P001

New rapid test for the characterization of carbapenemase-producing microorganisms.

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Introduction:
Rapid identification of carbapenemase-producing organisms (CPO) is important for patient management, infection control and epidemiology. When a CPO is detected, it is essential for timely containment and epidemiology to establish the exact class of carbapenemase of the strain. Since molecular testing is not available in every microbiology laboratory, a fast but reliable alternative is warranted. We evaluated the NG-Test CARBA 5 rapid immunoassay, which can confirm KPC-, OXA-, VIM-, IMP- and NDM-carrying strains in fifteen minutes.
Methods:
In total 30 well-characterized CPO and 10 non-CPO were tested. CPO were Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus mirabilis, Citrobacter freundii, Pseudomonas aeruginosaand Acinetobacterspecies, carrying KPC-, OXA-048-, VIM-, IMP-, NDM-, GES- and BIC-carbapenemase genes. Non-CPO were Enterobacteriaceaeand Pseudomonas aeruginosa, some of which known ESBL- or AmpC-producers. Of each strain a suspension was brought into the sample well of the test cassette, where after migration through the membrane containing labeled mouse anti-carbapenemase a red line is formed if the matching enzyme is present. A red control band indicates validity of the test.
Results:
All test results were valid. KPC, OXA-048, VIM and NDM carbapenemases were detected correctly. One strain with both KPC and VIM gave two clear red lines. GES, BIC and IMI, not detectable with this test, were negative. The three tested IMP-strains were negative, because these specific types (IMP-18, IMP-28) are not in the test's scope (IMP1, IMP-8, IMP-11). All non-CPO strains were negative.
Conclusion:
From a clinical point of view, for this challenging strain panel, the NG-Test CARBA 5 had a sensitivity of 80% and a specificity of 100%. However, the carbapenemases that were not detected, are rarely found. If results not in the test's intended use are excluded, sensitivity and specificity both were 100%. The test is easy to perform, with easy to interpret and quick results, and is a valuable asset to the confirmation of CPO.

P002

Rectal swab to determine appropriate prophylaxis prior to gold marker implantation.

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Introduction: During treatment of prostate cancer by irradiation, it is important to determine the exact position of the prostate. To this end fiducial gold markers are transrectally implanted into the prostate gland prior to image-guided radiotherapy. Because of the risk of infection by introducing enteral flora, patients receive prophylactic levofloxacin shortly before the procedure. In 2015-2016, eight cases of sepsis occurred following gold marker implantation. In most cases a fluoroquinolone-resistant Escherichia coliwas isolated from blood cultures. To evaluate for each patient the appropriate prophylaxis, we designed a study in which a pre-procedural taken rectal swab was cultured for fluoroquinolone-resistant Gram-negative rods.
Methods: Between April 2017 and May 2018 from all eligible patients a rectal swab was taken one week before gold marker implantation and sent in for culture. Of all isolated Gram-negative rods susceptibility testing for levofloxacin, amoxicillin-clavulanate, trimethoprim/sulfamethoxazole, doxycyline and fosfomycin was performed and reported. If one or more isolates tested resistant to levofloxacin, the next best choice of prophylaxis was administered. Additionally, each patient received a questionnaire in order to record adverse events of the implantation procedure, such as pain and fever.
Results: In total 82 patients were included in the study. From 9 (11%) patients one or more levofloxacin-resistant strain was cultured. In these cases cotrimoxazol (n=6), fosfomycin (n=2) and or ceftazidime i.v. (n=1) was administered. Of the 73 (89%) patients with fluoroquinolone-susceptible culture results, 68 received levofloxacin, five received ciprofloxacin. The response rate for the questionnaire was 94% (77 patients). Four patients reported (low) fever. On a scale of 1 (no pain) to 5 (very severe pain), 79% of the patients (n=61) experienced no pain, two patients reported severe pain. No cases of sepsis occurred during the study period.
Conclusion: Of the 82 patients in this study, for nine patients the antimicrobial prophylaxis was adapted according to culture results and for none of the included patients sepsis or other clinically relevant

adverse events were recorded. As a result, in our hospital, the culturing of a rectal swab has been implemented as a standardized part of the implantation procedure.

P003

Development of a genetic tool for Methylacidiphilum fumariolicum SolV

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Methane (CH_4) has become a compound of growing interest because of its implication in the global warming of the earth's surface. Methanotrophs are aerobic bacteria capable of using molecular oxygen to oxidize methane playing an essential role in the control of methane emitted in the atmosphere. Methylacidiphilum fumariolicum strain SolV isolated in 2007 from a geothermal environment in Italy is a thermoacidophilic verrucomicrobial methanotroph capable of growing at low pH and high temperatures. Its physiology has been extensively studied highlighting some exciting features like the ability to fix carbon dioxide (CO_2) into biomass via the Calvin-Benson-Bassham cycle, the possibility to use ammonium, nitrate or atmospheric nitrogen as nitrogen source and lanthanide-based methanol oxidation. Despite all the steps taken forward so far, Verrucomicrobia remain difficult to study, due to a lack of available genetic tools that would permit robust testing of hypotheses formulated from ecological and genomic data. For instance, Methylacidiphilum fumariolicum SolV possesses three copies of the pmo operon, which encodes the particulate membrane-associate methane monooxygenase (pMMO). Curiously though, pmoCAB3 was not expressed under any of the conditions tested so far. In order to generate knockout mutations of pmoCAB3 operon in strain SolV, this project aims to develop genetic tools enhancing opportunities to test the role of predicted gene function in the Verrucomicrobia phylum.

P005

Genotypically diverse Mycobacterium tuberculosis strains induce fundamentally different host responses in infected mice

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Introduction: The majority of TB cases are caused by Mycobacterium tuberculosisstrains of lineage 2 (East Asian/Beijing), lineage 3 (East-African Indian) and lineage 4 (Euro-American). Particularly lineage 2 mycobacterial strains belonging to the Beijing genotype have shown an aggressive global spread over the last century and have been associated with higher rates of treatment failure and disease relapse compared to other genotypes. In this study, we aimed to identify how these differences in virulence among modern Mycobacterium tuberculosislineages are reflected in host responses.
Methods: We infected BALB/c mice intratracheally with a Mycobacterium tuberculosisstrain belonging to lineage 2 (Beijing-1585), lineage 3 (EAI-1627) or lineage 4 (H37Rv). Disease progression was monitored up to 28 days post infection. We assessed mycobacterial loads, changes in cellular infiltrate composition in the lungs and cytokine expression at the protein and mRNA level in the lungs and bone marrow.

Results: Infection with Beijing-1585 and EAI-1627 resulted in 1.5 log higher lung mycobacterial loads at day 14 post infection compared to H37Rv infection. Beijing-1585 and EAI-1627 induced a marked influx of B-cells into the lungs and elevated pulmonary IL-4 protein levels compared to H37Rv, which induced a T-cell influx with higher IFN-γ and IL-17 levels. Myeloid cells in the lungs appeared functionally impaired upon infection with Beijing-1585 and EAI-1627 with reduced iNOS and IL-12 expression levels compared to H37Rv infection. This impairment might be related to the reduced expression in the bone marrow of IFN-γ, TNF-α and IFN-β in mice infected with Beijing-1585 and EAI-1627, already observed from the third day post infection onwards.

Discussion: Our findings indicate that the increased virulence of two clinical isolates compared to H37Rv is characterized by a fundamental differences in host responses, which already can be detected early during infection in lung as well as in bone marrow.

P006

Evaluation of the Atellica IM analyzer compared to the ADVIA Centaur XP analyzer for serologic

testing

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Background: The Atellica solution® is launched as the next fully-automated analyzer to replace the Centaur® immunoassay system for routine serologic testing by Siemens Health care diagnostics in Europe. Although the acridium esther technology and the reagents used in the immune assays are identical to the Centaur system, clinical validation in a routine diagnostic setting is mandatory. Materials/methods: For 10 different serologic assays (Hepatitis B virus: anti-HBE, HBE, HBS-antigen, anti-HBS, Hepatitis A virus: HAVT and HAM, hepatitis C virus: anti-HCV, HIV: CHIV, syphilis screening: SYF) the clinical performance and reproducibility was tested in our in-hospital routine laboratory. For each assay 40-50 patient sera were tested. The samples were carefully selected to obtain a mixed panel of positive, negative and possible cross-reactive patients for each disease state. Patient materials were prospectively analyzed at both ADVIA Centaur XP and Atellica IM and evaluated for concordance in quantitative (correlation coefficient >0.9)as well as qualitative (Cohens kappa >0.9) results. Reproducibility of patient sera with clinical relevant index values was tested in 3 different days. Results: Overall the tests showed a high level of correlation in quantitative results and met the criteria for acceptation although the concordance was not as high as was listed by the manufacturer. For several assays variation in performance between different lots of reagents was observed and this resulted in lower agreement between qualitative results for 2 assays (HAVT kappa 0.81, HCV kappa 0.87). Within lot reproducibility of clinical relevant sera with index values around the cutoff varied from 1 - 7,9%. The system was implementedin our fully automated daily routine diagnostic procedures. Evaluation after 3 months showed that reproducibility was not as stable as found in the verification process. This was partly explained by variation between reagents lots.
Conclusions: The Atellica system is suitable for the use of routine diagnostics for infection serology testing. Close monitoring of differences in quantitative and qualitative performance between different lots of reagents is needed.

P008

Crystal structure of the dihydrodipicolinate synthase: how to synthesize lysine in the acidothermophilic methanotroph Methylacidiphilum fumariolicum SolV

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Crystal structure of the dihydrodipicolinate synthase: how to synthesize lysine in the acidothermophilic methanotroph Methylacidiphilum fumariolicumSolV

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The amino acid lysine is synthesized in prokaryotes and plants, while it is an essential amino acid in mammals. Two dissimilar lysine biosynthesis pathways have evolved independently over time: the alpha-aminoadipate pathway and the diaminopimelate (DAP) pathway. In the latter pathway, all lysine biosynthesis routes are initiated by the enzyme dihydrodipicolinate synthase (DapA; EC: 4.3.3.7), which condenses L-aspartate-4-semialdehyde with pyruvate to form (2,4)-4-hydroxy-2,3,4,5-tetrahydrodipicolinate (HTPA). The enzyme is of pivotal importance for the entire biosynthesis pathway by means of negative feedback, since this enzyme is inhibited by allosteric binding of lysine, therefore regulating lysine production. Synthesis of lysine from HTPA can be established via four different routes: (1) the acetylase route, (2) the succinylase route, (3) the aminotransferase route and (4) the dehydrogenase route. The aminotransferase route was recently discovered in plants and later also in microorganisms such as methanococci, in which HTPA is converted to LL-2,6-DAP in a single step. Hereafter, meso-2,6-DAP is synthesized, which is the precursor molecule for peptidoglycan synthesis.

The methanotroph Methylacidiphilum fumariolicumSolV was isolated from a hot and extremely acidic volcanic ecosystem. This extremophile grows optimally at 55 °C, is able to grow below pH 1 and is dependent on rare-earth elements for growth. To investigate which of the four pathways for lysine biosynthesis is utilized by M. fumariolicumSolV, the closed genome was analyzed for lysine biosynthesis genes and compared to other known microbial genomes. Analysis revealed that M. fumariolicumSolV encodes for a fully operational aminotransferase route in the genome. In this way, HTPA can be converted directly to DAP by the enzyme LL-DAP aminotransferase and subsequently to meso-2,6-DAP

by the enzyme DAP epimerase for peptidoglycan synthesis. Phylogenetic analysis revealed clustering with other members of the Verrucomicrobia phylum.

With the use of Fast Performance Liquid Chromatography, DapA was co-purified together with a [NiFe] hydrogenase. The protein sample was divided over 480 different crystallization conditions utilizing the sitting drop method. A diffraction pattern of the microcrystals was obtained by X-ray crystallography, from which an electron density map could be inferred. The crystal structure of DapA was determined and revealed a homotetrameric enzyme, as a dimer of dimers. Preliminary analysis showed conservation of the structure in comparison with other microorganisms. More detailed structural analysis is needed to assess whether DapA of M. fumariolicumSolV contains structural novelties, for instance in terms of thermostability.

P009

COMPARISON OF SPF10-DEIA-LIPA SYSTEM VERSION 1 WITH TYPE-SPECIFIC QPCR FOR DETECTION OF HPV59 INFECTIONS, EVIDENCE FOR MISSING HPV59 INFECTIONS AND IMPACT ON VACCINE EFFECTIVENESS MEASUREMENTS

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IntroductionThe broad spectrum L1-based SPF10-DEIA LIPA system is widely used for HPV detection and typing in many epidemiological studies. This assay is known to be highly sensitive for most high risk HPVs but is less sensitive at detecting HPV59 infections. Here we investigated the sensitivity of this system on the detection of HPV59 infections and show the impact on vaccine effectiveness (VE) estimates. Methods Anogenital swabs collected in a biennial cross-sectional study among 16- to 24-year-old visitors to sexually transmitted infection clinics before and after introduction of HPV vaccination were tested using the SPF10-DEIA LIPA system. HPV59 viral loads were determined using a highly sensitive HPV59 qPCR viral load assay. HPV59 subtypes were determined by L1 sequencing. Results In total 6080 anogenital swabs collected in 2009, 2015 or 2017 were included in this study. All samples were tested with the SPF10-DEIA-LIPA system resulting in 194 HPV59 positive samples. Reanalysis of all samples with HPV59 qPCR identified 806 HPV59 infections. 614 HPV59 infections were not detected by with the SPF10-DEIA-LIPA system. Missed HPV59 infections had a significantly lower HPV59 viral load ($P<0.0001$). Associations of specific HPV59 subtypes and vaccination status of the participants in SPF10-DEIA-LIPA missed HPV59 infections were determined. Preliminary data suggest more missed infections in non-vaccinated participants and no differences in HPV59 subtypes in missed infections. VE measurements of the bivalent HPV vaccine against HPV59 infections based on both detection methods show a clear impact on the VE in preliminary results suggesting unmasking. Conclusion HPV59 infections are missed by the SPF10-DEIA-LIPA system.

P010

Evaluation of Two Sigma Transwab® Systems for Maintenance of Viability of Pathogenic Candida spp. Using the Clinical and Laboratory Standards Institute M40-A2 Standard

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An increase in healthcare complications due to fungal infections raises the requirement for Swab transport systems (STS) to efficiently recover and preserve pathogens of yeast origin. The Clinical and Laboratory Standards Institute (CLSI) M40-A2 protocol for evaluation of liquid-based swab transport systems for recovering organisms is used to assess the compliance and quality control of STS but at present does not address the recovery of yeast. The aim of this study was to compare the results of two commercial STS stored at room temperature and 4°C, over 48 h and their ability to recover and maintain viability of five clinical and reference strains of Candidaspp., including C. aurisNCPF 8971, C. albicansNCPF 3179, C. tropicalisNCPF 3111, C. parapsilosisATCC 22019 and C. glabrataATCC 2001. Findings from this study indicate that the STS used in this study are suitable for the collection and maintenance of the Candidaspp.tested, and is very suitable for the recovery of clinical C. auris.

P011

Diet induced differences in the gut microbiome composition in healthy humans

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Introduction: The Intestinal microbiota plays a central role in nutrient acquisition, protecting the host against invading pathogens and developing the immune system. In addition, the human microbiota has been implicated in the development of numerous diseases. Environmental factors such as lifestyle and diet are known to shape the microbiome. In this study, we aimed to detect structural differences in microbiota composition as a result of different diets.

Methods: Fecal samples from 149 healthy individuals in the Netherlands were selected from an existing collection derived from an earlier study on ESBL prevalence in vegetarians and non-vegetarians (Vegastudy). Samples were categorized in four different groups based on their diet: 1) meat consumers (n= 50), 2) fish consumers (n= 33), 3) vegetarians who do not consume fish or meat (n= 34) and 4) vegans who do not consume any animal derived products (n= 32). Groups were matched for gender, age, education level, having children at day-care, having pets, being ESBL-positive, travel abroad, living on a farm and degree of urbanization. Participants were excluded in case of antibiotic and proton pump inhibitor usage. All participants had given permission to use their fecal sample for further research. The microbiota composition was determined by 16s rRNA profiling. Sequencing data was analyzed using the Qiime2 and DADA2 pipelines. Statistical analyses were performed in R.

Results: The comparison of the relative abundances between the different diet groups revealed significant differences in the microbiota at both the family (n=10) and genus (n=13) levels. Most notably, there was a decrease of Lactobacillaceaeand treptococcaceaein vegans relative to meat consumers, which could be explained by the lack of dairy products in the vegan diet. No significant differences were found between the different diets by unsupervised analyses in either alpha diversity, using the Shannon diversity index, or in beta diversity using principal component analysis.

Conclusion: This study indicates that diet has an influence on microbiota composition in healthy individuals. In future studies, these sample will be analyzed with additional sequencing techniques to further unravel differences in both the microbiome as in the composition of the reservoir of resistance genes; the resistome.

P012

Exploring TTV as a biomarker of viral infection and rejection after kidney transplantation

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Introduction Torque teno virus (TTV) is a non-pathogenic virus infecting virtually everyone. TTV blood load has been proposed as a biomarker of immunity. Low TTV load could indicate under-immunosuppression with risk of allograft rejection, whereas high TTV load could signal over-immunosuppression with increased risk of infection.
Aim To explore whether TTV load measured in (future) kidney transplantation (KTx) recipients can predict kidney rejection and BK polyomavirus (BKPyV) infection, nowadays the most relevant infectious complication of KTx.
Methods A cohort of 389 patients transplanted between 2003-2012 in the LUMC sampled at baseline and 1.5, 3, 6, and 12 months post-KTx was retrospectively assessed for TTV load and occurrence of BKPyV viremia and acute kidney rejection. TTV and BKPyV loads were determined in blood plasma by quantitative PCR. Rejection was defined as installation of rejection therapy (methylprednisolone) within one year after KTx.
Results During follow-up, 27% recipients developed BKPyV viremia and 23% rejection. TTV detection increased from 84% at baseline (median load ^{10}Log 3.9; IQR 1.6) to 100%, peaking three months post-KTx (median load ^{10}Log 7.5; IQR 3.0; maximum load ^{10}Log 10.5). Landmark analyses showed no statistically significant association between TTV loads measured during follow-up and BKPyV viremia nor rejection. However, baseline TTV-positivity significantly increased the risk of developing BKPyV viremia post KTx (Hazard Ratio (HR) 2.0; CI: 1.0-4.0), where the highest baseline TTV loads produced the highest risk.
Conclusion The association between TTV loads obtained after KTx and BKPyV infection and rejection was weak, which might limit the use of TTV as a post-KTx biomarker of infection and rejection. Baseline TTV-positivity and load, however, were associated with development BK viremia after KTx, which

warrants further research into the scientific basis of this finding and into the application of this potentially predictive biomarker.

P013

Prevalence of all known human polyomaviruses determined by multiplex serology and quantitative PCR in blood of healthy blood donors

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Introduction The number of identified human polyomaviruses (HPyVs) has recently increased up to fourteen. In immunocompromised and elderly patients, HPyVs are known to cause disease, such as nephropathy (BKPyV), progressive multifocal leukoencephalopathy (JCPyV) and skin cancer (MCPyV), while healthy individuals remain persistently infected without symptoms. Whether latent HPyV infections are accompanied by viremia and whether blood-circulating HPyVs are potentially infectious, for example for transfusion-receiving immunosuppressed patients, is largely unknown.
Aim To determine the (sero)prevalence and load of all known HPyVs in blood of healthy individuals
Methods Lab-developed quantitative PCR and bead-based serology to detect HPyV-specific DNA and IgG antibodies, respectively, was validated and multiplexed. QPCR was performed on large volume (1 ml) DNA-extracts of serum from 1016 Dutch blood donors. PCR results with a Ct-value ≤ 40 were considered positive. The same sample set was analysed for the presence of HPyV VP1-specific antibodies.
Results For most HPyVs the seroprevalence was high (60-100%), except for HPyV9 (~20%) and HPyV12, NJPyV and LIPyV (<5%). The median number of HPyV coinfections was nine. HPyV DNA in blood was detectable in 5% of the population, with a maximum viral load of 452 copies/ml. The most prevalent polyomavirus was MCPyV, detected in 39 samples (3,8%), followed by JCPyV (0.5%), TSPyV (0.5%) and HPyV9 (0.4%). Other HPyVs were very rarely detected. Amplicon sequencing confirmed the presence of viral DNA. In the case of JCPyV, a strong correlation was observed between DNA detection and seropositivity as well as seroreactivity (IgG level) for the cognate HPyV.
Conclusions Except for some novel HPyVs, polyomavirus (co)infection is common in healthy individuals and occasionally accompanied by DNAemia, including pathogenic viruses such as JCPyV. Further study is needed to determine the presence and infectivity of intact HPyV in donor blood products, and the potential risk of HPyV infection for immunocompromised recipients.

P014

Bordetella pertussis in the Netherlands, 2015-2018; a sharp increase in prn-deficient isolates

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Introduction
In the Netherlands, pertussis notifications resurged since 1996, likely due to strain adaptation. To study the possible adaptations, Dutch medical microbiology laboratories are requested to submit their B. pertussissuspected samples to the RIVM for confirmation and molecular characterization. Here, we report on the surveillance data from 2015 to 2018.
Methods
All B. pertussissamples were cultured on charcoal agar plates and suspected colonies were subjected to MALDI-TOF MS en phenotypical tests for species confirmation. Confirmed B. pertussisisolates were characterized using whole genome sequencing using the Illumina MiSeq or HiSeq. The resulting data were used for identifying the pertussis toxin (ptxA, ptxP), fimbriae (Fim3) and pertactin (Prn) genotypes. Furthermore, core-genome MLST, using an in-house scheme consisting of 3,180 genes based on B. pertussisisolate B1917, was used to infer genetic relationships between the isolates.
Results
From 2015-2018, a Bordetella species could be culture-confirmed from 30% of the submitted samples, resulting in 219 Bordetella isolates of which 197 were B. pertussis. Other species identified were B. bronchiseptica(n=9), B. holmesii(n=7) and B. parapertussis(n=6). The average age of B. pertussiscarriers was 15 years, but most isolates (n=57) originated from persons younger than 12 months.
All 197 isolates had ptxA1 and 196/197 were ptxP3. The remaining isolate had ptxP1 and originated from 2015. For Fim3 genotypes, 78 isolates carried the Fim3-1 allele (39%), while 118 isolates had allele

Fim3-2 (60%). A single isolate harboured Fim3-4. The proportion between Fim3-1 and Fim3-2 fluctuated between the years from 50%-50% in 2015 to 24%-76% in 2018.
The majority of isolates (n=179) were prn-2, followed by prn-15 (n=3), prn-9 (n= 1) and prn-1 (n=1). No prn allele could be determined for the 13 remaining isolates.
In total, 30 prn-deficient isolates were present. In 2015, 15% (8/53) isolates were prn-deficient, while 12% (8/67) and 9.4% (3/32) of the isolates had this characteristic in 2016 and 2017. In 2018, an increase was seen and 11/46 (24%) of the isolates were prn-deficient. Sequence analysis showed that an inversion of ~22 kb in the promotor was the most frequently found (n= 15) cause of prn-deficiency followed by an insertion of the IS481 element in the prn-gene (n= 6).
A minimum spanning tree (MST) based on cgMLST, showed limited variation within the Dutch B. pertussispopulation with an average distance of 5 genes between two neighbouring isolates (range 0–56 genes). There was no clustering in the MST based on year of isolation or age, but there was a clear distinction between fim3-1 and fim3-2 isolates with a higher genetic diversity among fim3-1 isolates. Furthermore, all prn-deficient strains caused by the 22kb inversion clustered together and belonged to the fim3-1 branch of the MST.
Conclusion
The current Dutch B. pertussispopulation represents a homogenous group dominated by isolates that harbour ptxP3 and prn-2 as genotypes. The majority of prn-deficiency among B. pertussisisolates was caused by a large 22kb inversion, while the number and prevalence of prn-deficient strains in the Netherlands sharply increased in 2018.

P015

Incomplete species delineation results in inaccurate identification of Escherichia species based on whole-genome sequencing

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Introduction: Identification of bacteria through whole-genome sequence (WGS) analysis is increasing, and relies on the quality and comprehensiveness of genome databases for correct identification of species. Within the Escherichiagenus, four species are recognized; E. coli, E. fergusonii, E. albertiiand recently, E. marmotae. In addition, within the Escherichiagenus strains exist that have not been assigned to any of the species, but are termed ‘cryptic Escherichiaclades’. In public databases, these cryptic clade strains are often inappropriately entered as E. coliwhich might lead to misidentification of cryptic clades as E. colistrains. We analysed the presence of cryptic clades in the NCBI RefSeq and Enterobase databases, and explored if cryptic Escherichiaclades should be assigned to existing or new species, based on genomic similarity.
Materials/methods: We downloaded 292 Escherichiacryptic clade genomes from NCBI RefSeq and Enterobase, including metadata. We performed species identification on these strain genomes using Kraken2. We calculated genomic similarity between all strains, including reference Escherichiastrains using the average nucleotide identity (ANI) method. Furthermore, we extracted antimicrobial resistance gene profiles and virulence gene profiles.
Results: Strains from cryptic clades I through IV were wrongly identified as E. coliusing a genomic species assignment method. 29% and 81% of included cryptic clade strains have been erroneously entered as E. coliin the RefSeq and Enterobase databases, respectively. Cryptic clades were genetically different from existing Escherichiaspecies in varying degrees (range 89.0-96.7% ANI), with clade I being most similar to E. coli. Multiple cryptic clade genomes harboured clinically important genes, encoding carbapenemases, ESBLs, shiga toxin or enterotoxins. Notably, nearly 25% of included cryptic clade I strains harboured genes encoding shiga toxin.
Conclusions:
I) A large percentage of cryptic Escherichiastrains have been entered wrongly in the tested databases, possibly impeding their correct identification using WGS.

II) Based on genomic similarities we propose six distinct species within the Escherichiagenus: 1) E. coliand cryptic clade I; 2) cryptic clade II; 3) cryptic clades III and IV; 4) E. marmotae; 5) E. albertii; 6) E. fergusonii.

III) Finally, cryptic clade Escherichiamay harbour genes that could confer resistance or virulence and hence be a threat to human health.

P016

Method comparison of the ImmuView L. pneumophila and L. longbeachae Urinary Antigen test with the Alere BinaxNOW (Binax) assay in clinical urine samples.

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Objective: The objective of this study was to compare the clinical performance of the ImmuView L. pneumophilaand L. longbeachaeUrinary Antigen test to the Alere BinaxNOW Legionella Urinary Antigen Card (Binax, Alere Inc., Waltham, MA) using urine specimens from patients suspected of having pneumonia.
Method: A total of 100 frozen non-concentrated urine samples were tested. These samples were derived from 50 Legionnaires' disease (LD) cases, and from 50 clinical patients with a suspected lower respiratory tract infection who had tested positive in urine antigen tests, or blood or sputum culture for other pathogens (non-LD cases). In addition, a total of 200 urine samples derived from patients with a suspected lower respiratory tract infection were prospectively collected and tested with both tests. After defrosting of the frozen urine specimens, all samples were simultaneously tested by both the ImmuView test and the BinaxNOW. For the samples that showed a discrepancy between the tests (or in comparison with the case definition of the patient from whom the urine was derived), re-tested after heating at 95°C for 5 min and centrifugation for 15 min at 1000 g, or after concentration from 3 ml to 500µl. For the urine samples of five LD-cases and two non-LD cases the analytical sensitivity (limit of detection) and repeatability were examined. The urine samples of the five LD-cases were diluted with urine samples that were tested Legionella urinary antigen negative with both ImmuView test and BinaxNOW test.
Results: Of the 50 urine samples from LD-cases, 48 urine samples showed a positive result for L. pneumophilain the ImmuView test, and also tested positive with the BinaxNOW. Concentration and re-testing of the two negative samples did not change the result. All 50 urine samples from the non-LD cases showed a negative result for L. pneumophilain both the Immuview test and the BinaxNOW. This resulted in a sensitivity and specificity of both the ImmuView and the BinaxNOW of 98.0% (48/50) and 100% (50/50). Of these 200 non-frozen samples there were 3 samples that in both tests showed a positive result for L. pneumophila. The ImmuView test showed a lower detection limit compared to the BinaxNOW test in three of the five urine samples from Legionella cases (in either the first or the repeated tests). The analyses of reproducibility showed that for the 34 (diluted) samples that were tested at two consecutive times with the ImmuView test, 33 samples showed a consistent result for both the ImmuView and the BinaxNOW test. In addition, there were two non-concentrated urine samples that remained L. longbeachaepositive after heating in both the initial and the re-test with the ImmuView test. No other diagnostic tests were available to confirm the L. longbeachae diagnosis.
Conclusion: Both ImmuView and BinaxNOW showed a high sensitivity and specificity for the detection of Legionellaantigen in urine samples from clinical patients with a suspected lower respiratory tract infection. The ImmuView test may have detected two additional L. longbeachaepositive urine samples, although other diagnostic tests could not confirm this.

P017

Analysis of multidrug resistant Campylobacter coli in a Dutch teaching hospital using whole genome sequencing

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Introduction
Campylobacteriosis is one of the main foodborne related infectious diseases in the world. Resistance to antibiotics is seen frequently, though multidrug resistance for macrolides, fluoroquinolones and tetracyclines is not commonly described in the Netherlands. In this retrospective study multidrug Campylobacterisolates were derived from our laboratory information system and were subjected to whole genome sequencing to determine the genetic determinants for antibiotic resistance.
Methods
All cultured Campylobacterisolates from PCR positive faecal samples in our hospital between January 2016 and December 2018 were screened for erythromycin, tetracycline and ciprofloxacin susceptibility using agar disc diffusion. Retrospectively, isolates resistant for all three tested antibiotics were selected and sequenced using a MiSeq sequencer (Illumina). De novoassembly and error-correction was performed using SPAdes version 3.9.1. The presence of acquired resistance genes was identified by uploading assembled genomes to the ResFinder web-service of the Center for Genomic Epidemiology (version 3.1). Chromosomal mutations in gyrA, 23S, rplDand rplV, as well as the cmeR-cmeA intergenic

region, were analysed using MyDb finder web-service of the Center for Genomic Epidemiology (version 1.2) and aligned using AllignX (version 11.5.4) and MEGA (version 6.06).
Results
A total of 324 Campylobacter spp(C. jejunin=299, C. colin=22, and other Campylobacterspp n=3) were cultured and phenotypically tested. Four C. coliisolates tested resistant for all three antibiotics according to EUCAST guidelines. ResFinder identified a tet(O)-likeand a OXA-61-likegene in all isolates. Two isolates contained three different aminoglycoside resistance genes, aph(3')-III, ant(6)-Iaand aph(2'')-Ic-likegene. A gyrAalteration at T86I was confirmed in all four isolates. A 23srRNA A2075G point mutation was found in three of the isolates. No mutations associated with macrolide resistance in the rplDand rplVgenes were detected. No mutations in the cmeR-cmeA intergenic region known to lead to overexpression of the CmeABC efflux pumps were found.
Conclusion
Four of twenty-two C. coliisolates (18,2%) were phenotypically and genotypically confirmed multidrug resistant. Two of these isolates contained not only resistance mechanisms for macrolides, quinolones and tetracyclines, but also for broad spectrum penicillin's and aminoglycosides. This study confirms multidrug resistant C. coliisolates in human samples from the Netherlands using whole genome sequencing data.

P018
The genomic landscape of methanotroph Methylocella Tundrae T4 reveals the presence of two megaplasmids.
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Background - Methanotrophs are important in counteracting global climate change as they act as biofilter for the greenhouse gas methane. Methane is oxidized aerobically by the enzyme methane monooxygenase (MMO). Most methanotrophs use a particulate, membrane-bound copper containing form of this enzyme (pMMO), however some also possess a soluble, cytoplasmic iron containing form (sMMO). Methylocellaand Methyloferulaare the only methanotrophic genera known so far to possess only sMMO. The aim of this study was to understand the metabolic potential of Methylocella tundrae T4, isolated from an acidic arctic peatland [1].
Methods - We sequenced the genome of M. tundraeT4 using Illumina MiSeq and MinIon technology. The genome was assembled using Canu and polished with illumina data using Pilon. Annotation and manual curation was performed using MicroScope [2]
Results - We were able to construct the complete circular genome of 3.9 Mb and 2 mega plasmids of 315 Kb and 217 Kb. The genome contained indeed only an sMMO and no pMMO genes. The plasmids were not observed in previously published genome of the related methanotroph MethylocellailvestrisBL2. Surprisingly, one of the plasmids encoded genes for a second sMMO most likely involved in propane oxidation that Methylocella tundraeT4 may have acquired via horizontal gene transfer.
Conclusion - The complete genome of Methylocella tundraeT4 was obtained and can now be used to design experiments to study the metabolic potential in vivo.
[1] Dedysh SN, et al. (2004) Int J Syst Evol Microbiol 54:151–156.
[2] Medigue C, et al. (2017) Brief Bioinform 45:1–14.

P019
Exploratory interrogation of available metagenomics data sets to detect zoonotic parasites
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Introduction
Zoonotic parasites may be foodborne or depend on transmission via environmental sources, e.g. water or soil. Genetic markers are widely used for their detection and identification, using specific primers and subsequent sequencing, with Genbank Blast hits covering ≥250 nt and at 100% identity for reliable identification. Analysis of publicly available and rapidly expanding metagenome databases could become a powerful tool to study parasite occurrence and distribution in different environments.
Methods:

Molecular parasite markers were used to interrogate WGS sequencing projects from the publicly available metagenome database MG-RAST. For this purpose, a panel of zoonotic parasites was used including parasites found in human- or animal gut (Alaria, Sarcocystis, Hymenolepis, Echinococcus, Taenia, Cryptosporidium) and parasites that shed egg/oocyst stages into the environment, which can be found in water, soil, sludge, etcetera (Alaria, Baylisascaris, Hymenolepis, Echinococcus, Taenia, Toxocara, Toxoplasma).
Results
Cleanup and categorization of metagenomics sequencing projects in the MG-RAST database showed an uneven distribution of environment types , with 'host-associated', 'mat/biofilm' and 'soil' as most abundant types (>2200 metagenomes). Parasite marker sequences were queried in MG-RAST using most stringent settings (100% range and 100% identity), yielding 247 hits for 11 parasite species from nine environments. The vast majority of these hits were 18S sequences.
Most identified parasites were apicomplexan protozoa (Cryptosporidium, arcocystisand Toxoplasma gondii), of which the latter two were widely distributed between environment types. Cryptosporidiumspp. were most abundant in water, but were absent from waste water and sludge. Ascarid nematodes, mostly Toxocara caniswere most abundant in soil. Of the cestodes, only Hymenolepis diminuta(rat tapeworm) sequences were detected in the environments 'sediment' and 'human-oral', which is very surprising and highly improbable.
The latter finding illustrates that query results should be reviewed critically and query coverage and identity threshold values must be determined. A seemingly stringent threshold of 98% sequence identity resulted in a plethora of organisms after cross checking of the sequences in Genbank. Even 100% identity of a query sequence with a parasite sequence may not be specific enough to distinguish parasite genera, depending on query length and considered target.
Another challenge when querying public databases is quality of data. Metagenomic sequencing projects containing only amplified target sequences (usually 16S or 18S) may suffice for certain types of analyses, but are not discriminative enough to identify parasites at species level. Moreover, the available metadata (e.g. concerning description of origin and processing of samples) in current metagenomics databases is often insufficient or has errors, which requires curation before it can be used for analyses.
Conclusion
In conclusion, querying metagenomics datasets for zoonotic parasites is feasible but requires considerable optimization and validation efforts. This approach could provide access to huge and rapidly expanding datasets (i.e. metagenomics projects carried out worldwide for other purposes than parasitology). Metagenome analysis for parasites should be validated with control databases generated from various matrices spiked with parasites. Apart from accessing existing databases, this knowledge will also aid in designing novel dedicated metagenomics sequencing projects for detection and typing of parasites in different matrices.

P020

Plasmid similarities indicate a genetic link between Extended Spectrum β-Lactamase in livestock and the general Dutch population: A Whole Genome Sequencing story.

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The prevalence of carriage of Extended Spectrum β-Lactamase and AmpC β-lactamase producing Escherichia coli(ESBL-E) in the general Dutch population is approximately 5%. Over the past years, transmission of ESBL-E between animals and humans via direct contact has been reported. Moreover, transmission via the food chain was suggested. The contribution of livestock to ESBL-E carriership and human infections, however, remains unclear. To get a better understanding of this contribution 310 ESBL-E isolates obtained from livestock (broilers, laying hens, pigs, and goats), farmers and individuals from the general Dutch population were sequenced and analysed. Clonal as well as horizontal gene transfer through plasmids was studied.
Isolates selected had either a $bla_{CTX\text{-}M\text{-}1}$ (189), $bla_{CMY\text{-}2}$ (96), $bla_{SHV\text{-}12}$ (19), or $bla_{TEM\text{-}52}$ (6) gene. Whole genome sequencing (WGS) was performed. Plasmids were reconstructed and assigned to a plasmid (sub)type. Genes of plasmids with the same (sub)type were compared to establish plasmid similarities. More than 60% of the $bla_{CTX\text{-}M\text{-}1}$ carrying plasmids belonged to IncI1-ST3 obtained from different hosts (all hosts except goats), which confirms the important role of ST3 in the dissemination of this ESBL gene. Furthermore, the analysis suggests that ESBL-gene carrying plasmids were highly similar between isolates obtained from different farms, different farm animals and farmers, but also between farmers and individuals in the population at large, and more importantly, livestock and humans in the community at large. When investigating possible transmission events of ESBL-E it is important to study

horizontal plasmid transmission in addition to E. coliclonal transmission.

P021

Evaluation of the Atellica IM platform compared to the Immulite2000 platform for screening for recent infection with Toxplasma by the detection of IgM antibodies

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Background:
The Atellica solution® is launched as the next fully-automated analyzer to replace the Centaur® immunoassay system for routine serologic testing by Siemens Health care diagnostics in Europe. The new test panel includes a Toxoplasma IgM assay which makes use of a immunoglobulin class capture sandwich immunoassay with the acridium esther technology combined with the P30 antigen. We evaluated the clinical performance of this new assay compared to the Immulite Toxoplasma IgM assay in combination with IgG-avidity testing of positive samples.
Materials/methods:
For 58 patient samples the clinical performance and reproducibility was tested in our in-hospital routine laboratory. The samples were carefully selected to obtain a mixed panel of recent infection (n=21), negative and possible cross-reactive patients. Patient materials with known Toxoplasma IgG result (Centaur XP, Siemens), Immulite test results (Siemens) and Toxoplasma IgG-avidity test results (Biomerieux, Amsterdam Medical center, Amsterdam) were analyzed at the Atellica IM analyzer. Reproducibility of patient sera with clinical relevant index values was tested in 3 different days.
Results:
Both IgM assays showed a low level of correlation (correlation coefficient 0.51). Not all Immulite reactive samples showed reactivity in the Atellica assay. Interestingly all mismatched samples were shown to have high-avidity IgG antibodies which indicated that these samples were non-specific reactions in the immulite assay. All 21 samples of patient with recent infection were detected by the Atellica assay (sensitivity 100%). However, non specific samples in the Atellica assay were also found (specifity for recent infection was 76% including borderline reactive samples). Within lot reproducibility (variation coefficient) of sera with a low-, weak- or high antibody titer was 7.6%, 4.8%, 5.5%.
Conclusions:
The Atellica system is suitable for the use of routine diagnostic testing for screening for recent toxoplasma antibodies. The test shows a better specificity compared to the immulite test. Additionally monitoring of differences in quantitative and qualitative performance between different lots of reagents is mandatory after implementation in the daily routine diagnostic setting.

P022

i-4-1-Health: Prediction of antibiotics resistance from next-generation sequencing data

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[1]BioMérieux, Data Analytics, Sint-Martens-Latem, I-4-1-Health Interreg project partnership

Introduction: The i-4-1-Health project aims to better understand antibiotic resistance through identification of transmission routes of antibiotic resistant pathogens across sectors to prevent further resistance development. As bacteria spread easily, resistance will be charted in both healthy people and in the pig and poultry sector in the Flanders-Netherlands border region. In this work, we present the analysis pipeline developed to rapidly predict antibiotics resistance of a strain from next-generation sequencing (NGS) data.
Methods: The analysis pipeline was implemented on a BioNumerics® Calculation Engine and contains a reference database of validated genes known to confer resistance to certain antibiotics in Enterobacteriaceaeand Enterococci. This reference database was assembled from public databases from the Center for Genomic Epidemiology (CGE; www.genomicepidemiology.org), and the BetaLactamase DataBase (http://bldb.eu). The pipeline starts from the assembled genomic sequences and uses a blast-based approach to detect and identify the genes of interest. Detection parameters were set to 90% sequence identity and 60% sequence coverage.
To validate the analysis pipeline, publically available sequence reads from isolates with known antibiotic resistance phenotype and submitted by the US CDC were downloaded from NCBI's National Database of Antibiotic Resistant Organisms. Most of the isolates (98%) in the resulting set of 275 isolates showed resistance to beta-lactam antibiotics. 50 to 65% of the isolates was resistant to aminoglycosides, fluoroquinolones or trimethoprim-sulfamethoxazole, while only a third showed resistance to tetracyclines.

Two isolates in the set showed no resistance at all.
Results: On average, resistance to a certain class of antibiotics was correctly predicted in 82% of the cases, although quite some variation across antibiotic classes was observed. For instance, resistance to trimethoprim-sulfamethoxazole was predicted correctly in 93% of the cases, while resistance to fluoroquinolones was predicted correctly in only 61% of the cases. Within the i-4-1-health project resistance is reported as resistance to antibiotic classes. An isolate is considered resistant to a certain class when it shows resistance to at least one antibiotic in that class. The same approach was applied on the CDC data to allow direct comparison of results. This approach, however, may truncate results as the CDC panel considers less antibiotics compared to the i-4-1-health project. Resistance to other classes of antibiotics was predicted correctly in 84, 82 and 89% of the cases for aminoglycosides, beta-lactams and tetracyclines, respectively. In all other cases, no prediction result was obtained.

Conclusion: Given the fact that resistance results were summarized as resistance to classes of antibiotics and this may have led to truncated results, generally good predictions of resistance were obtained, although these varied per antibiotic class. Yet, reporting of resistance to an entire antibiotics class might be a too broad approach for some classes that contain multiple generations of antibiotics for which different frequencies of resistance are known, such as fluoroquinolones. At present, the pipeline predicts resistance only based on the presence of certain genes. More accurate results might be obtained when also antibiotics resistance originating from point mutations in certain genes is detected.

P023

i-4-1-Health: Microbial identification and contamination detection from next-generation sequencing data

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Introduction: The i-4-1-Health project aims to better understand antibiotic resistance through identification of transmission routes of antibiotic resistant pathogens across sectors to prevent further resistance development. As bacteria spread easily, resistance will be charted in both healthy people and in the pig and poultry sector in the Flanders-Netherlands border region. In this work, we present the analysis pipeline developed to rapidly determine which organisms are present in a next-generation sequencing data set.
Methods: The analysis pipeline was implemented on a BioNumerics® Calculation Engine and contains a reference database of validated Enterobactericeaeand Enterococcigenomes and their common contaminating bacterial species compiled from public data. A first step in the algorithm filters the most likely candidates from this database by comparing the k-mer profile of the reference genomes with the profile obtained from the raw sequencing reads of the sample to identify. In a second step the reads are mapped to these pre-filtered, candidate reference genomes. Corrected and normalized read count estimates are then calculated for each reference genome from which fractions of the organisms present in the sequencing data are estimated. Two approaches were taken to validate the pipeline. First, we assessed the performance of the pipeline in detecting in silicogenerated known mixes of organisms. These mixes contained two or three different Enterobactericeaeor Enterococcusspecies. Second, the pipeline was validated using approximately 300 samples from the i-4-1-health project for which a classical microbial identification using VITEK or MALDI-TOF had already been performed.
Results: The dominating organism in the sequencing data was correctly predicted in all cases, but a critical lower limit of the number of reads from the contaminating organism present in the mix was observed. More specifically, we observed that, in general, at least a number of reads corresponding with 0.1x coverage of the contaminating organism's genome should be present for correct detection. At a coverage of 30x for a 3Mbp genome and 150bp long reads, this means that contaminating organisms at a fraction as low as 0.67% of the total sequencing output can be detected. At a lower coverage the contaminating organism was not detected.
However, this minimum threshold depends on the organisms involved in the mix. Less closely related organisms (e.g. Escherichia coliand Klebsiella pneumoniae) were correctly detected when at least 0.67% of the total reads belonged to the contaminating organism. Whereas, for closely related organisms (e.g. E. coliand higella) at least 2.68% of the total reads should belong to the contaminating organism for correct detection.
Contaminants in binary (containing one contaminant) or tertiary (containing two contaminants) mixes were equally well detected at similar thresholds, while the fractions of contaminating organisms were predicted with maximum 0.5% deviation from the actual fractions.

Conclusion: Microbial identification and detection of contaminating organisms from next-generation

sequencing data was performed successfully. Contaminating organisms could be detected when the number of reads present in the data corresponds with at least 0.1x coverage of their genome. However, this number depends on the combination of organisms present in the mix.

P024

i-4-1-Health: Characterization and outbreak detection of antibiotic resistant E. coli strains through whole genome Multi Locus Sequence Typing (wgMLST)

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Introduction: The i-4-1-Health project aims to better understand antibiotic resistance through identification of transmission routes of antibiotic resistant pathogens across sectors to prevent further resistance development. As bacteria spread easily, resistance will be charted in both healthy people and in the pig and poultry sector in the Flanders-Netherlands border region. In this work, we present the analysis pipeline developed to type bacterial isolates from next-generation sequencing (NGS) data using a whole genome Multi Locus Sequence Typing (wgMLST) approach.
Methods: We developed a wgMLST scheme for E. coliby expanding the Enterobase core genome MLST (cgMLST) scheme developed by Marc Achtman and his team (Alikhan et al. 2018). to the pan-genome. In total, we identified 14,837accessory loci, complementing the 2,513 core loci. At the same time, the extended scheme also allows for the detection of subtypes or outbreak-specific markers, enabling more powerful classification and detection of outbreaks.
To validate, we re-analyzed publicly available NGS data from 1,411 E. colistrains collected in Dutch hospitals in the periods 2011-2014 (Kluytmans-van den Bergh et al. 2016) and 2012-2013, and in German hospitals and the Dutch community in 2010-2012 (Zhou et al. 2017). Using the NGS data, de novoassembly and typing by wgMLST was performed in BioNumerics. Two independent allele calling approaches were used to determine locus presence and to detect allelic variants in a quality-controlled manner, an assembly-free and a BLAST-based allele calling algorithm. Additionally, serotype, resistance and virulence genes were predicted from the NGS data by the BioNumerics E. coligenotyping plugin.
Results: The results obtained in each of the studies individually could be successfully replicated using our scheme. Both studies used a different, alternate ad hoc cgMLST and wgMLST approach. We obtained similar distributions of genetic thresholds as obtained by Kluytmans-van den Bergh et al. (2016) and a similar minimum spanning tree topology as Zhou et al. (2017) containing the same groups of closely related isolates.
When analyzing the combined dataset of the two studies, five clusters were detected containing strains differing in less than 30 loci from each other, from Dutch and German hospitals or the Dutch community. A cluster with strains from both Dutch and German hospitals was studied more closely. All 9 strains belonged to ST38 and the H18:O86 serotype. Interestingly, all strains from Dutch hospitals were predicted to be resistant to aminoglycoside, beta-lactam, macrolides and trimethoprim, while the strain from a German hospital only has predicted resistance to beta-lactam. German hospitals were sampled prior to Dutch hospitals indicating that the German strain could have acquired more resistance over time. This novel insight demonstrates the power of a universal wgMLST approach.

Conclusions: wgMLST allowed universal, rapid typing and detection of outbreaks in an international setting, using data from different sources and laboratories. Moreover, new data can be compared to previous data without the need of re-analyzing the previous data. This allows to rapidly gain new insights in the spread of bacteria.

P025

Potential mechanisms involved in the reduced antimicrobial efficacy of the synthetic peptide SAAP-148 in vivo

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Introduction
Antibiotic resistance among bacteria has become a global public health problem, complicating the treatment of infected (burn) wounds. To combat (resistant) bacteria, we investigated a novel synthetic antimicrobial peptide SAAP-148. This peptide was highly effective in vitroagainst a panel of multidrug resistant pathogens belonging to the ESKAPE panel. To demonstrate its potential for clinical use, we previously tested the efficacy of SAAP-148 using a rat model with partial thickness excision wounds

infected with Methicillin-resistant taphylococcus aureus(MRSA). However, repetitive daily treatment with SAAP-148 failed to cause a significant bacterial reduction compared to the placebo treated wounds. Therefore, we performed additional in vitroand ex vivoexperiments to determine the possible mechanisms involved in the reduced efficacy of SAAP-148 in this rat model.
Method
To test the possible interference of dressings on the efficacy of the peptide, the absorption of 100 µL SAAP-148 by 1 cm^2 of several wound dressings was determined after 1 h incubation at 37°C. Additionally, the efficacy of SAAP-148 containing wound dressings (20 µL 0.25%) was assessed using ex vivoexcisional wound models. The models were inoculated with 10^4, 10^5, 10^6, or 10^7 CFU MRSA for 1 h at 37°C and treated with SAAP-148 containing wound dressings for 1 h or with SAAP-148 in solution for 1, 4, or 24 h. Subsequently, the surviving bacterial numbers were determined. Furthermore, to mimic the wound environment, 10^5 CFU/mL MRSA in PBS, human plasma or burn eschar extract were exposed to increasing concentrations of SAAP-148 (0-0.1%). After incubation for 30 min at 37°C, the surviving bacterial numbers were determined.
Results
Wound dressings gauze, Mepilex border, Opsite Post-op and Cuticell absorbed >50% to nearly 100% of 100 µL SAAP-148 after 1 h incubation, whereas Tegaderm film absorbed only 25% of SAAP-148. Additionally, SAAP-148 was not sufficiently released from these wound dressings to reduce 10^5 CFU MRSA in ex vivowound models ($p<0.05$).
In the rat experiment, the high levels of CFU found in the wounds might have reduced efficacy of the peptide. Treatment of ex vivowound models with 20 µL 1% SAAP-148 for 1 h resulted in a significantly ($p<0.01$) higher number of surviving MRSA when wounds were inoculated with 10^7 CFU versus 10^5 CFU MRSA. Additionally, SAAP-148 treatment for 1 h or 4 h was more effective ($p<0.01$) than a 24 h treatment to reduce 10^5 CFU MRSA in ex vivowounds.
Finally, the wound environment also played a role as 10-times higher concentrations of SAAP-148 were required in 50% (v/v) plasma or 50% (v/v) burn eschar extract than in PBS to eradicate 10^5 CFU MRSA in vitro.
Conclusion
Although SAAP-148 showed a promising efficacy profile in vitro, the combination of several factors including the absorbance by the wound dressing, the presence of wound exudate, high bacterial load and long treatment intervals resulted in a reduced antimicrobial efficacy in vivo.

P026

Comparison of liquid swab transport systems for the recovery of wound pathogens and performance with molecular methods

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Introduction: Most wound swab specimens contain multiple bacteria, especially those collected from chronically infected sites. It is important, therefore, for a transport device to maintain the viability of all bacteria present without overgrowth which can prevent the detection of some of those microorganisms. This can result failure to diagnose the patient's condition. In this study, Sigma Transwab®PF and e-Swab were tested for their ability to maintain the viability of Pseudomonas aeruginosa, MRSA, treptococcus pyogenesand Bacteroides fragilis. Additionally, swabs were tested for their performance with molecular (PCR) methods.
Methods: Recovery studies were performed according to the M40-A2 swab elution method. 0.5 McFarland bacterial concentrations were prepared in sterile saline and diluted 1:10. Swabs were inoculated with 100µl of bacterial suspension, placed in their respective tubes of medium and held at 4ºC or 22ºC for 0h, 24h or 48h. After the holding period, swabs were processed by preparing serial dilutions and inoculated them onto agar plates. Following incubation of the plates, colonies were counted and used to calculate the concentration of bacteria in the liquid media.
For molecular testing, swabs in triplicates were inoculated with 0.5 McFarland concentration of MRSA and E. coli. DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen) according to manufacturers instructions. After extraction PCR was performed with 16S primers.
Results: For acceptable recovery based on CLSI M40-A2 standard, there should be no more than 1 log increase in cfu (for 4ºC) and no more than 3 log decrease in cfu (4ºC or 22ºC) for the specified holding period compared with time zero. Sigma Transwab® PF and e-swab met the acceptance criteria for . pyogenes, MRSA and B. fragilis. P. aeruginosais included as an indicator of overgrowth and is normally only tested at 4ºC. Sigma Transwab® PF met acceptance criteria even for the samples held at 22ºC. In contrast overgrowth was observed for e-swab samples inoculated with P. aeruginosaand the results were outside of the acceptable criteria. PCR was successfully performed from both swab systems.

Conclusions: Pseudomonas aeruginosais the most common microorganism present in the wound specimens, especially those collected from chronic wounds. Wounds are usually polymicrobial and it is important that Pseudomonas does not overgrow and compete with other bacteria. Failure in recovery or overgrowth of microorganisms at the preanalytical stage will have an adverse effect on the accuracy of the diagnosis. In this study both swab transport systems maintained the viability of the bacteria tested, but overgrowth was observed for P. aeruginosawith e-swab. Both swab types were tested with PCR with the successful amplification of the product, which proves that they are suitable for molecular testing, although any overgrowth could interfere with this.

P027

Laboratory developed quantitative hepatitis E PCR with the ELITe InGenius

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Introduction
Hepatitis E is increasingly recognized as a frequent cause of viral hepatitis in our region. In immune compromised patients it can cause severe chronic infection. Because serology is unreliable in immune compromised patients, PCR is used as a primary diagnostic method in this group. PCR is also used to monitor hepatitis E in plasma and stool to determine optimal treatment duration. A reliable and sensitive PCR is therefore an important diagnostic tool. Our goal was to set up an internally controlled, hepatitis E PCR for the ELITe InGenius with a lower limit of detection of 100 copies/ml in plasma. The ELITe InGenius platform was chosen to allow flexible sample-in-result-out diagnostics with minimal hands-on time.

Methods
A multiplex real-time one-step RT-PCR assay was composed for simultaneous detection of hepatitis E (Pas et al., 2012) and an internal control virus, equine arteritis virus (EAV, primers and probe designed by L.E.S. Bruijnesteijn van Coppenraet, personal communication). Extraction and PCR of plasma samples was performed on the ELITe InGenius (ELITechGroup) either with the 200 µl extraction protocol (200µl input, elution in 50µl) or the 1 ml extraction protocol (500µl sample input, elution in 100µl). For comparison, extraction was performed with both the EasyMAG (Biomerieux, 500µl input, elution in 110µl) and the MagNaPure 96 (Roche, 500µl input, elution in 100µl) followed by PCR on a LightCycler 480 (Roche). All PCRs were performed with TaqMan fast virus 1-step mastermix (Applied Biosystems). Quantification was performed based on the WHO international standard for hepatitis E.

Results
To investigate the consistency of replicates, seven standard curves were analysed for hepatitis E on the ELITe InGenius using the 200µl protocol. The correlation coefficient was 0,9816 and the efficiency of the mean standard curve was 2,05. The efficiency obtained by duplicate analysis of standard curves extracted with the MagNaPure 96 on a LightCycler 480 was 1.94.
Using the ELITe InGenius with the 200µl extraction protocol, 77,8% (7 out of 9) samples containing 100 copies/ml tested HEV positive. This sensitivity could be further improved to 100% (10 out of 10) by using 500µl sample input in the 1 ml protocol. Using 500µl input of plasma containing 100 copies/ml for the EasyMag resulted in HEV amplification in 44% (7 out of 16) of the PCR reactions on the LightCycler 480. After MagNaPure 96 extraction this was 80% (16 out of 20).

Conclusion
Our results show that quantitative hepatitis E PCR can reliably be performed on the ELITe InGenius with good sensitivity compared to established platforms for molecular diagnostics.

P028

Heterotrophic nitrification in Paracoccus spp.

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Nitrification is a key factor controlling the amount of bioavailable nitrogen in natural and man-made ecosystems. In soils, positively charged ammonium is retained while the negatively charged nitrification products nitrite and nitrate leach into the groundwater. Nitrification also controls electron acceptor availability for denitrification. Denitrifying organisms can respire nitrate, which is first reduced to nitrite with the subsequent stepwise reduction to nitric oxide, nitrous oxide, and dinitrogen. Out-diffusion of

these gaseous products leads to nitrogen loss from the ecosystem. Nitrous oxide furthermore is a potent greenhouse gas and incomplete denitrification has been shown to be a major source. Together, the interplay of nitrification and denitrification determine how much nitrogen is retained in an ecosystem.
In a similar fashion, wastewater treatment relies on the combined efforts of nitrification and denitrification to effectively remove excess nitrogen from wastewater. In such systems, the importance of autotrophic nitrifying archaea and bacteria is generally recognized, which is in stark contrast to our understanding of the role of heterotrophic nitrifiers. The contribution of heterotrophic nitrifiers to total nitrification remains a subject of controversy. Based on thermodynamic theory, heterotrophic nitrifiers should co-oxidize ammonium and organic substrates for energy conservation. However, studies on heterotrophic nitrification in Paracoccusspp. suggested that no energy is conserved from ammonium oxidation (Robertson et al., 1988; Robertson and Kuenen, 1990). In autotrophic nitrifiers, ammonium is oxidized to hydroxylamine and subsequently to nitrite by ammonia monooxygenase (AMO) and hydroxylamine monooxygenase (HAO), respectively. Both enzymes also appear to be present in heterotrophic nitrifiers, although the HAO appears markedly different in size and subunit composition compared to the enzyme of autotrophic ammonia-oxidizing bacteria.
The exact physiology and ecological importance of heterotrophic nitrification remain largely undefined. Several heterotrophic nitrifiers have also been shown to aerobically denitrify. This complicates accurate estimations of nitrification rates in complex systems as the nitrification products are directly denitrified and lost to the atmosphere. In this study, batch incubations with multiple strains of Paracoccus denitrificansand Paracoccus pantotrophuswere performed to characterize their phenotypes in response to ammonia. Preliminary results suggested that growth rate and yield and nitrification and denitrification rates in the presence of acetate, ammonium, and/or nitrate differ markedly between closely related Paracoccusstrains. This study aims to elucidate the mechanism underlying these phenotypic differences, including the open question of energy conservation by ammonia oxidation. Therefore, molecular methods in combination with comparative genomics will be used to identify and characterize the enzyme set catalyzing heterotrophic nitrification.

P029

Prevalence of Mycoplasma genitalium and macrolide resistance in Friesland and Noordoost polder (NOP)

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Introduction:
Mycoplasma genitaliumis regarded as a sexually transmitted infection. Adhesion to urogenital cells can lead to an inflammatory response, causing damage to the host cell. In this way it causes urethritis in men. In women, M. genitaliumis associated with cervicitis and pelvic inflammatory disease. However, high rates of asymptomatic infections are observed leading to longstanding debate on its clinical relevance.
Acquired resistance to antibiotics normally active against M. genitalium, such as the macrolide azithromycin, are found in a relatively high frequency of up to 45%. This is caused by specific point-mutations in the 23S rRNA gene. The objective of our study was to obtain information regarding prevalence of Mycoplasma genitalium, and resistance to azitrotromycin, in the province of Friesland and NOP.
Materials and Method:
By means of a specific PCR for detection of M. genitalium, a screening was performed on urogenital samples and urines from patients with or without STI related complaints, submitted to Izore for routine STI testing. Of the requests for STI testing, 67% contained clinical information. The S-DiaMGResTM kit (Diagenode) was used for detection of M. genitaliumand macrolide resistance.
Results:
In total 936 samples were tested. In 49 (5%) of them M. genitaliumwas found. Twelve (25%) appeared to be resistant for macrolide antibiotics, based on the detection of specific mutations in the 23S rRNA gene. Samples were categorized into different groups based on clinical information: samples from patients with (n=434) and from patients without (n=159) STI-related symptoms. Samples positive for Chlamydia or gonorrhea were excluded. No significant difference in M. genitaliumprevalence was found between the two groups.
Within the group with STI-related complaints, 37 men with urethritis-like symptoms were reported, of which 3 were M. genitaliumpositive (8%). Compared to 3% in the group of men without complaints there was not a significant difference (p=0.16, Fisher Exact Test).
Conclusion:
For the province of Friesland and NOP, M. genitaliumprevalence (5%) is within the range found in other

studies, in patients visiting STI clinics (3 – 13%). Macrolide resistance (25%) is relative low compared to resistance rates usually reported in literature (30 – 45%). Our findings on prevalences in relation to complaints support European guideline recommendations to perform M. genitaliumtesting only on patients with STI-related complaints.

P030

A novel plasma device for contaminated wounds: safety and bactericidal effect on human skin

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Introduction
Bacterial presence in wounds greatly increases the risk of infection and healing complications. A novel method to decrease the likelihood of infection and improve healing is Cold Atmospheric Plasma (CAP). CAP has been shown in various studies to provide a promising alternative (antimicrobial) treatment for wound healing. CAP devices generate an ionized gas with highly reactive species and electric fields. A newly developed flexible Dielectric Barrier Discharge (PLASOMA, Plasmacure) produces plasma in ambient air. We studied the safety and efficacy of this plasma device.
Methods
The bactericidal effect of plasma was tested on 10^5 CFU Methicillin Resistant taphylococcus aureus(MRSA) on agar, in collagen matrices and on ex vivo human skin by quantifying surviving bacteria. Safety was monitored by measuring cellular activity in skin biopsies, after multiple daily treatments. Possible double strand DNA breaks were monitored by staining tissue samples for γH2AX. Burn wound models were created by burning ex vivo human skin with a soldering iron for 10 sec at 95°C. Samples were treated with plasma for 2 min, for four times in 10 days and cultured air exposed for 3 weeks.
To determine the safety and disinfecting ability of plasma in humans, intact skin of the inner forearm of 23 healthy volunteers was treated 3 times for 20 sec. The contralateral arm was used for comparison. In 15 of these volunteers, both arms were contaminated with Pseudomonas aeruginosabefore plasma treatment. Pain, skin parameters and bacterial survival were measured.
Results
High reduction (4 log) of MRSA in vitro was reached in 1-2 minutes of plasma treatment on agar or in collagen matrices. Plasma treatment was less efficient on ex vivo skin samples, resulting in 0,5-1 log reduction. Increasing the distance between plasma pad and test sample from 0 to 1.4 mm did not affect the bactericidal effect of plasma. Plasma did not affect viability or DNA integrity of skin biopsies when used for 1-2 min. Repeated daily treatments of up to 4 times lowered viability of skin biopsies with 37%. Re-epithelialization and number of proliferative keratinocytes in ex vivo human burn wound models was not affected by repetitive plasma treatment.
During the clinical study no Serious Adverse Events (SAEs) related to the treatment occurred, while Adverse Events were graded mild and transient. Plasma resulted in warming up of the skin with 2,9± 0,8°C, increased redness (42% ± 39%) and a moderate level of pain (numerical rating scale 3,2 ± 2,1 on a 10-point scale), which was valued as acceptable by all participants. Plasma reduced the number of P. aeruginosa(log reduction 2,7 ± 1,1).
Conclusion
The novel CAP device quickly eliminated bacteria both in vitro and in vivo. Plasma did not affect viability, wound healing or DNA integrity of skin in vitro. The here tested plasma application has been found safe as it resulted in transient, moderate and acceptable pain and SAEs did not occur. Further testing will be done in a clinical trial on wounds in burn patients.

P031

Characterising the gastric and faecal proteome to unravel gastrointestinal function and maturation in preterm infants

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Introduction The nutritional requirements of preterm infants are unique and challenging to meet in

neonatal care, yet crucial for their growth, development and health. As such, it is relevant to increase understanding of how dietary inputs are being processed by the immature and developing gastrointestinal tract of preterm infants. In this study, we therefore investigated gastrointestinal function and maturation during early life of preterm infants in relation to gut microbiota development.
Methods Gastric aspirates of forty preterm infants and faecal samples of ten preterm infants from the same cohort were collected during the first two and six postnatal weeks of life respectively, and analysed with metaproteomics through LC-MS/MS. Moreover, gastric pH and protease activity was measured daily in the gastric aspirates.
Results Differences in the gastric proteome were mainly driven by human milk in enteral feeding (22.7%, p = 0.002) and sample pH (11.8%, p = 0.002), with digestive-related and immune-related proteins being significantly increased at times of human milk-predominated feeding. Composition of the faecal proteome was associated with gestational age (10.4%, p = 0.002) and postnatal age (6.3%, p = 0.002). Enzymes that were more abundant in very preterm infants compared to extremely preterm infants, include lactase, sucrase-isomaltose and maltase-glucoamylase. Generally, these enzymes increased in abundance with increasing postnatal age. As such, these enzymes could potentially serve as markers for gut maturation status. Quantity of various gut maturation markers was associated with the abundance of specific bacterial taxa in faeces.
Conclusion In conclusion, our data provides insight in the gastric and faecal proteome of preterm infants and presents a link to its association with gut microbiota development. Deeper understanding of gastrointestinal maturation and functioning in preterm infants in relation to microbiota development, and including the processing of their feedings, might contribute to the improvement of current nutrition support strategies.

P032

Phenotypic and genomic characteristics of Shigella spp. and Entero-invasive Escherichia coli (EIEC) isolates, collected during the Invasive Bacteria E. coli/Shigella Study (IBESS)

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Introduction: During 2016 and 2017, 15 Medical Microbiological Laboratories (MMLs) collected all higella spp.and EIEC isolates for the Invasive Bacteria E. coli/ShigellaStudy (IBESS). This is a report of the incidence and characteristics of these isolates.
Methods: MMLs sent their isolates, accompanied by their phenotypic antibiotic susceptibility patterns to the IBESS research group. Subsequently, isolates were identified based on phenotypic and antigenic properties, and serotyped using higellaand E. coliO-antisera. More than 80% of isolates were Whole Genome Sequenced using the Illumina MiSeq system. Traditional Warwick MLST typing and a core genome MLST phylogeny were performed using Ridom SeqSphere 5.0.0. with the EnteroBase Escherichia/Shigella cgMLST v1 scheme. The total incidence of higella spp. in the Netherlands in 2016 and 2017 was calculated using the national Shigellosis notifications. A multiplier of 53 was applied to correct for under-reporting and under-diagnosing (Haagsma, Geenen et al., 2013). The incidence of EIEC infections was calculated by extrapolating the fraction of higella spp. included in the IBESS study, with the same multiplier of 53.
Results: In total, 414 included isolates, from 411 patients, were identified as . sonnei(n=232), . flexneri(n=100), EIEC (n=64), provisional higella(n=10), . boydii(n=3) and 5 isolates of which the distinction between . flexneriand EIEC was unclear. IBESS included 40% of national notified Shigellosis; the national incidence of Shigellosis was calculated at 135/100.000 inhabitants per year. For EIEC this was 25/100.000 inhabitants per year. The . boydiiisolates were serotyped as serotypes 2, 8 or 11; the . flexneriisolates were mostly serotyped as 2a (53%) or serotype 6 (12%). Of the EIEC isolates, 38% were O1-O187 negative; the other EIEC isolates consisted of 16 different O-types, including O8, O10, O17, O48, O73, O109 and O141, which were not associated with the pathotype EIEC before. The EIEC isolates possessed the following typical E. coliphenotypic features, which are by definition negative for higella spp.: 30% were motile, 5% fermented salicin and 69% lactose, 8% hydrolyzed esculin, 45% carboxylated lysine, and 59% produced gas and indole. Regarding all higella app.and EIEC isolates, phenotypical resistance against the regular used antibiotics cotrimoxazole (73%) and ciprofloxacin (19%) was found. Most . sonneiisolates (78%) were ST152 (Warwick MLST), most . flexneriisolates (66%) were ST245 and, in contrast, EIEC isolates were evenly divided over 23 STs. In the cgMLST phylogeny, most EIEC isolates (n=45) formed six different clusters, while others (n=9) clustered with .

flexneriisolates, one EIEC clustered with . sonnei, and other EIEC isolates (n=5) were singletons. Conclusion: Among the isolates, . sonneiwas identified predominantly, followed by . flexneriand EIEC. The EIEC isolates were very diverse, evidenced by the many O-types and sequence types, and finally by the diverse clustering. For future research, virulence and resistance gene profiling and a more detailed analysis will be performed on all genomes.

P033

Comparison of two NAAT tests for bacterial vaginosis and candidiasis versus culture and microscopy (Nugent-score)

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Introduction
Bacterial vaginosis (BV) is characterized by discharge with a neutral vaginal pH, overgrowth of non-Lactobacillusspecies and the presence of leucocytes. Laboratory testing is traditionally performed by microscopy or culture of Gardnerella vaginalis. Microscopy is inexpensive and the reference method, but requires a slide at bedside and is prone to inter-observer variation. Culture and NAAT can be performed from a simple swab but are expensive in comparison. Culture is considered less accurate than NAAT or microscopy. The goal of this study is to compare 2 CE-IVD marked NAAT tests for BV and Candida albicansversus culture and microscopy.
Methods
200 clinical samples were included if both a swab and (for BV) adequate microscopy slides had been sent in for BV and candidiasis were available. These samples were tested with 2 NAAT kits for bacterial vaginosis and Candidaspecies: Atrida PCR-multiplex Bacterial Vaginosis and Candidakits, and Seegene Allplex Bacterial Vaginosis and Candidiasis kits. DNA was extracted with the Siemens Versant kPCR extraction module with sample preparation 1.0 reagents. Atrida PCRs were performed on an ABI7500 system, Seegene PCRs on a proprietary Biorad CFX96 system. The Nugent Scores of the slides were assessed by 2 technicians, blinded to the other's results, in order to estimate inter-observer variation. When their classification (normal, inconclusive or BV) differed, they reviewed together to obtain a consensus Nugent score. The consensus Nugent score was considered the reference method. In the comparison with culture, an inconclusive Nugent score was considered positive. Both NAAT-tests report similar result categories as the Nugent scoring system.
Results
The 200 BV-microscopy samples were classified concordantly in 169 cases (84,5%), in the 31 remaining cases a consensus was reached after re-evaluation. Unexpectedly, the concordance of this consensus score with the original (clinical) microscopy result, which was a similar consensus score, was only 81%. For 34 samples (17%) DNA extraction failed, presumably due to either insufficient sample quantity or viscosity. Thus, 166 samples were available for analysis. Concordance between culture and Nugent was 118/166 (71%). For the Atrida PCR the concordance was 128/166 (77%) and for Seegene 86/166 (52%). The number of inconclusive results with Nugent was 29, with Atrida 32 and Seegene 40. Atrida reported "bacterial load insufficient for analysis" in one sample (which was indeterminate in the Nugent score, negative with culture and Seegene). The raw data from Seegene were re-analysed (blinded) by Seegene with an updated version of the Algorithm (not yet CE-IVD) : 116/166 (70%).
For C. albicans, 42 cultures were positive and 124 negative. With culture as reference, the Atrida Candida PCR had 7 false-positives. Seegene indicated four false-negatives and one false positive (also positive with Atrida, indicating culture might have been false-negative).
Conclusion
The Atrida NAAT test is a better alternative than culture, if the consensus Nugent score is considered as the reference method. We noted that the inter-observer variation in this study was lower than in daily practice, possible due to the awareness of the participating technicians to the study design.

P034

Coculturing canonical and complete nitrifying microorganisms under substrate-limited conditions

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Introduction. Nitrification is a two-step process in which ammonia is first oxidized to nitrite, which is subsequently oxidized to nitrate. The process was already described by Winogradsky in 1890 and was generally considered to be catalysed by two functionally distinct groups of microorganisms: the

ammonia-oxidizing bacteria or archaea and the nitrite-oxidizing bacteria. In 2015, complete ammonia oxidation (comammox) to nitrate by a single organism was discovered (van Kessel et al. 2015; Daims et al., 2015). It was postulated that microorganisms performing the comammox process would only occur under conditions that select for lower growth rates but higher growth yields and would therefore outcompete canonical nitrifiers under substrate-limited conditions. Indeed, the only comammox microorganism for which physiological data is available has a high affinity for ammonia (Kits et al., 2017). Methods and results. To investigate nitrifying microorganisms under low substrate conditions, two coupled laboratory scale bioreactors were inoculated with biomass from a wastewater treatment plant. After several months of enrichment, both cultures were dominated by nitrifying microorganisms. Surprisingly, FISH and metagenomic analyses showed that canonical ammonia- and nitrite-oxidizing bacteria co-existed with comammox bacteria. Physiological characteristics including oxygen, ammonia and nitrite affinity of the obtained cultures are under investigation. In addition, pure cultures of the nitrifying microorganisms will be obtained. Conclusions. The obtained knowledge on the different groups of nitrifying bacteria will contribute to our understanding of niche differentiation between comammox microorganisms and other nitrifiers, which will eventually result in a better understanding of the factors influencing nitrification stability and efficiency in engineered systems.

P035

Case report: a goalkeeper's hip during kickboxing, pathophysiology of acute hematogenous osteomyelitis in adolescents

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Case report:

Staphylococcus aureus bacteremia of unknown origin in patients who recently suffered trauma and in persons involved in contact sports should alert physicians on the possibility of acute hematogenous osteomyelitis. In this case transient S. aureus bacteremia with hematogenous seeding to the epiphyseal plate after kickboxing related trauma and hematoma formation was responsible for development of osteomyelitis. A disease entity also known as the “goalkeepers hip” as it was first described in goalkeepers who developed osteomyelitis secondary to the impact of the iliac crest with the ground. This case was remarkable for the location where osteomyelitis developed and supports the theory that osteomyelitis starts in the osteoperiosteal area via an external periosteal route. Recognition, diagnosis and treatment of osteomyelitis is key to prevent bone destruction or septicemia.

P036

Nitrogen salvation by the early life gut microbiota: A metagenome study

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Nitrogen salvation by the early life gut microbiota: A metagenome study

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2. Danone Nutricia Research, Uppsalalaan 12, 3584 CT Utrecht, The NetherlandsAbstract
When human life begins, a gut microbiota develops dynamically. Notably, this microbiota can aid with digestion and thus supports the infant’s nutritional needs. To bolster up development of the body, the infant is in high demand of vitamins, amino acids and subsequent protein. For a large part, these nitrogenous compounds are products of microbial metabolism. However, it is unclear how the microbiota salvages the required nitrogen from breast milk. This complex bio-fluid holds several nitrogen sources, of which some are waste products of human metabolism. The aim of this study is to elucidate if the microbiota of the infant is equipped to utilize various sources of human milk nitrogen. This could lead to future insight on microbiota development and nutritional strategies. Recently, there has been an increase in infant gut shotgun metagenomics studies. These studies give insight into potential microbiota functionality. This study involves the screening of existing metagenome databases for genes involved with nitrogen salvation. Metadata on nutritional intake is used to connect the outcome to dietary nitrogen sources. This study shows that diet has a profound effect on infant gut nitrogen metabolism.
Theme: Infant gut microbial ecology.
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P037

Bacterial culture of body fluids and tissues: enrichment broths and prolonged incubation of agar plates: sense or nonsense?

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Introduction

In the routine bacteriology laboratory of Maastricht University Medical Center, media and incubation times for bacterial culture of body fluids and tissues were recently revised. According to the "Clinical Microbiology Procedures Handbook" (A.L. Leber, 2016), the incubation time of blood (BLO), chocolate (CHOC) and Schaedler(AN) agar plates was extended from 2 to 4 days. The use of enrichment broths is controversial and guidelines are lacking. Blood culture bottles have been shown to improve the sensitivity of bacterial culture of joint fluids. Therefore, we decided to use a pediatric blood culture bottle for aerobes (BD BACTEC™ Peds Plus™ medium)(PED) and a thioglycolate (THIO) for anaerobes. Because these changes had a major impact on laboratory workflow and incubator space, and the use of PED bottles was more expensive, we wanted to estimate the added value of this new laboratory routine.

Methods

Culture results of body fluids and tissues sent to the laboratory from June to December 2017 were analysed. Peritoneal fluids, tissues, biopsies, continuous ambulatory peritoneal dialysis (CAPD) fluid, pleural fluid, synovial fluid and other fluids were analysed seperately. For each positive culture was recorded from which medium and after which incubation time the microorganism(s) was/were isolated. Since orthopedic tissues were too numerous, 50 culture-positive orthopedic tissues were analysed seperately.

When samples were positive on BLO, CHOC, AN after >48 hours incubation or only in enrichment broth, medical records were scrutinezed to determine the clinical significance.

Results

1. Growth on BLO, CHOC, AN after 72 and 96 hours incubation.

361 positive samples were analysed. In 5 samples there was growth on BLO, CHOC or AN after >48 hours incubation. In all cases, the result had no clinical consequence (3 contaminations, in 2 samples earlier growth in PED).

2. Enrichment broths.

PED: 259 positive samples were analysed. In 95 (37%), only the PED was positive, of which 55 (21%) were true positives and 40 (16%) were considered contaminants. The ratio true positive/contamination was most favorable for peritoneal fluid (16/6), synovial fluid (6/3), tissue (28/10), and other sterile fluids (7/1). For CAPD fluid and pleural fluid, there were relatively more contaminants (1/4 and 2/10). For skin biopsies the 6 "PED only isolates" were all contaminants.

THIO: 167 positive samples were analysed. In 14 (8 %) only the THIO was positive. 4 had clinical significance (3 sonification fluids and 1 orthopedic tissue), 10 were contaminants.

20 (12%) samples were only positive in THIO and PED, of which 17 were true positives.

Conclusion

In our laboratory, extending the incubation time of agar plates of body fluids and tissues from 48 to 96 hours has no added value and will be abolished.

Since PED increases the true positives for synovial fluid, ascites, tissues, other body fluids and, in a lesser extent, for CAPD and pleural fluid, we will continue to use them for these samples.

For skin biopsies, PED yields only contaminants and will be omitted.

THIO will be exclusively used for sonification fluids and orthopedic tissue, as it has no added value in other samples.

P038

Prevalence of nasal carriage of methicillin-resistant Staphylococcus aureus in patients at hospital admission in the Netherlands, 2010 - 2017: an observational study

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Introduction

The MRSA Search and Destroy strategy relies on active screening of high-risk groups. However, a

substantial proportion of MRSA carriers found do not belong to the known risk groups, pointing to a reservoir of MRSA carriers in the community.

We determined the prevalence of MRSA nasal carriage upon hospital admission, among patients who were screened preoperatively for nasal . aureuscarriage between 2010 and 2017. Secondly, we aimed to evaluate the prevalence of MRSA carriers without the standard risk factors.

Methods
We conducted an observational study among patients who were screened preoperatively for nasal . aureuscarriage between 2010 and 2017. Samples of cardiothoracic patients were tested by PCR, other samples were cultured using chromogenic agar plates. A Poisson regression model with robust error variance was used to assess whether there was a trend in the prevalence of MRSA over time.
Results
In total, 31 093 nasal swabs were obtained from 25 660 patients. Three hundred seventy-five swabs (1·2%) had an invalid result . Therefore, 30 718 swabs (98·8%) were included in our analysis. Overall, . aureuswas detected in 7981/30 718 patients (26·0% 95%CI 25·5 – 26·5%) of whom 41 were MRSA (0·13% 95%CI 0·10 – 0·18%). The MRSA prevalence varied from 0·03% to 0·17% over the years without evidence of a changing trend over time (p=0·40). Results of the questionnaire revealed that 30 of the 41 patients (73·2%) had no known risk factors for MRSA carriage (0·10%; 95% CI 0·07 – 0·14%).

Conclusion
Our study revealed a sustained low prevalence of MRSA carriage upon hospital admission over seven years. This supports the effectiveness of the Dutch Search and Destroy policy, in combination with a restrictive antibiotic prescription policy.

P039

VICTORY: Validation of cellular tests for Lyme borreliosis

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Introduction
The current cornerstone of diagnostics for Lyme borreliosis (LB) is serology, however, this has several shortcomings. Antibody formation may be absent in the early phase of the disease, and once IgG-seroconversion has occurred, it can be difficult to distinguish between a past (cured or self-cleared) LB, and an active infection. It has been postulated that cellular tests for LB have both higher sensitivity earlier in the course of the disease, and are able to discriminate between a past and active infection. Several of such tests are already commercially available in Europe and patients present the test results in consultations with their physicians. Cellular LB tests lack thorough and independent validation, however.
Methods
We present the design of a comprehensive validation study for several cellular tests for LB. Our study will be a prospective two-gate case-control study. We will include patients who meet the European case definitions for either localized or disseminated LB. Four different cellular tests, either under development or commercially available, as well as two-tier serology will be performed around the start of antibiotic treatment and after 6 and 12 weeks. We will also include healthy controls without current LB and controls with potentially cross-reactive conditions (infectious or auto-inflammatory), both seronegative and seropositive for LB.
Conclusion
The findings of our study will help to better appreciate the utility of cellular tests in the diagnosis of Lyme borreliosis, and/or as a point-of-cure test.

P040

VDRL and anti-cardiolipin antibody detection is not useful as disease activity marker in Lyme disease

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Introduction
Lyme borreliosis is caused by the tick-borne spirochete Borrelia burgdorferi. Anti-Borreliaantibody detection is the cornerstone of diagnosis. However, anti-Borreliaantibodies remain positive after successful treatment and cannot be used to monitor disease activity. In syphilis, which is also a spirochetal disease, treponemal antibodies also remain elevated after treatment. Generally, non-treponemal cardiolipin based tests such as VDRL and RPR are used as disease activity markers. Based on the microbiological similarity between Borreliaspp. and Treponemaspp. we investigated whether cardiolipin-like serological assays can be used as disease activity marker in patients with Lyme borreliosis.
Methods
A selection of stored serum samples from patients with active Lyme disease and positive antibody responses to B. burgdorferiwere tested for positivity in the VDRL-test (Axis-Shield RPR-test, Biomerieux) as well as for presence of IgG and IgM against cardiolipin and β2-glycoprotein and for presence of rheumatoid factor (RF; EliA, Thermo Fischer).
Results
A total of 47 serum samples from 47 patients were included in this study. Clinical manifestations of Lyme disease included erythema migrans (n=16), acrodermatitis chronica atrophicans (ACA, n=8), arthritis (n=9 of which 1 also had ACA), lymphocythoma cutis (n=1), neuroborreliosis (n=13) and 1 patient with a tick bite in medical history without clinical symptoms. All samples were negative in the RPR-assay. The other assays were positive in a small number of patients, generally the observed titers were low. In total, there were only 11 patients with reactivity in any of the cardiolipin, β2-glycoprotein or RF assays.

Conclusion
Antibodies against cardiolipin-like antigens are not suitable as a disease activity marker for Lyme disease.

P041

Identifying multidrug-resistant microorganism carrying patients from Electronic Healthcare Records

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Introduction: Timely identification of patients who carry by multidrug-resistant microorganisms (MDRO) is crucial for proper healthcare services during hospitalisation, since MDRO are associated with increased morbidity, mortality and healthcare costs. To cover the knowledge gap between hospital admission and communication of laboratory test results a classical MDRO risk factor survey (RFS) is performed at the Jeroen Bosch Hospital (JBH) in the Netherlands. The applicability of this MDRO risk factor survey is questionable, since its performance is poor (area under the curve (AUC) = 0.505). However, JBH possesses a massive amount of complex data stored in Electronic Healthcare Records (EHR). It is likely that EHR data has captured hidden patterns and tends regarding the presence of MDRO. Since machine learning models are excellent tools for identifying complex patterns in complex data sources, we set out to train models on EHR data of JBH.
Methods: Logistic regression, decision tree and random forest models were trained on an EHR dataset of JBH. The dataset contained hospitalization history, patient descriptives and MDRO RFS responses. The response variable was obtained from microbiological test results. The dataset consisted of 95 features and 4591 patients, containing only 199 MDRO-positive cases (4.3%). Given the small amount of MDRO-positive cases, the response variable is heavily skewed. Machine learning models are likely to bias towards the majority class. To overcome this problem, over- and undersampled training datasets were constructed.
Results: The machine learning models trained on EHR data outperformed the classical MDRO RFS. The logistic regression, decision tree and random forest models scored on an unseen test set (n = 1148) an AUC of respectively 0.764, 0.737 and 0.762. Table 1 presents the confusion matrix of the three models. Although, the models outperform the classical MDRO RFS, the models produces many false positives (22.2% - 35.5%), resulting in patients being isolated unnecessarily.

Conclusions: Machine learning models outperform classical MDRO RFS in identification of MDRO-positive patients. However, the performance of the models was limited because of the small number of MDRO-positive cases. Consequently, the model produces many false positives, which results in unnecessary isolation measures which are a burden to the patient and wastes time and money. Future analyses should aim to reduce the number of false positives by exploring more data sources and adding more relevant features to the models, for example in the fields of prior medicine use, travel and globalization. In addition, future research could consider classification in multiple stages of hospitalization as addition of clinical features for each stage could enhance the performance of the models. Moreover, it might be relevant to explore the added value of graphed data structures and social network data.

P042

Prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis and Mycoplasma genitalium including relevant resistance-associated mutations

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Introduction
To diagnose STIs, typically only detection of Chlamydia trachomatis(CT) and Neisseria gonorrhoeae(NG) is performed. Trichomonas vaginalis(TV) and Mycoplasma genitalium(MG) have been added to the diagnostic algorithm, but are usually only tested when CT/NG remain negative and by persistence of symptoms. Therefore, the true prevalence of both CT, NG, TV and MG in a certain population is often unknown. Antibiotic treatment of NG and MG can be challenging. For NG, the first-choice treatment is ceftriaxon, that has to be administered intramuscularly. Ciprofloxacin is an oral option that can only be prescribed when susceptibility of NG is proven. Although in MG ~30% resistance is seen, azithromycin still remains the first-choice antibiotic. Cases of treatment failure with moxifloxacin, the second-choice drug, have been described. The aim of this study was 1) to determine the prevalence of both CT, NG, TV and MG in our population and 2) to determine the prevalence of fluoroquinolone resistance-associated mutations (RAM) in NG and MG and azithromycin RAM in MG.
Methods
As of December 10th 2018, all specimens tested for CT/NG in the Abbott M2000 were tested for the presence of TV and MG as well as for azithromycin RAM in MG using the RealAccurate® TVMGres PCR assay (Pathofinder). Correct extraction was monitored by the corresponding internal control. The presence of ciprofloxacin RAM in NG and moxifloxacin RAM in MG positive specimens was evaluated using prototypes of the newly developed real-time PCR assays 'NG-FQ^{res} qPCR' and 'MG-FQ^{res} qPCR' respectively (both NYtor). Both RAM assays are probe-based and yield a positive result when the corresponding RAM is present.
Results
Over a period of 1 month, 359 specimens were included in this study. Fourteen of these could not be evaluated for the presence of TV and MG, due to failure of the internal control. CT was found most often (8.9%, 32/359) followed by MG (3.2%, 11/345), NG (1.9%, 7/359) and TV (0.6%, 2/345). In 3 specimens, a co-infection was identified.
Five of the 7 NG positive specimens included in this study were tested for the presence of ciprofloxacin RAM, 2 of which tested positive. Retrospective analysis of 47 stored NG positive specimens identified 9 specimens harboring ciprofloxacin RAM, resulting in 21.2% overall.
Of the 11 MG positive specimens, 4 (36.4%) showed the presence of azithromycin RAM. Six of the MG positive specimens were tested in the MG-MOXI Res qPCR, none of them harbored moxifloxacin RAM mutations.
Conclusion
In our setting, this is the first time that the prevalence of all STI pathogens in suspected specimens was evaluated. Results are comparable with earlier Dutch studies: CT is found most often, followed by MG, NG and TV. The prevalence of azithromycin RAM in MG is also comparable with earlier studies. One-fifth of the NG positive specimens showed absence of the relevant ciprofloxacin RAM, whereas no moxifloxacin RAM were found in the tested MG positive specimens. Using these molecular assays for detection of relevant RAM makes fast and specific treatment recommendation for NG and MG infections possible.

P043

Harnessing Salmonella typhimurium to improve bladder cancer immunotherapy and vaccine development

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The use of bacteria in immunotherapy is interesting as they are metabolically active, are able to cross membranes and have intrinsic properties to trigger the immune system. For decades, the attenuated live vaccine against Mycobacterium tuberculosis has been the preferred therapy in treatment of non-muscle invasive bladder cancer. However, treatment discontinuation is high and 30% of patients have disease progressing into muscle invasive bladder cancer. Therefore, we investigate the potential of attenuated bacteria in vitro and in vivo for bladder cancer treatment. By using an auto-transporter platform, we are able to express target proteins, such as death receptor ligands, on the bacterial outer surface. In addition, we explore the use of engineered Salmonella enterica typhimurium in bladder cancer treatment. S. typhimurium preferentially accumulates in tumors and persist in antigen presenting cells. Notably, it has the ability to inject proteins directly into the host cytoplasm. We are currently working on increasing the IFN type I expression via stimulation of the cytosolic dsDNA sensing cGAS-STING pathway. This pathway has shown to have a key role in IFNβ production and directing the immune system to a Th1 response. Increasing IFNβ could be beneficial in improving cancer treatment but can also have a broader application in the development of vaccines

P044

Whole-genome sequencing based characterization of clinical Escherichia coli with mcr-1 plasmid-mediated colistin resistance in the Netherlands

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Background: Emerging multidrug-resistant Escherichia coliwith mcr-1plasmid-mediated colistin resistance are of global concern. This pilot study evaluates the characteristics of mcr-1 E. coliby whole-genome sequencing (WGS) in Dutch clinical settings.
Materials/methods: Colistin-resistant E. colicollected at Leiden University Medical Center (LUMC) between 2010 to 2017 were evaluated. Antimicrobial susceptibility testing was performed by VITEK2, E-test (bioMérieux) and colistin microdilution assay (Merlin Diagnostika). Multidrug-resistant E. coliwas defined following ECDC/CDC standards. Real-time PCR confirmed the presence of mcr-1genes. WGS data (Illumina platforms) analysis identified acquired resistance mutations and genes (ResFinder), Multi Locus Sequence Typing profiles (MLST) and core genome MLST (cgMLST) genotyping profiles (SeqSphere). Additionally, WGS-based cgMLST results were compared to four mcr-1E. coliprovided by tertiary hospitals from different geographic locations.
Results: We collected mcr-1E. colifrom six different patients at LUMC. All isolates were multidrug-resistant with resistance against at least five antimicrobial categories including fluoroquinolones (N=6), folate pathway inhibitors (N=6), 3rd generation cephalosporins (N=4) and aminoglycosides (N=3). WGS data detected multiple acquired chromosomal resistance mutations and plasmid-mediated antimicrobial resistance genes. Most clinical isolates belonged to different clonal complexes and included new complex types. We observed clonal similarity between two clinical strains (ST624) collected from different geographic locations.
Conclusions: WGS-based cgMLST confirmed a high diversity of multidrug-resistant mcr-1E. coliin Dutch clinical settings suggesting occasional introductions. Clonal similarity among clinical strains collected from different geographic locations suggests that antimicrobial resistance surveillance networks should include Enterobacteriaceaewith mcrgenes to evaluate potential sources and routes of transmission.

P045

Do vegetarians less frequently carry Extended-spectrum β-lactamase and/or plasmid-mediated AmpC-producing Escherichia coli/Klebsiella pneumoniae compared to non-vegetarians?

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Introduction: Extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC (pAmpC)-producing Escherichia coli/Klebsiella pneumoniae(ESBL-E/K) are frequently found on meat products in Dutch retail, especially on poultry. In addition, similar ESBL/AmpC-genes were found in isolates of meat

products, farm animals and humans. This suggests that meat consumption is an important risk factor for ESBL-E/K carriage in humans. In this study, we investigated if vegetarians are at lower risk to carry ESBL-E/K compared to persons who consume meat.
Methods: Participants were asked to send in a faecal sample and a questionnaire to collect information about their diet and risk factors for ESBL-E/K carriage, such as owning (farm) animals, antibiotic use, travel behaviour and hospitalization. Based on their self-reported dietary habits, participants were grouped as vegetarians, pescatarians (vegetarians who eat fish) and non-vegetarians (persons who eat meat at least 3 times per week). ESBL-E/K was cultured using selective enrichment/culture, and multilocus sequence types (MLSTs) were determined. ESBL/pAmpC-genes were analyzed using polymerase chain reaction (PCR) and sequencing. The risk of ESBL-E/K carriage in the three study groups were analysed using multivariable logistic regression.
Results: A total of 1542 participants were included in the analysis. Prevalence of ESBL-E/K carriage was 8.0% in vegetarians (63/785; 95%CI 6.3-10.1), 6.9% in pescatarians (27/392; 95%CI 4.8-9.8) and 3.8% in non-vegetarians (14/365; 95%CI 2.3-6.3). Multivariable analysis showed an OR for ESBL-E/K carriage of 1.8 for vegetarians (95%CI 0.9-3.8) and 1.3 for pescatarians (95%CI 0.6-3.0) compared to non-vegetarians. The predominant MLST was E. coliST 131 and the most prevalent ESBL genes were $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}1}$ in all diet groups. Independent risk factors for ESBL-E/K carriage were travel to Africa/Latin America/Asia (OR 4.4) or South/East Europe (OR 1.7) and rarely/never washing of hands before food preparation (OR 2.2).
Conclusion: Vegetarians and pescatarians do not have a lower risk of ESBL-E/K carriage compared to non-vegetarians, indicating that eating meat is not an important risk factor for ESBL-E/K carriage.

P046

Auranofin shows activity against M. abscessus in vitro and may point towards a potent molecular target

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Introduction:
Mycobacterium abscessuscauses difficult-to-treat, opportunistic pulmonary infections with high inherent antibiotic resistance. Since current recommended treatment only yields a cure rate of 25%, new treatment modalities are direly needed. One strategy includes repurposing of existing compounds. Auranofin, an anti-rheumatic agent, was shown to have broad-spectrum antibiotic capabilities, as well as activity against M. tuberculosis. Studies indicate it likely inhibits the thioredoxin reductase enzyme (TrxR), lowering an effective response to antibiotic-induced stress. To expand on this, we tested auranofin activity and pharmacodynamics against nontuberculous mycobacteria.
Materials/methods:
Minimum inhibitory concentrations (MICs) of auranofin were determined per CLSI guidelines for M. abscessusCIP 104536 and 38 clinical isolates, M. aviumATCC 700898, M. chimaeraDSM 44623, M.intracellulareDSM 43223 and 9 clinical M. aviumcomplex (MAC) isolates. Synergy between auranofin and drugs recommended for M. abscessusand MAC treatment was determined using the checkerboard and the fractional inhibitory concentration index (FICI) method. Time-kill kinetics assays were performed only for M. abscessusCIP 104536 with auranofin alone and combined with drugs showing low FICIs. For this, we incubated M. abscessusin aerated bottles and quantified colony forming units at pre-determined time points. For single-drug assays, we used two-fold serial dilution ranges starting at 32x MIC and ending at 0.25x MIC. For multi-drug assays, we screened concentrations from 2x MIC to 0.5x MIC for the partner drugs alone and in combination. For this, peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from healthy volunteers (Sanquin Bloodbank, Nijmegen, the Netherlands) and differentiated to human monocyte derived macrophages. Macrophages were infected with MAB CIP 104536 (multiplicity of infection (MOI) 1:1). Intracellular cfus were quantified at day 0, 1, 2 and 3.
Results:
The $MIC_{(50/90)}$ is 2/4 µg/mL for MAB and 64/64 µg/mL for MAC. Auranofin was most synergistic with linezolid (FICI = 0.375) and amikacin (FICI = 0.3125) against M. abscessus, but had no synergism with any drug against MAC. Time kill kinetics assays for M. abscessusCIP 104536 showed moderate bacteriostatic effect of auranofin at concentrations of 2x MIC, and bactericidal effect at higher concentrations. Both auranofin-linezolid and auranofin-amikacin combinations showed more activity in combination with auranofin than either drug alone, and 1x MIC and 2x MIC amikacin-auranofin combinations showed an initial 2 log kill with subsequent bacterial load increase over 21 days. Auranofin could not lower the intracellular bacterial burden of MAB.
Conclusions:

1) Auranofin and its target might be a potent lead in the development of drugs against MAB.
2) Due to poor penetration into macrophages, auranofin itself might not be usable in MAB therapy.

P047

Contact tracing for pan-resistant Klebsiella pneumoniae: timing is everything?

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Introduction
Carbapenemase producing Gram negative bacteria (CP-GNB) are sporadically found in The Netherlands. If patients that harbor CP-GNB were admitted without contact precautions, screening of contact patients with a rectal swab is advised by national guidelines (NVMM HRMO and WIP). However, timing of this screening is not specified. Recent spread of a pan-resistant Klebsiella pneumoniaein our hospital suggests that the current screening frequency may be insufficient and that proper timing is crucial.
Methods
Because of a previous outbreak with a pan-resistant, New Delhi Beta-lactamase (NDM)-positive Acinetobacter baumanniiwe perform screening on all patients admitted to the same ward whenever a patient harboring the epidemic strain is readmitted in our hospital. Screening is performed by NDM PCR until one week after discharge of the colonized patient. Positive NDM PCR results are confirmed by broth culture. Patients admitted to the Intensive Care Unit (ICU) on selective digestive tract decontamination (SDD) are screened by routine SDD cultures. CP-GNB are typed by Amplification Fragment Length Polymorphism (AFLP) to investigate possible transmission.
Results
A pan-resistant, OXA-48 and NDM-producing K. pneumoniaewas accidentally detected by NDM PCR screening in a patient without evident risk factors who was admitted to the nephrology ward. All other patients on this ward had negative NDM PCR results. A day before, a similar K. pneumoniaewas detected in SDD screening cultures of another patient on the ICU. This second patient had negative SDD cultures when admitted to the ICU four days earlier, but had been admitted to the nephrology ward before. Both strains were identical in AFLP typing. All patients admitted to the nephrology ward were screened again a week after these positive findings. Since no positive patients were detected, no major outbreak was suspected.
However, after one month this K. pneumoniae(identical AFLP type, OXA-48 and NDM positive) was detected anew from a urine sample of a third patient that had been tested negative in the initial screening, but was no longer admitted at the time of the second screening.

Discussion
We conclude that this pan-resistant K. pneumoniaewas transmitted to all three patients at the nephrology ward. The initial negative screening test in the second and third patients indicate that after transmission, colonization of the intestine takes some time to be detectable (by PCR) in rectal samples. Therefore, contact tracing using a single anal swab too soon after exposure is insufficient. The use of antibiotics might play a crucial role in this colonization. The NDM PCR proved very sensitive in an earlier Acinetobacteroutbreak when compared to culture using enrichment broth, rendering insufficient sensitivity of the PCR an unlikely explanation for the initial negative screening results. Ongoing transmission after initiation of infection control measures is also unlikely, as the second patient was moved to the ICU. Further research is needed to define optimal timing for contact tracing for different species. Pending evidence, we advise hospitals to consider performing extra screening at a later time point (e.g. a week) in case of contact screening.

P048

Deciphering the core metabolism of the nitrite oxidizer Nitrospira moscoviensis

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Nitrification is a tight interplay of ammonia-oxidizing microorganisms and nitrite-oxidizing bacteria that together convert ammonia via nitrite to nitrate. In biological wastewater treatment, nitrification initiates the removal of excess nitrogen compounds from wastewater. Members of the genus Nitrospiraare known main drivers of nitrite oxidation in such systems and have furthermore been identified in a large variety of natural habitats. The genus is phylogenetically and physiologically diverse with fascinating metabolic features, including a periplasmic nitrite oxidation machinery, the reductive tricarboxylic acid

(TCA) cycle for CO_2 fixation and a putative bd-like oxidase as novel type of terminal oxidase. However, extensive knowledge about the physiology of this genus is scarce.
A model nitrite oxidizer is Nitrospira moscoviensis,which was isolated from biofilm formed in a heating system pipe in Moscow. Genome-informed functional studies showed that N. moscoviensiscan grow aerobically on hydrogen and formate as alternative substrates, and can also oxidize these anaerobically using nitrate as electron acceptor.
Here, we investigated the transcriptome and proteome of N. moscoviensisincubated in a laboratory scale continuous stirred tank reactor system under nitrite-oxidizing conditions.As expected, genes for the reductive TCA cycle and the nitrite oxidoreductase, the enzyme catalyzing nitrite oxidation, were among the most highly expressed genes. Furthermore, our analysis allowed identification of the respiratory complexes expressed under autotrophic growth on nitrite. Since nitrite was the only nitrogen source available, it had to be reduced to ammonium for assimilation. Intriguingly, N. moscoviensislacks the canonical ferredoxin-dependent assimilatory nitrite reductase that is commonly found in other Nitrospiraspecies. Instead, a periplasmic octaheme cytochrome c(OCC) was postulated to reduce nitrite to ammonia and potentially link nitrite reduction to quinol oxidation and proton dislocation across the membrane. Indeed, this OCC was found to be highly expressed, along with two of the three ammonium transporters encoded in its close proximity, which is consistent with the requirement to import the periplasmically produced ammonium.
Taken together, our results give valuable insights into the metabolism of nitrite-oxidizing Nitrospiraby supporting and refining the genome-based metabolic model of N. moscoviensis.

P049

Clofazimine is on-par with the recommended Rifamycin in the standard combination regimen against a intracellular Mycobacterium avium complex in a Hollow Fiber Model

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Introduction
Mycobacterium aviumcomplex (MAC) bacteria cause severe opportunistic pulmonary diseases. The recommended treatment regimen is a combination of a rifamycin, ethambutol and a macrolide, of which only the macrolide shows in vitroactivity. Though evidence for microbiological response to the rifamycin and ethambutol is absent, they prevent macrolide resistance formation in MAC, in vitroand in vivo. Clofazimine, recently explored as a treatment modality for MAC infections, might replace either the rifamycin or ethambutol, preventing macrolide resistance while adding bactericidal activity to the regimen. Here, we investigated replacement of either ethambutol or rifabutin by clofazimine using an intracellular MAC hollow-fiber system.
Methods
THP-1 cells were infected with M. aviumATCC 700898 (multiplicity of infection 1:1), washed and brought into a hollow fiber system (HFS-MAC) to an initial inoculum of 5 log colony forming units (cfu)/mL. HFS-MAC was exposed once-daily to either a rifabutin-ethambutol-azithromycin (REM) mimicking the clinical standrad, a clofazimine-ethambutol-azithromycin (CEM), a rifabutin-clofazimine-azithromycin (RCM), or a rifabutin-ethambutol–clofazimine-azithromycin regimen (RECM). An untreated control was added. The conditions were performed in triplicate. All drug exposures were based on published pharmacokinetics studies. At pre-determined time points and over the course of 28 days, HFS-MAC was sampled for quantitative culture. Azithromycin resistance was monitored by inoculating agar plates supplemented with these drugs at 3 times the MIC.
Results
The rifabutin-free CEM regimen could not lower the bacterial burden below stasis. All other regimens achieved >3 log10 cfu/mL (>99.9%) kill by day 28. The recommended REM regimen had the lowest bacterial load at day 28 with 1.5 log10 cfu/mL. Adding clofazimine to the REM regimen (i.e. RECM) did not add activity to the CEM regimen and could not lower the bacterial burden further. For azithromycin, we only observed low resistance occurrence, never observing more than 10% resistant population by day 28. All regimens were able to suppress macrolide resistance evenly.
Conclusions
1) Clofazimine can’t replace rifabutin in an ethambutol-azithromycin-based regimen, as is evidenced by failure of lowering the bacterial burden below stasis.
2) Clofazimine can replace ethambutol, without a loss in activity or prevention of macrolide resistance.
3) Adding clofazimine to the recommended CEM regimen does not increase microbiological response.

P050

Investigating the role of Fc-mediated antibody effector functions in RSV disease: development of an antibody-dependent cell-mediated cytotoxicity (ADCC) assay

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Respiratory syncytial virus (RSV) infection is a major cause of severe respiratory illness requiring hospitalization in infants under 1 year of age. No vaccines are currently available and the determinants of severe disease remain elusive. For protection against invading pathogens, infants mainly rely on their innate immune response and the antibodies that they received from their mother during pregnancy. Traditionally, antibody-mediated protection against viral infections is thought to be mediated by direct binding of antibodies to viral particles, resulting in virus neutralization. However, in the case of RSV, virus neutralization titers do not provide an adequate correlate of protection, suggesting that other antibody-mediated effector functions are involved in protection. We therefore developed an assay investigating antibody-dependent cell-mediated cytotoxicity (ADCC) capacity of RSV-specific antibodies by NK cells. Using this assay, we have shown that plasma of infants hospitalized for RSV infection induces ADCC in primary NK cells. Although the capacity to induce ADCC varied between patients, no difference between the control group and RSV patients was found. These data indicate that the ADCC capacity of antibodies alone does not explain disease severity in infants. Current research is focused on obtaining additional antibody characteristics, including (antigen-specific) antibody titers, Fc-glycosylation pattern, and antibody-dependent cellular phagocytosis. We aim to reveal beneficial or harmful (combinations of) antibody characteristics that may protect from or render individuals susceptible to severe RSV disease. Additional research into Fc-mediated antibody effector functions is needed as the current lack of understanding of the mechanisms by which antibodies can protect against RSV infection and disease or, alternatively, contribute to disease severity, hampers the design of safe and effective vaccines against this virus.

P051

Inhaled pentamidine might be a strong asset in pulmonary non-tuberculous mycobacterial treatment

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Introduction
New, effective treatment regimens against non-tuberculous mycobacterial (NTM) pulmonary disease are needed. Development strategies include repurposing existing antibiotics.
Pentamidine can be used as an inhaled agent used in the prophylaxis of Pneumocystisinfection, with unclear mechanism of action and pharmacokinetics. Pentamidine shows synergy with other antimicrobials, mainly against multidrug-resistant gram-negative bacteria. Here, we investigate the effect and pharmacodynamics of pentamidine against NTM, both alone and in combination with drugs used in NTM treatment. Furthermore, we investigate pentamidine's ability to penetrate into primary human-derived macrophages infected with M. avium.
Methods
Minimum inhibitory concentrations (MICs) of pentamidine were determined per CLSI guidelines for M. abscessusCIP 104536 and 4 clinical isolates, M. aviumATCC 700898 and 4 clinical isolates, M. chimaeraDSM 44623 and M. intracellulareDSM 43223. Synergy was determined by checkerboard titration with drugs recommended for rapidly-growing mycobacteria (RGM) and slowly growing mycobacteria (SGM) treatment, respectively, using the fractional inhibitory concentration index (FICI). Time-kill kinetics assays were performed with pentamidine against M. abscessusCIP 104536 and M. aviumATCC 700898 alone and combined with drugs showing low FICIs. For this, we incubated the bacteria in aerated bottles and quantified colony forming units (cfu) at pre-determined time points. For single-drug assays, we used a two-fold serial dilution range from 0.25x MIC to 32x MIC. For multi-drug assays, we screened concentrations from 0.5x MIC to 2x MIC for the partner drugs alone and in combination. For ex vivoassays, peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from healthy volunteers (Sanquin Bloodbank, Nijmegen, the Netherlands) and differentiated to human monocyte derived macrophages. Macrophages were infected with M. aviumATCC 700898 (multiplicity of infection 5:1) and intracellular cfus were quantified at day 0, 3 and 6.
Results

MICs ranged from 8 to 32 µg/mL, with reference strains of M. abscessusand M. aviumshowing MICs of 32 µg/mL. Pentamidine was synergistic with amikacin against M. abscessus(FICI = 0.484 ± 0.271), and had initial synergy with clarithromycin (FICI = 0.438 ± 0.259), abrogated by erm(41)-induced macrolide resistance. For M. avium, slight synergy was noted with amikacin (FICI = 0.813 ± 0.240). Time kill kinetics assays for M. abscessusCIP 104536 showed an initial >2 log kill only for concentrations higher than 8x MIC. All arms showed sustained growth by day 2. For M. aviumATCC 700898, pentamidine alone and in combination with amikacin was highly bactericidal at combinations around 1x the MIC. Pentamidine lowered the bacterial burden inside macrophages by 3 log cfu (>99.9% kill) by day 6.
Conclusions
1) Pentamidine inhalation is an interesting lead for M. aviumbut not for M. abscessustreatment.
2) To incorporate it into the standard regimen against MAC, clinical evaluation and trials are needed.

P052

Synergism between tedizolid and other drugs opens up possibilities in non-tuberculous mycobacterial treatment

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Introduction:
Tedizolid is a next-generation oxazolidinone with potent activity against several strains of non-tuberculous mycobacteria (NTM), most notably M. abscessus(MAB) and M. aviumcomplex (MAC). However, systematic in vitropharmacodynamic studies that integrate tedizolid into current NTM treatment are absent. To fill this gap, we explore susceptibility of MAB to tedizolid and synergism between tedizolid and drugs recommended for MAB treatment.
Materials/methods:
Minimum inhibitory concentrations (MICs) of tedizolid were determined per CLSI guidelines for M. abscessusCIP104536 and 8 clinical isolates, M. fortuitum 6841and 2 clinical isolates, 2 clinical isolates of M. chelonae, M. peregrinumATCC700686, M. aviumATCC 700898 and 11 clinical isolates of MAC. Minimum bactericidal concentration (MBC) was determined by plating MIC wells on which we observed no growth on 5% sheep blood plates. Synergy was determined by checkerboard titration with clarithromycin, amikacin, tigecycline and cefoxitin for 8 isolates of rapidly-growing mycobacteria (RGM) and with clarithromycin, ethambutol, rifampicin, amikacin and minocycline for 4 isolates slowly-growing mycobacteria (SGM) including reference and clinical strains, with calculation of fractional inhibitory concentration indices (FICI). We defined synergy as a FICI <0.5. Intracellular potential of tedizolid was assessed in ex vivoassays. For this, peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from healthy volunteers (Sanquin Bloodbank, Nijmegen, the Netherlands) and differentiated to human monocyte derived macrophages. Macrophages were infected with MAV ATCC 700898 (multiplicity of infection (MOI) 5:1) and MAB (MOI 1:1). Intracellular cfus were quantified at day 0, 1, 2 and 3 for MAB and 0, 3 and 6 for MAV.
Results:
$MIC_{(50/90)}$ was 4/8 µg/mL for SGM and 4/8 µg/mL for RGM, in line with existing literature. Against MAB, tedizolid had an MIC/MBC ratio of 64, indicating a bacteriostatic effect. Lowest FICIs were observed between tedizolid and clarithromycin (FICI range 0.28 - 1, average 0.67) and between tedizolid and cefoxitin (FICI range 0.375-1, average 0.69) against MAB; synergy with clarithromycin was rapidly abolished by erm(41)-associated resistance. For SGM, lowest FICIs were observed between tedizolid and ethambutol (FICI range 0.625 – 1, average 0.72). In ex vivoassays, tedizolid could hold the intracellular bacterial burden static in macrophages infected with MAB, but did not prevent bacterial growth in macrophages infected with MAV.
Conclusions:
1) Tedizolid is an interesting option for MAC and MAB, but its true strength lies in a synergistic combination with other antibiotics recommended for NTM treatment.
2) Tedizolid alone, as well as these combinations, warrant further pharmacodynamic studies.
3) Tedizolid apparently penetrates into macrophages, but has better intracellular effect against MAB than against MAV.

P053

A PE/PPE substrate pair determines the system specific secretion of EsxB_1/EsxA_1 by the type VII secretion pathway in Mycobacterium marinum.

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Introduction
Type VII secretion systems (T7SSs) of pathogenic mycobacteria secrete a wide range of proteins that play important roles in both host-pathogen interactions and bacterial viability. There are four different classes of T7SS substrates; the Esx, the PE, the PPE and the Esp proteins. Some of these substrates are secreted as heterodimers and specific members of the Esx, PE and PPE protein families were shown to be dependent on just one of the five T7SSs (i.e.on ESX-1, ESX-3 or ESX-5) for their secretion. This raises the question how system specificity of these substrates is achieved. We have shown that the PE/PPE heterodimers specifically interact with their cognate EspG chaperones and this determines their destined secretion pathway. As both structural and pulldown analysis suggest that the EspG chaperones are unable to interact with the Esx proteins, the determining factor for system specificity of these substrates remains unknown.
Methods
Molecular cloning techniques were used to express the ESX-1 dependent substrate pairs PE35/PPE68_1 and EsxB_1/EsxA_1 in the model organism Mycobacterium marinum. We also used a variant of PPE68_1 to reroute this substrate, together with its partner substrate PE35, via the ESX-5 system. This variant contains the $EspG_5$ chaperone binding domain of an ESX-5 dependent PPE substrate. With protein secretion analysis, we determined secretion of the substrates and their rerouting via the ESX-5 systems in an ESX-1 mutant strain.
Results
In this study, we have investigated the secretion specificity of the ESX-1 substrate pair EsxB_1/EsxA_1 (MMAR_0187/MMAR_0188) in M. marinum. This substrate pair was hardly secreted when heterologously expressed. However, when this pair was co-expressed with the PE35/PPE68_1 substrate pair (MMAR_0185/MMAR_0186), which is produced from the same operon, secretion was established. Surprisingly, co-expression of EsxB_1/EsxA_1 with the PE35/PPE68_1 pair, carrying the $EspG_5$ chaperone binding domain, redirection via the ESX-5 system was established not only for the PE/PPE pair but also for the (wild-type) EsxB_1/EsxA_1 pair.
Conclusion
Our data suggest a secretion model in which the PE35/PPE68_1 substrate pair is a determinant factor in the system specific secretion of EsxB_1/EsxA_1.

P054

Comparison and dynamics of resistance gene content in canine and human Staphylococcus pseudintermedius.

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Introduction:
taphylococcus pseudintermediusis a major pathogen in dogs and can occasionally be found in human infections. In dogs . pseudintermediusis the major cause of pyoderma and is often methicillin resistant and/or multidrug resistant (MDR). In humans . pseudintermediusis an opportunistic pathogen found in elderly and immunocompromised patients and is primarily methicillin susceptible. Antibiotic resistance and in particular methicillin resistance in . pseudintermediusis mediated by gene clusters very similar to those of . aureus, leading to a risk of transmission of resistant bacteria between humans and dogs and of resistance genes between . pseudintermediusand . aureus.
This study compares the genomes of . pseudintermediusisolates from canine and human infections, focussing on resistance gene content, its dynamics and clonal distribution.
Methods:
Whole genome sequencing was performed on 97 methicillin-resistant . pseudintermedius(MRSP) isolates from dogs (2008-2018), 94 methicillin-susceptible . pseudintermedius(MSSP) from dogs (1993-2018) and 34 . pseudintermediusisolates from humans (2015-2018). Resistance genes and sequence types (ST) were identified using the batch upload pipeline from the Center for Genomic Epidemiology (CGE) .
Results:
Nearly all MRSP isolates (98%) were MDR, whereas only 38% of MSSP isolates were MDR isolates and in 5% no resistance gene was detected. MRSP showed a more diversified resistance gene content with a higher prevalence of genes present in both MRSP and MSSP. Comparison of the resistance genes in human and canine MSSP isolated in the same time period (2015-2018), showed that 59% of canine MSSP isolates were MDR and in 7% no resistance gene was detected. In human isolates significantly

less MDR isolates (47%) were present and 29% isolates contained no resistance gene. The single human MRSP isolate contained a resistance gene profile similar to MRSP isolates from dogs. Isolates from humans showed a resistance gene repartition similar to canine MSSP with a lower prevalence of tetM(40% in dogs, 23% in humans) and a slightly higher prevalence of ermB(41% in dogs, 47% in humans) and cat(pC221)(37% in dogs, 47 in humans). The increase in MDR in recent years in canine MSSP was linked to an increase in ant(6)-Ia, aph(3')-III,erm(B)and cat (pC221),also regularly found in human isolates. Those are reported to be carried on transposon Tn5405 and could indicate that the increase of MDR in MSSP is linked to the transfer of this transposon.
MRSP strains were mainly from known clonal complexes, whereas for MSSP no dominant ST or clonal complex could be identified, for human isolates one dominant clonal complex could be identified but various other STs where present.
Conclusions:
1. The dynamic resistance gene content in canine MSSP could be linked to transposon transmission.
2. Human isolates are less diverse and less often MDR than MSSP isolates from dogs and often carry no resistance genes.

P055

Diagnostic Accuracy of the Fujifilm/Wako (1-3)-β-D-glucan Assay in the Diagnosis of Pneumocystis jirovecii Pneumonia

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Background: Pneumocystis jiroveciiis an opportunistic fungal pathogen that can cause severe and life-threatening pneumonia (PJP), in immunocompromised patients. At present, the diagnosis of PJP pneumonia is performed on specimens obtained directly from the respiratory tract, either sputum or Broncho-Alveolar Lavage (BAL) fluid. Obtaining these samples may be invasive and difficult in patients that suffer from respiratory decompensation. β-D-glucan (BDG) is a major constituent of fungal and yeast cell walls that is released into the blood during PJP. A plasma BDG measurement for the diagnosis of PJP could potentially reduce diagnostic delay and reduce the number of invasive BAL procedures.
Materials and Methods: In a retrospective study, we identified 120 patients that underwent a BAL procedure following clinical suspicion of PJP from whom serum was available for BDG testing. Only serum samples obtained within ± 14 days of a BAL procedure were included. Furthermore, patients with confirmed candidemia or a positive serum galactomannan were excluded from the present study. Clinical diagnosis of PJP was established by reviewing individual cases, taking into account PCR results, cytology, co-morbidities, alternative microbiological diagnoses and response to treatment. BDG in serum samples was measured using the Fujifilm/Wako β-Glucan Test, on a Toxinometer MT-6500.
Results: 35 PJP infections were identified by case review. Cases were more likely to have a diagnosis of HIV, typical CT-findings and lower CD4+ cell counts compared to controls. Interestingly, in 16/85 negative subjects was Pneumocystis jiroveciidetectable by PCR. Median BDG values were higher in cases than in controls (2.4 vs 11.9 pg/ml, $p = 3x10^{-7}$). ROC curve analysis showed and AUC of 84.6%, with an optimal cutoff of 4 pg/ml, which is significantly lower than the value supplied by the manufacturer. The sensitivity, specificity, positive and negative predicted value were 74%, 96%, 90% and 90% at the optimal cutoff.
Discussion: The Fujifilm/Wako BDG assay shows robust diagnostic accuracy in the detection of PJP, albeit with a limited sensitivity. The test may be suitable as a rapid and minimally invasive procedure in patients suspect of having PJP. Negative test results should however always be followed up by further diagnostic procedures.

P056

Outer membrane vesicles decorated with S. pneumoniae proteins as broadly protective vaccine against pneumococcal colonization.

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Introduction
treptococcus pneumoniaecauses a variety of infectious diseases in humans, such as pneumonia, meningitis or sepsis. Prevnar, the available capsular polysaccharide vaccine, provides serotype-specific immunity, but covers only 13 of the 90+ different serotypes. Prevnar is effective at preventing invasive disease caused by these 13 serotypes but the effect on nasopharyngeal colonization, the source of

pneumococcal spread, is limited. Also, serotype replacement occurs, stressing the need for a broadly protective . pneumoniaevaccine that is capable of reduction of transmission to provide herd immunity. Outer membrane vesicles (OMVs) derived from Gram-negative bacteria attract increasing interest in the development of vaccines as they can be generated at low cost and have intrinsic adjuvant activity. We have developed a versatile platform, consisting of detoxified Salmonella Typhimurium OMVs that can be decorated on the surface with multiple antigens of choice at a high density by using the autotransporter Hbp. In addition to genetic fusion of the antigens to Hbp, we are also exploring enzymatic coupling of purified antigens to Hbp via the recently developed SpyTag/SpyCatcher protein ligation system. Since coupling occurs after translocation of the Hpb carrier, there is virtually no restriction to the size and complexity of proteins that can be coupled to OMVs.
Our aim is to test if conserved Streptococcal proteins can be coupled to OMVs and if the recombinant OMVs can be used as a broadly protective vaccine against pneumococcal colonization.
Methods
Conserved . pneumoniaeantigens were purified and coupled to Hbp on Salmonella OMVs through Spy-technology. C57BL/6 mice were intranasally (i.n.) or intramuscularly (i.m.) immunized three times with 8 OD units of recombinant OMVs (corresponding to ~4 µg total protein), at two-week intervals. Three weeks after the final immunization mice were challenged i.n. with 10^6 CFU of . pneumoniae. Three days after infection, colony forming units (CFUs) in the nasal cavity were measured.
Results
First, we tested if purified . pneumoniaeproteins can be coupled to the Hbp carrier, displayed on OMVs, via the SpyTag/SpyCatcher protein ligation system. Fusion of Hbp to the SpyCatcher did not hamper display on OMVs. Subsequent addition of purified proteins fused to the SpyTag domain lead to efficient covalent coupling to the Hbp-SpyCatcher carrier. This resulted in OMVs displaying conserved . pneumoniaeproteins at their surface at very high density.
Mice that received 3 intranasal vaccinations with the prototype vaccines, displaying pneumococcal proteins, showed lower colony forming units in the nasal cavity after . pneunoniaechallenge, compared to mice that received control OMVs. In contrast, intramuscularly administered Prevnar had little effect on the amount of nasal CFUs. The OMV platform is characterized by strongly elevated IL-17 levels, which are a known correlate of protection for pneumococcal disease.
Conclusion
From our work we can conclude that 1) proteins can be efficiently coupled to OMVs using the SpyCatcher/SpyTag system, and 2) OMVs displaying conserved . pneumoniaeproteins significantly reduce colonization in the nasal cavity after . pneumoniaechallenge. This makes recombinant OMVs a promising candidate for a vaccine against . pneumoniae.

P057

PCR ribotype 023: Comparable to other hypervirulent ribotypes of Clostridioides difficile?

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Introduction:
Clostridioides(C.) difficilePCR ribotype (RT) 027 and 078 are well-known hypervirulent strains. RT023 appears to have similar molecular characteristics as these strains, including a mutation in a toxin regulator gene and the presence of binary toxin genes. However, the severity and the outcome of C. difficileinfections (CDI) with RT023 are not known. Since 2009, 24 hospitals participate in the Dutch national surveillance program and register clinical and demographical data of hospitalized patients with CDI. Simultaneously, C. difficileisolates are sent to the National Reference laboratory. Here, we present clinical characteristics of CDI due to RT023, compared to other ribotypes, based on data of the National Reference laboratory for C. difficilein the Netherlands.

Methods:
Ribotyped isolates were divides into 6 groups: RT023, RT078/126, RT027, RT 014/020/295, RT001 and all ribotypes (except for RT023, RT027 and RT078/126). Clinical characteristics and outcomes were compared between RT023 and the other groups. Severe CDI were defined as: hypoalbuminemia (<20 g/L) and/or dehydration, fever (temperature of 38°C or higher) and leucocytosis (>15×10^9/L), pseudomembranous colitis or bloody diarrhoea. A complicated course was defined as admission to an intensive care unit, the need for surgical procedure or death.

Results:
In total, 5,359 patients with CDI were included in the sentinel surveillance in the period between May 2009 and February 2018. The proportion of RT023 was 2.4% (95% CI 2.0-2.8), encompassing 141 cases of CDI. CDI due to RT023 caused more severe disease, compared to CDI due to non-hypervirulent ribotypes, The severity of CDI was comparable to the hypervirulent RT027 and RT078/126 strains. Furthermore, CDI-related mortality of RT023 was similar to RT027 and RT078/126. Patients with CDI due to RT023 had more frequently a community onset of symptoms. Four sequenced RT023 strains contained a large transposon region and an unique trehalose genotype.

Conclusion:
1. CDI due to C.difficileRT023 leads to a clinical presentation with similar severity and results in comparable CDI-mortality as CDI due to the hypervirulent RT027 and RT078/126 strains.
2. Patients with CDI due to RT023 present more frequently with a community onset of symptoms.

P058

Analyzing human papillomavirus (HPV) 16 breakthrough variants with next-generation sequencing.

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Human papillomavirus (HPV) is a common sexually transmitted infection and the causative agent of invasive cervical cancer (ICC), the fourth most common female cancer worldwide. Currently, over 200 HPV types have been distinguished based on a >10% difference between HPV whole-genome (WG) sequences. Every type is sub-divided into lineages (1-10% WG sequence difference) and sub-lineages (0.5 – 1.0% WG sequence difference). All HPV isolates belonging to the same HPV type are considered variants. Interestingly, 90% of the ICC cases are caused by 13 high-risk (hr) types, yet 50-60% is caused by HPV16 and 20% by HPV18. Therefore, vaccination with a bivalent HPV vaccine targeting HPV16 and HPV18 was implemented into the National Immunization Program in 2009.
In response to the vaccination program, the biennial cross-sectional “PApillomavirus Surveillance among STI clinic YOungsters in the Netherlands (PASSYON)” study was set-up in 2009 to monitor HPV in a high-risk population. In this study, 16-24 year old STI clinic visitors provided a self-collected genital swab for HPV testing and answered a questionnaire including vaccination status. Though vaccination status was self-reported, 96% of the self-reported vaccinated candidates were serologically verified.
Despite tremendous reduction in HPV16/18 infections and therefore ICC risk in vaccinated girls, sporadic HPV16 breakthrough infections in vaccinated persons have been reported. Recent studies have shown that HPV16 comprises an extensive variant repertoire, consisting of four lineages (A to D) and a varying number of sub-lineages (A1, A2 etc.). Furthermore, HPV16 lineages express differential pathogenicity, regarding persistence and progression to cancer. In regard of this knowledge, we are interested if HPV16 variants in the breakthrough infections are phylogenetically different from HPV variants in non-vaccinated persons.
We carried out fragment-PCR (first development by van der Weele, 2018) on 13 DNA isolates from genital swabs belonging to candidates with HPV16 breakthrough infections. Primers were designed to span the entire 8 Kb genome of HPV16, after which the PCR products were sequenced with next-generation sequencing. The reads from each sample were assembled against a reference strain, K02718, to generate a WG consensus sequence. Thereafter, sequences were exported to Bionumerics 7.6.3 (AppliedMaths) for phylogenetic analyses.
We created a Maximum Parsimony tree with the HPV16 variants and the HPV16 reference lineages. In addition, we added 166 HPV16 WG sequences from HPV16 isolates obtained from unvaccinated women in another Dutch Study. Our results show that the HPV16 breakthrough infections, as well as the other HPV16 infections clustered with lineage A. The breakthrough infections did not cluster with a specific sub-lineage.
In conclusion, HPV16 breakthrough infections are similar to HPV infections found in non-vaccinated women. Currently, the HPV16 breakthrough infections found are all lineage A infections. Our results are supported by previous research, which states that lineage A is the most common in Europe and has the highest prevalence in squamous cell carcinoma (SCC), the most common histological presentation of ICC. In future research, we will carry out SNP analyses on the breakthrough consensus sequences and analyze the minor-variants.

P059

Superbacterial infections with influenza in 2017-2018, an atypical season?

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Introduction: The relatively long 2017-2018 influenza season was characterised by an unusual incidence of influenza B. The epidemic strained the healthcare system: with reduced staff availability and increased patient numbers, capacity problems occurred all-round and emergency rooms were closed. The high morbidity and mortality caused by influenza is compounded by commonly occurring superinfections with treptococcus pneumoniae, taphylococcus aureus, Streptococcus pyogenes, Neisseria meningitidisand Aspergillus fumigatus. A large number of influenza superinfections were observed in the clinic in 2017-2018, and we set out to evaluate how this related to the size of the epidemic and the influenza types (A/B) observed in different seasons. To this end, we used diagnostic data from four hospitals in the region Eindhoven (The Netherlands).
Methods: PAMM performs medical microbiology testing for four hospitals in the region Brabant South East (The Netherlands). Data on the number of positive influenza tests were extracted for the seasons 2015-2016, 2016-2017 and 2017-2018; the influenza type (A/B) was also recorded. For influenza-positive patients, blood and sputum culturing results were extracted. Presence of treptococcus pneumoniae, taphylococcus aureus, Streptococcus pyogenes, Neisseria meningitidisand Aspergillus fumigatuswas called a superinfection.
Results: The number of patients with confirmed influenza infections in hospitals in the region Eindhoven and the prevalence of bacterial superinfection illustrated that only the absolute, and not the relative, incidence of superinfections was higher in 2017-2018 than in the previous seasons:
In season 2015-2016, there were 342 influenza positives, from which 240 culture tests were performed** (70.2%), and 13 (7.1% of these culture tests) were positive.
In season 2016-2017, there were 371 influenza positives, from which 279 culture tests were performed** (75.2%), and 20 (8.6% of these culture tests) were positive.
In season 2017-2018, there were 755 influenza positives, from which 549 culture tests were performed** (72.7%), and 36 (8.2% of these culture tests) were positive.
Analyses of the percentages of superinfections for the three seasons, split per influenza type (A/B), also demonstrated that the relative incidence of superinfections was not increased in the 2017-2018 season.
Conclusions: The absolute number of bacterial superinfections in influenza cases was substantially higher in 2017-2018 than in previous seasons. However, corrected for the size of the epidemic, the proportion of influenza infections that were diagnosed superinfections, did not differ from the previous seasons. This kind of knowledge on influenza epidemiology aids healthcare providers in adequately preparing for upcoming influenza seasons.

P060

Unnecessary replicate microbiology diagnostics in primary and secondary care: a major problem?

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Introduction: Policy makers often state that a substantial part of diagnostic testing is unnecessarily repeated. This could result from multiple diagnostic service providers in a region, or limitations in test result visibility to different healthcare professionals. Relevant situations include a patient returning for a repeat visit to (another) general practitioner, or being referred to (another) hospital. Reducing unnecessary repeated testing would reduce healthcare costs. To assess the degree to which replicate tests are requested, we analysed data from our regional laboratory in primary and secondary care, for the three most requested types of tests.
Methods: PAMM performs medical microbiology testing for five hospitals and >450 general practitioners in the Eindhoven region, The Netherlands. We evaluated replicate requests over a two year period for the top three tests in a primary care setting (general bacterial culture on urine >15000/yr; STI diagnostics >8000/yr; faecal diagnostics >7000/yr); and within a secondary care setting (general bacterial culture on urine >20000/yr; hepatitis B >4000/yr; faecal diagnostics>4000/yr). Repeated testing was defined as a request for the same test on the same material for the same patient, within 1 week; or for STI or hepatitis B testing, within 4 weeks of the initial test request.
Results: We find that repeated diagnostics (some of which is even warranted) occurs much less often than frequently implied:

repeated tests in primary care:
-general bacterial culture of urine primary care tests are repeated for 0.9% and 1.5% of tests in primary

and secondary care settings, respectively.
- STI diagnostics primary care tests are repeated for 1.4% and 0.3% of tests in primary and secondary care settings, respectively.
- faecal diagnostics primary care tests are repeated for 0.2% and 0.3% of tests in primary and secondary care settings, respectively.

repeated tests in secondary care:
- general bacterial culture of urine secondary care tests are repeated in secondary care for 6.5% of tests.
-hepatitis B secondary care tests are repeated in secondary care for 1.6% of tests.
-faecal diagnostic secondary care tests are repeated in secondary care for 1.5% of tests.

Conclusions: Since privacy regulations obstruct the sharing of test results with other healthcare providers, and there are not proactive interventions on diagnostic requests, we were able to evaluate repeated testing requests in natura. We observe that the percentages, while not zero and thus necessitating improvement and interventions, indicate that there is a only minor gain to be obtained in health economic terms when considering the potential reduction of a small percentage from the total budget for microbiological diagnostics.

P061
Populating the tree: expanding our understanding of the metabolic versatility of environmental Nitrospira
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Introduction
Autotrophic nitrification, the stepwise oxidation of ammonia to nitrate via nitrite, is performed either in a mutualistic interaction of ammonia-oxidizing microorganisms and nitrite-oxidizing bacteria (NOB), or by complete ammonia oxidizers (comammox) that catalyze both nitrification steps on their own. All known complete nitrifiers belong to the genus Nitrospira, which previously was assumed to comprise only canonical NOB. This genus can be phylogenetically divided into at least six lineages and its members have been found in a wide range of habitats, including both natural and man-made systems. In biotechnological processes, such as drinking water and wastewater treatment, Nitrospiraare key nitrifiers that play a vital role in the removal of nitrogen compounds from these waters. Although distribution studies identified Nitrospira-like bacteria in a wide range of natural ecosystems, the majority of the genomes analyzed so far was obtained from isolates or metagenomes from engineered systems and belong to just two of the six known phylogenetic Nitrospiralineages.
Methods & results
To fill the knowledge gap that exists due to the biased phylogenetic distribution of genome-sequenced representatives of this important nitrogen-cycling clade, we sequenced and analyzed genomes of Nitrospiraspecies isolated from natural systems and affiliated with Nitrospiralineages not containing any genome-sequenced representatives. Subsequently, we compared these to publicly available genomes using a pangenomic approach. While the core genome of Nitrospiraincludes key pathways like the reductive tricarboxylic acid (rTCA) cycle for CO_2 fixation, all five respiratory chain complexes and the nitrite oxidoreductase for nitrite oxidation, the accessory genome enables the use of alternative substrates such as ammonia, hydrogen and formate as well as alternative nitrogen sources for assimilation, like nitrite, cyanate and urea.
Conclusion
This metabolic versatility opens the possibility to occupy different niches and to respond to fluctuating nutrient availability, which might be one reason for the global distribution and the high diversity of Nitrospira-like bacteria.

P062
Staphylococcal persisters during infection
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Introduction: Many bacterial species are known to cause recurrent infections, to which populations of persister bacteria have been suggested to contribute. Persister bacteria form a subpopulation of genetically identical bacteria that are tolerant to antibiotics, are non- or slowly growing, and retain the

capacity to quickly grow out and re-establish a bacterial population. The proportion of persisters within a bacterial population has been shown to increase upon passage through phagocytes. Whereas persisters that naturally occur in culture medium are predominantly dormant, almonellapersisters generated inside host cells have recently been shown to remain active, sense their environment, and manipulate host immunity [1]. Since recalcitrance of infection is also a major problem during infections with the Gram positive tapylococcus aureus,we wondered whether this population of active persisters might also exist amongst phagocyctosed . aureusand whether this could play a role in recalcitrant infections.
Methods: We quantified the amount of . aureuspersisters in populations before and after phagocytosis by purified, human neutrophils: these populations were incubated with different antibiotics for 20 hours, the antibiotics were washed away, and persisters were revealed by their ability to form colonies on agar plates. In addition, the MIC of the recovered colonies was determined to distinguish between (hereditable) resistance and (non-hereditable) persistence.
Results: The number of bacteria surviving antibiotic treatment and retaining the capacity to regrow was significantly higher in bacteria passaged through human neutrophils than in bacteria just grown in liquid medium. For 4 out of 5 tested antibiotics, no increase in MIC was observed.
Conclusion: Being phagocytosed by human neutrophils triggers . aureusto adopt a persister state in which the bacterium is tolerant to antibiotic treatment. This is a phenotypic adjustment, which is not inherited by their progeny.
[1] Stapels et al.2018 Science 362(6419):1156-1160

P063

Optimizing molecular surveillance of mumps viruses using non-coding regions: example of two 2018 mumps clusters

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Introduction
Mumps viruses continue to cause sporadic cases and outbreaks in countries with a high vaccination coverage for mumps, including the Netherlands. Currently, molecular surveillance is worldwide primarily focused on sequencing of the small hydrophobic (SH) gene. However, few studies have already shown that additional genes or regions contribute to the resolution of the sequence data in such a way that mumps cases that seem to be linked on basis of the SH sequence, appear to be linked to another source or chain of transmission. This was recently demonstrated on the basis of sequence data extracted from the sum of three non-coding regions (NCRs; total 1954 nt) between the N, P, M and F gene, which provided the most optimal sequence data for molecular surveillance (Jin et al, 2015; Gavilan et al, 2018; Bodewes et al,submitted). Although obtaining the complete genome could result in the most complete data, this is currently often hampered by the relative low amount of mumps virus RNA in clinical samples, especially in vaccinated cases (Gouma et al, 2016).
Methods
In the first half of 2018, a cluster of mumps cases occurred among adults in the eastern part of the Netherlands. In total 6 cases were reported and from 2 cases a clinical sample was available. In the second half of 2018 another cluster of mumps cases occurred in the southern part of the Netherlands, affecting mainly young adults. These cases were highly location clustered and mainly involved students from a university and their direct contacts, suggesting a single chain of transmission. From 9 of the 16 reported cases in this cluster a clinical sample was available. RNA was extracted from all samples and sequence data of the SH gene and the NCRs were obtained using RT-PCR followed by Sanger sequencing.
Results
All mumps viruses associated with both clusters belonged to the genotype G and SH sequences of both clusters were identical with each other. However, sequence analysis of the combined sequences of the three NCRs revealed clearly that the late 2018 cluster belonged to a different molecular group than the early 2018 cluster. Furthermore, some additional sequence variation was detected in a subcluster, suggesting that this sequence data might be used in the future to identify transmission chains. These results indicate that indeed NCR sequence data provide an increased sequence resolution.
Conclusion
For molecular surveillance of mumps viruses, sequencing of SH gene in combination with NCRs is currently the optimal approach.

P064

Using whole-genome sequencing in an outbreak investigation of Staphylococcus aureus

bacteremia on a neonatal intensive care unit

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Introduction
An increase in taphylococcus aureus(SA) bacteremia was seen on the Neonatology ward of a tertiary care center in the Netherlands. Eight neonates had had an SA bacteremia in six months, whereas the usual number of cases was 4-8 per year. We conducted an outbreak investigation to identify potential causes and sources and typed the strains by using whole genome sequencing (WGS).
Methods
The incidence of SA bacteremia on the neonatology ward was compared with earlier years. Epidemiological links and correspondence in clinical presentations were explored. Additionally, environmental samples were screened in search of a reservoir. The SA strains were studied for antimicrobial resistance and presence of virulence genes using ResFinder and VirulenceFinder (https://cge.cbs.dtu.dk) and typed using amplification fragment length polymorphism (AFLP) and subsequent core-genome multilocus sequence typing (cgMLST, SeqSphere Ridom GmbH). Hand hygiene and catheter-related practices were assessed by observational audits.
Results
The incidence of SA bacteremia was 0,6 – 1,6% in the 5 years preceding the increment. In 2018, the incidence was 2,2%. Clinical presentations were variable and spread along the ward with consecutive overlap in admission periods. cgMLST revealed related strains in one pair of patients, the other six patients had unrelated strains. No environmental sources were identified. Hand hygiene compliance rate was 76% in 2018 compared to 65% in 2016 and catheter-related practices were according to safety management system (VMS). Earlier outbreaks at the department lead to increased awareness and training in hand hygiene measures during this outbreak. After these training-efforts no further cases were identified.
Conclusion
WGS with cgMLST analysis was used to determine strain relatedness at high resolution and was useful in guiding efforts to elucidate the origins of a SA bacteremia increase. There were no indications found for a single reservoir of SA, leading to the increase in SA bacteremia cases. Suboptimal hand hygiene and poor environmental cleaning are the most likely causes of of this increase in SA bacteremia. Infection control measures taken after earlier outbreaks most likely prevented further SA bacteremia cases.

P065

Microbial community analysis of a Dutch drinking water treatment plant, with a special focus on microorganisms involved in N, C and Fe cycling

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In the Netherlands, both surface (35%) and groundwater (65%) are used for drinking water production. Anaerobic groundwater entering the drinking water treatment plant (DWTP) in Breehei, the Netherlands, contains high concentrations of ammonium (NH_4^+), iron (Fe), manganese (Mn) and methane (CH_4). Elevated concentrations of these chemical compounds in groundwater can cause severe problems during drinking water production and distribution. Microbial communities mediate the biodegradation of organic and inorganic matters, most prominently in drinking water filtration systems. However, they can also induce several serious problems, including biofilm formation and corrosion of water distribution pipes.
In this study we used high-throughput sequencing in combination with differential coverage and sequence composition-based binning to recover high-quality metagenome assembled genomes (MAGs) and to characterise the microbial communities involved in N, C and Fe cycling. Samples were collected during June 2016 and September 2018, from the primary and secondary sand-bed (PSB, SSB) filters, in addition to wall biofilm (WB). Besides, WB samples were also collected in May 2017. We recovered 50 MAGs with an estimated completion above 70% and with less than 10% redundancy. Metagenomic analyses indicated that microbial communities colonising the WB and the granular material of the PSB were dominated by bacteria that can utilise one-carbon compounds and were affiliated with the Methylococcaceaeand Methylophilaceae.The abundances of these bacteria drastically decreased in SSB samples. Iron oxidisers of the genus Gallionellawere highly abundant mainly in the WB, where colloidal iron deposition was observed. The different groups of nitrifying microorganisms were also among the most abundant microorganisms. Both canonical nitrite- and the newly discovered complete

ammonia-oxidising (comammox) Nitrospirawere recovered from all samples. Notably, these novel comammox Nitrospiradisplayed the highest abundance of all nitrifying microorganisms, particularly in the WB. The DWTP samples also harboured novel microorganisms, the metabolic role of which remain to be determined.
In conclusion, we demonstrated that the DWTP Breehei represents an interesting model system to study microbial community composition involved in drinking water production. The knowledge obtained here will help to advance our understanding of the role of microorganisms in the removal of contaminating compounds from groundwater and thus to safeguard human health.

P066

Unravelling oxygen survival and reduction by a mucosa-associated microbial community

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The mucus layer in the intestines is produced by specialized epithelial cells. Bacterial symbionts colonizing the mucosal layer, including the mucin-degrading bacterium Akkermansia muciniphila, are thought to stimulate the regulatory immune response and to produce metabolites useful in human metabolism. However, to be able to remain in the mucus layer, these bacteria have to be able to encounter the environmental factors. One of these environmental factors is the presence of oxygen, due to the close proximity to the host cells. Recently, A. muciniphilawas found to exploit these low oxygenated conditions. The oxygen-reduction capacities of A. muciniphilamay have a competitive advantage. We aim to study whether the de-oxygenation protects other bacterial species in the mucosal community that are not able to tolerate low amounts of oxygen. First, a selection of mucosa-associated species is made based on their oxygen tolerance and reduction capacities in combination with their metabolic activities. Then, the selected mucosa-associated synthetic community is co-cultivated in the presence of low oxygen concentrations in fermentor experiments. Preliminary results in oxygen reduction capacity experiments show that the ability to reduce oxygen and to what extend varies between mucosa-associated species. Therefore, we can conclude that the initial selection of mucosa-associated bacterial species is suitable for co-cultivations in fermentor experiments. The results from this study will provide more insight into the mechanisms by which the mucosal community is able to collaborate to survive in the presence of oxygen.

P067

Clostridioides difficile infections in the Netherlands: Incidence and clinical characteristics in the period of May 2017-May 2018.

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Introduction:
In 2005, the National Clostridioides (C.) difficileReference Laboratory was founded in the Netherlands. The foundation was stimulated by multiple outbreaks with the hypervirulent C. difficilePCR ribotype (RT) 027. Since then, the reference laboratory has offered ad hoc typing service for all microbiology laboratories in the Netherlands for typing of C. difficileisolates of patients with severe disease, or isolates from a suspected outbreak. Additionally, the National Reference Laboratory initiated a sentinel surveillance programme in May 2009 to monitor the incidence of C. difficileinfections (CDI) in 22 hospitals in the Netherlands. The results from the sentinel surveillance from the period May 2017 until May 2018 are presented here.

Methods:
Clinical characteristics and 30-day outcomes of hospitalized patients (>2 years old) that are diagnosed with CDI in participating hospitals are registered in the Dutch sentinel surveillance. Severe CDI is defined as: hypoalbuminemia (<20 g/L) and/or dehydration, fever (temperature of 38°C or higher) and leucocytosis (>15×10^9/L), pseudomembranous colitis or bloody diarrhoea. A complicated course is defined as: admission to an intensive care unit, the need for surgical procedure or death. The participating hospitals send all C. difficileisolates to the Reference Laboratory for further characterisation.

Results:
In total, 879 patients with CDI were included in the sentinel surveillance in the period between May 2017 and May 2018. The mean incidence rate of CDI was 2.90 CDI cases per 10.000 patient-days, which was similar to previous years. The proportion of patients with severe CDI and the proportion of patients with a complicated course were also comparable to previous years. Severe CDI was seen in 20.4% of the patients. Of 797 patients with a reported 30-day outcome, 86.8% had an uncomplicated course, 0.4% needed surgery as a consequence of CDI, 0.3% was admitted to the ICU because of CDI and 12.5% of the patients died within 30 days (n=100). Twenty five deaths (3.1%) were due or contributable to CDI. Compared to the start of the surveillance, symptoms of CDI had more frequently a community-onset. This was seen in 45% of the cases, while healthcare facility onset of symptoms was seen in 55% of the cases.
The most prevalent RT that was found was RT014/020 (20.9%), similar to the previous year. The proportion of CDI cases due to RT002 had increased significantly compared to previous years and was now the second most frequently encountered RT (11.9%). The hypervirulent RT027 was found in 1.2% of samples, which was similar to previous years.

Conclusion:
1. Compared to previous years, the mean incidence of CDI has not changed in the Netherlands.
2. The proportion of community-onset cases has increased compared to the start of the surveillance.
3. Severe CDI was seen in 20.4% of the patients.
4. The overall mortality rate within 30 days was 12.5%, with a CDI-related mortality rate of 3.1%.
5. With a proportion of 20.9%, RT014/020 was the most frequently encountered RT.

P068

Isolation and GMP-compliant culturing of a non-toxigenic Clostridioides difficile strain for application in a controlled human infection model

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Introduction: Clostridioides difficileis considered part of the normal human gut microbiota, but is also the main causative agent of nosocomial diarrhoea. Why do some people get colonised or infected with this bacterium, while others don't? The answer may lie in gut microbiota composition differences. To improve our understanding of the role of gut microbiota composition in C. difficilecolonisation, we aim to administer non-toxigenic C. difficile(NTCD) spores to healthy volunteers in a controlled human infection model. Therefore, a NTCD strain needs to be isolated from human faeces, and spore production has to comply with Good Manufacturing Practices (GMP). Brain-Heart-Infusion (BHI) broth, a medium that is commonly applied for C. difficilegrowth in vitro, cannot be applied due to the risk of prion disease. Therefore, a new semi-synthetic medium for C. difficilegrowth and spore production needs to be defined for application in a controlled human infection model.
Methods: Stool samples from 117 healthy individuals were cultured directly on C. difficileselective media and after ethanol shock on selective C. difficilemedia and antibiotic containing agar plates. Plates were read after 3 and 5 days of anaerobic incubation at 37°C and suspicious colonies were tested for the presence of GluD gene with PCR to confirm the identification of C. difficile. Isolates were PCR-ribotyped and toxigenicity was determined via a multiplex PCR for TcdA, TcdB and binary toxin genes. Growth of the NTCD strain was performed on minimal medium (CDMM), tryptic soy broth (TSB), Dulbecco's Modified Eagle Medium (DMEM) and Chicken and Beef Broth (CBB) from Maggi, using BHI medium and a strain collection-derived NTCD as reference. Bacterial growth and spore production were determined via OD600 measurement and plate-counting, and via plate-counting after ethanol shock respectively.
Results: For 117 cultured faeces samples 2 were positive for C. difficile. One was a NTCD strain with PCR ribotype 780. Growth kinetics and spore production were normal on BHI medium as compared to the strain collection-derived NTCD. CDMM, DMEM and CBB did not allow for growth and spore production. The NTCD strains grew and produced spores using TSB. However, further investigations showed that the used casein in this medium cannot be guaranteed to be appropriate for human consumption and therefore doesn't comply with GMP.
Conclusion: Colonisation rate of healthy individuals with C. difficilewas 1,7%. Culturing C. difficileon GMP-complying media remains challenging. Other media will be defined and tested, including modified-TSB, to assure a human consumable growth medium for spore production to be applied in a controlled

human infection model.

P069

Nosocomial bloodstream infection rates: exploration of a quality indicator of infectionprevention in the hospital

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Introduction: Outcome indicators for the quality of infectionprevention in the hospital are scarce and/or time consuming to collect. The proposed rate of bloodstream infections (BSI) by Highly Resistant Microorganisms is so low in the Netherlands that it has no use as a tool for focussing interventions and comparison between wards and hospitals.
Methods: From January 2015 up to January 2019 BSI in patients admitted to the hospital > 2 days were prospectively registered by the infectionprevention department. Positive blood culture data are derived form the Laboratory of bacteriology. Data on source of BSI, ward, specialism and other patient characteristics were collected form the EPD. Rates of BSI were calculated as number of BSI per 1000 nursing days.
Results: Rates of nosocomial BSI vary considerably between wards (0,2 - 6,0 per 1000 nursing days) and specialisms (0,2 - 4,5 per 1000 nursing days). Also, the distribution between sources of nosocomial BSI is variable for different wards. Time trends of nosocomial BSI are shown per ward and can be used for monitoring.
Conclusions: Rate of nosocomial BSI can be used to monitor the performance of wards over time. Analysis of the source of BSI per ward can focus interventions to decrease nosocomial BSI rates. BSI rates can be used to compare (wards /specialisms from) hospitals.

P070

Biodegradation of Contaminants of Emerging Concern in Wastewater Treatment Plants

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Frequently used chemicals such as pharmaceuticals and personal care products are more and more detected at trace levels in surface and groundwater all over the world, including drinking water. Despite their low concentration, many studies indicate that they have a negative impact on aquatic life and possibly human health. Therefore, they are being called contaminants of emerging concern (CECs). Wastewater treatment plants (WWTPs) are crucial to remove these pollutants before they reach the surface waters, but WWTPs have initially not been designed for that purpose. Our aim is to better understand the removal of CECs during nitrification, the first step of nitrogen removal in WWTPs. More specifically, our aim is to identify the metabolic pathways involved in the conversion and removal of CECs for which different experiments are being performed. Bioreactors were inoculated with samples from Groesbeek municipal WWTP in order to identify bacteria able to degrade CECs under nitrifying conditions; DNA was extracted before and after exposure to CECs to determine which bacterial species in the activated sludge are able to utilize CECs as carbon and energy source; Furthermore degradation experiments with the model nitrifying bacteria such as Nitrosomonas europeaare planned to understand the mechanisms of CEC degradation by ammonia oxidizing bacteria. The experimental data of these studies will be used to model the fate of a suite of CECs and will enable us the redesign of WWTPs to better bioremediate micropollutants.

P071

Retrospective comparison of two chemokine CXCL13 assays for the diagnosis of Lyme neuroborreliosis

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Introduction: In the Netherlands, Lyme neuroborreliosis (LNB) is a common manifestation of disseminated Lyme borreliosis, with an annual incidence rate of 2.6 (95% CI 2.4-2.8) per 100,000 inhabitants. The diagnosis of LNB consists of clinical symptoms (e.g. meningoradiculitis or paresis) as well as laboratory findings, such as an elevated number of leucocytes in the cerebrospinal fluid (CSF), and evidence of intrathecally produced Borrelia-specific antibodies. Unfortunately, the level of intrathecal

Borrelia-specific antibodies can be too low for detection in the early stages of the disease. Furthermore, the decision to treat often precedes the time point at which test results for intrathecal Borrelia-specific antibodies become available. Therefore, new diagnostic markers are needed with a high sensitivity in the early phase of the disease and a shorter turnaround time. Recent studies have shown that the B-cell chemokine (C-X-C motif) ligand 13 (CXCL13) can be a useful marker for the diagnosis of early LNB, even though elevated levels have been found also in other diseases.
Methods: We evaluated two assays for the detection of CXCL13 in CSF: the Quantikine CXCL13 ELISA (R&D Systems, Minneapolis, MN, USA) and the Luminex based recomBead CXCL13 assay (Mikrogen GmbH, Neuried, Germany). Retrospectively, we performed these assays on all consecutive patients for which CSF and blood sample pairs were sent to our laboratory between August 2013 and June 2016 and for which enough volume (≥ 1250 µl CSF and ≥ 110 µl serum) was available to perform this study and another study (that will be published separately). Patients were divided into six groups based on the likelihood of having LNB or other diseases. We also included six additional definite LNB patients from before August 2013 and after June 2016. The CXCL13 concentrations in CSF were measured and the results were evaluated through receiver operating characteristic (ROC) curve analysis in which the results of the definite LNB patients were compared with those of non-LNB infectious disease patients and non-infectious disease patients.
Results: In total, 152 consecutively collected patients were included, as well as six additional definite LNB patients; 7/158 (4.4%) were classified as LNB, 11/158 (7.0%) as probable LNB, 24/158 (15.2%) as possible LNB, 15/158 (9.5%) as non-LNB infectious disease patients, 47/158 (29.7%) as non-infectious disease patients, and 54/158 (34.2%) as unknown disease patients. In general, CXCL13 concentrations measured with the recomBead CXCL13 assay were higher than with the Quantikine CXCL13 ELISA, and this is exemplified by the median CXCL13 concentrations of the probable LNB patients; these were 69.7 pg/mL (11.3-378) and 10.7 pg/mL (6.35-233), respectively. ROC curve analysis showed that the Quantikine CXCL13 ELISA and the recomBead CXCL13 assay performed equally well to diagnose LNB (area under the curve (AUC): 0.985, and AUC: 0.988, respectively) (P value = 0.628). Both assays had a sensitivity of 100.0% and a specificity of 96.8% for detecting LNB.
Conclusion: This retrospective study showed that both the Quantikine CXCL13 ELISA and the recomBead CXCL13 assay had a good performance and that CXCL13 is a useful tool in the diagnosis of LNB.

P072

Detection of BORSA carriage among health care workers at NICU and paediatric ward as an incidental finding of a MRSA contact tracing and screening procedure

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Background/Introduction
During a MRSA contact tracing and screening investigation we encountered an incidental finding with two Borderline Oxacillin Resistant S.aureus(BORSA) positive screening cultures among Neonatal Intensive Care Unit (NICU) health care workers (HCW). Clear guidelines and evidence from literature on how to handle BORSA occurrence in this vulnerable population is lacking. This has led to further investigations and in this report we describe the number of screened HCWs, the infection prevention measures and the screening and follow up of NICU patients. The NICU in our tertiary medical centre has on average 800 yearly admissions.
Methods
An infection control team was installed after discovery of two BORSA carriers among NICU HCW. The team appointed all additional infection control measures and evaluated new findings.
All NICU HCW were screened for BORSA carriage both in nares and throat with two swabs and the four swabs were simultaneously incubated in a non-selective broth and plated on a MRSA chromogenic agar. Positive HCW were excluded from clinical work and were offered a .aureusdecolonization treatment. All patients who were admitted when BORSA carriage among personnel was discovered were screened using a throat swab. To assess the likelihood of direct transmission among HCW isolates were analysed using AFLP and whole genome sequencing (WGS).
Results
127 HCW were screened for BORSA at the NICU, 3 of them were positive. Because 1 HCW worked at both the NICU and the general Paediatric ward an additional 77 HCW from the Paediatric ward were screened, 2 of them were BORSA positive.
Patient screening started with all 10 neonates admitted at the moment of discovery of the positive HCWs. Because all neonates were BORSA negative it was decided that BORSA carriers could resume their work. During a 7 week follow-up period NICU patients received a weekly BORSA throat screening

swab to monitor transmission. 72 patients were screened ranging from 1 to 9 cultures per patient, with a total of 138 cultures. No BORSA positive patients were discovered. In the same period 125 clinical cultures were performed. No BORSA related infection was found. After 7 weeks of follow up no spread from HCW to patients had occurred and the BORSA screening program was discontinued.
AFLP showed that the strains from 2 HCW at the NICU were related, and the strain from the HCW that worked at both the general ward and NICU was unrelated. WGS data analysis with core genome MLST confirmed the relatedness as shown with AFLP of the 2 NICU strains as these belonged to the same cluster and sequence type, the 3 other strains were unrelated.
Conclusions
During a 7 week follow up period no transmission from BORSA positive HCW to neonates were observed in either screening or clinical cultures and spread among HCW was excluded using cgMLST analysis. This has led to the conclusion that in case of BORSA carriership among HCW no additional measures are needed. More vigilance and experience is needed to design adequate evidence based interventions for the future in this vulnerable population.

P073

Improved reproducibility of two commercial enzyme-linked immunosorbent assays to determine intrathecal Borrelia-specific antibodies by a simple optimization of the assay procedure

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Introduction: In the Netherlands, the incidence of Lyme borreliosis has quadrupled in the last two decades and the annual incidence rate of Lyme neuroborreliosis (LNB) is 2.6 (95% CI 2.4-2.8) per 100,000 inhabitants. The laboratory diagnosis of LNB includes detection of intrathecally produced Borrelia-specific antibodies, which can be established by measurement and comparison of the relative amount of specific antibodies in serum and cerebral spinal fluid (CSF). Many of the available LNB tests are based on enzyme-linked immunosorbent assays (ELISA). The reproducibility of ELISAs can be affected by the 'drift effect', the 'edge effect' and environmental conditions. Therefore, we investigated the reproducibility of two commercial ELISAs for the determination of intrathecal Borrelia-specific antibodies.
Methods: The Enzygnost II Lyme Link VlsE/IgG (IgG-ELISA) and the Enzygnost II Borreliosis/IgM (IgM-ELISA) (Siemens Healthcare Diagnostics, Marburg, Germany) were performed on the DS-2 ELISA robot, according to the instructions of the manufacturer (Dynex technologies, Kowloon, Hong Kong). These ELISAs consist of independent vertical "strips" of each eight test wells of which minimum one to maximum 12 can be used in one ELISA tray (maximum capacity 96 wells). One IgM-ELISA and two IgG-ELISA experiments, testing four strips, were performed. In these experiments, a negative control was tested in one well of strip 1 and two wells of the strip 4 (A1, G4 and H4). The optical density (OD) of a single dilution of the positive kit control was measured 29 times in the remaining wells of strips 1-4.
Results: The coefficients of variation (CV) of testing the positive control 29 times, of the IgM-ELISA and the first IgG-ELISA were 8.9% and 8.2%, respectively. It was noted that the ODs in the outer wells of the ELISA tray were higher than the ODs in the inner wells. To investigate whether this was caused by the edge effect, a second IgG-ELISA was performed and, in this experiment, and extra strip (strip 5) was added, filled with distilled water. The average of the differences between the ODs of the positive control in the wells in strip 3 and strip 4 was 0.150 in the first IgG-ELISA and -0.009 in the second IgG-ELISA ($p = <0.0001$).
Conclusion: Our study showed that the reproducibility of these two Enzygnost II BorreliaELISAs is mostly affected by the variation in OD values between the outer and the inner wells of the ELISA tray, with higher OD values in the outer wells, presumably caused by the edge effect. In practice, when determining whether a patient has a pathological antibody index, which involves calculation of the quotient of the CSF-serum Borrelia-specific antibodies, this could lead to erroneous results.
Our study suggests that the edge effect can be countered by avoiding the outer wells for measurements and by adding a "dummy" strip with water. The edge effect may apply to other ELISAs as well and, therefore, this aspect should be investigated when implementation validations for new ELISAs are performed.

P074

Four weeks of sugar beet pectin supplementation did not alter faecal microbiota composition in young adults and elderly

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The world population is aging. As a consequence, elderly suffer from an increased incidence of age-related diseases, some of which have been associated with alterations in their gut microbiota. However, the gut microbiota of elderly has so far received limited attention and hence, the impact of aging on its composition dynamics, and how it is influenced by dietary modification is largely unknown. In this study, we aimed to investigate whether the microbiota of elderly is different from young adults as well as to what extent the gut microbiota responds to the supplementation of pectin in elderly and young adults. We performed a randomized double-blind placebo-controlled study with a parallel design in 52 young adults (18-40y) and 48 elderly participants (65-75y). Each subject either consumed 7.5g of pectin or maltodextrin as control twice per day for 28 days. Before and after the intervention, faecal samples and several physiological parameters were collected. For microbiota profiling, DNA was isolated from faecal samples followed by HiSeq Illumina sequencing of PCR-amplified V5-V6 variable regions of the 16S rRNA gene. Baseline samples were used to compare microbiota composition between the young adults and elderly age groups, while the impact of pectin supplementation was studied by comparative analysis between pre- and post-intervention samples. The microbiota of young adults and that of the elderly was very similar. Nevertheless, the abundance of five minor genera (Enterorhabdus, Ruminiclostridium6, Mogibacterium, Coriobacteriaceae_uncultured, LachnospiraceaeUCG-008) showed to have predictive strength in classifying the microbiota to young adults or elderly. Furthermore, twenty-eight days of sugar beet pectin supplementation did not alter the faecal microbiota composition and diversity in both elderly and young adults.

P075

Nitrite comproportionation as novel interaction mechanism between anammox and comammox bacteria

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Biogeochemical cycling of nitrogen has been altered due to increased anthropogenic nitrogen input, resulting in eutrophication and biodiversity loss. Removal of nitrogen from wastewater partially alleviates these effects, but is an energy-demanding process as nitrification requires intensive aeration. For a long time, nitrification was considered to be a two-step process performed by separate microorganisms: ammonia oxidising prokaryotes (AOP) and nitrite oxidising bacteria (NOB). Recently, complete ammonia oxidation (comammox) by a single organism belonging to the genus Nitrospira was discovered. First, comammox was identified in an enrichment culture originating from a recirculating aquaculture system biofilter. This enrichment culture was used as inoculum for a new bioreactor running on a defined medium with low ammonium, nitrite and nitrate concentrations and hypoxic conditions. Interestingly, FISH images acquired from flocs growing in this bioreactor showed small clusters of anammox and comammox bacteria in close proximity, indicating interaction. Colocalisation of anammox and comammox is counterintuitive at first thought as both anammox and comammox compete for ammonium and nitrite as substrates. However, we conjecture that under hypoxic conditions comammox partially oxidizes ammonium to nitrite in order to provide anammox with its substrate such that community productivity is maximized. Besides that, comammox could perform nitrite comproportionation to form nitrite from ammonium oxidation coupled to nitrate reduction, which recycles the nitrate produced by anammox. Here, we show formation of $^{29}N_2$ and $^{30}N_2$ upon incubation with ^{15}N-labelled ammonium in the presence or absence of nitrite and nitrate respectively, which suggests comammox and anammox act together in the formation of dinitrogen gas. Furthermore, the effects of increasing oxygen concentrations on the formation rates of $^{29}N_2$and $^{30}N_2$ demonstrate the conditions in which anammox and comammox may transition from cooperation into competition. These findings lead to a better understanding of the interaction between anammox and comammox bacteria, which could eventually be applied for effective and sustainable nitrogen removal from wastewater.

P076

The LymeProspect study into long-term effects of Lyme borreliosis in adults and children: first descriptive results of 150 prospectively followed patients with erythema migrans

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Introduction
Between 0-48% of patients treated for Lyme borreliosis report disabling persistent symptoms. The aim of the LymeProspect study is to prospectively determine the prevalence of persistent symptoms in adults and children with confirmed Lyme borreliosis after antibiotic treatment. Moreover, we aim to assess microbiological, immunological, genetic, clinical, cognitive-behavioral, and epidemiological determinants for development of these symptoms.
Methods
Adult patients and children with confirmed erythema migrans (EM) or disseminated Lyme manifestations were included at the initiation of antibiotic treatment. During one year follow-up, participants were subjected to online questionnaires and provide blood samples. A subset of adult patients underwent skin biopsies. The occurrence and severity of persistent symptoms was measured with questionnaires based on Dutch population norm scores. Potential determinants of persistent symptoms were assessed by serology, cellular immune responses, polymerase chain reaction (PCR) for Borreliaand other tick-borne pathogens, gene expression arrays and validated questionnaires on symptoms (CIS, CFQ, SF-36-pain, PHQ-15, and specific questionnaires for children), disabilities, comorbidity and cognitive-behavioral variables.
Preliminary results
Until October 2018, over 70 children and around 1100 adults were included. Inclusion of adults has stopped and 94% of them presented with EM. This report describes the clinical, immunological, and laboratory data from the first 150 adult patients, of whom 52% were female and their median age was 54 years (range 23-81). Nearly all patients (136/138; 98.5%) were diagnosed by the GP as EM, 80.4% were classified as typical and 18.1% as atypical EM. Independent assessment of pictures of the skin lesions showed that 30% lack the characteristic central clearing. Median diameter was 10 cm (range 5-30) and the median time between development of EM and inclusion in the study was six days (range 0-83). Doxycycline was prescribed in the majority of cases (122/136) with a median duration of 10 days (range 10–30). Baseline C6 ELISA (IgM/IgG) was positive in 63.3%; seroconversion occurred in 4% within six weeks. Serological results did not differ significantly between patients with or without central clearing. Clinical data and serology will be presented, as well as cytokine responses.
Conclusions
EM in Europe relatively often presents without central clearing, which may complicate the diagnosis. This preliminary analysis describes clinical, microbiological and immunological features of 150 adult EM patients in the Netherlands. The LymeProspect study will provide important insights into the long-term effects of Lyme borreliosis and determinants for persisting symptoms in both adults and children. Importantly, the inclusion of children will continue in 2019.

P077

Carriage of Neisseria meningitidis detected in young adults by testing orpharyngeal swabs and saliva using conventional culture and molecular diagnostic methods

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Introduction: Meningococcal carriage precedes invasive meningococcal disease (IMD). The current outbreak of IMD caused by serogroup W strains in the Netherlands highlights the importance of meningococcal surveillance. However, comprehensive data on the carriage of meningococci of this and other serogroups is lacking. The current study evaluated use of oral fluids and molecular diagnostics for the detection of meningococcal carriage in young adults.
Methods: Oropharyngeal swabs and saliva and were collected from 150 students of the University of Applied Sciences Utrecht in the fall of 2018. All samples (n=300) were instantly cultured on meningococcus-selective-plates and meningococcal isolates were identified with MALDI-TOF. DNA extracted from all bacterial growth harvested from cultured plates was tested in meningococcus-specific qPCRs targeting metAand ctrA.Samples were considered positive for meningococci when signals for

metAand ctrAwere <40 C_T and <35 C_T, respectively. Statistical analysis was performed using the McNemar's test and Spearman rank test, a p<0.05 was considered as significant.
Results: In total, 67 (45% of 150) of students were identified as carriers of meningococcus by any of the methods used. Thirty-seven students (25% of 150) were culture-confirmed for meningococcal carriage, while 64 students (43% of 150) were confirmed as meningococcal carriers using qPCR-based detection. qPCR-based detection with metAand ctrAas targets for encapsulated meningococci was highly concordant for both oropharyngeal swabs and saliva (Spearman rank, p<0.0001). A comparison between culture-confirmation and qPCR-based detection on oropharyngeal swabs and saliva showed that qPCR-based detection yielded significantly more meningococcal carriers than culture-confirmation of oropharyngeal swabs and saliva (McNemar's, p<0.0001). We have observed a significant difference (McNemar's, p<0.0001) in the number of students identified positive in saliva (20% of 150) and oropharyngeal swabs (37% of 150) with qPCR-based detection. Furthermore, qPCR-based detection of meningococcal carriage on students identified resulted in a comparable carriage prevalence as a combination of both PCR-based detection and culture-confirmation (McNemar's, p=0.25).
Conclusion: We observed a relatively high meningococcal carriage prevalence among students in the Netherlands. qPCR-based meningococcal carriage detection with cultured oropharyngeal swabs resulted in significantly higher yield of students identified as carriers than with cultured saliva samples or culture-confirmation on saliva and oropharyngeal swabs with MALDI-TOF. Our study demonstrates the increased sensitivity of molecular surveillance methods and PCR-based detection.

P078

Genome mining and antimicrobial screening of novel sponge-associated Flavobacteria

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Introduction: Discovery of new effective drugs remains a major challenge in the light of the antimicrobial resistance crisis. Marine sponges are considered as a gold mine of pharmaceutically interesting metabolites that appear to be mainly produced by their symbiotic microorganisms. Nevertheless, the real metabolic biodiversity is inaccessible as most sponge-specific microbes are yet uncultured and many biosynthetic gene clusters of previously cultured species remain cryptic.
Objectives: Explore the genomes of several representatives of the Flavobacteriaceaefamily, including 7 newly isolated and sequenced sponge-associated strains in terms of their secondary metabolic profile and investigate their antimicrobial potential.
Methods: Both cultivation-independent and cultivation-dependent methods were employed. Whole-genome sequencing of the seven sponge-associated Flavobacteria was followed by de novogenome assembly and annotation. Prediction of the protein-encoding genes of the draft genomes was performed using Prokka. Identification of the secondary metabolite biosynthetic gene clusters was done using the AntiSMASH web platform. The antimicrobial bioactivity of the isolates was tested by disc diffusion assays against 6 reference strains.
Results: Exploration of the secondary metabolism of the sponge-associated strains revealed that most biosynthetic gene clusters were non-homologous to previously reported ones highlighting the novelty of the studied strains. A wide variety of biosynthetic gene clusters were found to be related to the production of antibacterial, antifungal, antioxidative and cytotoxic compounds. However, screening tests against 6 indicator strains showed no antimicrobial activity supporting the fact that many biosynthetic pathways are silent under certain laboratory conditions.
Conclusions: Considering the hints derived from the flavobacterial genomes, application of different growth conditions or selection of other indicator strains might lead to activating their clusters and unlocking their antimicrobial potential.

P079

No quantification cycle (Cq) differentiates colonization from infection in the diagnosis of Pneumocystis jirovecii pneumonia using the real-time polymerase chain reaction (PCR) technique

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Introduction: The real-time polymerase chain reaction (PCR) technique has high sensitivities (97 – 99%) and specificities (94 – 90%) in the diagnosis of Pneumocystis jirovecii. Different quantification cycle (C_q)

cut-off values have been proposed to distinguish colonization from infection. However, these are laboratory and region specific due to the biological nature of the pathogen and the PCR target kits used in differing laboratories. This study aimed to determine a cut-off C_q value to distinguish colonization from infection of P. jiroveciiin the province of Friesland, by using the mean C_q value of immunocompetent individuals as a reference; it was hypothesized that these individuals would have a higher C_q value than the study group. Furthermore, C_q values in the study group were compared with the number of specific clinical symptoms to assess correlation. It was hypothesized that samples from patients with few clinical symptoms would have a higher C_q value than samples with many clinical symptoms (strongly indicative for Pneumocystis jiroveciipneumonia).
Methods: A retrospective study was conducted at medical microbiology laboratory Izore of Bronchial alveolar lavage (BAL) samples, bronchial washes, sputum and swabs from the naso- or oral pharynx sent for real-time PCR diagnosis of Pneumocystis jiroveciipneumonia (PJP) between September 2015 and February 2018. Rest samples of BAL or bronchial washes from immunocompetent patients, without clinical suspicion of PJP, were collected to form the reference group. These were patients with chronic illnesses or suspected for lung conditions for which further diagnosis using BAL or bronchial washes was required. 242 samples (150 study group and 92 reference group) were collected. Symptoms of fever, cough, dyspnoea and clinical findings of low oxygen saturation, arterial hypoxia, chest x-ray or computed tomography abnormalities were used as symptoms associated with the clinical diagnosis of PJP in the study group. To assess correlation between these symptoms and C_q value, samples in the study group were divided into two groups depending on accessibility of medical records. Group A consisted out of samples with 3 or less symptoms gathered from Izore's application form and group B consisted out of 4 - 7 symptoms gathered from medical records.
Results: In the study group, 54 samples (36%) were termed PJP positive with a mean C_q value of 27.87 (SD ± 3.40). In the reference group, 3 samples (3%) were PJP positive with a mean C_q value of 30.36 (SD ± 4.07). These were termed as colonization.
There was no significant difference between the mean C_q values of the immunocompetent reference group and the immune incompetent PJP positive group (P= 0.48). In the study group, there was no significant difference in C_q value between group A and B (P= 0,59).
Conclusion:
No C_q value was found to differentiate colonization from infection in the diagnosis of PJP using the real-time PCR and there was no correlation between number of symptoms and C_q
There is a low prevalence of colonization (3%) in the immunocompetent yet chronically sick population.

P080

Multicenter proficiency test of bacterial outbreak analyses performed in The Netherlands.

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Introduction

The increasing use of whole-genome sequencing (WGS) for outbreak investigations shows great promise for infection control and mitigation by enabling timely analyses not limited by borders or institutional catchment areas. To deliver on this opportunity, easy and reliable exchange of uniform data is necessary. However, applying WGS in a diagnostic setting requires specific expertise and dedicated hardware. Additionally, there is a vast amount of commercial and open-source bioinformatic tools available. We performed a multicenter study to compare the outcomes of WGS data analyses in a range of pipelines employing different tools and procedures, where participants followed their regular procedures for WGS in a bacterial outbreak investigation.
Methods
The benchmark data were supplied by 3 centers, that each selected ten paired-end Illumina sequencing samples from their existing data. The benchmark consisted of 10 methicillin-susceptible taphylococcus aureusstrains (supplied by Radboud University Medical Center), 10 Enterococcus faecium(University Medical Center Groningen), and 10 Klebsiella pneumoniae(University Medical Center Utrecht).

Participating centers were asked to analyze these data using their current outbreak analysis approach, which differed per center with in-house developed scripts or pipelines.
Results
In total 9 centers participated in the proficiency test: 9 analyzed the K. pneumoniaeset, 8 analyzed the . aureusset and 8 analyzed the E. faeciumset. Outcomes for the K. pneumoniaeand the E. faeciumsets were concurrent between all centers, identifying 3 outbreak clusters of each 2 samples and 1 outbreak cluster of 5 samples, respectively. For the . aureusset the results were not concurrent between the centers, mainly due to differences in read cleaning strategy and quality thresholds: 2 centers excluded samples for further analyses and 1 indicated which ones would have been excluded, whereas others analyzed all samples. A variety of cleaning and trimming tool were used among the participating centers. SPAdes is the most applied assembly tool (7/9 centers), whereas for outbreak analyses many different tools were used from which 6 out of 9 applied commercially available tools.
Conclusion
This study resembles the real-life situation where data preprocessing is required to deal with differences in data format and naming, prior to read cleaning and assembly. Outbreak conclusions are difficult to compare, emphasizing the need for aligned analysis strategies between centers. For the samples that were included, similar results were found across the different participating centers, regardless of the use of read cleaning/trimming, assembly, commercial or open-source applications. However, data quality assessment did affect the reported outcomes. Furthermore, centers with access to bioinformaticians preferably select open-source tools whereas centers with less bioinformatic expertise tend to choose commercial tools. Although the outcomes are concurrent there still is a gray area on what to define as outbreak. Besides all the concurrent outcomes there still is a gray area on what can be defined as outbreak and what not. We conclude that, to avoid missing or incorrectly reporting outbreaks, predefined quality parameters should be applied in order to standardize analyses, setting a bar for minimal requirements, and enabling reliable exchange and reporting of outbreaks.

P081

Development of antibodies that enhance immune clearance of Staphylococcus aureus biofilms

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Development of antibodies that enhance immune clearance of taphylococcus aureusbiofilms

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Implant associated taphylococcus aureusinfections are often difficult to treat due to the formation of biofilms on prosthetic material. Biofilms are bacterial communities adhered to a surface with a self-made extracellular polymeric substance that surrounds resident bacteria. In contrast to planktonic bacteria, bacteria in a biofilm are in an adherent, dormant state and are insensitive to most antibiotics. In addition, bacteria in a biofilm are protected from phagocytic cells of the immune system, such as neutrophils. Therefore, complete surgical removal and replacement of the prosthetic implant is often necessary to treat this type of infections. In this study, we aim to evaluate whether monoclonal antibodies recognizing biofilm structures can enhance recognition of . aureusbiofilms via the human immune system.
Methods
We produced recombinant monoclonal antibodies (mAbs) specific for wall teichoic acid (WTA, a glycopolymer of the . aureuscell wall) and polymeric-N-acetyl-glucosamine (PIA/PNAG, a major constituent of the Staphylococcusbiofilm extracellular polymeric substance). Using flow cytometry and ELISA-based methods we determined the binding of these antibodies to planktonic cells and in vitrobiofilms. Following incubation with IgG/IgM depleted human serum we determined whether antibodies recognizing biofilm can activate the human complement system. Confocal microscopy was used to visualize the location of antibody binding in the biofilm 3D structure.
Results
We show that human IgGs directed against WTA and PNAG bind to . aureusbiofilms in a dose-dependent manner. This interaction was specific since no binding was observed for control antibodies (recognizing the hapten DNP). Upon incubation with human serum, anti-WTA and anti-PNAG antibodies could react with the human complement system and deposit C3b molecules on the biofilm. Direct comparison showed that anti-WTA antibodies are more potent in inducing complement activation than

anti-PNAG antibodies.
Conclusion
Having established that the here used antibodies can bind biofilms and subsequently activate the complement system, we will now study how the deposition of complement affects recognition and clearance of biofilms by phagocytic immune cells.

P082

Exploring microbe-immune crosstalk: immunoglobulin coating of vaginal microbiota.

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Introduction: As knowledge on the composition of the vaginal microbiome is accumulating due to high-throughput sequencing, more interest is directed to microbe:immune crosstalk. Local immunoglobulins (Igs) play an important role as mediator of mucosal immunity and homeostasis and by interacting with the bacteria IgA shapes the composition of the microbiota. Alterations in IgA coating of commensal gut bacteria are associated with inflammatory bowel disease and Clostridium difficileinfection. In the vaginal mucosa, IgG is the dominating antibody, while at other mucosal surfaces IgA is most prevalent. Little is known about the role of local Igs in the female genital tract and the influence on the vaginal microbiome. In a small pilot and proof-of-principle study, we aim to characterize the IgA and IgG coating of vaginal bacteria using a combination of flow cytometry based techniques and microbiota profiling.
Methods: Vaginal swabs were collected from healthy, not pregnant volunteers. IgA and IgG coating of vaginal bacteria was visualized with fluorophore-conjugated secondary antibodies and measured with flow cytometry. The bacterial composition was analyzed with bacterial profiling technique IS-pro, using fragment variability of the 16S-23S rDNA intergenic spacer region.
Results: Vaginal swabs of seven healthy volunteers were testen. Lactobacillusspecies dominated the vaginal microbiota in six samples and these contained large numbers of IgA and IgA/IgG coated bacteria. One sample with increased diversity showed little IgA coated bacteria and more IgG coated bacteria. The proportions of Ig coated bacteria varied among individuals.
Conclusion: Vaginal bacteria show varying amounts of IgA, IgG and IgA/IgG coating between different healthy volunteers. These first results indicate there might be an association between Ig coating and microbiota composition. The high amount of Ig coated vaginal commensals demonstrate the importance of local Igs in the mucosal environment. Disturbances of the coating might lead to increased local inflammation and can possibly identify women at risk for diseases of the reproductive tract and adverse pregnancy outcomes. This promising combination of techniques enables researchers to look beyond microbiota composition and investigate the interaction with the host. These results indicate a putative role of Igs in the maintenance of a healthy microbiome.

P083

An investigation of the compatibility of a new molecular transport medium with a PCR analytical platform.

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Introduction: In recent years the introduction of self-contained cartridge systems for the rapid identification of target pathogens using nucleic acid amplification has transformed the ability of clinical microbiology laboratories to provide accurate and timely diagnostic data, allowing correct treatment to be commenced immediately, and also preventing unnecessary treatments. When culture is not required, it can be convenient and safer to transport the specimen in a medium which effectively kills the pathogens without disrupting the DNA or RNA so that analysis is still possible. If such a system is used, however, it is essential to know that the medium will be compatible with such an analysis.
This study we investigated the ability of Sigma MM to recover analytical quality DNA from specimens spiked with MRSA, while demonstrating that the bacteria had been killed. The PCR analysis was performed using Cepheid GeneXpert PCR analyser and Cephid Xpert MRSA. The results for both PCR and culture were compared with those for identical specimens inoculated into Sigma Transwab, a widely used transport swab device with liquid Amies.
Methods: 0.5 McFarland bacterial concentrations were prepared in sterile saline and diluted 10^{-1},10^{-2}, and 10^{-3}. Aliquots of 100µl for each dilution were inoculated directly into the tubes of 6 Sigma Transwabs and 6 Sigma MM, giving a total of 18 inoculated devices for each type of device. A further 2 devices of each type were inoculated with 100µl of sterile saline as negative controls. After a holding time of 24 hours all devices were tested by inoculating into the test kits for GeneXpert, and by plating onto MRSA

Chromogenic agar plates. Specimens for GeneXpert were processed according to the manufacturer's instructions. All agar plates were incubated at 37^{O}C for 18 hours.
Results: All inoculated devices were correctly identified by the GeneXpert PCR system as containing MRSA. There were no false positives with any of the negative controls. On the agar plates MRSA was recovered as colonies for all the Sigma Transwabs, but there was no growth from any of the Sigma MM devices.
Conclusions: This study has shown that the Sigma MM devices were compatible with the GeneXpert PCR analyser for MRSA. There was no interference with the chemistry. The Sigma MM devices were also completely effective at killing the MRSA at all concentrations, rendering the specimens non-infective.

P084

Anaerobic degradation of sulfated polysaccharides by two novel Kiritimatiellales strains isolated from Black Sea sediment

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The marine environment contains a large diversity of sulfated polysaccharides and other glycopolymers. Saccharolytic microorganisms degrade these compounds through hydrolysis, including the hydrolysis of sulfate groups from sugars by sulfatases. However, thus far no sulfatase-rich marine anaerobes are known. In this study, we aimed to isolate marine anaerobes using sulfated polysaccharides as substrate. Anoxic enrichment cultures were set up with a mineral brackish medium, which was inoculated with anoxic Black Sea sediment from 2100 m depth and incubated at 15°C (in situT = 8°C) for several weeks. Community analysis by 16S rRNA gene amplicon sequencing revealed the enrichment of Kiritimatiellaeotaclade R76-B128 bacteria with the sulfated polysaccharides fucoidan and iota-carrageenan as substrate. We isolated two strains, F1 and F21, which would represent a novel family within the order of the Kiritimatiellales. They were capable of growth on various mono-, di- and polysaccharides, including fucoidan. The desulfation of iota-carrageenan by strain F21 was confirmed quantitatively by an increase in free sulfate concentration. Strains F1 and F21 represent the first marine sulfatase-rich anaerobes, encoding more sulfatases (521 and 480, 8.0% and 8.4% of all coding sequences, respectively) than any other microorganism currently known. Specific encoded sulfatase subfamilies could be involved in desulfating fucoidan (S1_15, S1_17 and S1_25) and iota-carrageenan (S1_19). Both strains encoded a single anaerobic sulfatase-maturating enzyme which could be responsible for post-translational modification of formylglycine-dependent sulfatases. Strains F1 and F21 are potential anaerobic platforms for future studies on sulfatases and their maturation enzymes.

P085

Bacterial survival in clinical serum samples from patients with bacteraemia.

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Introduction: Although sepsis is often associated with bacteraemia, little is known about how bacteria behave in clinical blood samples taken for routine diagnostics. This study assesses bacterial survival, measured by bacterial growth, in routine clinical serum or plasma samples from adult patients with sepsis.
Methods: This experimental laboratory study included clinical serum or plasma samples from 12 adult patients with blood culture proven E. coli, . aureusor . pneumoniaesepsis, admitted to the university hospital Radboudumc during a period of two months in 2018. Samples collected up to 3 days prior to blood culture collection were eligible for inclusion, resulting in multiple samples per patient. The samples had been stored at 4^{o}C for a maximum of 7 days. From each sample, two aliquots were taken for inoculation in brain-heart infusion bouillon (BHI) and sheep blood agar plates (BP), and a third baseline aliquot was immediately stored at -20^{o}C. Survival was assessed daily for 5 days from inoculation, through both inspection of visual growth and quantitative polymerase chain reaction (qPCR) i.e. increase in bacterial load. Primary outcome was bacterial load measured in copies per 200ul serum or plasma sample.
Results: Serum or plasma samples were collected from a total of 12 bacteraemic patients, including 8 E. coli, 1 . aureusand 3 . pneumoniaepositive patients. In total, samples from 4 patients (33.3%) showed

visual growth, corresponding with the blood culture isolate. From these 4 samples, 2 . pneumoniaesamples also displayed increase in bacterial load by qPCR. The remaining 2 samples concerned growth by culture of E. coliwhich could not be confirmed molecularly, due to a high background signal resulting from recombinant E. coliDNA in the qPCR master mix. All 3 samples inoculated within 24h of collection showed visual growth, while the remainder had had a delay of at least 48 hours before inoculation and showed 11.1%% positivity (1 out of 9).The 7 samples that did not show visual growth also did not display molecular growth.
Conclusion: In bacteraemic patients, detection and cultivation of bacteria from serum or plasma samples taken for routine diagnostic purposes is possible. Time delay between serum or plasma collection and inoculation, resulted in a decline in bacterial viability.

P086

Validation of Chlamydia trachomatis specific gene transcripts as a more accurate marker for bacterial viability.

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Introduction. The nucleic acid amplification test (NAAT) is the current gold-standard for routine Chlamydia trachomatis(CT) diagnostics, as it is highly specific and sensitive for the detection of CT nucleic acids (DNA and/or RNA). However, many NAATs detect genetic material without discriminating between viable (i.e., able to cause infection and disease) and non-viable (remnant DNA) CT. Previous studies demonstrated that CT NAAT positive test results can be detected up to 8 weeks post-treatment. Yet, the clinical relevance of post-treatment CT NAAT positivity is unclear. In such cases, additional information regarding CT viability may be beneficial.
In contrast to the relatively stable DNA molecules, messenger RNA (mRNA) molecules are short-lived and, therefore, could be a valuable marker for CT viability. However, a previous study demonstrated large variability in the decay rate of CT gene transcripts, with a maximum half-life time of ~5000 min. Therefore, it is essential to evaluate the decay rate of the different mRNA targets when choosing a suitable marker for CT viability.
We aimed to validate potential CT mRNA targets as a more accurate marker for CT viability. Therefore, we evaluated the decay rate of CT specific gene transcripts which are expressed during the different life stages of CT propagation.

Methods. Confluent HeLa cell monolayers were inoculated with the laboratory strain CT svD (DSMZ-19411). Infected cells were incubated for 24h at 37°C with 5% CO_2, until the mid-phase of the CT life-cycle. Infected cells were treated with 10ug/ml rifampicin to stop de novogene transcription, and total RNA was isolated at 5 different time points: at 0 min (T_0, no treatment), 10 min (T_1), 30 min (T_2), 60 min (T_3), 180 min (T_4), and 24 hours (T_5) after treatment. Total RNA was isolated using the FavorPrep™ Tissue Total RNA Mini Kit (Favorgen). Following DNAse treatment, RNA was converted to cDNA using the iScript™ cDNA Synthesis Kit (Biorad) and used as a template to amplify fragments of the CT 16S(early), murG(early), omp2(mid), and hctA(late) genes. Primers targeting the Human GAPDH gene were used as an internal control of the cDNA synthesis and reproducibility of mRNA preparation. Experiments were performed in duplo.

Results. Investigation of different transcripts from the 16S, murG, and omp2genes showed almost no decay over the assessed time period (increase in cycle of quantification [Cq] value <1), while transcripts from the hctAgene showed an increase of Cq 3.0 (min-max; 2.9-3.0) within the first 30 minutes. Furthermore, transcripts from the hctA gene were undetectable within 24 hours after rifampicin treatment.
Conclusion:
1. The detection of CT hctAgene transcript shows the most promising results as a potential marker for CT viability.
2. Future research should evaluate the time to clearance of mRNA molecules in patients after being treated.

P087

Limited increase in the number and proportion of capsular switches in the Dutch invasive pneumococcal population 2004-2016.

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Introduction:
Streptococcus pneumoniae is a human pathogen and a primary cause of invasive bacterial disease worldwide. To reduce the incidence of invasive pneumococcal disease (IPD) in The Netherlands, a 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2006 followed by a switch to 10-valent vaccine (PCV10) in 2011.Changes in serotype distributions over the years after vaccine introduction are well documented, but it is unclear what effect vaccine-induced pressure has on capsular switching. The aim of this study was to assess the distribution of capsular switching events in the invasive pneumococcal population by comparing the serotypes and genetic background of isolates from pre-PCV, post-PCV7 and post-PCV10 periods.
Methods:
A total of 4,773 isolates where obtained from blood or CSF by The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM, Amsterdam) over the pre-PCV (n= 1,154, years 2004-2005), post-PCV7 (n= 1,775, years 2008-2011) and post-PCV10 (n= 1,844, years 2013-2016) periods. All isolates were serotyped by Quellung reaction and the genetic background was established via Multiple Locus Variable number of tandem repeat Analysis (MLVA). A selection of 45 isolates from the top-3 MLVA-complexes prone to capsular switches were subjected to whole genome sequencing using the Illumina HiSeq of which 26 isolates were suspected new variants (capsular switches) and remaining 19 isolates were selected as ancestral strains. Comparative analysis was done using core-genome MLST based on 1,210 genes.
Results:
In total, MLVA showed 57 MLVA-complexes (MCs) containing 3,839 isolates, while the remaining 934 isolates were singletons. Fifteen of the 57 MCs harbored isolates (n=45, 1,2%) that had a different serotype than the MLVA Complex bound serotype, indicating possible capsular switches. Of the 45 isolates, 8 originated from the pre-PCV period, while respectively 13 and 24 isolates came from the post-PCV7 and post-PCV10 periods, but the increase of capsular switches over time was non-significant (Fisher exact P 0,1438). The three MCs containing the most isolates suspected of capsular switching were MC09V (n=13), MC12F (n=5) and MC14 (n=8) and together these three MCs comprised 56% of the total number of possible switches. The thirteen MC09V isolates switched to 4 different serotypes (ST) namely ST14 (n=4), ST19F (n=3), ST15A (n=3) and ST24F (n=3). For MC12F the isolates switched to two serotypes, specifically ST07F (n=3) and ST09N (n=2) and all MC14 isolates switched to ST19F (n=8). CgMLST based on 1,210 genes of the 45 isolates showed that all isolates grouped in the minimum spanning tree in the same clusters as with MLVA, thereby confirming the capsular switches.
Conclusion:
In this study, we showed that capsular switches occur within the Dutch invasive pneumococcal population based on MLVA and cgMLST. However, the number and proportion of capsular switches remains very low and only slightly increased over time. Although capsular switches occurred in more than 25% of the MLVA complexes, some MCs seem to be more to prone to capsular switching than others. Furthermore, the isolates in some MCs switched to multiple other serotypes e.g. MC09V whereas others switched to only a single ST such as MC14.

P088
Contact Tracking in Infection Control: There Must Be a Better Way
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Introduction: In order to control outbreaks and prevent spreading of multi-drug resistant micro-organisms (MDRO), it is essential to perform contact tracking. Yet, in many cases this is one of the most time consuming work processes in the daily practice of Infection Control Practitioners (ICPs). An ICT solution could support this process and add efficiency. In this study, we assessed the effects of automatization in contact tracking and outbreak management.
Methods: First, the different steps in the work process of contact tracking were analysed to decide which ones could be automated. Second, various ways of presenting data were evaluated to identify an optimal way to support contact tracking around an index patient or involving large cohorts of patients. An external software provider was engaged to build a new soft-ware application that brought data together from different sources, such as the hospital electronic patient records system (EPR) and the laboratory information system (LIMS). Through integration with the source systems, (near) real-time data could be accessed.

Results: Contact tracking involving two patients who were unexpectedly found to be colonized with vancomycin-resistant enterococci (VRE), was used to test the application. Contact patients were automatically identified based on information from the EPR. Culture results of the contact patients were received real-time from the LIMS and automatically processed and displayed. A message was automatically generated and sent to the ICP when new data, such as a new VRE-positive patient, became available. Up-to-date overviews, such as the amount of positive contact patients and how they relate to the index patient, could be generated at any time. Furthermore, it was possible to automatically add a label in the EPR for all contact patients. The module also allowed for automatically sending a letter to groups of patients, but this function was not tested in this pilot.
Conclusions: With the use of the newly developed software module, majority of the administrative tasks involved in contact tracking could be automated. Contact tracking therefore became more efficient (~80% time reduction) and more reliable (automated inclusion of contact patients and processing of culture results). Moreover, automatization supported other aspects of the ICP work process, such as data transmission within the team and securing that the necessary tasks were executed. This example shows that automatization in infection control is not only desirable but also feasible, and we are currently using this module in our daily routine. Additional modules are being developed, including automated signalling of potential outbreaks.

P089

Inquiry of Antibiotic Resistance Genes in Global Bacterial Genome databases.

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Antibiotic resistance (ABR) is a global health threat, increasingly rendering antibiotic therapies ineffective. Resistance to antibiotics can occur either by mutations in existing genes or by acquisition of novel genes by means of horizontal gene transfer. The latter mechanism facilitates the exchange of resistance genes between human commensals or environmental bacteria and clinical pathogens.
To increase our understanding of the distribution of ABR genes, we categorized resistance genes in all publicly available genome sequences from bacterial isolates retrieved from distinct reservoirs (such as human, cattle, water). We used the Comprehensive Antibiotic Resistance Database to annotate resistance genes in the genome repository PATRIC (>115,000 genomes) using strict settings, after which we cluster all sequences with CD-HIT (sequence identity threshold set to 100% and query coverage to 80% of the sequence length). Genic metadata was used to assign resistance genes to reservoirs such as human, wastewater, soil, water, cattle, chicken, pig, food, pets and other. The resulting database enables coupling of ABR genes to bacterial species, environmental reservoir, and, when available, phenotypic features such as antibiotic resistance.
We focussed on carbapenemases (CPM) as this class of ABR is of particular concern in the clinic. Carbapenemases were identified in the list of ABR genes from PATRIC genomes based on homology (100% similarity over 100% of their length) with all known CPM genes. For this purpose we used two CPM lists: the beta-lactamase database (BLDB) and the TypeNed database. The BLDB is an international database containing 566 CPM protein sequences and TypeNed is a national database containing 274 different CPM genes identified in the genomes of clinical isolates obtained through surveillance for CPM producing Enterobacteriaceae (CPE) from hospitals in the Netherlands.
CPM genes were identified in ~8000 genomes (roughly 7% of the total) and from the 566 types in the BLDB database, 203 were detected in the PATRIC genome database, with these genes present in 95 bacterial species. As expected, CPMs were mostly detected in A. baumaniiand K. pneumoniaederived from human clinical sources, but they could also be detected in several other bacteria, mostly E. colifrom non-clinical environments such as poultry, pets and wastewater. Some CPM containing isolates were Vibriospecies, which are not present in the TypeNed database. In the Netherlands, the CPM gene blaVIM-2 has been detected only in P. aeruginosa, but the genome database inquiry also detected a few other bacterial species carrying this gene isolated from poultry, pets and wastewater. Non-clinical isolates carrying bla-OXA-48 were detected in water and wastewater.
In conclusion, database queries using CPM genes from human clinical isolates allow us to track potential reservoirs of resistant pathogens, but also to identify non-pathogenic species carrying these CPM genes.

P090

Mycoplasma genitalium in clients visiting a sexually transmitted infections (STI) clinic: prevalence and resistance to azitromycin.

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Background
An association between Mycoplasma genitalium(MG) and male urethritis and possibly pelvic inflammatory disease has been reported. Treatment is hampered by increasing resistance to azitromycin. We assessed the prevalence of MG in clients visting the Hague and Amsterdam STI clinics and of resistance to azitromycin.
Materials/methods
Samples from 1494 clients in Amsterdam and 1536 clients in The Hague, all visiting the STI clinic, were tested for urogenital MG. In addition, anal samples were tested from MSM, from women from The Hague and from high-risk (anal sex, symptoms, notified and/or sexworker) women from Amsterdam. Presence of MG, Chlamydia trachomatis(CT) and Neisseria gonorrhoeae(NG) was assessed using TMA assays (Aptima, Hologic). Statistical analysis was done with Fischer exact tests. Genotypic azitromycin resistance testing was perfomed by a newly developed qPCR using locked nucleic acid probes and confirmed by partly sequencing the 23SrRNA gene.
Results
We successfully tested 939 men who have sex with women (MSW), 1025 men who have sex with men (MSM) and 1277 women. MG was detected in 8.4% of urines of MSW and in 6.4% of urines of MSM. In women, MG was found in 11.0% of vaginal samples. Of the anal samples of MSM and women 15.4% and 10.0% were positive. In total, 20.1% of MSM and 13.2% of women were thus positive for MG on any anatomical location. Among MSM positive for MG, only 10.3% were positive for both urine and anal samples and 67.5% in the anal specimen only. Among MG-positive women of whom both vaginal and anal samples had been tested, 61.9% were positive in both samples and just 16.1% in the anal sample only. In Amsterdam, the MG prevalence in MSW, MSM and women was 9.9%, 24.4% and 14.8%, respectively, and exceeded the MG prevalence in The Hague (6.7%, 15.9% and 10.9%). Prevalence of CT in MSW, MSM and women (vaginal samples only) was 12.3%, 7.2% and 10.5%, respectively, and of NG this was 2.3%, 4.9% and 1.0%. Correlations between MG and NG in MSM and MSW were significant ($p<0.001$), but correlations between MG and CT were not. Testing for azitromycin resistance was succesfully performed in 50 (34%) urines, 81 (32%) anal samples and 75 (53%) vaginal samples. Resistance could be shown in 34 (68%) urines, 48 (59%) anal samples and 37 (49%) vaginal samples.
Conclusions
MG is highly prevalent in clients visiting an STI clinic. MG is also frequently present in anal specimens from MSM and women, and especially in MSM this is frequently the only location where MG is detected. In women presence of MG in anal samples is common, but infrequent in the absence of vaginal MG. MG prevalence among STI clinic clients in Amsterdam is higher than in The Hague. NG is more frequently found in MG-positive specimens. Resistance to azitromycin is very high, hampering the use of macrolides. Information on the clinical relevance of MG and azitromycin resistance is urgently needed.

P091

Increased Dissemination and Parallel Evolution of Antimicrobial Resistance in Salmonella enterica serovar Paratyphi B variant Java from Poultry in Latin America and Europe

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Introduction
Isolates of almonella entericaserovar Paratyphi B variant Java (here referred as Java) from poultry are known to carry Antimicrobial Resistance (AMR) genes and belong to Multi Locus Sequence Type (ST) 28. The objective of this study was to investigate the evolutionary relatedness of Java-ST28 from multiple Latin American (LA) and European (EU) countries.
Methods
Ninety-three Java-ST28 strains were selected from previous studies from Colombia (n=52), Costa Rica (n=15), Guatemala (n=5) and the Netherlands (n=21) and subjected to whole genome sequencing. Additional sixty-nine genomes were collected from Enterobase. Characterization of AMR was made with the ResFinder database. Time resolved phylogeny and effective population size (N_e) were inferred using Bayesian Evolutionary Analysis Sampling Trees (BEAST) and Bayesian skyline plot.

Results
A clear phylogenetic distinction was observed between EU and LA Java strains. EU strains exhibited gyrase mutations conferring resistance to fluoroquinolones. In turn, LA strains carried the qnrB19gene conferring reduced-susceptibility to quinolones. Resistance to β-lactams was mainly mediated by bla_{TEM-1B} in EU and by bla_{CMY-2} in LA. Molecular clock was estimated at 1.7 single nucleotide polymorphisms/genome/year [Confidence Interval (CI):1.44-2.0]. Evolutionary separation was observed between strains from EU and LA and dated to 1987 (CI: 1978-1988) with BEAST. $N_e$in EU increased sharply in 1995 (CI: 1992-1998) and in LA in 2005 (CI: 2001-2007).
Conclusions
I) Java-ST28 from LA and EU form two distinct clades. II) The estimated years of $N_e$increase in EU are in accordance with literature reports. III) The EU and LA clades have acquired resistance to fluoroquinolones and β-lactams independently, indicating parallel evolution of AMR in both regions.

P092

Validation of Clostridium difficile ribotyping directly on faecal samples

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Introduction: Clostridium difficileis the most common cause of nosocomial diarrhoea. Hospital outbreaks occur regularly due to the high number of bacterial spores excreted by symptomatic patients. To detect potential transmission between patients, bacterial strain typing is performed. The most widely used typing technique for C. difficileis PCR ribotyping. With this method, strains are distinguished based on number and lengths of the ribosomal 16S-23S intergenic spacer (IS) regions. Ribotyping is usually performed on cultured strains of C. difficile. The objective was to develop a ribotyping method that could be applied directly on faecal DNA and would eliminate the need for culture. Optimally, the technique should be as sensitive as qPCR, as this would make it possible to use the technique as a first-line diagnostic assay. Our secondary objective was to combine direct ribotyping with C. difficiletoxin genes detection. Furthermore, we aimed to validate our protocol on a large reference set of C. difficilepositive stools.
Methods: We designed a primer set with high specificity and sensitivity to perform ribotyping directly on total faecal DNA. Primers for detection of toxin A, B and binary toxin genes were adapted from previously published work by the study group of Persson et al. PCR protocols were optimized. DNA isolation was performed with MagNaPure96. PCR ribotyping and toxin detection was applied on 65 faecal samples with positive qPCRs for C. difficiletoxins and on their 65 corresponding cultured strains. Cp-values ranged between 27.2 and 38.9. In addition we tested 2 faecal samples that were positive for both C. difficileand Campylobacter. Fourteen control samples were used; these were negative for C. difficilebut positive for other enteropathogens, such as Campylobacter, almonellaor norovirus. PCR products were analysed by capillary gel electrophoresis using an ABI 3500 machine. We used TIBCO Spotfire to visualise DNA fragment peak profiles. Clustering was performed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA), with cosine correlation as distance measure and average value as ordering weight.
Results: Ribotype peak profiles were observed in all 67 C. difficilepositive faecal samples and strains, indicating high sensitivity (n=67/67). No peaks were observed in C. difficilenegative samples, resulting in a specificity of 100% (n=14/14). Profiles or faecal samples and strains were highly similar. By cluster analysis based on ribotype peak profiles, samples with identical ribotypes could be easily distinguished from others. Furthermore, toxin genes were detected in all C. difficilepositive and none of the C. difficile negativesamples. Presence or absence of toxin A, B and/or binary toxin in the different ribotypes corresponded to literature data.
Conclusion: C. difficileribotyping directly on faecal DNA is feasible, with sensitivity comparable to qPCR and could allow rapid detection of bacterial transmission between patients. This may result in more timely application of infection control measures and could potentially limit C. difficileoutbreaks.

P093

Impact of antibiotic-use during the first week of life on the intestinal microbiota development in infants

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Introduction
Neonatal microbial colonization drives postnatal gut maturation and supports the development of the immune system, thus possibly affecting (long-term) health. The babies' first encounter with a dense bacterial population happens during birth and the microbial exposure of the newborn is determined by the delivery mode. The exact impact of early life factors on later gut microbiota colonization and health remains unclear especially when disruptive factors like antibiotic use also come into play. The aim of this study is to investigate the impact of antibiotic use during the first week of life on the developing intestinal microbiota in term infants.
Methods:
We investigated the microbiome composition of stool samples that were collected within the observational cohort of the INCA study. In total 436 term infants were recruited, of whom 151 received antibiotic treatment during their first week of life for suspected neonatal infection (AB+). Clinical outcomes of the infants were registered continuously during the first year of life and feces was sampled at nine time points from birth until two years of age. An additional maternal fecal sample was collected after birth. A subset of 1677 fecal samples (496 AB+) were analyzed using 16S Illumina sequencing and Bifidobacterium-specific ITS sequencing.
Results:
The development of the infant intestinal microbiota follows a temporal pattern that is mainly driven by consecutive dominance of Proteobacteria during the first week of life, followed by Actinobacteria up to 6months and finally Firmicutes at 2 years of age. During this development, the infant's microbial community characteristics, such as alpha diversity, move towards that of the adult maternal fecal samples. Antibiotic administration during the first week of life affects the temporal pattern of the intestinal microbiota differently depending on the delivery and feeding mode. Antibiotic administration during the first week of life seems to delay the normal intestinal microbiota development, especially delaying the peak of Actinobacteria in vaginally born babies that are being breastfed.

Conclusion:
Clinical interventions around birth like antibiotic treatment can affect the microbiota development in early life depending on the delivery and feeding mode of the infant. The possible health implications of these microbiota changes need to be further investigated and considered for cost-benefit analyses of medical interventions.

P094

Prediction of plasmid and chromosomal contigs from short read assembly data using machine learning

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Introduction:Antimicrobial resistant (AMR) genes in bacteria are often carried on plasmids. Since these plasmids can spread the AMR genes between bacteria, it is important to know if the genes are located on highly transferable plasmids or in the more stable chromosomes. Whole genome sequence (WGS) analysis makes it easy to determine if a strain contains a resistance gene, however, it is not easy to determine if the gene is located on the chromosome or on a plasmid as genome sequence assembly generally results in 50-300 DNA fragments (contigs). With our newly developed prediction tool, we analyze the composition of these contigs to predict their likely source, plasmid or chromosomal. This information can be used to determine if a resistant gene is chromosomally located or on a plasmid. The tool is optimized for 19 different bacterial species, including Campylobacter, E. coli,and almonella, and can also be used for metagenomic assemblies.
Methods:The tool identifies the number of chromosomal marker genes, plasmid replication genes and plasmid typing genes using CheckM and DIAMOND Blast, and determines pentamer frequencies and contig sizes per contig. A prediction model was trained using Random Forest on an extensive set of plasmids and chromosomes from 19 different bacterial species and validated on separate test sets of known chromosomal and plasmid contigs of the different bacteria.
Results:Prediction of plasmid contigs was nearly perfect when calculated based on number of correctly predicted bases, with up to 99% specificity and 99% sensitivity. Prediction of small contigs remains difficult, since these contigs consists primarily of repeated sequences present in both plasmid and chromosome, e.g. transposases.
Conclusion:The newly developed tool is able to determine if contigs are chromosomal or plasmid with a very high specificity and sensitivity (up to 99%) and can be very useful to analyze WGS data of bacterial genomes and their antimicrobial resistance genes.

P095

Comparison of two Lateral Flow Assays for the diagnosis of Invasive Pulmonary Aspergillosis

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Introduction
Invasive pulmonary aspergillosis (IPA) is a severe opportunistic infection in immunocompromised patients, and is especially associated with patients receiving allogeneic stem cell transplantation. Early diagnosis is essential, as timely treatment can prevent disease progression. Two rapid lateral flow assays (LFAs) have recently become available, made by OLM Diagnostics and IMMY.
Methods
We performed a retrospective analayis of bronchoalveolar lavage fluid (BALf), obtained from adult patients with hematological malignancies that were suspected for IPA. All patients had host criteria for IPA and radiological abnormalities on computed tomography (CT). A final diagnosis of EORTC/MSG "probable" IPA was obtained by case review, taking into account galactomannan values, disease progression and more likely alternative diagnosis.
Results
66 patients were included, of whom 35 tested positive for galactomannan in BALf and were consequently classified as having 'probable' IPA. For the IMMY LFA, a successful test result was obtained in all samples. Sensitivity was 86% and Specificity 81%, with positive and negative predictive values both 83.3%. For the OLM LFA, two samples gave persistent technical failures due to obstruction of the device. Sensitivity of the assay was 82%, specificity 94%, and positive and negative predictive values were 93% and 83% respectively.
Conclusion
Both types of LFAs provided good diagnostic accuracy, with minor differences in apparent calibration in terms of sensitivity and specificity.