated pulmonary aspergillosis (CAPA): A multicentre, observational cohort study

Drs. Nico Janssen[1,2], MSc C Jacobs[3], MD M Ergün[1,4], MD, PhD K van Dijk[5], MD, PhD BJA Rijnders[6], MD, PhD JA Schouten[7], MD JB Buil[1,4], MD, PhD M Bourgeois[8], MD, PhD N van Regenmortel[9], MD, PhD P Lormans[10], MD, PhD R Nyga[11], MD, PhD F Tamion[12], MD, PhD S Nseir[13,14], PharmD, PhD RJM Brüggemann[1,15], MD, PhD FL van de Veerdonk[1,2], MD, PhD J Wauters[3,16], MD, PhD PE Verweij[1,4]

[1]Radboud University Medical Center, [2]Radboud University Medical Center, [3]University Hospitals Leuven, [4]Radboud University Medical Center, [5]Amsterdam University Medical Center, location VUmc, [6]Erasmus MC University Medical Center, [7]Radboud University Medical Center, [8]AZ Sint-Jan Brugge-Oostende, [9]ZNA Stuivenberg, [10]AZ Delta Hospital, [11]Amiens University Hospital, [12]Rouen University Hospital, [13]Lille University Hospital, [14]University of Lille, [15]Radboud University Medical Center, [16]KU Leuven

Introduction

Soon after the outbreak of the coronavirus disease-2019 (COVID-19) pandemic, concerns were raised about invasive pulmonary aspergillosis (IPA) occurring in patients with severe COVID-19 admitted to the intensive care unit (ICU), in analogy to influenza-associated pulmonary aspergillosis. Studies published so far have employed different patient populations and case definition criteria in relatively small cohorts. We set out to investigate the incidence of COVID-19-associated pulmonary aspergillosis (CAPA), its impact on patient outcomes and its potential risk factors.

Methods

A partially prospective/partially retrospective observational cohort study was performed during the first wave of the COVID-19 epidemic in one cohort in eight Dutch and Belgian ICUs (discovery cohort) and one cohort in three French ICUs (validation cohort). CAPA was defined according to the European Confederation of Medical Mycology and International Society for Human and Animal Mycology (ECMM/ISHAM) CAPA classification.

Results

In the discovery cohort, 519 patients were included and 279/519 (53.8%) were classifiable according to the ECMM/ISHAM criteria. The remaining 240 could not be classified as necessary diagnostic tests and procedures had not been performed. Ultimately, 42/279 (15.1%) patients were classified as suffering from CAPA (6/279 [2.2%] proven CAPA, 32/279 [11.5%] probable CAPA and 4/279 [1.4%] possible CAPA). Presence of any European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) host factor was not significantly different between patients with CAPA and those with CAPA excluded (13/39 [33.3%] versus 31/166 [18.7%], p = 0.053), nor was systemic corticosteroid use before ICU admission (7/39 [17.9%] versus 14/160 [8.8%], p = 0.141). However, in the CAPA versus the CAPA excluded group, use of other T or B cell immunosuppressants (7/42 [16.7%] versus 12/233 [5.2%], p = 0.014), chronic obstructive pulmonary disease (COPD; 8/42 [19.0%] versus 19/237 [8.0%], p = 0.042) and HIV/AIDS (3/42 [7.1%] versus 1/237 [0.4%], p = 0.011) were more prevalent. In-ICU mortality was significantly higher in those with CAPA versus those with CAPA excluded (22/42 [52.4%] versus 81/237 [34.2%]). After correction for covariates, CAPA was not independently associated with in-ICU death (adjusted odds ratio [aOR] 2.03; 95% confidence interval [95% CI] 0.94–4.37). In the validation cohort, 304 patients were included and 209/304 (68.8%) were classifiable. Ultimately, 21/209 (10.0%) were classified as probable CAPA (2 with tracheobronchitis, 19 with pulmonary CAPA). Here, no significant differences between the CAPA and CAPA excluded groups were found regarding presence of any EORTC/MSGERC host factor, COPD or HIV/AIDS, but bronchiectasis was more frequent (2/21 [9.5%]

versus 1/188 [0.5%], p = 0.027). In-ICU mortality in the CAPA group was 9/21 (42.9%) versus 46/185 (24.9%), but did not reach statistical significance. Here, too, CAPA was not independently associated with in-ICU death (aOR 1.53; 95% CI 0.51–4.55).

Conclusion

1. CAPA affects 10.0–15.1% of classifiable patients with COVID-19 admitted to ICU.
2. COPD, HIV/AIDS, bronchiectasis and use of immunosuppressants other than corticosteroids are potentially risk factors for the development of CAPA.
3. CAPA is associated with high in-ICU mortality, although differences were not significant in the validation cohort.

P68

Intestinal microbiota composition and diversity are not associated with treatment response in metastatic colorectal cancer patients treated with capecitabine chemotherapy

Janine Ziemons[1,2], J. Ziemons[1,2], J. de Vos-Geelen[1,3], Dr. L. Valkenburg-van Iersel[1,3], A.C.L. Wildeboer[1,2], A. Vievermans[2], Dr. G.J. Creemers[4], Dr. A. Baars[5], Dr. J.H.M.J. Vestjens[6], N.G. Le[7], D.J.M. Barnett[7,8], Dr. S.S. Rensen[2,9], Dr. J. Penders[7,9], Prof. Dr. M.L. Smidt[1,2]

[1]Maastricht University, [2]Maastricht University Medical Centre, [3]Maastricht University Medical Centre, [4]Catharina Hospital, [5]Hospital Gelderse Vallei, [6]VieCuri Medical Centre, [7]Maastricht University Medical Centre, [8]Maastricht University, [9]Maastricht University

Introduction

Capecitabine is the standard systemic therapy for metastatic colorectal cancer (CRC). Previous pre-clinical research indicated that the intestinal microbiota is able to potentiate anti-tumour efficacy of capecitabine and that capecitabine treatment impacts intestinal microbiota composition and diversity. This study aims to explore changes in intestinal microbiota composition during capecitabine treatment and its association to treatment response.

Methods

Patients with metastatic CRC treated with capecitabine were prospectively enrolled in a multicentre cohort study in the Netherlands. Patients collected a faecal sample and completed a questionnaire before, during and after three cycles capecitabine. Several clinical characteristics, including tumour response and toxicity were recorded. Intestinal microbiota composition and diversity were analysed by amplicon sequencing of the 16S rRNA V4 gene-region.

Results

In total, 33 patients were included. After three cycles of capecitabine, six patients (18%) achieved a partial response (PR), 25 (76%) showed stable disease (SD), and one (3%) experienced progressive disease (PD). Based on this, six patients were classified as responders and 26 patients as non-responders. Microbial diversity (α-diversity), community structure (β-diversity) as well as bacterial abundances on genus and phylum level were not significantly different between responders and non-responders and were not significantly affected by the course of three cycles of capecitabine.

Intra-individual shifts of intestinal microbiota composition after recent antibiotic treatment were observed in some patients.

Conclusion

This is the first clinical study with longitudinal intestinal microbiota sampling in patients with metastatic CRC, exploring the effect of capecitabine on the intestinal microbiota and vice versa. The main conclusions are that:

(1) Intestinal microbiota composition and diversity before, during and after three cycles of capecitabine were not associated with treatment response and did not significantly change during the course of chemotherapy.

(2) The use of antibiotics during capecitabine treatment had strong effects on gut microbiota composition in some patients.

(3) Microbial dysbiosis at baseline, complex and extensive medical histories as well as major effects of antibiotics could potentially explain the absence of clear capecitabine-induced effects in this cohort of metastatic colorectal cancer patients.
(4) In the future, changes in intestinal microbiota composition and diversity during capecitabine treatment should be evaluated in studies with larger and more equal group sizes between responders and non-responders.

P69

The epidemiology of colistin-resistant Enterobacterales in humans in the Netherlands: a prospective matched case-control study

Drs. Karuna Vendrik[1,2], A de Haan[1], DW Notermans[1,3], P Bijkerk[1], A Schoffelen[1], S de Greeff[1], L Wielders[1], M Mennen[1], A Hendrickx[1], E Kuijper[1,2], L Schouls[1]

[1]National Institute for Public Health and the Environment (RIVM), [2]Leiden University Medical center, [3]Amsterdam University Medical Centers, location Academic Medical Center

Introduction: Multidrug-resistant Gram-negative bacteria are rapidly emerging worldwide. Last-resort treatment options, such as the polymyxin colistin, are used more frequently. Unfortunately, colistin-resistance is increasing. This study aimed to obtain insight into the prevalence and epidemiology of colistin-resistance in the Netherlands.

Methods: A 6-month surveillance project was performed with a prospective matched case-control design on the occurrence, geographic distribution, population dynamics and risk factors of colistin-resistant Enterobacterales. The project was part of a pan-European multi-centre study of the European Centre for Disease Prevention and Control. Twenty-two Dutch laboratories with 32 associated hospitals were invited to send a maximum of five colistin-resistant Escherichia coli or Klebsiella pneumoniae (ColRE) isolates to the National Institute for Public Health and the Environment (RIVM). As control group, colistin-susceptible isolates (ColSE) were collected that matched with the ColRE isolates on species, location of collection (hospital/community) and patient material. Clinical and epidemiological data were collected by a web-based questionnaire. At the RIVM, the minimum inhibitory concentration (MIC) for colistin was assessed by broth microdilution (BMD) and mcr-1 to mcr-8 genes were detected by PCR. Fifty ColRE and 50 ColSE isolates were analysed by next-generation sequencing, enabling whole-genome multi-locus sequence typing (wgMLST) and the identification of resistance genes, including mcr-9 and mcr-10.

Results: Eighteen laboratories submitted 72 ColRE isolates (75% E. coli and 25% K. pneumoniae) with matching ColSE isolates that met the study inclusion criteria. Most isolates were derived from urine samples (76%). Furthermore, 58% were collected in hospitals, 33% in general practices and 8% in other healthcare facilities. The median age was 74 years in the ColRE and 72 years in the ColSE group and 74% was female in both groups. Clinical infection due to ColRE was reported in 78% of patients in the ColRE group, and 74% in the ColSE group. Of patients with ColRE isolates, 21% had received colistin in the previous 6 months, significantly more than the ColSE group in which patients had received colistin in 3% (p= 0.017). The use of other antibiotics was not significantly different (73% versus 56%, p= 0.081). Twenty-three percent of ColRE and 13% of ColSE isolates were from patients residing in long-term care facilities (p= 0.140). The majority of ColRE isolates was detected by participating laboratories by automated antimicrobial susceptibility testing. Notably, 46 of 118 isolates classified by submitting laboratories as ColRE, were excluded based on the MIC for colistin as assessed by BMD (39%). The mcr-1 gene was detected in five ColRE isolates (7%), and the mcr-9 gene in two ColRE isolates (3%). wgMLST analysis revealed diverse populations for E. coli and K.pneumoniae with only one small genetic cluster of 2 K. pneumoniae isolates.

Conclusion: Colistin-resistance is not rare in the Netherlands since at least 72 ColRE were observed in 18 of 22 participating laboratories in a period of six months. Only 7 of the 50 ColRE isolates carried mcr genes, indicating that colistin resistance in the Netherlands is predominatly caused by chromosomal mutations. Importantly, patients with ColRE had used more colistin compared with patients with ColSE.

P70

A Multicenter Comparison of the Performance of Nine Commercial Borrelia Serology Screening Assays

Drs. Ing. B.J.A. Hoeve-Bakker[1,2], M. Jonker[1], Dr. A.H. Brandenburg[3], Dr. P.M. den Reijer[4], Dr. F.F. Stelma[5], Dr. A.P. van Dam[6], Ing. T. van Gorkom[1,2], Dr. S.F.T. Thijsen[2], Dr. K. Kremer[1]

[1]National Institute for Public Health and the Environment (RIVM), [2]Diakonessenhuis Hospital, [3]Izore Centre for Infectious Diseases Friesland, [4]Gelre Hospitals Apeldoorn, [5]Radboud University Nijmegen Medical Centre, [6]Public Health Service (GGD) of Amsterdam

Introduction

Recently, three enzyme-linked immunosorbent assays (ELISAs) for the detection of Borrelia-specific antibodies have been taken off the market. Consequently, almost half (n=24) of the ISO15189-accredited medical microbiological laboratories (MMLs) in the Netherlands were forced to implement a new Borrelia serology screening assay. This requires both insight into how currently available diagnostics compare and thorough diagnostic assay validation. Therefore, we organized a retrospective study to assess the performance of nine Borrelia serology screening assays on a well-defined study population of patients and controls.

Methods

Nine different ELISA or chemiluminescence immunoassay (CLIA)-based assays were compared using sera of 196 individuals, derived from 74 Lyme borreliosis (LB) patients, 74 healthy volunteers, and 48 cross-reactivity patients. The LB patients were diagnosed with erythema migrans (EM; n=11), Lyme neuroborreliosis (LNB; n=35), Lyme arthritis (LA; n=20) and acrodermatitis chronica atrophicans (ACA; n=8). LB was defined following the Dutch CBO guidelines and LNB following the European Federation of Neurological Societies (EFNS) guidelines.

Borrelia serology screening assays were selected based on a literature review and availability of CE marking under the new in vitro diagnostic (IVD) Regulation (EU) 2017/746. The assays detected IgM and IgG, either in two separate assays or combined in one assay, by using whole cell antigens supplemented with recombinant VlsE (n=2), recombinant antigens (n=6), or a combination of both (n=1).

Results

The overall sensitivity of the assays for clinically diagnosed LB patients ranged between 91.9% and 98.6% (IgG and IgM results combined). The sensitivity was better for patients with LNB, LA or ACA (range: 97.1% to 100%) than for EM patients (range 54.5% to 90.9%).

The positivity rates in the two control groups varied by group and by assay. Among the 74 healthy individuals, the positivity rates ranged between 8.1% and 29.7%, which is in line with the seroprevalence in the Netherlands.

The positivity rates of the assays among the cross-reactivity patients showed the broadest range (22.9% to 64.6%). These rates seemed to correlate with the type and number of different antigens used in the respective assays, and this should be further investigated. Intra- and inter-assay variation fell within the respective manufacturer's specifications.

Conclusion

1. All evaluated Borrelia serology screening assays performed comparably with respect to early and late disseminated Lyme borreliosis.
2. Considerable variation was observed in terms of cross-reactivity.
3. Further technical validation of the assay of choice is recommended to adhere to the ISO15189 criteria.

P71

Empirical treatment of periprosthetic joint infection after revision arthroplasty: worrisome rate of bug-drug mismatches

Drs Karin Veerman[1,2], Drs. D Telgt[1,2], Dr. WHM Rijnen[2], Dr. JHM Goosen[1], Prof. Dr. HFL Wertheim[2]
[1]Sint Maartenskliniek, [2]Radboudumc

Introduction
Periprosthetic joint infection (PJI) is a feared complication of total joint arthroplasty of the hip or knee. Debridement, antibiotic treatment and implant retention (DAIR) is an effective treatment of early (<90 days) PJI. Despite the lack of recommendations in recent guidelines, cefazolin is often used as empirical treatment awaiting cultures in suspected early PJI. Although gram positive skin flora are well known causative micro-organisms in PJI after primary arthroplasty, less is known about causative micro-organisms of PJI after revision arthroplasty. The aim of this study is to analyze whether the current empirical treatment recommendations are adequate based on microbiology data of early PJI after revision arthroplasty of the hip and knee.

Methods
This is a retrospective study in the Sint Maartenskliniek (SMK) and Radboudumc in Nijmegen. We identified DAIR procedures performed within 90 days after revision arthroplasty of the hip or knee (index) between 2012 and 2020. Patients were included if index revision was for aseptic reason and PJI was diagnosed based on tissue cultures taken during DAIR. Success rate of DAIR was calculated if follow-up details of at least one year were available in the EMR.

Results
We identified 97 early PJIs treated with DAIR in 93 unique patients: 65 in the SMK and 28 in the Radboudumc. PJI was most frequently caused by Staphylococcus epidermidis (n=57), Gram-negative bacilli (n=31) or Enterococcus spp. (n=13). Polymicrobial infection was diagnosed in 38 PJIs. Cefazolin monotherapy was used as empirical treatment in 63 DAIRs. Single dose cefazolin prophylaxis followed by clindamycin or flucloxacillin was administered in 21 and 3 DAIRs respectively. On three occasions, antimicrobial agents that also covered Gram-negative bacilli were used because of sepsis.

In 74% (95% CI: 0.65-0.83) of all DAIRs there was a mismatch between the empirical antimicrobial treatment and causative micro-organisms of PJI. Mismatches were due to Gram-negative bacilli in 42%, Enterococcus spp. in 10% and Staphylococcus spp. in 49% of the cases. If vancomycin would be the empirical treatment this can potentially reduce the mismatch from 74% to 32% (95% CI: 0.23-0.41). Vancomycin and ciprofloxacin combination therapy can reduce the proportion of mismatches to 1% (95% CI: 0.00-0.03).

For 69 DAIRs we had one year follow up data. Success rate was significant reduced by mismatch: 95% vs. 65% (OR: 0.11, 95% CI: 0.01-0.88, p=0.02). Reviewing longer term follow-up data (mean 43 months), there was a trend towards reduced infection-free survival when mismatches were present: 85% vs. 63% (OR: 0.30, 95% CI: 0.08-1.18, p=0,09).

Conclusion
There is a high number of mismatch between susceptibility of micro-organisms causing early PJI and empirical antimicrobial treatment after DAIR. This mismatch was mainly caused by cefazolin resistant Staphylococcus epidermidis and Gram-negative bacilli. This appears to strongly determine the success rate of PJI after revision arthroplasty. There are indications that empirical treatment with vancomycin can

improve clinical outcome. A prospective study is needed to assess whether broader spectrum empiric antimicrobial treatment will improve clinical outcome without increasing issues due to toxicity and other side effects (resistance).

P72

Frequent detection of Shigella in MSM also in the absence of clinical symptoms

Dr Alje Van Dam[1,2], Drs JF Braam[1], Ing M Hoogeland[1], Prof.Dr. HJC De Vries[1,3], Dr SM Bruisten[1]
[1]Amsterdam Health Service, [2]Amsterdam UMC, [3]Amsterdam UMC

Introduction: Shigellosis can present as a severe bloody diarrhea and is a reportable infectious disease. About 22% of reported shigellosis cases in the Netherlands occur in men who have sex with men (MSM). Not all Shigella infections result in clinical symptoms and not all persons with clinical symptoms of shigellosis are diagnosed. We performed a study among MSM visiting the STI clinic in Amsterdam to assess the prevalence of Shigella.

Methods: From March to June, 2020, anal swab samples taken from MSM routinely visiting the STI clinic to detect Chlamydia trachomatis and Neisseria gonorrhoeae were additionally tested pseudonymously for the presence of Shigella by PCR on the ipaH gene. Consecutive samples from MSM who reported no diarrhea, or diarrhea during last month, or diarrhea at visit of clinic were included. Predefined minimal numbers of inclusion of these groups were 150, 100 and 50, respectively. During the same months the frequency of Shigella as assessed by PCR in routinely tested samples sent by general physicians was assessed.

Results: We included samples from 214 MSM without diarrhea, 109 MSM who recently had diarrhea and 68 MSM who reported diarrhea at visit of the clinic. The total number of samples positive for Shigella was 13/389 (3.3%), of whom 6/212 (2.8%) had no diarrhea, 4/107 (3.7%) recently had diarrhea and 3/68 (4.4%) had diarrhea at clinic visit. Positive samples were more frequently found in persons using or recently having used PREP (10/152), compared to no PREP (2/163) or being HIV-positive (1/74) (p=0.02, chi square test). In comparison, only 11/774 (1.4%) routinely tested fecal samples sent by general physicians during the study period were positive for Shigella.

Conclusion: Shigella infections without symptoms or with minor symptoms are relatively common in MSM. More detailed studies should focus on the risk of transmission from these persons to others, leading to symptomatic infections.

P73

Detection of intrathecal antibody synthesis to diagnose enterovirus infections of the central nervous system

Syriam Sooksawasdi Na Ayudhya[1], Gregorius Sips[1,4], Susanne Bogers[1], Lonneke Leijten[1], Leo Smeets[2], Andrea Bruning[3], Kimberley Benschop[4], Katja Wolthers[3], Debby Van Riel[1], Corine GeurtsvanKessel[1]
[1]Viroscience department, Erasmus Medical Center, [2]Reinier Haga Medisch Diagnostisch Centrum, [3]Amsterdam University Medical Center, [4]National Institute for Public Health and the Environment

Background
Enterovirus D68 (EV-D68) re-emerged in 2014. Besides causing predominantly severe respiratory disease in young children, it is associated with a wide range of neurological complications, of which acute flaccid myelitis (AFM) occurs most frequently. Diagnostics to confirm EV-D68 associated CNS complications are challenging since viral RNA is rarely detected in cerebrospinal fluid (CSF). Virus-specific EV-D68 antibodies have been detected in the CSF of patients with EV-D68-associated CNS complications, but in these analyses the blood-CSF barrier function was not assessed. We evaluated the use of a commercial Enterovirus (EV) ELISA kit, to determine an antibody index (AI), by using paired CSF and serum samples in patients with EV-D68 associated AFM. Subsequently, we compared the detection of EV-specific antibodies in EV-D68 infected patients with virus neutralization test (VNT).

Methods
EV-specific antibodies were detected by a commercial SERION ELISA classic EV IgA, IgM, IgG according to the manufacturer's protocol. For the assay validation, paired CSF/blood samples were included from patients with EV-associated CNS disease (n=6, CSF PCR positive)
In addition, we tested 3 paired CSF/blood (serum) samples from AFM patients (with positive EV-D68 PCR in respiratory samples). Virus specific IgG, total IgG and total albumin in blood and CSF were used to calculate the AI. In addition, we compared the commercial EV ELISA with VNT against a recent EV-D68 isolate (clade B3).

Results
We were able to determine a virus-specific IgG AI in 5 out of 6 patients with confirmed EV-associated CNS disease (83%), and in 1 out of 3 patients with EV-D68-associated AFM (33%) using a commercial EV ELISA kit. Five patients had an AI > 1.5 suggesting local intrathecal antibody production, including one patient with EV-D68-associated AFM. Further assay comparison showed that EV-D68 neutralizing antibodies could be detected by VNT earlier after disease onset compared to EV specific antibodies measured by the ELISA kit.

Conclusion
Intrathecal antibody detection combined with AI-calculation can be used as a diagnostic tool for EV-D68-associated CNS disease. Since EV-D68 viral RNA in CSF is rarely detected by PCR, AI-serology is highly recommended for diagnostics, and underscores the value of routine collection of paired CSF/blood samples. Detection of EV-D68 antibodies using VNT is more sensitive than the commercial ELISA and the development of a EV-D68 ELISA with an increased sensitivity will support future diagnostics.

P74

Outbreak of Arcanobacterium haemolyticum in chronic wounds: lessons regarding care transition.

Marjan J. Bruins[1], M.J. Bruins[1], Msc S.V. van Rossum-de Vries[2], Dr J. Oskam[3], Dr G.J.H.M. Ruijs[1], Dr S.B. Debast[1]
[1]Isala, [2]Isala, [3]Isala

Introduction
An aging population and comorbidities such as diabetes and vascular problems contribute to the increasing occurrence of chronic wounds, defined as skin lesions that do not heal spontaneously or take more time to heal than is considered normal. In the Netherlands in recent years wound expertise centres (WECs) have been established in hospitals. According to the national trend to transfer care from the hospital to primary care, in our region they work in close cooperation with a regional home wound care organization, of which specialized nurses provide wound care at the patients' homes. Since the beginning of 2016, we saw a marked increase of A. haemolyticum in cultures of chronic wounds. This study describes the investigation into the source of the outbreak and the infection prevention measures that were taken to control it.

Methods
The Outbreak Management Team coordinated by the Laboratory of Clinical Microbiology and Infectious Diseases of Isala hospital, Zwolle, the Netherlands, researched the current chain of care. To find potential transmission routes a survey of literature and medical records was performed and samples were taken from care workers, the environment and items used for wound care, such as ointments, dressings, curettes, forceps, etc. for microbial culture. Routine practices during wound care were audited and wound care nurses were interviewed. Based on these results, infection prevention measures were implemented in a bundled approach, involving education, better aseptic wound care conditions and hygienic precautions. To assess the effectiveness, before and after the implementation two rounds of wound cultures and A. haemolyticum PCRs were performed among all patients in home care.

Results
Although A. haemolyticum isolates from wound care patients were found to be identical by molecular typing, a definite outbreak source could not be determined among employees, environment or items used during wound care as no A. haemolyticum was isolated. However, the A. haemolyticum PCR was positive in samples taken from three different forceps, used by two nurses on several patients. In the screening rounds before and after the implementation of infection prevention measures the proportion of A. haemolyticum positive cultures decreased significantly from 14% to 2%. Among 57 patients newly in home wound care tested in the second screening (n=57) and among subsequent hospitalized and WEC patients no new cases were found.

Conclusion
The outbreak of A. haemolyticum was caused by re-using contaminated instruments on multiple patients. Through the implementation of strict(er) infection prevention measures and re-education of all employees involved the outbreak was controlled. To guarantee a successful and high-quality transition of care, a chain-transcending view is required, with early attention to the risks (and opportunities) in the field of infection prevention.

P75

Aspergillus fumigatus outbreak possibly caused by construction work on the corridor of an intensive care unit: analysis using whole genome sequencing

MSc Amber Hendriks[1,2], S.J.P. Hiel[3], T. Bosch[1], J.P.M. Coolen[2], S. Kuiling[1], J. Zoll[2], P. Anrochte[4], S.M.T. Camps[4], P.E. Verweij[1,2], L. van Dommelen[5]

[1]National Institute for Public Health and the Environment (RIVM), [2]Radboud University Medical Centre, [3]Máxima Medical Centre, [4]Máxima Medical Centre, [5]Stichting PAMM

Introduction

Whole genome sequencing (WGS) for typing strains related to an outbreak is already widely used in bacteriology and virology, but scarcely in mycology. We evaluated the use of WGS for genotyping of Aspergillus fumigatus isolates from a presumed outbreak of invasive aspergillosis cases in an intensive care unit (ICU). In late September 2020, a sudden rise in positive selective gut decontamination surveillance cultures for molds, mostly A. fumigatus, was detected in several patients. The rise coincided with construction work within the corridor ceiling of the ICU. Construction work has been associated with outbreaks of invasive aspergillosis cases previously. Several interventions took place, with cessation of the construction works being the first. WGS was used to map A. fumigatus strains both from patients and environmental samples.

Methods

After detecting the outbreak, fungal culture was added to all surveillance and/or respiratory patient samples. Environmental samples (air samples) were taken for fungal culture throughout the ICU using 1000L/sample. WGS was performed on seven patient samples and two air samples on a NextSeq 550 using the Illumina DNA prep and a NextSeq High Output kit. The raw reads were trimmed with Trimmomatic, and FastQC was used as quality control. A Split Kmer Analysis (SKA) was used to determine single nucleotide polymorphisms (SNPs) based on kmers without using a reference. A phylogenetic tree was created using IQ-TREE and visualized using iTOL.

Results

Surveillance and/or respiratory samples were taken from nine patients staying in the ICU during the construction work period. Three of which were diagnosed with invasive aspergillosis (IA) and four patients had colonisation with A. fumigatus or A. flavus. Of the three patients with IA (all A. fumigatus), two patients had underlying COVID-19 pneumonia and one patient was immunocompromised (chemotherapy for urothelial carcinoma).

After cessation of construction work and cleaning and disinfection of the ICU no newly colonized or infected patients were detected in the ICU. Air samples showed no more than two colony forming units/1000L, which was considered acceptable. A. fumigatus strains from seven respiratory samples from six patients (including two strains from before the outbreak) and two from air samples from different patient rooms were used for WGS. Three samples were from two patients with IA. Analysis of the genome sequence indicated that none of the strains were related to each other. The isolates with the most similarity differed more than 9,000 SNPs and the most unrelated strains differed more than 40,000 SNPs. The two strains isolated from environmental samples were also not comparable with each other and had a difference of nearly 20,000 SNPs.

Conclusion

The Aspergillus outbreak was likely caused by release of A. fumigatus spores during construction work, as no new cases were observed after cessation of the construction. The lack of genetic similarity of isolates suggests accumulation of Aspergillus spores in the hospital environment, rather than a source that supported growth and reproduction of A. fumigatus. WGS was found to be a suitable technique for examining inter-strain relatedness of A. fumigatus in the setting of outbreak investigation.

P76

SARS-CoV-2 serologic prevalence among health care workers in a hospital setting

Marja Konstantinovski[1], A. Bijsterbosch[1,6], S.H. van Lidth de Jeude[4,5], Dr. C.F. Melissant[3], Dr. M.R. Batstra[2]
[1]ReinierHaga Medisch Diagnostisch Centrum, [2]ReinierHaga Medisch Diagnostisch Centrum, [3]VitaSana, [4]Leiden University, [5]Ordina Business Consulting, [6]Hogeschool Rotterdam

Introduction: In March 2020 the Dutch healthcare system was confronted with the SARS-CoV-2 pandemic. This new disease led to many concerns among Health Care Workers(HCW) with regards to their safety. This study aimed to monitor the disease incidence and parameters predisposing HCW to a SARS-CoV-2 infection at a regional hospital in the Netherlands.

Methods: HCW were included in the study cohort for prospective follow up during April-July 2020. HCW from different departments were included, both from COVID and non-COVID care. HCW received 5 invitations to provide a serum sample and to submit a questionnaire to monitor disease symptoms and exposure factors for COVID. The serum was analyzed for SARS-CoV-2 antibodies using the Maglumi IgM and IgG and Wantai total Ab serology kits.
To compare the serologic response among HCW to the general population a control group from a single employer in the same region was used. This employer continued most of their activities during the lockdown period as it was designated vital for the food production chain by the government. The control group provided a single questionnaire and serum sample after the first wave, in September 2020. The serum was analyzed for SARS-CoV-2 antibodies using the Wantai total Ab serology kit.

Results: 427 HCW (70% female, age average 43 years SD±12) were included in the study, 79% of the visits were completed and 70% completed at least the first (April ’20) and the last visit (July’20). In the control group 419 persons(46% female, age average years 42 SD±13) were included for a single visit.
4,9% (21/427) of the HCW and 4,5% (19/419) of the control group had a serologically proven COVID based on Wantai results. This difference in seroprevalence was not significant. No difference in seroprevalence was found for HCW at COVID or non-COVID departments. There was a significant relation($P<0,05$) between occurrence of COVID related symptoms and seroconversion however the PPV of symptoms was low with 12%.

Conclusion: Seroprevalence for SARS-CoV-2 after the first wave was similar among HCW with occupational exposure to COVID patients and non-health care workers. This implies that if infection control policies are observed the occupational exposure for HCW is manageable and HCW can safely be employed in caring for COVID patients.

P77

Evaluation of rapid SARS-CoV-2 antigen testing of symptomatic health care workers.

Marjan J. Bruins[1], Dr C Oliveira dos Santos[1], Drs M Spoelman-Lunsche[3], Ing M.I. van den Bos-Kromhout[2], Dr S.B. Debast[1]
[1]Isala, [2]Isala, [3]Isala

Introduction

RT-PCR is the gold standard for the detection of SARS-CoV-2, has a high sensitivity but is performed in batches and takes 4-5 hours. For health care workers (HCWs) with COVID-19 associated complaints rapid testing is warranted to prevent transmission and ensure continuity of care. Recently developed lateral flow antigen assays take only 15-30 minutes, do not need any equipment and are suited for point-of-care testing. In a prospective study we evaluated the use of the Abbott Panbio COVID-19 Ag Rapid Test to exclude or confirm COVID-19 in our hospital's HCWs and to determine its sensitivity for this specific group with (mostly) early complaints.

Methods

Between November 2020 and February 2021 all HCWs who were tested for COVID-19 by PCR because of COVID-19 related complaints were asked to participate. After signed informed consent a second nasopharynx swab was taken for rapid testing. We also registered when the complaints had started. The Panbio test was performed on site at room temperature by a trained laboratory technician and read after 15 minutes incubation according to the manufacturer's instructions. Test results were compared to the qualitative RT-PCR results determined with the Hologic Panther Fusion System. Of positive samples cycle threshold (Ct) values were obtained using an in-house quantitative real-time COVID-19 PCR.

Results

In total 1101 SARS-CoV-2 tests were included in the study, of which 84 were PCR positive (prevalence of 7.6%). Of these, 48 were antigen test positive. Overall sensitivity was 57.1%. If the complaints had started within the last day, sensitivity was only 47.1%. If the onset had been two or three days ago, sensitivities were 63.0% and 66.7% respectively. Most false negative results occurred in HCWs with Ct values above 30, consistent with early (up to one day) or longer lasting (seven days or more) complaints. The test was easy to read. Positive test lines differed in intensity but gave no dubious results. There were no false positive results, specificity was 100%.

Conclusion

According to our results, the Panbio COVID-19 Ag Rapid Test was mainly false negative during early or late infection. The short turnaround time and easy performance of the assay are major advantages, but the sensitivity proved to be too low for it to be used as a diagnostic test in an algorithm for timely identifying COVID-19 positive HCWs.

P78

Evaluation of SARS-CoV-2 antigen assay based testing strategy of HCW in a hospital setting

Marja Konstantinovski[1], S. Popping[1,2], D. Schriel[4], H. Bakker[3], J. Hoogenboom-Beuving[1]
[1]RHMDC, [2]Erasmus Medical Center, [3]GGD Haaglanden, [4]Reinier de Graaf Gasthuis

Introduction:
Health care worker (HCW) testing for SARS-CoV-2 should aim to identify infectious cases and reduce nosocomial transmission. Turnaround times of the golden standard PCR test may be as long as 24 hours, causing unnecessary absence and understaffing of crucial care. Antigen tests (ag-test) can be performed with a much shorter turnaround time, but with lower sensitivity. This study aims to evaluate a SARS-CoV-2 ag-test based strategy for HCW in a regional hospital during October -December 2020.

Methods:
A SARS-CoV-2 testing site was realized on the premises of the hospital. All HCW could register for a same day test.
Trained staff used a single swab to take both a nasopharyneal and orophanryngeal sample for combined antigen and PCR-test. A SARS-CoV-2 antigen assay was performed using the Panbio COVID-19 ag rapid test device (ABBOTT). Afterwards the swab was inserted into a lysis buffer for RT-PCR testing according to an in-house protocol. To assess whether a significant loss of viral load due to this method occurred, CT values before and after implementation of the ag-test were compared. Sensitivity and specificity of the ag-test were evaluated HCW could resume their work if the ag-test was negative, but were instructed to wear a surgical mask until the result of the PCR test was available. If subsequently the PCR test was positive, HCW went on sick leave.
To assess whether HCW with false negative ag-test results caused secondary transmission, infection control reports and patients files were evaluated.

Results:
Ag-test sensitivity 70% ~95% CI(60.5 – 78.3) and specificity >99% ~95% CI(98.9 – 100) was based on 777 PCR confirmed samples in the prospective validation phase.. Further analysis of the HCW set (N=352) showed that sensitivity for samples with low CT values(<30) was 88% compared to 22% for CT values of >30.The mean CT value was 21 for true positive and 31 for false negative ag-test results.
CT value distribution before and after implementation of the ag-test showed no significant differences. It was decided that since specificity was >99% the confirmatory PCR for positive results would be discontinued after the validation phase.
In total 1786 antigen and 1725 PCR tests were performed. 148 HCW tested positive in the ag-test.
Contact tracing for the 57 (3,2%) HCW that tested false-negative identified no evidence of onward transmission in the waiting period between the ag and PCR test result. Infection control reports were missing for 14 people.

Conclusion:
Using a single swab for both PCR and antigen testing is a reliable, cost efficient and patient friendly method. Sensitivity of the ag-test strongly depends on the viral load, as measured in CT values. False-negative results are more common in samples with CT>30.
A HCW testing strategy for SARS-CoV-2 based on a combined antigen and PCR test improves continuity of care in the hospital by eliminating waiting times for PCR results, without increasing the risk of nosocomial transmission.

P79

Evidence of cat-to-human transmission of Staphylococcus felis

Dr. G.J.S. Sips[2], Dr. M.A.M van Dijk[1], Dr. M. van Westreenen[2], Dr. L. van der Graaf-van Bloois[1], **Dr. Birgitta Duim[1]**, Dr. E. M. Broens[1]
[1]Utrecht University, Faculty of Veterinary Medicine, [2]Erasmus University Medical Center

Introduction

A 58-year-old woman presented to the outpatient emergency department with symptoms of dorsal wound leakage and mild inflammation following laminectomy. Wound samples were cultured and revealed growth of skin flora with dominance of white non-hemolytic colonies which were identified as Staphylococcus felis by MALDI-TOF MS. With a diagnosis of local wound infection, the wound was surgically closed, and the patient was discharged in good condition with an antibiotic regimen of oral flucloxacillin. S. felis is a frequently isolated Staphylococcal species on the skin and oral mucosa of healthy cats and has not been associated with human infections. In this study we sampled the owner's cats to further investigate the potential source.

Methods

Swabs were taken from three cats from multiple body sites and were submitted to the Veterinary Microbiological Diagnostic Center of Utrecht University. Samples were enriched in Mueller-Hinton broth with 6.5% NaCl followed by culturing on sheep blood agar. Suspected colonies were confirmed as S. felis using MALDI-TOF MS with the Bruker database. We sequenced the human and all feline isolates with Illumina NextSeq. Reads were assembled using SPAdes, a core-genome alignment using Parsnp with 29 publicly available S. felis genomes was performed to construct the genome phylogeny. The genomes were annotated with Prokka and orthology was predicted using Roary. Antimicrobial resistance genes were predicted using ResFinder.

Results

Cat #1 tested positive at all three sites, cat #2 at the axilla and oral mucosa and cat #3 at the perineum and oral mucosa. S. felis is often susceptible to most antimicrobials, and the only detected resistance gene was a Streptogramine A/lincosamide encoding gene in isolates of cat #1. Comparison of the S. felis core genomes showed that the isolates from each individual cat clustered separately, but the genome of the human isolate was identical to genomes of isolates from cat three.

Conclusion

Whole genome analysis revealed sequence identity of the human isolate with isolates of one of the cats demonstrating evidence for cat-to-human transmission and a zoonotic potential for S. felis.

P80

False amoxicillin/clavulanic acid susceptibility in Bacteroides fragilis using gradient strip tests

Dr Rob Rentenaar[1], Ing. B. Bovo-Heijmans1[1], J. Diggle[2], Dr. A.C. Fluit[1], Dr. M. Wootton[2]
[1]UMC Utrecht, [2]Public Health Wale, University Hospital of Wales,

Introduction: Repeatedly, too low minimal inhibitory concentration (MIC) results were obtained in Bacteroides fragilis quality assessment strains, using gradient strip tests with a ratio of amoxicillin:clavulanic acid of 2:1. We aimed to find the most accurate available gradient strip tests for susceptibility testing of amoxicillin/clavulanic acid in B. fragilis in comparison with agar dilution with EUCAST methodology and breakpoints.
Methods: Twenty-seven clinical B. fragilis isolates were investigated using gold standard EUCAST amoxicillin/clavulanic acid agar dilution (fixed clavulanic acid concentration at 2 mg/L, with increasing amoxicillin concentrations) in one laboratory (PHW) as well as two commercial gradient strip tests: XL (ratio), or AMC (fixed concentration) in two laboratories (PHW and UMCU)
Results: Using agar dilution (fixed concentration), 19 isolates were susceptible, 1 isolate was susceptible increased exposure (I) and 7 isolates were resistant. Categorical agreement of the gradient strip tests with agar dilution (fixed concentration) was 70% for XL (ratio) and 89% for AMC (fixed concentration). Very major error rates in comparison with agar dilution (fixed concentration) were 100% and 0%, respectively.
During 5 years of XL (ratio) usage in the UMCU, among 307 consecutive B. fragilis isolates, 295(96%), 11(4%) and 1(0%) were S, I and R, respectively. In contrast, after switching to AMC (fixed concentration) usage, in a period of 5 months, among 27 consecutive B. fragilis isolates, 21 (74%), 1 (4%) and 6 (22%) were S, I and R, respectively.
Conclusions: EUCAST breakpoint usage in amoxicillin/clavulanic acid susceptibility tests for B. fragilis should be accompanied by EUCAST methodology. When using alternative methods such as gradient strip test, a higher degree of alignment with EUCAST methodology such as using fixed clavulanic acid concentration improves precision.

P82

In Search of Novel Antimicrobials from Fungal Resources

Xudong Ouyang[1,2], Jelmer Hoeksma[1], Prof.dr. Jeroen den Hertog[1,2]
[1]Hubrecht Institute, [2]Leiden University

Antimicrobial resistance is a major threat to human health. New antimicrobials with distinct working mechanisms are required to combat bacteria that have become resistant to all known antimicrobials. Fungi are considered to be an excellent source since they produce a large number of unstudied compounds as their secondary metabolites. In our previous study, we have tested more than 10,000 fungal supernatants on different bacteria and found 381 hits with antimicrobial activity. Here, we used Bacillus subtilis as a read-out to identify active compounds and to investigate their killing mechanisms. The active compounds from 45 selected fungi were purified by ethyl acetate extraction and preparative HPLC. Then the pure compounds were identified with a combination of chemical analyses, including LCMS, UV-Vis spectroscopy/spectrophotometry, high resolution mass spectrometry and NMR. 10 compounds were successfully identified including two poorly described antimicrobials. Furthermore, an imaging-based method, dynamic bacterial cytological profiling, was applied to rapidly identify specific antibacterial Mode of Action (MoA). This method utilized high-resolution fluorescent microscopy to observe morphological changes of bacterial cells in response to antibacterial compounds exposure and facilitated comparison of cytological profiles with those treated with control antimicrobials. Our results demonstrate that one of the novel antimicrobials has a unique profile compared with all the control antibiotics, suggesting a novel MoA, which may provide a lead for therapeutic drug discovery.

P83

Does Trichomonas hurt? A five-year comprehensive full-region study

Matthijs Berends[1], Dr J Weel[2], D Scoop[2], Dr T Schuurs[2]
[1]Certe, [2]Izore

Introduction
Trichomonas vaginalis (TVG) is the etiological agent of trichomoniasis, the most prevalent non-viral sexually transmitted disease worldwide and a widespread, global health concern. The relatively mild symptoms have historically led to this disease being under diagnosed, and under researched. However, growing evidence that TVG infections are associated with high morbidity in both men and women, has increased the efforts to diagnose and treat patients harbouring this parasite. In this study, we aimed to assess this burden of disease.

Methods
Between 1 May 2012 and 31 January 2017, we routinely screened all patients for TVG where any STD diagnostics were requested by a GP, gynaecologist or social health care service in the region. All samples were tested using real-time PCR. The patient catchment area covered the whole of Friesland, one of the twelve provinces of the Netherlands, comprising ~645,000 inhabitants. All requesters were asked to supply clinical information about their patients.

Results
In total, 47,735 patients were screened, of which 69% was female. The median age was 27 (IQR: 22-38). Out of 67,774 samples, 540 (0.8%) were positive for TVG, with a median cycle threshold (Ct) of 22.47 (IQR: 18.33-29.15). As a comparison, 9.7% tests were positive for Chlamydia trachomatis (CTR) and 1.4% was positive for Neisseria gonorrhoeae (NGO). Compliance of the supply of clinical information was 87%. TVG was found in both women (489/540, 91%) and men (51/540, 9.4%), although the median DNA load was almost 300 times higher in women (Ct 21.6) then in men (Ct 29.8). In women, presence of vaginal discharge and/or irritation was predictive only for presence of TVG and, opposingly, predictive for absence of both CTR and NGO.

Discussion
To our knowledge this is the first full-region approach to study on the prevalence of TVG in relation to clinical complaints. Although our study results corroborate previous findings in the Netherlands, they contrast the high prevalences seen worldwide. Vaginal discharge appeared to be predictive for presence of TVG, and this reason for clinical burden is probably easily overlooked by physicians. Additional, in-depth analyses will be necessary to further specify how TVG infections are associated with other disease states.

P84

More symptomatic infections and higher bacterial load in Neisseria gonorrhoeae and Chlamydia trachomatis co-infections compared to Neisseria gonorrhoeae single infections.

Helke Van Dessel[1], dr I.H.M. van Loo[1], B.M.J.W. van der Veer[1], Professor C.J.P.A. Hoebe[1,2], dr N.H.T.M. Dukers-Muijrers[1,2], Professor P.H.M. Savelkoul[1], dr P.F.G. Wolffs[1]
[1]Mumc, [2]South Limburg Public Health Service

Introduction
Neisseria gonorrhoeae (NG) and Chlamydia trachomatis (CT) are the most prevalent bacterial sexually transmitted infections. NG and CT co-infections are reported around 5% in high risk groups. Data whether CT and NG co-infections are more symptomatic compared to NG only infections hardly exist. Moreover, the influence of CT co-infection on NG bacterial load is not studied in a large population. Therefore, we quantified NG bacterial loads in a large population of women, heterosexual men, and men who have sex with men (MSM) in oropharyngeal, anorectal, and genital samples in relation to CT co-infection and reported symptoms.
Methods
The study population includes data and samples of NG positive patients who visited the South Limburg public health service STI clinic between January 2012 and July 2017.
Samples were screened for NG and CT with the cobas 4800 (Roche Diagnostics, Basel, Switzerland). NG positive samples with data on sexual orientation and symptoms were included. For quantification of NG load a standard curve was made for each specimen type.
The Chi-square test was used for comparison of symptoms and the Mann-Whitney test for pairwise comparisons of bacterial load.

Results
Of the 1326 included NG positive samples, 205 (15.5%) were CT positive, of which 48 (23.4%) were from women, 33 (16.1%) from heterosexual males and 124 (60.5%) from MSM. 1121 (84.5%) were CT negative, of which 234 (20.9%) were from women, 126 (11.2%) from heterosexual men and 761 (67.9%) from MSM.
Of the NG and CT positive samples and NG only samples, 104 (50.7%) resp. 372 (33.2%) were anorectal swabs, 39 (19.0%) resp. 102 (9.1%) vaginal swabs, 54 (26.3%) resp. 102 (9.1%) urine samples and 8 (3.9%) resp. 421 (37.6%) oropharyngeal swabs. Mean load per sample for NG and CT positive samples were: anorectal swab 4.9 ±1.1 log10 CFU/ml, vaginal swab 4.5 ±0.8 log10 CFU/ml, urine sample 4.7 ±0.8 log10 CFU/ml, oropharyngeal swab 3.6 ±1.0 log10 CFU/ml. In NG only samples, mean load per sample were: anorectal swab 4.3 ±1.3 log10 CFU/ml, vaginal swab 4.2 ±1.2 log10 CFU/ml, urine sample 4.5 ±1.0 log10 CFU/ml, oropharyngeal swab 3.1 ±1.0 log10 CFU/ml. In anorectal swabs, the NG bacterial load was significantly higher in samples positive for NG and CT compared to samples positive for NG only ($p<0.001$).
When coinfected with CT, vaginal swabs and urines were more often associated with genital symtoms and anorectal swabs more often associated with proctitis. This difference was statistically significant for anorectal samples ($p=0.02$), of which 87% were from MSM.

Conclusion
This is the first study to describe the association of CT-coinfection in NG infection, in relation to reported symptoms and NG load in a large group of NG infected patients.
NG and CT co-infections demonstrated a higher bacterial load of NG in anorectal samples compared to NG only.

Proctitis was significantly more reported in NG/CT co-infected anorectal samples compared to NG- only anorectal samples.
Whether NG/CT-interaction causes the higher NG load or whether high risk sexual behaviour results in both co-infection and higher NG load remains to be established.

P85

Optimizing duration of outage of healthcare personnel using PCR cycle threshold value of SARS-COV-2 during COVID-19 epidemic in a university hospital in the Netherlands

MD Leonie Bank[1], MD D Hanssen[1], MD M Slaats[1], Prof. P Savelkoul[1,3], Dr. F van Tiel[1], R Olivers[2], Dr. I H M van Loo[1,3]

[1]Maastricht University Medical Center, [2]Maastricht University Medical Center, [3]Maastricht University Medical Center

Introduction: Since March 2020, the pandemic of COVID-19 has reached not only patients, but also health care workers (HCWs), causing shortage of staff thereby increasing pressure on hospitals even further. We present our testing policy implemented during the first wave, which aimed to minimize risk of spreading COVID-19 while maximizing employability of HCWs.

Methods: In the period March 11th 2020 until July 1st, all symptomatic HCWs were tested for SARS-COV-2 using RT-PCR of nasopharyngeal-throat swab 48 after onset of complaints. Personnel with severe complaints (fever, dyspnea, malaise) received a ban from working and were swabbed only after these severe complaints had resolved for 24 hours. Starting March 29th, in mildly symptomatic HCWs with PCR with cycle threshold (CT) ≥ 30 work ban was lifted if HCW was fit for work. Additional measures entailed working with a surgical mask and clinical personnel was deployed only on COVID-19 wards. PCR was repeated until a negative result was obtained. In order to assess the effect of this policy on employability of HCWs, difference in number of days between onset of symptoms and CT ≥ 30 and negative PCR were compared. Groups were divided based on time between onset of symptoms until first PCR-test: tested after up to 3 days (early testers), and tested after 7 days (late testers)

Results: In total, in the studied period 1139 episodes underwent testing at the outpatient testing clinic. A total of 277 HCWs were tested positive. After implementation of follow-up policy, 182 cases were retested until a negative PCR result was obtained. Early testers group comprised of 59 cases, late testers comprised of 53 cases.

Overall in positive HCWs, the median time until PCR CT value ≥ 30 was 13 days (IQR 8 – 17), and the median time until negative PCR was 25 days (IQR 18 – 33). This resulted in a median difference of 10 days (IQR 7 – 18 days). In the early testers group the median time until CT ≥ 30 was 10 days (IQR 3 – 16), median time until negative PCR was 22 (IQR 16 – 30). Median difference was 10 days (IQR 3 – 17). In the late testers group, the results were 16 (IQR 13 -21), 29 (IQR 23 – 39) and 12 days (IQR 7-19) respectively.

Conclusion: Our policy led to return to work of HCWs 10 days earlier using Ct ≥ 30 as cut-off, compared to using negative test result, in the early testers group. For the late testers group, the median difference was 12 days.

P86

No increased risk for non-Hodgkin lymphoma after Q fever: results from a 16 year retrospective population-based analysis incorporating the 2007-2010 Dutch Q fever outbreak

Drs. Jesper Weehuizen[1], dr. S.E. Roeden[1], drs. S.J. Hogewoning[2], dr. W van der Hoek[3], Prof. dr. M.J.M. Bonten[4], Prof. dr. A.I.M. Hoepelman[1], dr. C.P. Bleeke-Rovers[5], dr. P.C. Wever[6], Dr. J Oosterheert[1]
[1]University Medical Center Utrecht, [2]Netherlands Comprehensive Cancer Organisation, [3]National Institute for Public Health and the Environment, [4]University Medical Center Utrecht, [5]Radboud University Medical Center and Radboud Expertise Center for Q fever, [6]Jeroen Bosch Hospital

Introduction: A causative role of Coxiella burnetii in the pathogenesis of non-Hodgkin lymphoma (NHL) has been suggested, though supporting studies suffered from considerable risk of bias. We aimed to better quantify this association by assessing the risk of NHL after Q fever in the entire Dutch population over a 16 year period.

Methods: We performed a retrospective population-based linked analysis. The incidence of NHL in the entire Dutch population from 2002 until 2017 was studied and modeled with notified acute Q fever cases as determinant. The primary outcome measure was the adjusted relative risk for NHL after acute Q fever.

Results: Between January 2002 and December 2017, 266,050,745 person years were observed, with 68,485 persons diagnosed with NHL, reflecting a crude incidence rate of 25.7 cases per 100,000 person years. In total, 4,310 persons were diagnosed with acute Q fever, with a highest yearly incidence rate of 14.2 cases per 100,000 person years in 2009. The adjusted relative risk for NHL after acute Q fever was RR 1.01 (95% CI 0.97 – 1.06, p = 0.50), and 0.98 (95% CI 0.89 – 1.07, p = 0.60), 0.99 (95% CI 0.87 – 1.12, p = 0.85) and 0.98 (95% 0.88 – 1.08, p = 0.67) for subgroups of diffuse large cell B-cell lymphoma, follicular lymphoma, or B-cell chronic lymphatic leukemia, respectively. Modeling with lag times (1-4 years) did not change interpretation.

Conclusion: Although the Netherlands suffered the largest Q fever outbreak ever recorded, there is no evidence for association between acute Q fever and NHL.

P87

Evaluation of 18 commercial serological assays for the detection of antibodies against SARS-CoV-2 in paired serum samples.

Drs. Daniëlle Hanssen[1], MD M Slaats[1], MD M Mulder[1], Prof. P H M Savelkoul[1,2], Dr. I H M van Loo[1,2]
[1]Maastricht University Medical Center, [2]Maastricht University

Introduction
A variety of serological tests have been developed to detect the presence of antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We evaluated the performance of 18 commercially available SARS-CoV-2 antibody assays.

Methods
Early (six to eight days after the start of symptoms) and late sera (>14 days) were included from ICU patients (n=10 and n=16, respectively) and health care workers (HCW) (n=5 and n=9, respectively), together with 22 negative controls. The sensitivity, specificity and area under the curve (AUC) were determined for the 18 assays, 9 of which were Enzyme-Linked ImmunoSorbent Assays (ELISAs) of five different manufacturers, 1 ElectroChemiLuminescence ImmunoAssay (ECLIA) and 8 point-of-care tests (POCT).

Results
In >14 days samples, the Vircell IgG and Wantai Ig ELISA's had superior sensitivity compared to the other ELISA's (96%). Furthermore, the Roche Ig, the Epitope Diagnostics IgM, Wantai IgM, Euroimmun IgG and IgA all showed a specificity of 100%. For the IgM and IgA assays, the Euroimmun IgA test showed the highest sensitivity in early samples: 46.7% (23.5-70.9) to 53.3% (29.1-76.5). The POCT of Boson Biotech and ACRO Biotech showed the highest sensitivities: 100% and 96% (83.5-99.8), however these POCTs have impaired specificity (<90 %). The POCT of Orient Gene Biotech, VOMED Diagnostics, and Coris Bioconcept showed highest specificities (100%). In general, all tests performed better in patients admitted to the ICU (i.e. patients with severe symptoms).

Conclusion
We conclude that the Wantai Ig and Vircell IgG ELISA's may be suitable for diagnostic and population screening purposes. The IgM/IgA tests performed poorer than their IgG/Ig counterparts, but may have a role in diagnoses of SARS-CoV-2 in a population in which the background seroprevalence of IgG high, and IgM and/or IgA may distinguish between acute or past infection.

P88

Antibodies against SARS-CoV-2 in healthcare workers and clinical symptoms as putative antibody production prediction.

Drs. Daniëlle Hanssen[1], Bsc K Heijgele[1], MD L E A Bank[1], MD M Slaats[1], MD M Mulder[1], Associate Professor J Penders[1,2,3], Prof. P H M Savelkoul[1,2], Dr. I H M van Loo[1,2]
[1]Maastricht University Medical Center, [2]Maastricht University, [3]Maastricht University

Introduction

In addition to molecular testing, serologic testing of health care workers (HCWs) might be of value for mitigation strategies and return-to-work decision making. The aim of this study was to gain insight in the development of antibodies to SARS-CoV-2 in infected HCWs and to explore possible clinical predictors of the IgM, IgA, IgG and IgT antibody response.

Methods

In this retrospective analysis, data from PCR-confirmed COVID-19 HCWs (n=156) was included. At time of initial COVID-19 PCR (T=0), a questionnaire including HCWs characteristics and symptoms was collected. Sera were collected at 2 timepoints: the first serum at the time of the initial PCR (T=0), and the second at least 21 days after the initial serum (T≥21). The Wantai SARS-CoV-2 IgM ,Wantai SARS-CoV-2 Ab ELISA (IgT), Euroimmun IgA, and Euroimmun IgG ELISA were used to determine antibody responses to SARS-CoV-2 infection. Logistic regression analysis was performed to assess associations between antibody responses and patient characteristics.

Results

156 HCWs were included with a mean age of 40 years (SD 13). The mean time from onset of symptoms until the initial serum (T=0) and the follow up serum (T≥21) was 7 days (SD 5), and 38 days (SD 12), respectively. At T=0, 34% (37/109) of the HCW showed IgM, 21% (21/108) IgA, 13% (14/108) IgG and 45% (49/109) IgT. At T≥21, 78% (107/137) of the HCW tested positive for IgM antibodies, 51% (69/135) for IgA, 72% (97/135) for IgG and 96% (132/137) for IgT.
The most common reported symptoms were coughing (106/155; 68.4%), rhinorrhoea (110/156; 70.5%), and headache (95/156; 60.9%). We found no correlation between reported symptoms and antibody responses. However we observed higher IgM responses in males (p= 0.008).

Conclusion

At T≥21 a substantial proportion of HCWs developed IgM (78%), IgG (72%), and IgT (96%), antibodies. Interestingly, we observed IgA seroconversion in about only 50% of the HCWs. Furthermore, we observed higher IgM responses at T≥21 in male HCWs.

P89

Long lasting immune response in hospitalized patients with COVID-19, a 6-month follow-up study

Msc Michiel Slaats[1], Dr. F. Magdelijns[2], Dr. A. Oude Lashof[1,3], B. van der Veer[1,4], L. Liu[3], R. Posthuma[5,7,8], S. van Santen[6], Dr. I. van Loo[1,4]

[1]Maastricht University Medical Center (MUMC+), [2]Maastricht University Medical Center (MUMC+), [3]Maastricht University Medical Center (MUMC+), [4]School for Care and Public Health Research Institute (CAPHRI), [5]Maastricht University Medical Center (MUMC+), [6]Maastricht University Medical Center (MUMC+), [7]Centre of expertise for chronic organ failure (CIRO+), [8]NUTRIM School for Nutrition and Translational Research in Metabolism

Introduction

The corona virus disease 2019 (COVID-19) has spread over the world at an alarming rate. To prevent further spread, vaccines are developed, for which an adequate protective immune response is essential. However, duration of and protection against infection is yet unknown. Therefore, we evaluated the antibody responses in ICU and general ward COVID-19 patients at, during and after hospital admission with follow-up until 6 months.

Methods

Sera of patients admitted to the Maastricht University Medical Centre, the Netherlands, between March through June 2020, due to SARS-CoV-2 infection were retrospectively analysed. Height of IgG and IgM antibodies in serum were measured using semi-quantitative ELISA's, at different time points during admission (day of admission, week 1, week 2, week 4) and at follow-up (between 8 and 31 weeks after admission). Optical density (OD) was used as an indicator of antibody response and differences over time were analysed. The interval between start of illness and serum sample date was calculated for optimal comparison.

Results

At admission, IgM was detectable in 68.3% (56/82) and IgG in 37.8% (31/82). IgM rose rapidly to a positivity rate of 97.7% at 2 weeks. After this peak a decline was seen and 50.5% were negative for IgM at follow up. IgG rose later and eventually positivity rate of IgG increased to 95.2% (100/105) at follow up. Despite a decrease in ratios, IgG antibodies were still detectable after 6 months. ICU patients had significant higher IgM at the first week of admission, compared to patients admitted to the general ward. (p=0.002). However no differences were seen in IgM or IgG at follow up.

Conclusion

Initial antibody response of hospitalized COVID-19 patients consist largely of IgM, which peaks around 3 weeks and eventually disappears. In 95.2% IgG antibodies are seen; IgG shows a peak around 6 weeks and remain detectable at least 6 months and it will most likely remain detectable for over a year. While the height of IgM is initially higher in more severely ill (ICU) patients, no differences were seen in IgM or IgG after a month.

P90

Antibiotic prophylaxis in transrectal prostate biopsy: resistance data based on rectal screening

Mw. Sofie Tops[1], Dr. E. Kolwijck[1], Prof. dr. H.F.L. Wertheim[1]
[1]*Radboudumc*

Introduction: Several classes of antibiotics are proven effective as perioperative prophylaxis in transrectal prostate biopsy (TPB). Antibiotic prophylaxis with fluoroquinolones (FQ) was the first choice for many years due to effective penetration into prostate tissue and low resistance rates. However, increasing FQ resistance in gram-negative bacteria resulted in a significant increase up to 6% in infectious complications after TPB. Guiding antibiotic choices based on individual susceptibility testing of rectal flora may reduce infectious complication rates. Moreover, surveillance of local resistance data is useful to investigate the suitability of oral empirical antibiotic prophylactic regimens in TPB. In this study, the resistance rates of gram-negative bacteria in rectal cultures for different antibiotics are shown. These rectal cultures were collected in the contex of a prospective randomized multicenter trial (PRO-SWAP) on targeted prophylaxis in TPB.
Methods: Rectal cultures were collected between 11 February 2018 and 19 January 2021 using Eswab (Copan, Murrieta, CA). One drop of ESwab medium was plated on a Colombia blood agar (growth control) and four selective screening agars. Each selective agar consisted of a MacConkey medium with vancomycine plus one of following antibiotics: ciprofloxacin 0.5 mg/L, trimethoprim 2 mg/L, fosfomycine 4 mg/L + glucose-6-phosphate 25 mg/L or mecillinam 2 mg/L + amoxicillin/clavulanic acid 8 mg/L. The inoculated plates were incubated at 36 °C for 48 hours. Growth on the selective agar plate(s) demonstrated rectal carriage with gram-negative bacteria that had a higher mean inhibitory concentration (MIC) than the antibiotic concentration in the agar. In this situation, we agreed that it was not safe to prescribe the antibiotic in the agar as prophylaxis for TPB. All Enterobacterales, Acinetobacter species or Pseudomonas aeruginosa, identified by MALDI-TOF, were defined as clinically relevant.
Results: In total, 1275 rectal culture results related to 1275 TPBs were obtained. Growth was observed on 15.3% of the agars with ciprofloxacin, 53.1% of the agars with trimethoprim, 62.4% of the agars with fosfomycin and 44.3% of the agars with mecillinam + amoxicillin/clavulanic. Growth was observed on 100% of the growth controls. In 49.7% of cultures with growth on the agar with ciprofloxacin, growth was also present on the other three selective agars.
Conclusion: Based on these resistance data, ciprofloxacin remains the best empirical oral perioperative prophylactic choice for TPB. However, since ciprofloxacin is not effective in 1 in 7 patients, culture-guided prophylaxis might be an effective strategy to select alternative oral antibiotics to reduce infectious complications after TPB.

P91

Surveillance of azole resistance in Aspergillus fumigatus in the Netherlands renewed

Drs Rebecca van Grootveld[1,2], Drs A Hendriks[2], Dr T Bosch[2], Dr A Schoffelen[3], Dr S de Greeff[3], Prof Dr E Kuijper[1,2], Prof Dr P Verweij[2,4]

[1]Leiden University Medical Center, [2]National Institute for Public Health and the Environment, [3]National Institute for Public Health and the Environment, [4]Radboud University Medical Center

Introduction

In 2013 surveillance of azole resistance in Aspergillus fumigatus was initiated due to increasing azole resistance. In ten hospitals (five university medical centres and five teaching hospitals) isolates are screened for azole resistance with a four-well agar plate (VIPcheckTM) and resistant isolates are sent to the reference laboratory Radboudumc for MIC testing and sequence analysis of the cyp51a gene. The current surveillance is suboptimal because of a number of reasons: the geographical distribution of surveillance hospitals in the Netherlands, all isolates are screened including isolates that are clinically irrelevant, no clinical information is collected and culture-negative cases of invasive aspergillosis are not included. For these reasons the resistance surveillance is being renewed.

Aims of new forms of surveillance

To gain insight into: 1) the magnitude of the problem of azole-resistant A. fumigatus, 2) changes over time of azole-resistant A. fumigatus phenotypes and genotypes, 3) characteristics of patients with invasive aspergillosis, 4) the disease burden of invasive Aspergillus infections

Performance of new forms of surveillance

All patients with a positive A. fumigatus culture will be included and evaluated. From these patients we will collect basic information: date of birth, sex and postcode. Only isolates from patients with a suspected fungal infection and treated accordingly, or azole-resistant isolates will be sent to the reference laboratory Radboudumc for MIC testing and sequence analysis of the cyp51a-gene. In addition, RIVM will perform whole genome sequence analysis of the isolate. Patients who are treated with antifungals and have a classifiable (EORTC/MSGERC, AspICU, IAPA, CAPA) disease will be included in an in-depth analysis to collect information about clinical characteristics, predisposing host factors, antifungal treatment and outcome of the infection. Similar as in the basic surveillance, clinical isolates from patients included in the in-depth analysis will be characterized by MIC testing, sequence analysis of the cyp51a-gene and whole genome sequence analysis. Once the intensified surveillance is established, also culture-negative invasive aspergillosis cases will be included.

Who will perform the surveillance?

The basic surveillance will be performed by the local medical microbiologist. For the in-depth surveillance, support of a physician will be provided.

How will the data become available?

A yearly report with surveillance data will be published in Nethmap and data from the in-depth analysis will be published in a medical journal. Sequencing data can be requested from the RIVM and will be archived in the

European Nucleotide Archive (ENA) database. Participating hospitals will receive reports on resistance phenotypes and resistance markers, as well as a phylogenetic tree of all sequenced A. fumigatus cultures.

P92

Follow-up of carriership of multidrug resistant micro-organisms (MDRMO) by taking periodic swabs.

Msc Marguerite Bruijning[1], Ing. H.C.M. Dogterom-Ballering[1], Dr. K.E. Veldkamp[1]
[1]*LUMC*

Introduction
Anti-microbial resistance is increasing worldwide. Dutch Regional Antimicrobials Stewardship teams are working on measures to prevent the spread. Therefore MDRMO carriers are labeled in the electronical medical record (EMR). Primary goal of this study was to follow-up patients carriership by taking rectal swabs at predefined time intervals to see if the notification in the EMR could be removed. Secondary goal was to create a system in which patients are known with their own carrier status and able to communicate the carrier-status when needed.
Participating patients treated at the LUMC (Leiden University Medical Center) which were new or known carriers of MDRMO received a letter with information of their carriership which they could show to other health care institutions to be sure the right precautionary measures could be taken.

Methods
Between June 2019 and December 2019 patients were informed through a letter written by their doctor if they were MDRMO carrier. Simultaneously with this letter, patients received a brochure and after informed consent, participating patients received after 1 month, 3 months, 6 months and 13 months an envelope with swab materials, a cover letter and instructions how to take and to send the swab to the lab. Patients received the results of the cultures by letter or email. After 2 negative cultures one month apart for Extended Spectrum Beta Lactamase Enterobacteriaceae (ESBL), 12 (3 at a time) consecutive in 13 months for Carbapenemase Producing Enterobacteriaceae (CPE) and 20 (5 at a time) consecutive negative cultures in 13 months for Vancomycin Resistant E.faecium (VRE) the notification in the electronic file would be removed.

Results
In 6 months time 98 patients (56% female) were included in this study. Of 62 patients we were able to complete the data in one year time. For 20 patients the last cultures are still expected and they were excluded in these results. After one year 16 (26%) fulfilled the criteria to lift the MDRMO notification. Most patients were carrier of an ESBL or FA resistance MDRMO.
Twenty three (37%) of the participants started and ended the study with the same micro-organism in four cultures and 21 (34%) patients had varying results in their cultures.

Conclusions
After a 13 month follow up period are 26% of the participants are no longer carrier of MDRMO according to the predefined regional criteria, and their notification could be removed. Thereby we prevent unnecessary isolation measures and associated drawbacks. Participating patients were generally satisfied to know their MDRMO status according to the emails and phone calls we received on a for the purpose reserved address and consultation hour. Also patients were willingly to take and send in the cultures since only 6 were lost to follow up. Transfer of carriership data is important to be able to take precautions to prevent spread of resistance. Secondly unnecessary preventive measures should be prevented, this study shows that patients can play an active role in this mission.

P94

Signal-based, tailored, and (re-)interpretable protein sub-cellular localization predictions in Gram-positive bacteria through a novel meta-predictor

Dr. Stefano Grasso[1,2], Dr. T van Rij[2], Prof. JM van Dijl[1]
[1]UMCG, [2]DSM B.V.

Introduction: Subcellular localization (SCL) is a critical aspect of protein function and the potential application of proteins either as drugs or drug targets, or in industrial and domestic applications. However, the experimental determination of protein localization is time-consuming and expensive. Therefore, various localization predictors have been developed for particular groups of species. Most currently available SCL predictors rely on finding homologs of proteins of interest and assigning to them the SCL of the best hit. Despite its efficiency, this approach relies on database annotations, which may be poor, and on how many homologs from closely related species have been studied. Intriguingly, despite their major representation among biotechnological cell factories and pathogens, a meta-predictor based on sorting signals and specific for Gram-positive bacteria was still lacking. Lastly, with the recent explosion of machine- and deep-learning, the emphasis on interpretability and data reusability drastically reduced. We thus present the GP4 SCL meta-predictor, which addresses these issues.
Methods: GP4 (Gram-Positive Protein Prediction Pipeline) combines multiple tools, each specific for different sorting signals and compartments. GP4 aims at mimicking protein sorting as it would happen in a bacterial cell. Since GP4 is not homology-based it has a broad applicability and does not depend on annotated databases with homologous proteins. In fact, its applicability is mainly within the phylum of Firmicutes, but it works also for Actinobacteria. Being a sorting-signal based predictor, GP4 is particularly suited also for heterologous or engineered proteins, as well as completely synthetic organisms. A webserver running GP4 is available at: http://gp4.hpc.rug.nl/
Results: Our benchmark analysis highlights the improved performance of GP4 compared to other widely-used SCL predictors. Additionally, GP4 presents features that are usually overlooked by other tools. Novelty elements include: improved cell-wall protein prediction, including differentiation of the type of interaction, prediction of non-canonical secretion pathway target proteins, separate prediction of lipoproteins, and better user experience in terms of parsability and interpretability of results. Non-canonical usage may include: little studied or novel species, synthetic and engineered organisms, and re-use of prediction data to develop custom prediction algorithms.
Conclusion: Due to its superiority in detecting extracellular and cell-wall proteins, GP4 can probably help in the identification of novel targets for drugs against pathogenic Firmicutes and Actinobacteria. This is a consequence of its design, where prior knowledge on genomes or proteins is not necessary. The main limitation of GP4 is our overall understanding of protein sorting and our ability to in silico mimic it. Altogether, we advocate a paradigm shift in the development of SCL predictors, as meta-predictors exploiting specific strengths and compensating for weaknesses of the individual tools have proved superior. Nevertheless, a stronger effort in developing sorting signal detectors should be made, yet without disregarding usability and parsability. Lastly, standalone versions that do not rely on webservers, and with standardized outputs that are easy to programmatically read and parse will be necessary to foster the field in this direction.

P95

The zebrafish embryo as a novel infection model for Neisseria meningitidis

Yvonne Pannekoek, Ing. Kim Schipper, Arie van der Ende

Background
Neisseria meningitidis, the meningococcus, has to overcome the host innate immune system to develop severe invasive meningococcal diseases like meningitis and septicemia. The polysaccharide capsule is the most important virulence factor; unencapsulated meningococci do not survive in the bloodstream. The immune system of the zebrafish embryo resembles that of mammals. Two days post-fertilization the innate immune system is fully functional, while the adaptive immune system does not develop before 4 weeks post-fertilization.
Due to its transparency and the availability of a wide range of available fluorescent tools, the zebrafish embryo infection model offers the unique ability to study host-pathogen interaction in real time. We explored the zebrafish embryo as a potential meningococcal infection model to study host-pathogen interaction
Methods
Wildtype meningococcal strain H44/76 (serogroup B) and its unencapsulated isogenic variant HB-1 were transformed to express mCherry from the chromosome. Zebrafish embryos were collected within 2 hours post-fertilization (hpf) and kept at 28 °C in embryo medium. Bacteria (~5x103 cfu) were injected in the fish caudal vein at 28 hpf and zebrafish embryos (20-40/experiment) were monitored for survival up to 96 hours post-infection (hpi). Location of meningococci in the embryos was assessed by fluorescence microscopy. Transgenic zebrafish embryos with eGFP expressing neutrophils (mpo:eGFP) were used to study the interaction between neutrophils and the meningococcus. Neutrophils were quantified by estimating the level of green color in images taken from defined areas in the embryos using the image processing and analysis program ImageJ.
Results
Zebrafish embryos killing by H44/76 was dose dependent. 50% of the embryos were killed within 96 hpi when infected with an inoculum between 4x103 and 8x103 cfu. At 24hpi, 33% of the embryos were dead, while in the remaining embryos meningococci were observed throughout the body (58%) or the tail only (10%) and often showed pericardial edema. At 48 hpi, 43% of the embryos were dead, 23% had cleared the infection, while 18% of the embryos had systemic infection, 8% showed meningococci in the head only and 10% in the tail only. The survival at 96 hpi of embryos infected with the wt or the unencapsulated HB-1 was 51 ± 2 %, and 84 ± 5%, respectively, while 93 ± 4 % of mock infected embryos survived ($p<0.05$).
Reculture of H44/76 meningococci after 1-4 hpi indicated multiplication in the zebrafish and meningococci were still cultured from the embryos at 24 hpi. In contrast, HB-1 could be cultured up to 4 hpi, but at 3 and 4 hpi the number of cfu was 20-fold lower than that of H44/76 ($p<0.022$). At 24 hpi, the number of neutrophils was 80% lower in H44/76 infected embryos than in mock infected embryos ($P<0.0001$). In HB-1 infected embryos the number of neutrophils was 3-fold higher than in H44/76 infected embryos ($P<0.0001$).
Conclusion
The zebrafish embryo is a promising infection model to study the pathogenesis of invasive meningococcal disease. This model will be useful for medium-throughput screening. The polysaccharide capsule is also in zebrafish an important virulence factor.

P96

Predicting neonatal early onset sepsis: a 14-year cohort study

Alieke Van Der Hoeven[1], MT van der Beek[1], E Lopriore[2], SJ Steggerda[2], V Bekker[2]
[1]Leiden University Medical Center, [2]Leiden University Medical Center

Introduction
Neonatal early onset sepsis (EOS) has a high mortality if treatment is not initiated promptly. Since signs and symptoms can be very subtle and non-specific, treatment is started in many infants. Early prediction of the absence of a culture proven sepsis in infants would significantly reduce the time of antibiotic treatment and hospitalization. We hypothesized that if clinical suspicion is low, C-reactive protein (CRP) is low and the blood culture does not show growth 24 hours after onset of suspicion of infection, it is safe to stop the antibiotics.

Methods
We retrospectively reviewed data from 2007 until 2020 of infants with a suspicion of EOS, either based on risk-factors or clinical signs. We collected blood culture results, CRP and, in infants where time to positivity (TTP) of the blood culture was ≥ 24 hours, clinical parameters.

Results
The blood culture showed growth of a pathogenic microorganism in 50 of 4120 included infants (1.2%). Median TTP was 14.4 hours, and eight infants (16%) had a TTP ≥ 24 hours (range 24.03-44), of whom seven infants had a raised CRP and/or had clinical symptoms of infection within 24 hours. If antibiotics had been stopped at 24 hours, the infant without signs of infection and a low CRP would have received adequate treatment since antibiotics given at 24 hours will be effective for another six hours.

Conclusion
A combination of blood culture results, CRP and clinical signs of infection within 24 hours after suspicion of infection can rule out EOS.

P97

Posaconazole for prevention of invasive pulmonary aspergillosis in patients with severe influenza admitted to the intensive care unit: A randomised, open-label, phase IV, proof-of-concept trial

Drs. Nico Janssen[1,2], MD L Vanderbeke[3,4], MD, PhD DCJJ Bergmans[5], MD, PhD M Bourgeois[6], MD JB Buil[1,7], MD, PhD Y Debaveye[8,9], MD, PhD P Depuydt[10], MD S Feys[3,4], MD, PhD G Hermans[3,9], MD O Hoiting[11], MD B van der Hoven[12], MSc C Jacobs[3], PharmD, PhD K Lagrou[4,13], MD V Lemiale[14], MD P Lormans[15], MD, PhD J Maertens[4,16], MD P Meersseman[3,4], MD, PhD B Mégarbane[17,18], MD, PhD S Nseir[19,20], MD JAH van Oers[21], MD M Reynders[22], MD, PhD BJA Rijnders[23], MD, PhD JA Schouten[24], PharmD, PhD I Spriet[25,26], PhD K Thevissen[27], MD, PhD AW Thille[28], PharmD R Van Daele[25,26], MD, PhD FL van de Veerdonk[1,2], MD, PhD PE Verweij[1,7], MD, PhD A Wilmer[3,4], PharmD, PhD RJM Brüggemann[1,29], MD, PhD J Wauters[3,4]

[1]Radboud University Medical Center, [2]Radboud University Medical Center, [3]University Hospitals Leuven, [4]KU Leuven, [5]Maastricht University Medical Center, [6]AZ Sint-Jan Brugge-Oostende, [7]Radboud University Medical Center, [8]University Hospitals Leuven, [9]KU Leuven, [10]Ghent University Hospital, [11]Canisius-Wilhelmina Hospital, [12]Erasmus MC University Medical Center, [13]University Hospitals Leuven, [14]Saint-Louis Hospital, [15]AZ Delta Hospital, [16]University Hospitals Leuven, [17]Lariboisière Hospital, [18]University of Paris, [19]University Hospital Lille, [20]University of Lille, [21]Elisabeth-TweeSteden Hospital, [22]AZ Sint-Jan Brugge-Oostende, [23]Erasmus MD University Medical Center, [24]Radboud University Medical Center, [25]University Hospitals Leuven, [26]KU Leuven, [27]KU Leuven, [28]University Hospital Poitiers, [29]Radboud University Medical Center

Introduction

Invasive pulmonary aspergillosis (IPA) characteristically affects immunocompromised patients. However, IPA has been increasingly recognized as a complication in patients with severe influenza admitted to the intensive care unit (ICU), occurring in 16–19% of this population and associated with increased mortality. We investigated whether antifungal prophylaxis with posaconazole (POS) could reduce the incidence of influenza-associated pulmonary aspergillosis (IAPA).

Methods

A randomised, open-label, phase IV, proof-of-concept clinical trial was performed comparing 7 days of intravenous POS prophylaxis (POS arm) with no prophylaxis and standard-of-care only (SOC arm) in adult patients admitted to ICU with respiratory insufficiency due to influenza in 12 Belgian, Dutch and French participating centres. All patients suffered from influenza as proven by polymerase chain reaction (PCR), had influenza symptoms for ≤ 10 days and underwent mycological diagnostic work-up within 48 hours of ICU admission. Patients with IAPA on admission were excluded from the study as they required antifungal therapy. The primary endpoint was the incidence of IAPA (according to the modified AspICU criteria) at ICU discharge in patients who did not already suffer from IPA at inclusion (modified intention-to-treat [mITT] population). This trial was registered at ClinicalTrials.gov, NCT03378479.

Results

Between 1 December 2017 and 31 March 2020, 252 critically ill influenza patients were screened for inclusion at the participating centres. Ultimately, 88 patients were included in the study and were randomised to either POS (43/88 [48.9%]) or SOC (45/88 [51.1%]). IAPA was diagnosed within 48 hours of ICU admission (early IAPA) in 15/88 (17.0%), with tracheobronchitis in 4/15 (26.7%); these patients were excluded from the mITT population. Of the remaining 73 patients in the mITT population, 37/73 (50.7%) were randomised to the POS arm and 36/73 (49.3%) to the SOC arm. Incidence of proven and putative IAPA at ICU discharge (late IAPA) was 2/37 (5.4%) in the POS group and 4/36 (11.1%) in the SOC arm (p = 0.32). IAPA in the two patients in the POS arm was diagnosed after completing the full 7-day prophylactic course.

In total, IAPA occurred in 21/88 (23.9%) included patients. Overall, ICU mortality was 12/21 (57.1%) in all IAPA patient versus 12/67 (17.9%) in all non-IAPA patients (p = 0.001). POS prophylaxis was prematurely discontinued in 9/37 (24.3%) patients.

Conclusion

1. IAPA incidence in respiratory insufficient patients admitted to ICU was 21/88 (23.9%).
2. IAPA was diagnosed within 48 hours of ICU admission in 15/21 (71.4%, early IAPA).
3. IAPA was associated with a high in-ICU mortality (57.1%), even after early diagnosis and immediate antifungal therapy.
4. The high incidence and mortality of early IAPA indicates that patients with severe influenza require immediate antifungal therapy on admission to the ICU. POS prophylaxis might be beneficial to prevent late IAPA.

P98

Dissemination of a mosaic transposon carrying fourteen different antimicrobial resistance genes driven by a polyclonal outbreak in two hospitals.

Casper Jamin[1], M De Vries[1], W Van Der Zwet[1], E Heddema[2], Prof. C Hoebe[1,3], Prof. P Savelkoul[1], L Van Alphen[1]
[1]Maastricht UMC+, [2]Zuyderland Medical Centre, [3]South Limburg Public Health Service

Introduction
Bacterial antimicrobial resistance (AMR) is a worldwide threat and nosocomial spread of multiresistant bacteria is frequently observed. The introduction of whole genome sequencing (WGS) allows for high resolution typing of resistant bacteria and obtaining a detailed genomic profile such as AMR genes and plasmids. Unfortunately, assembly of complete chromosome and plasmid sequences using short-read WGS is impossible, thus a hybrid approach combining long read sequencing data must be employed for its complete reconstruction.
An initial cluster of three patients, carrying clonal extensive drug resistant (XDR) Enterobacter cloacae, was identified. Further surveillance revealed more colonized patients, but also hospital environmental samples to be colonized with phenotypically similar E. cloacae. In this same period comparable drug resistant E. cloacae and Klebsiella pneumoniae were found in a hospital in the region. We aimed to identify possible transmission links of the different XDR strains found in both hospitals.

Methods
In total 15 Enterobacter strains from our hospital and 5 Enterobacter and 8 Klebsiella strains from the neighbouring hospital were subjected to WGS. Furthermore, a selection of 6 genetically diverse isolates was subjected to longread sequencing. Transposon phylogeny was determined using TETyper.

Results
Short read sequencing revealed that the Klebsiella pneumonia strains formed a single clonal complex, while the Enterobacter cloacae strains belonged to various sequence types. In both hospitals, in total three outbreak clusters were identified, yet no interhospital transmission was observed. Various non-clonal strains of both hospitals were carrying the same set of genes conferring resistance to tetracyclines, trimetroprim, (fluor)quinolones, aminoglycosides, chloramphenicol, sulfonamides and beta-lactams. Unfortunately, short read assembly generated a fractioned assembly and most AMR genes were located on small contigs. Using long read sequencing a 41kbp transposon was identified. This region was extremely dense in AMR genes and transposases, as it coded for 14 resistance genes and 19 transposases from various families. In the strains subjected to long read sequencing, this transposon was found twice on the chromosome and in four strains on plasmids with completely different k-mer profiles and origin of replication. Three conserved regions of 7, 15 and 19 kbp were identified. Although these regions were conserved, the orientation and order of these regions showed rearrangements in various strains. Even clonal isolates showed rearranged transposon regions. Typing of the transposon on single nucleotide polymorphism (SNP) level using short-reads, revealed seven variants among the 28 isolates. One variant was indistinguishable among the 2 Klebsiella and 12 Enterobacter isolates found in both hospitals.

Conclusion
These results show the different layers of complexity in the dissemination of antimicrobial resistance. Firstly, three different small outbreaks clusters of two species were observed, all having the same set of

AMR genes. Secondly, completely different plasmid backbones were found, excluding the possibility of plasmid transmission. Finally, typing the transposon on SNP level revealed possible spread of this single mosaic structured transposon in different isolates in two hospitals. The dissemination of such a transposon is probably driven by antibiotic pressure and thus enabling bacteria to survive the most common used antimicrobial agents used in our hospitals.

P99

Antibody index as a tool for the diagnosis of neurological complications of COVID-19

Marc Shamier[1], Ms. Y.C. Crijnen[2], Dr. M. Titulaer[2], Ms. S. Bogers[1], Dr. C.H. Geurtsvankessel[1]
[1]Erasmusmc, [2]ErasmusMC

Introduction
Growing numbers of reports support an association between neurological disease and SARS-CoV-2 infection. Provisional case definitions for COVID-19-associated neurological disease were proposed by Ellul, including inflammatory disease of the central nervous system (CNS) due to a direct viral effect or an immune-mediated mechanism and cerebrovascular disease(1). A recent study on 33 deceased patients provided evidence for SARS-CoV-2 neuroinvasion through PCR and immunohistochemistry. SARS-CoV-2 RNA was demonstrated in the olfactory bulb, suggesting a potential port of entry into the CNS(2). Antibody Index serology is a useful tool for the diagnosis of viral infections of the central nervous system, especially in later stages when viral genome can no longer be detected in CSF(3). We investigated the utility of SARS-CoV-2 antibody index for the diagnosis of neurological complications of COVID-19.

Methods
Paired CSF and serum samples were collected of patients with PCR proven SARS-CoV-2 infection and neurological symptoms. SARS-CoV-2 IgG titers were measured in serum and CSF using an immunofluorescence assay. To correct for blood-CSF barrier dysfunction, albumin and IgG were measured. Antibody indexes were calculated as previously described by Reiber(4). When CSF and serum sample volumes were sufficient for additional analysis, we screened for autoantibodies associated with auto-immune encephalitis. A SARS-CoV-2 PCR was performed on all CSF samples. To assess the likelihood of a SARS-CoV-2 associated etiology, clinical and serological data were interpreted by a neurologist and a clinical virologist. The results were compared to antibody indexes of mortem controls without neurological disease.

Results
Nine patients with proven SARS-CoV-2 infection and neurological symptoms were included. The median age was 65 years. Neurological manifestations included encephalopathy, encephalitis and seizures. No SARS-CoV-2 RNA was demonstrated in CSF. Intrathecal SARS-CoV-2 IgG production was demonstrated in 3 patients with impaired consciousness. The antibody indexes of these patients ranged from 1.9 to 21.5, versus 0.06 to 0.3 in post-mortem controls. 5 patients were screened for the presence of autoantibodies, there were no signs of auto-immune encephalitis. Several patients with a low SARS-CoV-2 antibody index had metabolic disturbances (uremia, hepatic dysfunction) as an alternative explanation for the neurological symptoms.

Conclusion
1. The demonstration of intrathecal SARS-CoV-2 specific IgG production can provide evidence for a causal relationship between neurological disease and SARS-CoV-2.
2. Because of the systemic inflammation in COVID-19 and the comorbidity associated with severe disease, alternative explanations such as metabolic disturbances and auto-immune encephalitis should be ruled out.

References
1. Ellul MA, Benjamin L, Singh B, Lant S, Michael BD, Easton A, et al. Neurological associations of COVID-19. Lancet Neurol. 2020 Sep;19(9):767–83.

2. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nat Neurosci. 2020 Nov 30
3. Shamier MC, Bogers S, Yusuf E, Berge JCEM, Titulaer M, Van Kampen JJA, Geurtsvankessel CH. The role of antibody indexes in clinical virology. Under review. December 2020.
4. Reiber H, Peter JB. Cerebrospinal fluid analysis: Disease-related data patterns and evaluation programs. J Neurol Sci. 2001; 184: 101-122.