ABSTRACTS POSTER PRESENTATIONS

P001

Reproducibility between two readout methods of a commercial broth microdilution assay for *Pseudomonas aeruginosa* isolates from patients with Cystic Fibrosis

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Introduction: Broth microdilution (BMD) and, possibly, some automated susceptibility tests are reliable methods for susceptibility testing of colistin in Gram-negative clinical isolates, whereas disk diffusion and gradient strip tests are unreliable. *Pseudomonas aeruginosa* isolates from Cystic Fibrosis (CF) patients may be slowly growing and frequently render automated susceptibility testing unsuitable, leaving BMD as the sole option for antimicrobial susceptibility testing (AST) of colistin. In this study, reproducibility between the VIZION™ readout system of a commercial BMD method was compared to visual readout for susceptibility testing of colistin and four beta-lactam antibiotics in *P. aeruginosa* isolates from patients with CF.
Methods: Fifty-six unique *P. aeruginosa* isolates from respiratory secretions from CF patients were characterized phenotypically. AST was performed using commercially available microdilution plates with freeze-dried antibiotics (EURGNCOL, Sensititre™).
Results: *P. aeruginosa* isolates were phenotypically diverse and displayed slower growth rates compared to quality control isolates. When comparing VIZION™ with visual readout, colistin exact MIC agreement was 82% and 95%, essential MIC agreement was 98% and 100%, categorical agreement was 98% and 98% and reliability (weighted kappa) was 0.95 (95% CI 0.91-0.99) and 0.99 (95% CI 0.97-1.00) after 24 and 48 h incubation, respectively. For all five antibiotics, the total number of errors (using VIZION™, in comparison with visual readout) decreased from 15 (5%) to 10 (4%) after 24 and 48 h incubation, respectively.
Conclusion: Sensititre commercial BMD seems suitable for AST in *P. aeruginosa* isolates from CF patients. Reproducibility between the VIZION and visual readout increases after prolonged incubation of 48 hours.

P002

Outpatient parenteral antibiotic therapy (OPAT) for *Staphylococcus aureus* bacteraemia: experience from a UK teaching hospital

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What is known and objectives: We describe failure rates of 79 patients with *Staphylococcus aureus* bacteraemia (SAB). We describe both initial outcomes up to four weeks following completion of OPAT and undertake a survival analysis on our cohort (median follow up period of 73 weeks).
Patients and methods: A Prospectively maintained registry of all patients attending OPAT was examined for cases of SAB. Patient case records were reviewed, data extracted and entered into a SPSS database. Only the first episode of OPAT treated SAB was included for each patient. Diagnosis, demographics, comorbidities, microbiology and treatment were recorded, as well as adverse reactions to antimicrobials, and case records were examined for evidence of failing initial prescribed OPAT therapy and up to 24 months of follow up.
Results: Seventy nine cases of SAB were identified. The overall success rate following initial OPAT was 91.4%. There was a range from 86.7% for patients with bone and joint infections (BJI) to 100% for patients with an endocarditis or infected venflon related SAB. The failure rate after 4 weeks following completion of OPAT was 22.2%. There were no factors significantly associated with poor initial outcome. Nonetheless, longer duration of inpatient stay prior to commencing OPAT did appear to convey some increased risk of early failure at a level approaching significance (OR: 1.018; 95%CI 1- 1.037; p= 0.056). Factors that were somewhat approaching significance included being aged over 60 years old, a pre-existing diagnosis of diabetes and bone and joint infection (BJI) as the source of the bacteraemia, but were not significant in a multivariate model. On survival analysis, there was a statistically significant difference in survival distributions according to choice of antimicrobial ($X2(2)= 7.723$, p= 0.021) and whether the patient had a pre-existing cancer diagnosis ($X2(1)= 4.417$, p= 0.036). Cox regression analysis suggests that patients receiving teicoplanin experience worse outcomes than those receiving ceftriaxone (B= 1.490; Exp(B)= 4.435; 95%CI 1.410- 13.947). Patients with a cancer diagnosis also appeared to be at greater risk of treatment failure (B= 1.052; Exp(B)= 2.864; 95%CI 1.020- 8.038). Nevertheless due to the small number of failure cases further studies with larger cohort are required in order to assess whether these are indeed risk factors for treatment failure.
Conclusions: SAB can be successfully managed through OPAT. Despite a broad definition of treatment failure, failure rates both within 4 weeks of completion of OPAT and during the longer follow up period were low. There were indications that a longer inpatient stay prior to OPAT might be a risk factor for early OPAT failure. It is possible that teicoplanin may be a less optimal choice of antibiotic than ceftriaxone, although it is difficult to draw firm conclusions on the basis of a small sample. The same conclusion must be applied to cases with a pre-existing diagnosis of cancer. Diabetes, old age and BJI as a source of bacteraemia were not associated with poorer outcomes during the follow-up period, although

greater vigilance for such patients may be indicated during the initial OPAT treatment phase.

P003

Evaluation of the BD MAX Check-Points CPO assay for rapid detection of carbapenemase genes in Gram-Negative bacilli in spiked rectal swabs

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Introduction: Rapid identification of patients carrying carbapenemase-producing organisms (CPO) is important to prevent spread of these organisms. The BD MAX Check-Points CPO assay (Check-Points, Wageningen, The Netherlands) is a new automated real-time PCR which is developed to detect the most prevalent carbapenemase genes (KPC, OXA-48, VIM, IMP and NDM) directly from rectal swabs. Due to the low-prevalence of CPO in The Netherlands we evaluated the assay with contrived rectal specimens.
Methods: The contrived specimens were prepared by spiking 83 well-characterized isolates (72 Enterobacteriaceae, 9 *Pseudomonas aeruginosa* and 2 *Acinetobacter* species) carrying the assays target genes (15 IMP, 19 VIM, 15 NDM, 18 KPC, 14 OXA-48, 1 OXA-48/NDM and 1 OXA-48/VIM) and 17 isolates (14 Enterobacteriaceae, 1 *Pseudomonas aeruginosa* and 2 *Acinetobacter* species) without these genes into negative rectal swabs. The negative rectal swabs were prepared by dipping eSwabs (Copan) into a fecal sample and confirmed negative by BD MAX Check-Points CPO (BDCPO) and culture. The spiked samples were prepared at concentrations of 1 to 2 X the Limit of Detection (LoD), LoD ranging from 4.8 X 10^3 CFU/mL to 64 X 10^3 CFU/ml depending on the carbapenemase gene, and at a concentration of 0,5 X 10^7 CFU/mL for negative isolates. Each sample was tested with BDCPO and cultured overnight on ChromID Carba and ChromID OXA48 (bioMerieux). Cultured colonies were tested by disk diffusion using the CLSI M100-S27 criteria for ertapenem, imipenem and meropenem. Before testing the samples were de-identified, furthermore the BDCPO and culture were performed by two different technicians on different locations.
Results: All 83 CPO isolates were cultured and found Intermediate or Resistant for at least one of the tested carbapenems. Of the 83 isolates 17 (20.5%) were cultured on ChromID OXA48, 74 (89.2%) on ChromID Carba, 8 (9.6%) isolates were cultured on both media. BDCPO correctly identified all CPO positive rectal swabs and also all carbapenemase genes were correctly identified. Both the sensitivity and specificity of BDCPO was 100%.
Conclusions: The BD MAX Check-Points CPO is an accurate and rapid test to identify rectal colonization with CPO.

P004

Prevention of neonatal deaths - Evaluation of the new Sigma GBS® transport system for *Streptococcus agalactiae* screening

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Introduction: *Streptococcus agalactiae* is the main cause of neonatal meningitis and can cause septicaemia and pneumonia. It colonises the vagina in about 20- 30% of pregnant women and can be transmitted from mother to baby before or during labour. Nearly 10% of the babies affected by infection do not survive and those who recover may be left with permanent conditions. In order to prevent neonatal infection antibiotics can be given to women during labour or to the babies after birth. These women can be identified by microbiological screening, involving taking a swab from the vagina or rectum. Unfortunately, many countries still have not recognised the scale of the problem and do not have screening policies in place. The use of a selective broth proved to increase the method selectivity by 60-90%. The purpose of this study was to evaluate the new Sigma GBS® selective medium with clinical isolates of *Streptococcus agalactiae* collected from pregnant women. The medium aims to maintain or increase the number of *S. agalactiae,* while inhibiting the numbers of other bacteria under the same conditions.
Methods: Sigma GBS was tested with 20 clinical isolates of GBS in order to assess the enrichment abilities of the medium. The medium was also tested for its ability to inhibit *E. coli.* Low concentrations of *Streptococcus agalactiae* and high concentration of *E. coli* were prepared and media were inoculated in triplicate. Samples were tested at time zero and after 24h incubation at 37ºC and RT. Colonies were counted after incubation and compared against time zero samples. **Results:** The number of viable cells of *Streptococcus agalactiae* isolates increased after 24h of incubation from very low 10^2 cfu/ml to too numerous to count after incubation at 37ºC. The medium was able to maintain the viability of *S. agalactiae* at RT over 24h of incubation and inhibit *E. coli* at both temperatures even with high concentrations of bacteria.
Conclusions: Many countries introduced screening programmes and as a result of this the number of GBS infections in newborn babies has fallen significantly (around 80% on average). However, in some European countries, routine screening for GBS is not offered and the incidence is increasing. When direct agar plating is used instead of selective enrichment broth, as many as 50% of women who are GBS positive have false negative culture results. Sigma GBS® is a transport device able to maintain and increase (at RT and 37ºC) the number of *S. agalactiae* and inhibit other bacteria, which increases the sensitivity of the GBS screening therefore improves outcomes for mother and baby.

P005

Validation of an innovative real-time PCR assay for the simultaneous detection of *Mycoplasma genitalium* and macrolide resistance-associated mutations with ELITe InGenius®

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Background: Mycoplasma *genitalium* causes sexually transmissible infections and infected patients are commonly treated with the macrolide azithromycin. As macrolide resistance is becoming quite common, and could concern up to 40% infected patients, the empirical azithromycin treatment is impaired. In the present study, we described the validation of an innovative real-time PCR assay, Macrolide-R/MG ELITe MGB® Kit, based on Pleiades probes melt curve analysis for *M*. *genitalium* and macrolide resistance-associated mutations detection, in combination with ELITe InGenius, a fully automated sample-to-result solution.
Materials/methods: Macrolide-R/MG ELITe MGB® Kit is a monoreagent, ready-to-use, real-time PCR assay that detects in one single reaction *M*. *genitalium* and five single nucleotides polymorphisms of the 23S rRNA gene associated with macrolide resistance: A2058G, A2058T, A2058C, A2059G, and A2059C. The performance of the prototype were validated by testing a large panel of *M. genitalium* positive clinical samples, initially characterized with a PCR assay for the detection of *M*. *genitalium* infections, and a combined 23S rRNA gene PCR/sequencing assay to identify the mutations. Then analytical and clinical performance of the full system, in combination with ELITe InGenius, was assessed.
Results: The newly developed assay has demonstrated an excellent correlation with expected results on clinical samples. The complete analytical and clinical validations with the complete system as a sample-to-result solution is ongoing.
Conclusions: Macrolide-R/MG ELITe MGB® Kit used in combination with ELITe InGenius is an innovative solution that could be easily implemented in the routine diagnostic workflow to guide antibiotic treatment of *M. genitalium* infection.

P006

Database development for Matrix-assisted laser desorption/ionization-time of flight mass spectrometry for identification of *Shigella* species and *Escherichia coli*

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Introduction: *Shigella* species can cause gastro-enteritis and are known to be genetically related to *Escherichia coli*. Entero-invasive *Escherichia coli* (EIEC) is a pathotype of *E. coli* that can cause the same disease as *Shigella species* and is even more related with *Shigella* due to the same genetic code for the infection mechanism. This relation hinders distinction and identification of *Shigella species* from *E. coli* in general and particular from EIEC. Laboratories frequently use Matrix-assisted laser desorption/ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for identification of bacteria, in which a protein profile of unknown bacteria is compared to a database. The currently available databases are not able to distinguish *Shigella* from *E. coli*. This study assesses the ability of MALDI-TOF MS for the distinction of *Shigella species* and *E. coli*, including EIEC, in a routine setting by developing a customized database.
Methods: A set of 562 isolates was used, consisting of 37 *S. dysenteriae*, 157 *S. flexneri*, 32 *S. boydii*, 232 *S. sonnei*, 62 EIEC, 22 other *E. coli* pathotypes from human origin, and 20 other *E. coli* pathotypes from animal origin. These isolates were identified by biochemical profiling and serotyping, and were equally divided according to species and origin into a training set and a test set. Ethanol/formic acid extraction with silica beads was performed on all isolates in the training set, and extracts were run on a Microflex LT instrument (Bruker Daltonics). Main Spectrum Profiles were created to build a customized database. This database was tested with the test isolates, performing the direct transfer procedure (DTP) and ethanol/formic acid extraction with silica beads, both spotted in duplicate. In this experiment, the highest scoring species above log-score 2.000 was considered as correct identification. ,
Results: Using the DTP procedure, 50% of *E. coli* was identified as *Shigella* and 4% of *Shigella* was identified as *E. coli*. For the extraction procedure, these percentages were 31% and 2%, respectively. Applying the DTP procedure, 45% of *S. dysenteriae*, 91% of *S. flexneri*, 7% of *S. boydii*, 96% of *S. sonnei*, and 50% of *E. coli* isolates were identified in concordance with classical methods. For the extraction procedure, the percentages of concordance with classical methods were 75% *S. dysenteriae*, 95% *S. flexneri*, 0% *S. boydii*, 97% *S. sonnei* and 69% *E. coli*. Overall, 15% of duplicates result in different species designation with small log-score differences, using DTP. Using the extraction procedure, 10% of duplicates result in different species designation.
Conclusion: The customized database is not applicable for correct distinction, as half of *E. coli* isolates were identified as *Shigella*. Above 90% of *S. flexneri* and *S. sonnei* isolates were correctly identified, however, other species are falsely identified as *S. flexneri* and *S. sonnei*. On top of this, in 10-15% of isolates, duplicates result in different species designations, indicating that a correct identification is a sheer change. For future research, model generation and biomarker assignment for identification with MALDI-TOF MS are tested with these sets of isolates.

P008

High prevalence of *Mycoplasma genitalium* and *Trichomonas vaginalis* in patients visiting the gynaecology department

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Introduction

Sexually transmitted diseases (STDs) are caused by different pathogens, mainly including Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma genitalium (MG) and Trichomonas vaginalis (TV). Although CT and NG are well-known as cause of STDs and included in national STD programs, MG and TV are lesser well-known and not a part of standard STD screening. Since both MG and TV can cause serious infections in women and are associated with adverse pregnancy events and infertility, we aimed to determine the prevalence of MG and TV in comparison to CT and NG in women visiting the gynaecology department.

Methods

From August to October 2017, all urogenital samples collected for diagnosis of STD at the gynaecology department of HMC-Westeinde, Den Haag, were included in this study. Samples were tested by transcription mediated amplification on the Hologic Panther system, using the commercially available assays for CT/NG (Aptima Combo), MG and TV (all Hologic). Specimens with invalid test results were repeated once. The prevalence is calculated as the number of positive patients of the total group with valid test results. For MG the physicians were advised to perform a test of cure 3-4 weeks after treatment.

Results

A total of 919 specimens from 573 patients were tested. MG was identified most often and tested positive in 5.1% of the patients, followed by CT (3.5%), TV (2.8%) and NG (0.2%). Four patients had co-infections of CT/MG (2), CT/MG/TV (1) and CT/NG (1). Test of cure after initial treatment with azithromycin was performed for 15 MG positive patients and was found positive for 2. Invalid test results as a result of inhibition was found in 0.2% of the MG samples. For both the CT/NG and TV assay no internal control is included.

Conclusion

MG was found most often in urogenital specimens from patients included in this study. This is in contrast to most other studies in which CT is most prevalent. Moreover, a high number of patients was identified TV positive. Since both MG and TV are associated with adverse pregnancy events, standard STD screening at this gynaecology department should include MG and TV as well.

P009

Epidemiology and detection of *Tropheryma whipplei* in healthy individuals in the Netherlands

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Introduction: *Tropheryma whipplei* is the causative agent of a variety of clinical manifestations including classic Whipple's Disease, chronic localized infections and acute infections. Carriage of *T. wipplei* has been found in stool samples of healthy individuals in Europe with variable prevalences (1-4%). PCR is widely used as a first-line diagnostic method. Risk factors of carriage are largely unknown.

Objectives: Determine the value of 3 commonly used PCR targets for the detection of *Tropheryma whipplei* , the prevalence of *T. whipplei*, risk factors, and short-term carriership in a large group of healthy individuals.

Methods: Faecal samples of 1675 healthy individuals drawn from municipal population registries in the Netherlands were analyzed using 3 commonly used targets in a *T. whipplei* multiplex PCR. The multiplex PCR was repeated when only one target was positive. Sequencing of PCR products was performed when samples still had one positive target after repeat testing. Samples were considered positive when 2 targets were positive or when sequencing of the PCR product of a single positive PCR target showed *T. whipplei* DNA.

All participants completed a questionnaire. Chi-squared and Fisher's exact tests were used to screen 43 variables for correlation with *T. whipplei* carriage. Benjamini-Hochberg correction was used to adjust for multivariate testing. P-values of <0.05 were considered significant. Uni- and multivariate logistic regression analysis were used to determine the odds-ration (OR) of variables that had significant p-values.

Repeat samples were obtained from part of the participants at 1 and 6 months after the first collection. 24 sets that contained at least 1 positive sample were tested to determine the short-term (1-6 months) carriage, of which 22 were usable.

Results: A total of 109 samples had at least two targets positive in the PCR after repeat testing, but 10 samples still had only one positive PCR target. Sequencing showed *T. whipplei* DNA in 9 of those.

A prevalence of 7.04% (Confidence interval (CI): 5.89-8.40) (118/1675) was found.

Smoking in the household was the only risk factor found after Chi-squared and Fisher's exact tests with Benjamini-Hochberg correction (p=0.043). Smoking in the household (OR: 2.72 (95%, CI 1.50-4.73)) and the age group 5-12 years (OR: 4.70, CI 1.61-15.74) compared to young adults (aged 18-40) were risk factors after logistic regression analysis, whereas the use of antibiotics in the past 8 weeks was a protective factor (OR: 0.24, 95% CI 0.04-0.78) for *T.whipplei* carriage.

Short-term carriage was seen in 13 of 22 (59%) sets.

Conclusion:

1. Tropheryma whipplei is a common bacterium in healthy individuals in the Netherlands.
2. The use of all three targets in a multiplex PCR is recommended in a clinical setting.
3. Single target positive samples can be false-positive. Sequencing of single-target positive samples should be performed to confirm the presence of *whipplei* DNA.
4. Smoking in the household and young age are risk factors whereas the use of antibiotics is a protective factor.
5. Short-term carriage is variable.

P010

Bacteriophage inhalation therapy in cystic fibrosis patients with chronic pulmonary *Pseudomonas aeruginosa* infection (BITCOIN study): study protocol for a double-blind placebo-controlled cross-over pilot study

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Introduction:
Cystic fibrosis (CF) is a genetic disorder that is characterized by chronic bacterial pulmonary infections. *Pseudomonas aeruginosa* is one of the most prevalent pathogens in these infections. It is extremely difficult to eradicate *P. aeruginosa* from the lungs of CF patients. Consequently, more than half of all patients with CF are chronically infected with *P. aeruginosa*, which is associated with a more rapid decline in pulmonary function and increased mortality, despite the chronic use of inhaled antibiotics. Therefore, there is an unmet need for a more adequate therapy against a chronic infection with *P. aeruginosa.* Currently, there is renewed interest in the use of bacteriophages for the treatment of bacterial infections. The research question addressed in this pilot study is whether nebulization with bacteriophages with in vitro activity against patient-specific *P. aeruginosa* isolates reduces bacterial load of *P. aeruginosa* in the airways of CF patients and improves patient pulmonary function and well-being.
Methods:
This is a double-blind placebo-controlled cross-over pilot study. In total, 12 patients with stable CF and chronic pulmonary *P. aeruginosa* infection will be included. Subjects will receive 28 days of bacteriophage inhalation therapy and 28 days of placebo inhalation therapy in a random order, with a wash-out period between the two cycles and follow-up period after the last cycle of 28 days. Participants will be followed-up on a bi-weekly basis. The primary outcome will be the bacterial load of *P. aeruginosa* in sputum cultures. Secondary outcomes will include pulmonary function (forced expiratory volume in 1 second, FEV1), quality of life, the safety and tolerability of bacteriophage inhalation therapy, *in vitro* sensitivity of isolated *P. aeruginosa* for the bacteriophage cocktail and the prevalence and levels of serum bacteriophage antibodies.
Conclusion:
Chronic pulmonary *P. aeruginosa* infections in CF patients are a major cause of morbidity and mortality, despite chronic use of inhaled antibiotics. It is therefore important to study alternatives for antibiotics, one of which could be the use of bacteriophages. In this pilot study, bacteriophage inhalation therapy will be assessed for its efficacy in reducing *P. aeruginosa* bacterial load from the airways of CF patients.

P011

Reduced sensitivity of vancomycin/ampicillin broth enrichment for the detection of VanB positive vancomycin resistant *Enterococcus faecium* by culture

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Introduction:
Detection of vanB vancomycin resistant *E. faecium* (VRE) by selective culture with vancomycin containing media is challenging because low vancomycin MIC's (even below 8 mg/L) occur in some vanB VRE isolates. VanB expression may be inducible leading to higher vancomycin MIC's and clinical resistance, therefore detection of those vanB VRE may be important. Several studies demonstrated synergism between ampicillin and vancomycin in VRE. Nevertheless, the current Dutch national guideline for the detection of VRE (NVMM Guideline HRMO VRE) recommends that ampicillin may be added to selective vancomycin containing enrichment broth cultures. We hypothesized that combining vancomycin and ampicillin in broth enrichment culture of vanB VRE impairs sensitivity in comparison with broth enrichment culture with vancomycin alone.
Methods:
Ten *E.faecium* isolates of different multilocus sequence types occurring in the Netherlands (table) and with vancomycin MIC's between 4 and 64 mg/l were selected (consensus MIC of measurements with broth microdilution and ETEST®). Two concentrations of each isolate were inoculated in three types of Enterococcoselä enrichment broths containing either: 1) vancomycin 6 mg/l + ampicillin 16 mg/l; 2) vancomycin 6 mg/l only and 3) vancomycin 6 mg/l and aztreonam 75mg/l. Inoculation densities were evaluated using counting plates.
All inoculated broth cultures were evaluated after 24 h incubation at 35°C for brown black color change. Furthermore, 100µl of broth culture was sub-cultured onto tryptic soy blood agar containing 5% sheepblood, for 24 hours, to evaluate growth recovery.
Results:

After 24h incubation of the *high* inoculum density broth cultures (aiming at 10e4 CFU VRE/inoculum), only 3/10 (30%) of VRE isolates demonstrated a yellow to brown/black color conversion of the Enterococcoselä broth media (table). In none of the ampicillin + vancomycin enriched broth cultures, a brown/black color change was observed. Upon subculture from the vancomycin + ampicillin broth cultures, 9/10 (90%) of VRE isolates could be recovered, compared to 10/10 of subcultures from vancomycin alone broths or vancomycin + aztreonam broths (table). With a *low* inoculum (aiming at 10e2 CFU VRE/inoculum), after 24 h incubation, only 2/10 (20%) of VRE isolates demonstrated a brown/black color change (table). Upon subculture 6/10 (60%) of VRE isolates could be recovered from vancomycin + ampicillin broth in comparison with 10/10 (100%) from the vancomycin only broth or 9/10 (90%) from the vancomycin + aztreonam broth (table).

Conclusion:

Selective vancomycin + ampicillin enrichment cultures have decreased sensitivity for the detection of vanB VRE isolates circulating in Dutch hospitals in comparison with vancomycin broth enrichment alone. The recommendation that ampicillin can be added to vancomycin in enrichment cultures should be omitted from the Dutch national guideline for the detection of VRE (NVMM Guideline HRMO VRE). Aztreonam may be a reasonable alternative additional selective agent for broth enrichment cultures, without important synergism with vancomycin in VRE. Brown/black color conversion after 24 hours of Enterococcoselä broth incubation is not sensitive for the presence of vanB VRE.

P012

An ELISpot assay, measuring *Borrelia burgdorferi* B31-specific interferon-gamma secreting T-cells, cannot discriminate active Lyme neuroborreliosis from past Lyme borreliosis; a prospective study in the Netherlands

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Introduction: Two-tier serology testing is most frequently used for the diagnosis of Lyme borreliosis (LB); however, a positive result is no proof of active disease. To establish a diagnosis of active LB, better diagnostics are needed. Despite the lack of published studies on clinically validated cellular assays, various laboratories offer these assays for the diagnosis of LB. These assays include the enzyme-linked immunospot (ELISpot) assay and the lymphocyte transformation test (LTT) and thus the clinical validation of these assays is urgently needed.
Methods: We investigated the utility of a standardized ELISpot assay by isolating peripheral blood mononuclear cells (PBMCs) of various well-defined groups comprising both treated and untreated patients and healthy controls. The number of *Borrelia*-activated T-cells secreting interferon-gamma (IFN-γ) was measured to investigate whether the ELISpot can be used as a marker for disease activity. *Borrelia burgdorferi* strain B31 and different recombinant antigens were used to stimulate the T-cells.
Results: We included 33 active and 37 treated Lyme neuroborreliosis patients, 28 healthy individuals treated for an early manifestation of LB in the past and 145 untreated healthy individuals. The median number of *B. burgdorferi* B31-specific IFN-γ secreting T-cells/2.5x10^5 PBMCs did not differ between active Lyme neuroborreliosis patients, treated Lyme neuroborreliosis patients and treated healthy individuals (6.0 (interquartile range (IQR): 0.5-14.0), 4.5 (IQR: 2.0-18.6), and 7.4 (IQR: 2.3-14.9), respectively) (p 1.000); however, the median number of *B. burgdorferi* B31-specific IFN-γ secreting T-cells/2.5x10^5 PBMCs among untreated healthy individuals was lower (2.0 (IQR: 0.5-3.9)) (p ≤0.016).
*Conclusion: The*Borrelia ELISpot, measuring the number of B. burgdorferi B31-specific INF-γ secreting T-cells/2.5x10^5 PBMCs, correlates with exposure to the *Borrelia* bacterium, but cannot be used for the diagnosis of active Lyme neuroborreliosis.

P013

Asymptomatic and symptomatic patients have a similar Neisseria gonorrhoeae load suggesting comparable transmission potential

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Introduction: *Neisseria gonorrhoeae* (NG) is one of the most common bacterial sexually transmitted infections (STI). Currently, there is limited data on transmission of NG in relation to symptoms and bacterial load. A previous model study estimated that asymptomatic carriers account for 89% of the onwards transmission. Other studies have shown that a higher bacterial load is linked to symptomatic urethral and anorectal infections in men who have sex with men (MSM). However to date, no study has determined NG bacterial load in a large mixed group (MSM, heterosexual men, and women) at all relevant anatomical sites (urethral, vaginal, anorectal, and oral). Here we describe NG bacterial load per sample site in relation to symptoms, sexual orientation, and age.
Methods: The study population includes data and samples of NG positive patients who visited a STI clinic for screening between January 2012 and May 2016. In this timeframe, 642 patients were NG positive accounting for 814 consultations. Consultations in which symptom data was missing (7%, 58/814) were excluded. In total, 1022 samples from 598 patients accounting for 756 consultations were NG positive (218 urine, 107 vaginal, 356 anorectal, and 341 oral samples). All samples were screened for NG with the cobas 4800. Bacterial load was determined by interpolation of a standard curve. Univariate and multivariable linear-regression models were used to assess associations of determinants (symptoms,

sexual orientation, and age) with NG load per sample site
Results: In 23.8% (180/756) of the consultations the patients reported symptoms at one or more sample sites. Using univariate linear regression a higher mean bacterial load was observed in urine and anorectal samples from symptomatic patients, approximately one and two crossing-threshold value respectively. Vaginal and oral samples from symptomatic patients did not have a significant higher mean bacterial load. Furthermore, the range of loads per sample site was similar between symptomatic and asymptomatic patients. Also, women appear to have a lower anorectal and oral load compared to MSM. Age was only significantly associated with load in oral swabs in which younger patients have a higher load.
Multivariable analyses were adjusted for age and the significantly associated determinants in univariate analyses. The associations in both urine and anorectal samples remained similar for samples from symptomatic patients. However, the lower load in women of oral samples attenuated and became non-significant but the association remained similar in anorectal samples. The association of a higher load in younger patients of oral samples remained similar.
Conclusion: We showed that urine and anorectal samples from symptomatic patients have a higher mean bacterial load but this difference is almost within the accuracy of a PCR. Together with frequent asymptomatic patients (76.2% of the consultations), the similar bacterial load could explain the high contribution of asymptomatic carriers in the model study. However, it is unclear which other factors might influence transmission or whether it is solely dependent on NG bacterial load. Therefore, more research on the role of load and strain specific characteristics in symptoms and transmission is needed to improve NG control.

P014
MALDI TOF for identification of single microbial cells directly in clinical samples
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In the past years MALDI TOF (Matrix-Assisted Laser Desorption/Ionization Time of Flight) has rapidly emerged as a standard diagnostic technology used in most clinical microbiology laboratories. The reason for its success is that MALDI TOF – compared to classical technologies used – reduced the time needed for identification of clinical isolates obtained from 10 hours to 10 minutes, *but only after time consuming culture and isolation steps.* Combined with its relative ease of use and little infrastructural requirements it has led to one of the most rapid and successful transformations in the clinical microbiology laboratory work processes in the last decades.
Current MALDI TOF however has the shortcoming that it uses *bacterial isolates* as input, that prevents it from 'ticking all the boxes' necessary to become the ultimate diagnostic technology of choice for clinical microbiology laboratories. Current innovations try to move away from the classical methods requiring culture of the pathogens, most of them using (fully automated) rapid multiplex PCR technology.
We followed a unique approach by developing MALDI-TOF for single cell use (DigiTOF®), skipping all culture and isolation steps, whilst maintaining the advantages of conventional MALDI TOF. Direct analysis of patient samples by DigiTOF® technology basically requires interfacing of three innovative processes, which have successfully been completed i.e. (i) isolate and prepare individual cells from clinical samples, (ii) interrogate cells one by one by the mass spectrometer and (iii) reliably interprete the individual cell MALDI TOF spectra.
After proof of concept was obtained, DigiTOF® Beta prototypes were built recently. Presently, the fully automated prototype is used to develop the first databases, for urinary tract infections and for prosthetic joint infections, to be able to clinically investigate the DigiTOF technology for diagnosis of these infections.

P015
Direct *Clostridium difficile* ribotyping on faecal samples for real-time infection control
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Background: *Clostridium difficile* is notorious for its potential to cause hospital outbreaks and therefore monitoring its spread in the hospital is paramount for adequate infection control. At our institution we routinely type cultured *C. difficile* isolates by Amplified Fragment Length Polymorphism (AFLP) analysis to monitor hospital epidemiology. We developed a protocol for simultaneous detection and direct ribotyping of *C. difficile* in fecal samples for real-time infection control and applied this technique during a *C. difficile* outbreak at our institution.
Methods: We optimized FAM-labelled interspace-region profiling (IS-pro) primers and PCR protocol for direct *C. difficile* ribotyping on faecal samples. DNA extraction was performed using the MagnaPure system. The PCR reaction products were analysed by capillary gel electrophoresis using an ABI 3500 sequencer, which allows for a quantitative read-out of fragment lengths, thereby generating the ribotype peak profiles. The peak profiles of 11 clinical faecal samples obtained from patients with diarrhea during an outbreak were compared to the profiles of the corresponding 11 cultured isolates, which were typed by AFLP (n=6 outbreak isolates, AFLP-type AT17120, PCR ribotype 017). In addition we directly ribotyped a fecal sample before an isolate was cultured.
Results: In 11 of 11 faecal samples a ribotype peak profile was generated that corresponded to the peak profile of the cultured *C. difficile* isolates. Six direct ribotype profiles corresponded to the ribotype 017 profile, confirming the outbreak by this ribotype. The results of direct faecal ribotyping corresponded to the results of classical AFLP typing of cultured strains. Direct ribotyping of one faecal sample allowed us to allocate the sample to the outbreak-cluster, before the isolate was cultured and typed.
Conclusions: Direct capillary gel electrophoresis ribotyping on faecal samples without prior culture of *C. difficile* is

feasible. This results in a lower turnaround time for ribotyping test results, thereby enabling infection control consultants to monitor *C. difficile* hospital epidemiology more closely and in real-time.

P016

Molecular point-of-care diagnostics for *Clostridium difficile* in 20 minutes

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Background: *C. difficile* is the main causative agent of antibiotic-associated diarrhoea both in heathcare settings and in the community. The symptoms vary from mild diarrhoea to life-threatening pseudomembranous colitis. Rapid diagnosis is required for initiation of infection control measures and appropriate antibiotic treatment. The Roche cobas® Cdiff assay for the Liat® enables a diagnostic result in 20 minutes.
Materials/methods: Between A prospective study was performed between January 2017 and January 2018. In total, 75 patients who tested positive for *C. difficile* using an in- house developed real-time tcdB PCR were included in the study. For every positive patient, a random negative sample was included as well. Once the diagnostic PCR was positive, a feces toxin assay (VIDAS) was applied on the same day and a culture was performed to characterise the strains.
Results: Out of 75 previously identified positive samples, 32 samples were also positive by VIDAS representing 32 patients with laboratory confirmed CDI. In total, 58 were culture positive and resulted in 20 different *C. difficile* ribotypes, but no hypervirulent Type 027 was detected. Of 75 positive TcdB tested samples, 57 positive with the Liat. Of 32 samples from laboratory confirmed CDI, all samples.were positive by Liat. Of 75 negative tested PCRs, none was positive by Liat assay.
Conclusions: Compared to our in-house developed PCR, the Liat® Cdiff test has sensitivity of 76% which increased to 100% when laboratory confirmed CDI was used as standard. The 20 minutes turn-around-time provides the potential to point-of-care testing so that adequate infection control measures can be initiated promptly.

P017

Bacterial Community Composition Along a Methane Gradient

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Methane is a potent greenhouse gas that contributes to global warming 30 times greater than CO_2. It is mostly produced via biogenic activities, but its release into the atmosphere is mitigated by CH_4 oxidizing microorganisms. Typically, natural and cultivated wetlands are major sources of CH_4 while forests and meadow soils are sinks. This study focused on the differences in the methanotrophic community between adjacent paddy and meadows. We used 16S rRNA gene amplicon sequencing and CH_4 flux measurements to analyze the bacterial community composition and related CH_4 emissions in bordering paddy and meadow fields. Unexpectedly, neither CH_4 fluxes measured in the paddy and meadow nor the methanotrophic community composition were significantly different, calling for caution when including paddy and meadow areas into global CH_4 flux calculations. Finally, integrating data from the current study and from the literature, we propose a conceptual model offering explanations as to how paddy and meadow fields may not play their traditional roles as sources and sinks of CH_4.

P018

High quality draft genome sequence of '*Candidatus* Methanoperedens BLZ2', a nitrate-reducing anaerobic methane-oxidizing archaeon enriched in an anoxic bioreactor

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Introduction: Methane that escapes to the atmosphere acts as potent greenhouse gas. Global methane emissions are mitigated by methanotrophs, which oxidize methane to CO_2. '*Candidatus* Methanoperedens spp.' are unique methanotrophic archaea that can perform nitrate-dependent anaerobic oxidation of methane. A high quality draft genome of only 85 contigs from this archaeon is presented here.
Methods: DNA was extracted from a bioreactor highly enriched in '*Ca.* Methanoperedens BLZ2' and sequenced with an Illumina Miseq protocol. Reads were assembled *de novo* using the CLC Genomics Workbench. Contigs were binned using MetaBat (1), yielding a draft genome composed of 85 contigs (N_{50} = 74.304 bp). Quality was assessed using CheckM (2). Annotation was done using Prokka (3) and also as previously described (4).
Results: Based on the presence of 228 specific marker genes, the draft genome was 99.35% complete and only 4.58% contaminated. The final sequence had a GC content of 40.3% and was composed of 3.74 Mb with 4041 putative open reading frames. The 16S rRNA gene in this genome was 100% identical to that found in '*Ca*. Methanoperedens BLZ1' and 95% identical to '*Ca*. M. nitroreducens ANME2d' (5, 6). In accordance with previous data all genes necessary for reverse methanogenesis were present, including *mer* (encoding F_{420}-dependent 5,10-methenyl-H_4MPT reductase). Among enzyme complexes presumably involved in electron transfer reactions nitrate reductase transferring electrons to the terminal acceptor nitrate is of special interest. It could be demonstrated that nitrate reductase was present as canonical (NarGHI) and non-canonical version (NarGH, potential membrane anchors HCOIIa, HCOIIb, NapH, cytochrome subunit Orf7 and chaperone NarJ, being the similarity to '*Ca*. Methanoperedens BLZ1' between 92 and

100%). Both were encoded in two clusters present in close proximity to each other on the same 129 kb-long contig. The subunit composition of other important soluble and membrane-bound enzyme complexes was largely in accordance with existing data.
Conclusion: Nitrate-dependent methanotrophic archaea not only consume the greenhouse gas methane but also connect the biogeochemical cycles of carbon and nitrogen. Due to their fastidious nature and slow growth they have long escaped discovery. Accurate genomic data as presented here helps to reveal the metabolic potential of these important microorganisms.
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P019
I-4-1-Health: Creating the platform for cross-border charting, characterization and outbreak detection of antibiotic resistant *E. coli* strains
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Introduction
The i-4-1-Health project aims to better understand antibiotic resistance through identification of contamination routes of antibiotic resistant pathogens across sectors to prevent further resistance development. Resistance in *E. coli,* the most common Gram-negative pathogen in humans, is of particular concern. 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 propose the analysis framework developed for rapid next-generation sequencing (NGS) data analysis, as illustrated on publicly available data. i-4-1-Health is funded within the Flanders-Netherlands Interreg V program, financially supported by the European Regional Development Fund.
Methods
To accommodate rapid exchange of big data, a network running on a client-server platform empowered by BioNumerics was established empowering seamless interaction between local BioNumerics clients and an international server database. This combination allows for real-time data exchange both at the local and international level. The centrally stored data makes it possible to perform rapid genotyping and comparison of bacterial sequences and to link geographically different clusters of illness.
To validate we analyzed publicly available NGS data from 1,411 *E. coli* strains 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 novo assembly and typing by whole genome MLST (wgMLST) was performed in BioNumerics. Additionally, serotype, resistance and virulence genes were predicted from the NGS data by the BioNumerics genotyping tool.
Results
Strain genotyping took 3 days using BioNumerics on a common desktop computer. 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’ve acquired more resistance over time.
Conclusions
Rapid exchange of large NGS datasets and results was enabled by a client-server platform empowered by BioNumerics. This setup and integrated data analysis allows to map antibiotic resistance in livestock farming, the general population and health care, to link geographically different clusters for outbreak detection and to monitor antibiotic resistance across borders.
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P020

Predicting disease activity in Crohn's disease based on intestinal microbial profiling

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Introduction:
Crohn's disease (CD) is an idiopathic, chronic inflammatory disease of the gastrointestinal tract, being characterized by periods of disease exacerbation and periods of remission of disease activity. Timely recognition and treatment of these exacerbations of disease activity is important because a chronic state of mucosal inflammation can lead to various complications. Current techniques used for disease monitoring in CD are invasive, expensive techniques. Therefore, non-invasive biomarkers have been increasingly used to monitor disease activity, such as C-reactive protein and fecal calprotectin. Unfortunately, these biomarkers are not specific for CD induced mucosal inflammation. Microbiota-based disease monitoring and disease prediction holds great promise for the future. Current research on microbiota-based disease monitoring is based on data obtained by DNA sequencing. Although continuously improving, sequencing techniques are laborious, time-consuming and expensive. Therefore, an alternative to sequencing has been developed for microbiota analysis: IS-profiling. The aim of this study is to investigate the potential of the IS-pro technique as a prediction tool for disease activity in CD.
Methods: Repeated fecal samples were collected from 71 CD patients, resulting in 87 active disease and 98 remission samples . Gut microbial profiles were obtained with the IS-pro technique. The IS-pro technique discriminates bacterial species by the length of its' 16S-23S ribosomal interspace fragment. Two classification algorithms were used for disease activity prediction: nearest shrunken centroid classification (NSCC) and group regularized ridge regression (GRRR).
Results: Based on distance from the class centroids **(**shrinkage value Δ2.35)**,** the top 5 most discriminating species were Unknown Bacteroidete (nucleotide length 453), *Parabacteroides distasonis*, *Escherichia coli, Barnsiella* spp. and Unknown Bacteroidete (nucleotide length 439). NSCC showed an AUC of 89% without shrinkage and with shrinkage 66%. With GRRR the prediction accuracy without selection of bacteria was 87%. With a model selection of 100 bacteria, the prediction accuracy was 82%.
Conclusion: Relapse samples can be discriminated from remission samples with an accuracy of 66% to 87%, depending on the model used**.** Microbiota-based prediction of CD activity by means of IS-profiling holds great promise for the future.

P021

Reduced risk of BK polyomavirus infection in HLA-B51 positive kidney transplantation recipients

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Introduction Identification of HLA types and T cell epitopes that influence the course of BK polyomavirus (BKPyV) infection after kidney transplantation (KTx), including development of BKPyV-associated nephropathy (BKPyVAN), can be useful for patient risk stratification and possibly vaccine development.
Methods In a retrospective cohort of 407 living kidney donor-recipient pairs, donor and recipient HLA class I/II status was correlated with the occurrence of recipient BKPyV viremia and BKPyVAN within one year post-KTx. Relevant HLA types were systematically analyzed for candidate peptide epitopes in silico.
Results None of the 78 HLA types tested increased the risk of BKPyV viremia and BKPyVAN in our cohort. A significant reduction of BKPyV viremia ($p = 0.002$) and BKPyVAN was observed in HLA-B51 positive KTx recipients (KTRs). Multivariate analysis showed that HLA-B51-positivity, found in 36 KTRs (9%), reduced the risk of viremia approximately 5-fold (HR 0.18, 95% CI: 0.04 – 0.73, $p = 0.017$). Four HLA-B51-restricted putative cytotoxic T lymphocyte epitopes were identified, including the previously described HLA-B supermotif-containing peptide LPLMRKAYL, encoded by two relevant T-antigen proteins and previously shown to be highly immunogenic.
Conclusion HLA-B51 positive KTRs seem less susceptible to BKPyV infection, which might be explained by efficient presentation of a particular BKPyV-derived immunogenic peptide.

P022

Proteomes of cells and spores of an IPTG-inducible kinA strain compared to wild-type *Bacillus subtilis*

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Introduction
Bacillus subtilis can form metabolically dormant spores that are able to survive harsh environmental conditions. Spores may survive treatments used to microbiologically stabilize foods, thus causing food spoilage and food borne disease risks. A phosphorelay controls the initiation of sporulation, starting with expression and auto-phosphorylation of the kinase KinA. KinA transfers phosphoryl groups to Spo0A, through Spo0F and Spo0B. Accumulation of Spo0A~P_i above a given threshold then triggers sporulation. Both our own current studies as well as previous research has shown that inducing *kinA* expression in *Bacillus subtilis* bearing *kinA* under control of an IPTG responsive *hyperspank* promoter ($P_{hy\text{-}spank}$) triggers sporulation independently of the culture medium being nutrient poor or nutrient rich. With this strain a time

resolved detailed proteomic study of spore formation becomes possible as sporulation can be induced in all cells simultaneously by the addition of IPTG. In order to be able to compare the proteome of $P_{hy\text{-}spank}$ *kinA* with wild-type (WT) *B. subtilis* the base-line proteomes of both their cells and spores must be established. Here we report on such studies.
Method
A defined, MOPS-buffered medium, was used to metabolically label all proteins with ^{15}N by replacing the sole nitrogen source $^{14}NH_4Cl$ with $^{15}NH_4Cl$. A one pot method that can process all the soluble and insoluble proteins without significant protein loss, was used (Swarge et al., under final revision). $P_{hy\text{-}spank}$ *kinA* and WT were labelled with ^{14}N and ^{15}N respectively. Identical amount of cells and spores were mixed to prepare peptides samples. Electrospray Ionization(ESI) -FTICR Mass spectrometry was used to quantitatively analyze tryptic protein digests.
Results
1174 and 1173 proteins were identified from respectively cells and spores. 749 cellular proteins and 785 spore proteins were identified in three biological replicates and form a robust set of proteome identifications. As we mix vegetative cells and spores of both the $P_{hy\text{-}spank}$ *kinA* strain and the wild-type in a one to one ratio the theoretical ration of ^{14}N and ^{15}N peptides should be close to one if a given protein is present in similar amounts in cells or spores of both. For vegetative cells we observed for 497 proteins ^{14}N / ^{15}N ratios between 0.9 and 1.1. For 122 proteins the ratios were higher than 1.1 with a maximum of 15.7 (!). We observed for 130 ^{14}N / ^{15}N protein ratios lower than 0.9. For spores we found that only 243 protein ratios ranged from 0.9 to 1.1. Most (636) protein ratios ranged from 0.7 to 1.8. 109 protein ratios were higher than 1.8 with a maximum of 71.76 (!). 37 ^{14}N / ^{15}N protein ratios were lower than 0.7.
Conclusion
The $P_{hy\text{-}spank}$ *kinA* strain, suitable for the homogeneous induction of sporulation, displays compared to control cells, characteristic significant differences in protein expression in vegetative cells as well as spores. In the currently ongoing time resolved proteome analyses using IPTG induced spore formation in the $P_{hy\text{-}spank}$ *kinA* strain, the here described differences set the baseline for comparisons.

P023

An in silico survey of *Clostridium difficile* plasmid epidemiology

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Background: *Clostridioides difficile* (*Clostridium difficile)* is an important enteropathogen, causing more than 120.000 infections per year in the European Union. In recent years, epidemic subtypes have emerged, without characterized genomic determinants (e.g. acquired virulence genes). We recently discovered that plasmids might be more widespread in *C. difficile* than prior acknowledged. As plasmids are known carrier of virulence determinants in other species, we conducted an in-silico survey of publicly available *C. difficile* whole genome sequencing data with the goal to discover new plasmids.
Materials/methods: The National Center for Biotechnology Information database was queried for Sequence Read Archive runs of *C. difficile* consisting of paired-end Illumina data. The resulting ~5400 runs were downloaded, analyzed with KmerGenie and assembled with Velvet. The assembly graph was parsed to detect replicons that were clearly separated from the chromosomal assembly. All separated replicons with a coverage exceeding 1.5 times that of the chromosome were considered as plasmids or other epichromosomal replicons. Multi Locus Sequence Types (STs) were derived via MLSTcheck.
Results: 5336 genomes could successfully be assembled, and 1067 epichromosomal replicons could be retrieved. Most samples (452) contained one epichromosomal element, with six being the most found. 100 elements could be assigned to the technical spike-in phage phiX, and 600 to known plasmids and phages of *C. difficile*. Within the remaining 367 elements, 234 could be clustered into eight bigger subgroups. Functional annotation revealed mainly plasmid replication and DNA processing/maintenance related functions, but one plasmid group harbored penicillin resistance functions (BlaR1 peptidase and penicillinase repressor).
The distribution of plasmids also revealed that STs belonging to ST 7 and 8 (166 samples) had on average more than 1 plasmid. STs belonging or related to ST 1 and 11, which correspond to the clinically relevant PCR ribotypes 027 (American Pulse-field type NAP1) and 078 (NAP7/8), contained hardly any plasmids (24 within 1821 samples).
Conclusion: The occurrence of plasmids within *C. difficile* has long been overlooked. We show a potentially relevant bias in distribution of plasmids within the species, and hope to unravel in the future which functions are relevant for plasmid maintenance and virulence.

P024

Screening and isolation of antimicrobial secondary metabolites from sponge-associated microorganimsms

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Introduction: The rapid increase of drug-resistant bacteria leads to major threats to animal and human health. Therefore, alternative strategies to find novel antibiotics are emergent to develop. Marine sponges are known as a prolific source of bioactive compounds; however, many of them are produced by their associated microorganisms. In addition, sponges produce only minor quantities of bioactive compounds resulting in challenges in mining these bioactive compounds. Therefore, searching for the bioactive compounds from sponge-associated microorganisms will be a good strategy for mining novel antibiotics.

Methods: We isolated bacteria from sponges using seven different media, and then screened antimicrobial isolates against nine reference strains including Gram negative bacteria (*Escherichia coli*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Vibrio parahemolyticcus*), Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Rhodococcus* sp.), and fungi (*Aspergillus niger*, *Candida albicans*). The isolate with the highest activity was targeted for isolation and purification of antimicrobial compounds using chromatographic methods. The chemical structure of purified compounds was elucidated by spectroscopic analyses (ESI-MS, ^{1}H NMR, ^{13}C NMR, HSQC, HMBC). The minimum inhibitory concentration (MIC) of the compounds against reference strains was determined by the broth microdilution method.
Results: Out of 496 isolates from sponges, 92 isolates exhibited antimicrobial activity against at least one of reference strains. The majority of isolates (70) showed antimicrobial activity against one reference strain, 10 isolates showed activity against two reference strains. Finally, eight and four isolates showed activity against three and four references, respectively. From a selected isolate (*Bacillus* sp.), five secondary metabolites were extracted and purified including (**1**) Cyclo (L-Pro-L-Tyr), (**2**) Macrolactin H, (**3**) Macrolactin A, (**4**) 15, 17-Epoxy-16-hydroxy macrolactin A, and (**5**) mix of macrolactin B/C. The bioassay tests showed that compounds **2**, **3**, **4** exhibited activity against a wide range of reference strains, whereas compounds **1** and **5** showed only activity to a few reference strains with weak activity.
Conclusion: Our study revealed microorganisms associated with sponges as potential producers of antibiotics. Extraction of secondary metabolites from sponge-associated microorganisms might be developed as an alternative strategy for mining novel antibiotics instead of the sponges themselves, thereby avoiding negative impacts on ecology and environment.

P025

Transcriptomics of a synthetic co-culture performing chain-elongation from syngas

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Syngas, a mixture of CO, H_2, and CO_2, can be generated via gasification of any carbohydrate material. Fermentation of syngas by carboxydotrophic microbes allows its conversion to value-added chemicals. Organisms involved in the fermentation of syngas use the CO or H_2 in the gas as electron donor, fixing CO_2 to the final end products. Currently acetate and ethanol are relatively well established products from syngas fermentation and there is interest to broaden the scope towards the production of more complex products. However, genetic engineering of carboxydotrophic organisms and the knowledge of their metabolism is rather limited, making it difficult to 'create' strains producing these products. A possible way to broaden the scope of products is via co-cultivation of microbes which can make use of each other's products. We established a co-culture of *Clostridium autoethanogenum*, a well-known carboxydotrophic acetogen, together with *Clostridium kluyveri*, a well characterized organism employing the reverse β-oxidation pathway. *C. autoethanogenum* uses the syngas to produce a mixture of acetate and ethanol. *C. kluyveri* subsequently uses these products to perform chain elongation. This results in a co-culture producing a mixture of C4 and C6 acids and alcohols using syngas as a sole substrate.
Using a transcriptomics approach and studying the co-culture behaviour in a chemostat-system we have attempted to unravel the functioning of this culture and how its production spectrum can be optimized. Results indicate that production of hydrogen by *C. kluyveri* stimulates the metabolism of *C. autoethanogenum*, resulting in more alcohol formation. This has a subsequent effect on the production levels of C4 and C6 products by *C. kluyveri*. This type of interactions is important to for optimization production and tuning of current and future synthetic co-culture designs.

P026

Characterization of a putative triheme from anammox bacteria reveals a non-canonical heme binding site.

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Anaerobic ammonium oxidizing (anammox) bacteria use nitrite as terminal electron acceptor and produce about half of the dinitrogen gas that is released into the atmosphere. Their metabolic pathway comprises three steps and involves a cyclic electron flow. First, a putative nitrite reductase converts nitrite into nitric oxide, and requires one electron. The second step is catalysed by hydrazine synthase (HZS). This enzyme requires three electrons for the reduction of nitric oxide to hydroxylamine. Subsequent condensation of hydroxylamine with ammonia yields hydrazine. Finally, oxidation of hydrazine to dinitrogen gas yields four low potential electrons that are fed into the quinone pool. From there, three electrons are carried to HZS by a putative *c*-type cytochrome. A small, soluble cytochrome within the HZS gene-cluster is hypothesized to be the electron donor for hydrazine synthesis. The amino-acid sequence of the corresponding gene product is conserved among different anammox genera and contains three canonical *c*-type heme binding sites. We purified this protein to homogeneity from biomass of the anammox bacterium *Kuenenia stuttgartiensis*, and characterized its spectroscopic features. Mass-spectrometry reveals that this putative triheme actually contains a fourth heme, which appears to be attached to a unique "CXCH" heme binding motif.

P027

Number of ESBL *E. coli* on filet américain and minced meat: absent and low

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Introduction:
Human exposure of ESBL producing *E. coli* (ESBL-EC) is possible through various routes. As meat is found to be contaminated with ESBL-EC (prevalence depending on the meat product) eating meat could be one of the transmission routes for humans. In a previous study, ESBL-EC exposure to humans via different meat products was explored and calculated in a Quantitative Microbiological Risk Assessment (QMRA) model by Evers et al. (2017). This model takes into account storage, preparing conditions, hygiene in the kitchen, but also bacterial prevalence and counts on meat among others. The results showed that although poultry meat is most often contaminated, meat products that are eaten raw (mainly beef products like filet américain) lead to the highest human exposure of ESBL-EC through eating meat. However, the lack of knowledge about bacterial concentrations on meat other than chicken meat (about 9 CFU/g) was an important data gap. The goal of this project was to determine ESBL-EC prevalence and concentrations on filet américain and minced meat (beef) to make the model more accurate.
Methods:
The filet américain (n=100) and minced meat products (n=100) were each obtained during two different time-points. Filet américain was bought from 17 different supermarket chains (which represented eight different meat packing industries) and 21 different butchers. The minced meat products were only bought in supermarkets (16 different supermarket chains, representing 17 different meat-packing industries). Products were bought in Amersfoort, Amsterdam, Den Haag, Ede, Nijmegen, Vlaardingen, and in and around Utrecht. Per sample, 25 gram of meat was mixed and incubated with 225 ml Buffered Pepton Water (BPW) overnight at 37°C. The next day, 10 µl was incubated on Brilliance *E. coli* and coliform selective agar with (BECSA+) and without (BECSA) 1 mg/L cefotaxime. From all samples with growth on BECSA+ again 25 gram was taken and mixed with 25 ml BPW. From this mixture 2 times 1 ml (representing 1 g of meat) was incubated on BECSA and BECSA+ plates (14 cm). The plates were incubated 4 h at 37°C and subsequently 18-24 h at 44°C. Typical *E. coli* colonies (purple) were collected and phenotypically confirmed for ESBL-production by disk diffusion test.
Results:
All 100 filet américain samples were negative for ESBL-EC (prevalence 0%; 95CI:0-4%), whereas 8/100 minced meat samples were ESBL-EC positive (8%; 95%CI 5-15%), however the ESBL-EC counts were low (<2 cfu/g meat). One meat-packing industry was responsible for 5/8 positive samples.
Conclusion:
The estimated prevalence of ESBL-EC contaminated filet américain products is less than 4%. Minced meat is more often contaminated, however the risk for exposure to humans is relatively low, due to the low numbers of ESBL-EC present in this product and the fact that minced meat is also often well cooked. Still, transmission through consumption, handling the meat and cross-contamination in the kitchen is possible. These quantitative results are a first step in improving the accuracy of the QMRA model by Evers et al. (2017).

P028

Differential gene expression of *Aspergillus fumigatus* and *Aspergillus niger* interacting with epithelial lung cells

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Introduction: *Aspergillus fumigatus* is the main causative agent of aspergillosis. Most infections occur in immunocompromised individuals, indicating an efficient clearance of conidia by the pulmonary defence system in immunocompetent individuals. Infections by other aspergilli like *Aspergillus niger* can occur, but to lesser extent. Previous studies showed that *A. fumigatus* and *A. niger* behave differently in the presence of type II alveolar A549 epithelial cells. *A. fumigatus* is more efficiently internalized by the A549 cells and shows a delay in germination, when compared to *A. niger*. The hyphae of *A. fumigatus*, that escaped the epithelial cells grow parallel to the epithelium, while the *A. niger* hyphae grow away from the epithelial cell layer. This study focusses on the gene expression of *A. fumigatus* and *A. niger* after co-cultivation with A549 cells. Our hypothesis is that the difference in lifestyle between the two aspergilli is also observed in the gene expression profiles.
Methods: RNA of the co-cultivation of the A549 cells with *A. fumigatus* or *A. niger* was isolated and sequenced. The obtained RNA sequences were analysed with custom R and python scripts to obtain the differentially expressed genes and GO terms.
Results: The obtained RNA sequences show big differences in the global gene expression of *A. fumigatus* and *A. niger* upon contact with A549 cells. A total of 545 and 473 genes for respectively *A. fumigatus* and *A. niger* were differently expressed when compared to growth in absence of A549 cells. Of these genes only 53 (~10%) were shared between both species. The different response was also illustrated by the fact that only 4 GO terms were shared between the differentially expressed genes of both gene sets. Genes described in hypoxia regulation and heat shock were found up-regulated in *A. fumigatus* and their homologs in *A. niger*. The *A. fumigatus* thioredoxin reductase and allergen genes were found up-regulated in this fungus, but homologous genes were down-regulated in *A. niger*. After co-cultivation with *A. fumigatus* 62 genes were up and 47 genes were down-regulated in the A549 cells. Co-cultivation with *A. niger* resulted in 17 up and 34 down-regulated genes. GO term related with the immune response were down-regulated in the A549 cells upon exposure to *A. fumigatus*, but not in the case of *A. niger*. This is a strong indication that *A. fumigatus*

reprograms the A549 cells to be immunologically less alert.
Conclusion: Our dual transcriptome analysis supports earlier observations of a markedly difference in life style between *A. fumigatus* and *A. niger* when grown in presence of type II epithelial lung cells. These results show an important difference in gene expression, amongst others the downregulation of immune response genes in epithelial cells by *A. fumigatus* and not by *A. niger*.

P029

The histone-like protein HupA binds to DNA and associates with the nucleoid in *Clostridium difficile*

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The maintenance and (re-)organization of the chromosome plays an important role in the development and survival of bacteria. Histone-like proteins are architectural proteins that bind DNA, modulate its conformation and by doing so affect a variety of cellular processes [1]. No histone-like proteins of *C. difficile* have been characterized to date.
Here, we investigate aspects of the *C. difficile* HupA protein, a homologue of the histone-like proteins HUα and β proteins of *Escherichia coli*. HupA is a small thermostable protein that is present as a homodimer. It binds to the DNA without strong sequence specificity. Preliminary data suggest that binding induces a conformational change of substrate DNA and may lead to compaction of the chromosome *in vivo*. Fluorescence microscopy shows co-localization of HupA with the nucleoid of *C. difficile*.
The present study is the first to characterize a histone-like protein in *C. difficile* and opens the way to study the role of chromosomal organization in DNA metabolism and other cellular processes.

Reference

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P030

Seroreactivity of human polyomaviruses in the healthy Dutch blood donor population

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Introduction The number of human polyomaviruses (HPyVs), small double-stranded DNA viruses that cause persistent asymptomatic infection, has rapidly increased to 14. In immunocompromised and elderly patients, some HPyVs cause disease, such as nephropathy (BKPyV), progressive multifocal leukoencephalopathy (JCPyV), hair follicle dysplasia known as trichodysplasia spinulosa (TSPyV) and Merkel cell carcinoma (MCPyV). Seroprevalence data in the Dutch blood donor population is currently lacking.
Methods IgG-seroreactivity for 14 different HPyVs was analyzed using sera from healthy Dutch blood donors (N=1044). Glutathione S-transferase (GST)-Viral Protein 1 (VP1) fusion proteins, expressed for each polyomavirus in E. coli, were bound to glutathione-casein coupled Luminex fluorescent beads and used as antigen. VP1-directed antibodies were detected by biotinylated goat anti-human IgG and streptavidin-R-phycoerythrin. Seropositivity cut-off thresholds were determined by analyzing serum samples from children (n=36) between 10 and 15 months old.
Results All donors were seropositive for at least four HPyVs. The average number of infecting HPyVs was nine (8.7 ± 1.6 SD). Seroprevalence was high for many polyomaviruses across all age categories (18-29; 30-39; 40-49; 50-59; 60-69), even above 90% for BKPyV, the Karolinska Institutet polyomavirus (KIPyV), the Washington University polyomavirus (WUPyV) and the Malawi polyomavirus (MWPyV). Seroprevalence ranged from 60-90% for JCPyV, MCPyV, HPyV6, HPyV7, TSPyV and Saint Louis polyomavirus (STLPyV), while HPyV9 seroprevalence was approximately 20%. The seroprevalence of HPyV12, New Jersey polyomavirus (NJPyV) and Lyon IARC polyomavirus (LIPyV) was low, ranging between 4-6%. A significant increase ($P<0.05$) between seropositivity and age category was seen for KIPyV, MCPyV, HPyV6 and HPyV7. For BKPyV and TSPyV the intensity of the measured seroreactivity decreased with age.

Conclusions

HPyV infections are common in the Netherlands
The average number of infecting HPyVs per individual, 9, is high
Primary HPyV infection occurs in childhood, evidenced by high adult seroprevalences
Low seroprevalence of HPyV12, NJPyV and LIPyV suggests that humans are not the primary host
Age-related patterns of seroreactivity are HPyV-specific suggesting different modes of exposure
PCR testing will reveal whether healthy blood donors are viremic for HPyVs

P031

Temperature effects on aerobic methanotrophs in thermokarst lake sediments

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Permafrost covers a quarter of the Northern hemisphere land surface and stores twice the amount of carbon currently present in the atmosphere. Global warming leads to an increase in permafrost thaw causing thermokarst lake formation. These processes influence organic matter decomposition rates by disrupting local hydrological and soil conditions, and have the potential to alter microbial CO_2 and CH_4 emissions. However, the effect of global warming on both methane producing and consuming microbial communities in thermokarst environments is not yet well explored.
Here, we investigated the effect of increasing temperature on the activity of aerobic methanotrophs enriched from thermokarst lake sediments from Barrow, Alaska. Sediment slurries were diluted with freshwater mineral medium, amended with methane and oxygen and incubated under 4°C or 10°C. When needed, cultures were transferred to fresh medium. The activity of the enrichment cultures was followed over time and the microbial community composition was investigated with 16S rRNA gene amplicon sequencing.
The maximum CH_4 consumption potential after 64 days of incubation was 216 (± 18) µmol CH_4 / g dry weight per day at 4°C and 250 (± 2) µmol CH_4 / g dry weight per day at 10°C. Community analysis revealed that enrichment cultures were dominated by members of Methylococcaceae (20%), Flavobacteriales and Sphingobacteriales. Other less abundant groups included known methylotrophs. The metabolic role of Flavobacteriales and Sphingobacteriales is surprising and will be further investigated. Future research will focus on the co-occurrence of methanotrophs with non-methane consuming methylotrophs and heterotrophs. A bioreactor system will be set up to study microbial interactions under controlled conditions under different methane and oxygen concentrations. The data obtained from these experiments will help to better understand changing microbial interactions and activities under warming conditions.

P032

Microbial communities and their networks in iron-rich methanic sediments of the Bothnian Sea

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The Bothnian Sea is an oligotrophic environment characterized by low salinity with high concentrations of reactive iron, methane, ammonium and low sulfate. Our previous analysis indicated active iron dependent anaerobic oxidation of methane in these sediments [1].
Here, we analyzed the sediment microbial community composition at various sampling sitesin the Bothnian Sea over a vertical scale in high-resolution. 16S rRNA amplicon sequencing and analysis revealed stratification of both bacterial and archaeal taxa along the geochemical gradients of iron, sulfate and methane. Two coastal sites had more similar communities than the central basin site located at a greater depth. The dominant groups of *Archaea* were comprised of *Thaumarchaeota*, *Bathyarchaeota*, *Woesearchaeota*, *Euryarchaeota* and unclassified archaeal lineages. Bacterial community was dominated by various lineages of *Gammaproteobacteria*, *Anaerolineales*, *Bacteroidetes*, *Flavobacteriales* and *Desulfobacterales.*
To gain a greater insight into the metabolic networks within the iron-rich methanic zone located below the sulfate-methane transition zone (SMTZ), we performed the total metagenomic sequencing of sediment-derived DNA. The analysis of assembled and binned contigs along with the directed BLAST-based analysis of functional biomarkers revealed the importance of several fermentative and respiratory pathways involved in nitrogen-, methane- and sulfur cycles. Our results indicated flexible metabolic capabilities of major microbial players, which can adapt to changing redox conditions and which distribution is most likely governed by the quality of available organic substrates.
Reference
[1] Egger, Rasigraf et al 2015 doi: 10.1021/es503663z

P033

Evaluation of the Cobas® Liat® and Panther Fusion® PCR systems for rapid detection of influenza virus

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Introduction Rapid identification of influenza virus A (Flu-A) and influenza virus B (Flu-B) is critical for infection control practice and reduces costs. We performed a comparative evaluation of two fully automated molecular platforms for the detection of these pathogens: the Cobas® Liat® PCR system (Roche Molecular Diagnostics) and the Panther Fusion® system (Hologic).
The Cobas® Liat® PCR system is a point of care system with a turnaround time of 20 minutes. Samples are analyzed one at a time. The Panther Fusion® system is a random access system with a capacity of 60 samples (12 slots of 5 tests each) and a turnaround time of 2.7 hours.
For evaluation, a collection of 14 clinical samples found influenza positive in routine clinical testing, as well as the QCMD 2017 influenza panel were tested on both platforms. Results of the clinical samples were compared to our in house real time PCR test.
Methods 14 nasopharyngeal swabs (3 in virus transport medium, 11 in eSwab medium) were selected of which 11 swabs were positive for Flu-A and 3 swabs were positive for Flu-B. For 2 Flu-B samples a 10-fold (10^{-1} and 10^{-2}) dilution series was made in Eswab medium to obtain a total set of 18 samples with a Ct-range of 25-36.

For evaluation of the Cobas® Liat® PCR system the influenza A/B & RSV assay was used with an input volume of 200 µL. For the Panther Fusion® system (Flu A/B/RSV assay) 500 µL of each sample was added to a Panther Fusion® specimen lysis tube (with 710 µL solution), of which 360 µL was used for the analysis.
Results 9 Flu-A positive samples tested positive on the Cobas® Liat® PCR system, the Panther Fusion® system and in the in house PCR. Two samples tested positive on the Cobas® Liat® PCR system only with estimated Ct values of 34.0 and 34.8.
The 3 Flu-B positive samples tested positive in the Cobas® Liat® PCR system and the Panther Fusion® system, while only 2 samples were positive in the in house PCR test. The 10-fold dilution serie of Flu-B positive sample 1 (10^{-1} and 10^{-2}) tested positive in all assays. The 10^{-1} dilution of Flu-B positive sample 2 tested positive in the Cobas® Liat® PCR system (estimated Ct-value 34.0) and the Panther Fusion® system (Ct-value 37.7), but negative in the in house PCR. The 10^{-2} dilution tested negative in all assays.
Both Cobas® Liat® PCR system and Panther Fusion® system scored 100% for the QCMD 2017 influenza panel.
Conclusion Compared to our in house PCR tests, both commercial systems were slightly more sensitive. Both systems are useful for rapid detection of influenza and can be easily implemented in daily routine diagnostics.

P034
Microbial community of the volcanic area at Pantelleria Island, Italy
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Volcanic ecosystems are characterized by high temperature, low pH and emission of the greenhouse gasses methane and carbon dioxide. Despite these hostile conditions, microbial activity is observed in these ecosystems. Microbes are involved in the elemental cycles of carbon, nitrogen and sulfur. The aim of the project is to obtain a fundamental understanding of the microbial ecology of extremely acid terrestrial volcanic ecosystems. Pantelleria Island, south of Sicily, Italy, is a volcanic area, characterized by a high soil temperature (50-100°C), low pH (2.5-4.5) and methane, hydrogen and carbon dioxide emissions. Hydrogen and methane fractions in the gas are decreasing towards the surface. Oxygen is only measured in the top layer of the soil, up to 20 cm. The measured gas profiles can be described as the combined effect of mixing with air and microbial consumption. In order to link the physiochemical properties of the ecosystem to the microbial community, metagenome studies are performed. Using two different DNA extraction methods and Illumina sequencing, 30 genomes of over 90% completeness could be assembled. Many of the microorganisms in this extreme environment are only distantly related to known, cultivated bacteria. Furthermore, based on metagenomics analysis, the most abundant microorganism is a methanogen, belonging to the *Methanocella* genus. In addition, aerobic methanotrophs, belonging to the *Verrucomicrobia* and *Gammaproteobacteria*, are found. This research shows that the volcanic area of Pantelleria Island hosts a variety of novel microorganisms and many play an important role in the carbon cycle.

P035
Germination proteins and spore structure in *Bacillus cereus*
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Spore-forming pathogen *Bacillus cereus*, widely existing in nature, has a crucial role in food spoilage and may lead to foodborne disease. Hence the food industries aim at increasing our knowledge of *B. cereus* endospore structure stress resistance and germination mechanisms. These protective structures consist of a core with the genetic material encaged by protective layers that are composed of an inner membrane, a peptidoglycan containing cortex, the proteinaceous coat, and the equally protein rich exosporium. Together they provide the spore with unique resistance and functional properties.
In order to understand *B. cereus* spore germination, the organization of the germinant receptor proteins in the inner membrane is currently analyzed. Â Previous studies have indicated that the GerR receptor plays a dominant role in the germination of *B. cereus* strain ATCC 14579 and the CotE protein is important for coat and exosporium assembly. We will confirm these observations and extend them to the spatially resolved localization of the GerR receptor in the inner membrane using reporter proteins with GFP, mCherry and m mTurquoiseÂ Â fluorescent reporter proteins. In particular, we address whether there are GerR protein foci analogous to the germinosome GerA and GerK protein protein foci seen in *Bacillus subtilis*.Â
Our lab has developed a ‘one-pot’ spore processing method for mass spectrometric analysis of proteins from all spore layers, which enables time lapse proteome analyses in spore formation and germination of the insoluble and soluble spore protein layers while avoiding sample loss. Here, we plan to apply this method on *B. cereus* to analyze the composition and structure of the spore layers, in particular their protein cross-linking. To this end we will use differential labelling with heavy nitrogen (^{15}N) in order to follow the progression of spore protein cross-linking during sporulation. We will do this analogous to the work done by Abhyankar et al. in *B. subtilis*[1]. As there is no sporulation medium for *B. cereus* in which ammonium is the single nitrogen source, we will make our own 99.9% ^{15}N labelled yeast-extract and use this as nitrogen source in sporulation medium, hence labeling the developing *B. cereus* spores. Preliminary data shows that sporulation can be completed at 30â• °C within 48 hours in the yeast extract based medium. In comparison, using regular Chemically Defined Growth and Sporulation (CDGS) medium the process takes 96 hours at 30â• °C. The spores obtained in the yeast extract based medium showed the same heat stress resistance as those obtained in CDGS. Our

final aim is to get insight in the structural organization of the spore inner membrane, cortex, outer membrane, coat, crust and exosporium layers in order to explain the mechanistic basis of (heat) stress resistance and spore germination kinetics in *B. cereus*.

Reference

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P036

Engineering a bacterium to produce hydrazine from wastewater -the rocket challenge-

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Hydrazine is a highly reactive molecule used as a rocket fuel due to its substantial reducing power. It is produced chemically by the Olin Raschig process. The only organisms known to (biologically) produce hydrazine are the anaerobic ammonium oxidizing (anammox) bacteria. Anammox bacteria are important players in the global nitrogen cycle and are applied in wastewater treatment for the removal of nitrogen compounds. Anammox bacteria convert the substrates ammonium and nitrite to dinitrogen gas via the intermediates nitric oxide and hydrazine. They perform this reaction in three consecutive steps: nitrite is converted to nitric oxide by a nitrite reductase (Nir), nitric oxide is then combined with ammonium to form hydrazine by hydrazine synthase (Hzs), and finally, hydrazine is converted to dinitrogen gas by hydrazine dehydrogenase (Hdh). In this project, we aim to transfer the two genes responsible for the conversion of nitrite and ammonium into hydrazine, *nir* and *hzs,* into the bacterial host *Wolinella succinogenes*. Besides, we aim to control the expression of both genes by means of an expression cascade that includes inducible promotors. By using this engineered host we could produce the rocket fuel hydrazine in a sustainable way from waste products.

P037

Making a 3D model of the polygonal anaerobic methanotroph *Methylomirabilis* sp. and localization of the nitric oxide dismutase enzyme

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Methylomirabilis bacteria perform the anaerobic oxidation of methane (AOM) coupled to denitrification. It is proposed that these bacteria denitrify nitrite to nitric oxide and use a novel enzyme “nitric oxide dismutase” (NOD) to convert nitric oxide to nitrogen gas and oxygen. This internally produced oxygen is then used for the oxidation of methane to carbon dioxide. Next to their unusual metabolism, the ultrastructure of *Methylomirabilis* is also remarkable: the cells have an atypical polygonal cell shape with sharp edges that run along the cell length and end in a cap-like structure at the cell poles. It is hypothesized that this polygonal cell shape is formed by a surface protein layer (S-layer).
With this study we aim to gain more insight into the unusual polygonal cell structure of *Methylomirabilis* sp. by performing electron tomography on serial sections to obtain a complete 3D image and model of a cell. In addition, we will investigate the location of the novel NOD enzyme in the cell through immunogold localization.

P038

Preliminary results of the Invasive Bacteria *E.coli-Shigella* Study (IBESS study)

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Introduction: The bacteria *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei* and Entero-invasive *Escherichia* (EIEC) all can cause gastro-enteritis and are difficult to distinguish in the laboratory. Due to regulations for infectious disease control, laboratories are obligated to notify infections with *Shigella spp* towards public health services. Infections with EIEC do not have this notification obligation. To gain insights in diagnostics, incidence, health outcomes and economic consequences of infections with EIEC and to compare them with *Shigella spp*, the Invasive Bacteria *E.coli-Shigella* Study (IBESS study) was initiated. This is a report of preliminary results.
Methods: Inclusion in the IBESS study started at 1 January 2016 and ended 1 January 2018, targeting a sample size of 1100 inclusions, a response percentage of 60% patients and 440 cultured isolates. Included were patients with gastro-enteric complaints, of which *Shigella spp* and/or EIEC was detected in their fecal sample, which was submitted to one of 15 participating medical microbiological laboratories (MMLs) for regular diagnostics. The MMLs performed their regular diagnostics, and sent a fecal sample, a DNA-extract and, if applicable, the selected *Shigella*/EIEC isolate to the IBESS group. Specific Shigella agar plates were sent to the IBESS group for extended culture protocols if an isolate could not be obtained. All isolates were identified and serotyped. Epidemiological and clinical data from patients was collected by an infectious disease nurse if consent from the patient and their physician was obtained.
Results: In total, 1211 patients were included. In 416 inclusions, 419 isolates were cultured, consisting of 234 *S. sonnei*, 102 *S. flexneri*, 65 EIEC, 10 provisional *Shigella*, 3 *S. boydii*, and 5 isolates of which a definite identification could not be made. Data collection from patients was completed for 1087 inclusions, of which 730 were willing to participate, resulting in a response percentage of 67.2%. The IBESS study reached a reasonable geographic distribution, except for the

regions Gelderland, Rotterdam, Haaglanden and the north of Noord-Holland, probably due to the absence of participating MMLs in these areas. 48.2% of patients were male, age was not normally distributed with a median of 38 (IQR: 25-55) years. 79.5% of patients reported ingestion of possibly contaminated food or water, and 75.3% of them had a recent travel history. The most reported symptom was diarrhea by 97.1% of patients. 77.1% of patients held bed rest, and 44.5% took a leave of absence from work or school. Most patients (92.7%) visited their general practitioner (GP). Conclusion: The IBESS study achieved more inclusions than targeted. The response percentage for the IBESS study is high with 67.2%, while the number of cultured isolates is lower than expected. The incidence for EIEC in the Netherlands is lower than *S. sonnei* and *S. flexneri*, however it is also possible that with the currently used diagnostics, *Shigella spp.* is more easily detected in stool samples. The comparison of infections with EIEC to infections with *Shigella spp* regarding health outcomes and economic consequences, will be made when all the collected epidemiological and clinical data from patients is available.

P039

Unraveling the phylum Planctomycetes

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Introduction
Members of the phylum Planctomycetes, a member of the PVC (Planctomycetes-Verrucomicrobia-Chlamydiae) superphylum, have in the past been described to lack peptidoglycan and to possess a compartmentalized cell plan. However, both assumptions have recently been challenged turning most of the previous hypotheses of the phylum upside down. Nevertheless, Planctomycetes still have many unusual features. They divide FtsZ-independently by an extraordinary polar budding mechanism. Associated with this atypical trait is a life style switch from sessile mother to planktonic daughter cells. Also, planctomycetal genomes encode numerous giant genes and bear the genomic potential for secondary metabolite production.
With relatively few isolates being publicly available, the whole phylum is notably under-sampled. Therefore, we focused our efforts on broaden the strain and data basis for future insights.
Methods
For this purpose, we sampled multiple aquatic habitats around the globe and isolated about 80 new planctomycetal strains from various biotic and abiotic surfaces. To allow detailed analyses we produced closed as well as high-quality draft genomes of the novel isolates as well as high-quality images.
Results & Conclusion
Most of these strains represent novel taxa up to the order level, capturing an unpreceded degree of diversity. In total, we brought more novel Planctomycetes into pure culture than currently described species of this phylum exist. The exploration of the gained genomic information not only enables deep insights into the taxonomy of the phylum and its subtaxa, but especially into their unique cell biological characteristics and their promising secondary metabolism.

P040

Implementation of point-of-care testing and a temporary influenza ward in a Dutch hospital: trial protocol

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Introduction The seasonal influenza epidemic poses a significant burden on hospitals, both in terms of capacity and costs. To increase efficiency of patient care, we decided to implement a point-of-care test (POCT) for influenza on the Emergency Department (ED) of the Jeroen Bosch Ziekenhuis, a tertiary teaching hospital in ‘s Hertogenbosch with 683 beds. In parallel, a separate ward for influenza-positive patients was established. We hypothesize that shortening the time to diagnosis will decrease unnecessary infection control measures and lead to shorter time from ED presentation to hospital admission. A separate ward for influenza-positive patients could reduce nursing workload and capacity problems and increase infection control.
Methods A PCR-based point-of-care-test for influenza A/B/RSV with a turnaround time of 20 minutes (Cobas Liat, Roche Molecular Diagnostics) was placed at the ED and at the Microbiology laboratory reception desk. Two nose/throat (NT) swabs per patient are collected in Eswab medium (Copan Diagnostics). One swab is used for direct POCT at the ED or microbiology laboratory reception desk, depending on time and day: during office hours, influenza testing is performed by the laboratory reception personnel; during weekends and nightshifts, ED nurses perform the test. Both have been trained by lab technicians. The second NT swab is used for confirmatory testing at the laboratory using a Panther Fusion Flu A/B/RSV test (Hologic). At least the first 75 ED-samples and 15 reception desk-samples will be tested pairwise; after that, random pairwise testing will be performed. Furthermore, the temporary influenza ward has been equipped with 15 beds for all influenza-positive patients, except for those requiring special care at another ward (e.g. intensive care or hematology). After five days, these patients are either dismissed or moved to another ward.
Results are expected after the 2017-2018 influenza season. We will describe our experiences in the process of implementing the test and setting up the influenza ward. We will compare the performance of the POCT in daily practice at the ED with the laboratory test and describe its sensitivity and specificity. Further endpoints include time to diagnosis, time from ED to hospital admission, length of hospital stay, use of antibiotics and costs, all retrospectively comparing the influenza season of 2016-2017 with that of 2017-2018.
Conclusion These data will learn us whether implementing an influenza POCT at the ED is possible and whether a temporary influenza ward leads to increased efficiency of patient care, with possible secondary benefits such as a

decrease in costs and decreased unnecessary use of antibiotics.

P041

Molecular analysis of the nitrite-oxidation system of *Nitrospira moscoviensis*

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Nitrite-oxidizing bacteria (NOB) catalyze the second step of nitrification, the formation of nitrate from nitrite, and play a significant role in the nitrogen cycle. Members of the genus *Nitrospira* appear to be the most diverse and widespread NOB. They also have been found to be the predominant NOB in wastewater treatment systems. Despite the environmental and biotechnological importance of *Nitrospira*, we lack knowledge on the composition of their nitrite oxidation system. Further it is not known how electrons derived from nitrite oxidation are channeled into the respiratory chain for energy conservation.
The nitrite oxidation system of *N. moscoviensis* has been demonstrated to be located in the periplasm. It was suggested the nitrite oxidation system could be membrane associated and form periodically arranged hexagonal particles, which were observed on the cytoplasmic membrane of *N. moscoviensis*. Genome analysis of *Nitrospira* revealed a nitrite oxidoreductase (NXR) that is more closely related to the enzyme of anaerobic ammonium-oxidizing bacteria than to the *Nitrobacter* NXR. The *N. moscoviensis* genome encodes multiple copies for the catalytic NXR alpha and beta subunits, but no *Nitrobacter*-like membrane anchored gamma subunit could be identified. Several candidate genes for either a soluble or membrane anchored gamma subunit were proposed, but their involvement in the NXR complex awaits experimental confirmation.
In this study we investigated the NXR subunit composition and localization. Immunoblotting of *N. moscoviensis* with antibodies raised against the NXR of *Kuenenia stuttgartiensis* resulted in labelling of the NXR alpha and beta subunits, as confirmed by MALDI-TOF. Additionally, a third band with the size of 30 kDa was labelled, which might represent the NXR gamma subunit. To localize the NXR, immunogold labelling and cell imaging by EM was performed on *N. moscoviensis* cells and spheroplasts. This confirmed the localization of the NXR in the enlarged periplasmic space. Interestingly, no labelling of spheroplasts was observed, indicating a soluble nature of NXR.
A soluble NXR gamma subunit candidate was identified as one of the dominant proteins in the *N. moscoviensis* soluble fraction by SDS-PAGE separation and MALDI-TOF identification. The gene corresponding to this candidate protein was highly expressed in biomass grown under stable conditions in a continuous stirred tank reactor. Complexome analysis showed that the NXR alpha and beta subunit co-migrated with 3 additional proteins on a blue native gel, one of which corresponds to the size of the soluble gamma subunit candidate. Based on these observations we propose the NXR to be a soluble periplasmic complex consisting of three subunits, although we cannot exclude weak interactions with complexes of the membrane-bound respiratory chain.

P042

Fast progression in the whole genome sequencing project of *Mycobacterium tuberculosis* at the RIVM; implications for the laboratory diagnosis of tuberculosis

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Background: In 2016, a four-year nationwide project was initiated, in which all *Mycobacterium tuberculosis* complex cultures, isolated in the Netherlands and received at the RIVM, are subjected to whole genome sequencing (WGS). This technique is applied simultaneously with the regular laboratory diagnosis of tuberculosis (TB) to facilitate a well-founded comparison of its functionality. This project focusses on the application of WGS for 1) the identification of (sub)species and genotypes within the *M. tuberculosis* complex, 2) detecting resistance mutations to predict antibiotic resistance, and 3) determine the genetic distance between isolates to follow transmission.
Methods/Results: In 2016, there were 535 *M. tuberculosis* complex isolates available to compare WGS with the traditional variable number of tandem repeat (VNTR) typing regarding epidemiological investigations. In 2016, 13% of cases were clustered by WGS in comparison to 25% with VNTR typing, while the confirmation of suggested epidemiological links by municipal health services (MHSs) was 31% for VNTR clustered isolates but increased to 57% for WGS clustered isolates. This will increase the efficiency in epidemiological investigations.
Next to this, in close collaboration with Oxford University in England, a new and more reliable algorithm has been developed to identify the (sub)species and genotype families within the *M. tuberculosis* complex. The more exceptional (sub)species in addition to genotype families in the complex can be identified by this algorithm.
In a large international study including approximately 650 Dutch samples, coordinated by Oxford University, the predictive value of mutations detected by WGS in drug resistance associated genes was examined on basis of a database of more than 10,000 sequences. The negative predictive value (prediction of drug sensitivity) was so high (98%) that in the near future it will be possible to stop with 90% of the phenotypic drug susceptibility tests in the Netherlands.
Conclusion: Starting in 2018, all MHSs will be provided with information on clustering of TB cases on basis of WGS, in addition to clustering on basis of VNTR typing. Furthermore, WGS will replace the initial phenotypic drug susceptibility testing and will be applied for identification of all known (sub)species of the *M. tuberculosis* complex and to detect genotype families in the very near future. This will reduce the cost of the laboratory diagnosis of TB significantly.

P043

Purification and characterization of the mycosin protease of the ESX-5 secretion system of pathogenic mycobacteria.

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Introduction:
Mycobacteria, such as *Mycobacterium tuberculosis*, require a set of specialized secretion systems for bacterial viability and virulence, the Type VII secretion systems. Pathogenic mycobacteria have up to five of these systems, ESX-1 to ESX-5, three of these systems have now been shown to be involved in either nutrient uptake, immune modulation and/or pathogenicity. The composition of the 1.5 MDa, membrane-embedded, ESX-5 system has been characterized, consisting of four conserved membrane components, $EccB_5$ to $EccE_5$. More recently, the first electron microscopy structure of the ESX-5 secretion channel has been obtained, showing a sixfold symmetry and a size that allows the translocation across the inner membrane. However, there is a fifth conserved component, MycP, or the mycosin protease. This periplasmic protein is not an integral part of the core secretion complex, although it is essential for secretion. It was found that MycP loosely associates with the ESX complex and thereby stabilizes it. In addition to this, only one proteolytic substrate is known for any of the mycosins; EspB for $MycP_1$. In order to obtain a better biochemical understanding of $MycP_5$ we tried to purify it from *Escherichia coli*, however all attempts resulted in insoluble, instable or not properly folded proteins.

Methods & Results:
Alternatively, in this study we used the fast-growing and non-pathogenic *Mycobacterium smegmatis*, for the efficient overproduction and purification of $MycP_5$. In addition to this we introduced and purified the entire ESX-5 complex from *M. smegmatis*, allowing us to rapidly and efficiently screen the effect of different mutations, in order to identify the potential interaction partner(s) of $MycP_5$ within the ESX-5 secretion complex.
We were able to successfully overproduce soluble $MycP_5$ from *M. smegmatis*, both periplasmic and cytoplasmic. And after purification we set out to identify potential proteolytic substrates using a peptide library. Furthermore our results show that, by introducing specific mutations, we can enforce a stable interaction between $MycP_5$ and the ESX-5 secretion complex.

Conclusion:
Our results show that *M. smegmatis* is an efficient bacterium for the overproduction of proteins from pathogenic and slow-growing mycobacteria. Furthermore we confirmed that $MycP_5$ does interact with the secretion channel but that this interaction is lost during the purification of the native complex from e.g. *M. tuberculosis*.

P044

Outer Membrane vesicles of *E. coli* mediated resistance to ampicillin by carrying resistant genes and proteins

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Introduction: Antibiotic-resistant bacteria are one of the biggest threats in modern medicine. Understanding the mechanisms of resistance as well as the transmission of resistance genes is crucial for the development of new classes of antibiotics. Outer Membrane vesicles (OMVs), which are released by Gram-negative and -positive bacteria, have been found to play crucial roles in bacterial pathogenicity. Recent studies suggested that MVs are involved in the protection against antibiotic-mediated killing.
Objectives: Here we hypothesize that OMVs contribute to antibiotic resistance. First we aim to demonstrate the presence of resistant genes and functional enzymes within outer membrane vesicles. Then we will investigate whether OMVs can protect susceptible *E.coli* from antibiotics-meditated killing.
Methods: One *E.coli* strain with plasmid encoding the beta-lactamase CTX-M-15 resistance gene and susceptible *E.coli* strain were used. Antibiotic susceptibility profiles of the strains were determined using a VITEK®2 system. OMVs were isolated from bacterial cultures by a combination of ultrafiltration and size exclusion chromatography. The presence of OMVs was confirmed by tunable resistive pulse sensing in addition to the Bradford assay to determine the protein content. PCR and nitrocefin assays were used to detect resistance gene and active beta-lactamase, respectively. Disc diffusion test and microtiter plate test were used to investigate the efficacy of antibiotics and protection respectively when ampicillin was pre-incubated with OMVs derived from resistant or susceptible bacteria.
Result and conclusion: Our data show that *E.coli* releases a significant amount of OMVs during 18 h of culturing. Also, we could demonstrate the presence of DNA and most importantly resistance genes and functional beta-lactamase protein inside the MVs. Interestingly, OMVs-derived from resistant bacteria decrease the efficacy of ampicillin and enhance the growth of susceptible *E.coli* when exposed to ampicillin comparing with OMVs- derived from wild type *E.coli* or PBS. This finding emphasizes the contribution of OMVs in antibiotics resistance as an important virulence factor for bacterial surviving.

P045

Stress-induced release of cell wall-deficient cells in filamentous actinomycetes

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The cell wall is a shape-defining and stress-bearing structure that envelopes almost all bacteria and helps to protect them in fluctuating environments. We here show that hyperosmotic stress has a dramatic and unexpected effect on the morphology of filamentous actinomycetes, leading to the extrusion of a new cell wall-deficient (CWD) cell type. These cells reinitiate peptidoglycan synthesis and are able to revert to the mycelial mode-of-growth. Sporadically, we detected that CWD cells gained the ability to proliferate in the wall-deficient state as so-called L-forms, which coincided with the accumulation of mutations. For decades, such L-forms have been generated in laboratories under highly specialized conditions, invariably aimed at interrupting cell wall synthesis. Given that formation of these CWD cells is common in filamentous actinomycetes, our work thus highlights the formation of CWD cells as a natural adaptation to environmental stress.

P046

Clinical validation of a dedicated Flu/RSV assay on the new geneLEAD VIII platform

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Introduction
Microbiology laboratories have been diagnosing viral respiratory tract infections by molecular methods using batch wise testing for years. During the winter months, rapid availability of in particular influenza and RSV results will improve patient management. Random access, sample in result out platforms have become available, reducing the time-to-result. Recently, the geneLEAD VIII platform (Diagenode Diagnostics, Liege, Belgium) was launched, enabling testing of 1-8 samples simultaneously. This platform provides results within 2 hours with 10-15 minutes hands-on time. The performance of a new InfluenzaA/B+RSV assay (R-DiaFluRSV™) on this geneLEAD VIII platform was established.
Methods
During the 2017 and 2018 winter season, samples submitted for viral respiratory pathogen testing were subjected to the geneLEAD VIII platform and compared to both the lab developed tests (LDT) of the LUMC and the CE-IVD cleared influenza A/B R-gene® and RSV/hMPC R-gene® assays (Argene, BioMerieux). After the prospective part, stored samples were used to ensure at least 80 influenza positive and 100 RSV positive swabs, nasal washes and nasopharyngeal aspirates.
Results
The first 88 samples resulted in for 6 influenza A , 14 influenza B and 14 RSV positive samples whereas 53 samples were negative for these targets. However, using the extended LDT panel of the LUMC, eight samples were positive for hRV, six for hMPV, two for HCoV-HKU1, one for HCoV-OC43 and one for hPIV4 (*NB: completed study results will be presented at the meeting in March*). No discrepant results were observed so far.
Conclusion
The R-DiaFluRSV™ assay on the geneLEAD VIII platform enables diagnostic testing for Flu/RSV with reduced hands on time and provides results in a clinically relevant timeframe. Results are in concordance with the Argene assays and the LUMC lab developed test.

P047

Structure of a mycobacterial type VII secretion system membrane complex

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Introduction
Type VII secretion (T7S) systems are used by mycobacteria to translocate a wide range of proteins across their distinct and complex cell envelope. Pathogenic mycobacteria, including *Mycobacterium tuberculosis*, have up to five of these systems termed ESX-1 to ESX-5, each having its own crucial role in viability and/or virulence. T7S does not resemble other known specialized secretion systems and the actual mechanism of membrane transport is still largely unknown. As a first step in this analysis, we have previously isolated the ESX-5 membrane complex from *Mycobacterium marinum* and determined that this macromolecular machinery has a size of ~1.5 MDa and consists of four membrane components, i.e. EccBCDE (Houben et al., Mol Microbiol 2012). The aim of this study is to understand the mechanism of protein transport across the mycobacterial cell envelope by elucidating the structure and further characterizing the T7S membrane channel.
Methods
We have reconstituted, isolated and analysed the ESX-5 membrane complex by negative stain single particle electron microscopy and a suite of biochemical and biophysical techniques.
Results
To more easily analyse the structure and function of T7S systems, we reconstituted the ESX-5 system in the avirulent and fast-growing mycobacterial species *Mycobacterium smegmatis* that lacks ESX-5. The reconstituted system proved to be active and was efficiently secreting specific ESX-5 substrates. Importantly, different components were relatively

overexpressed and the complex was properly assembled in the membrane. We subsequently purified the ESX-5 membrane complex to homogeneity and analysed the isolated particles through negative stain electron microscopy. This resulted in the first structural images of a T7S membrane channel at 13 Å resolution. The ESX-5 complex has a six-fold symmetry with the four components present in an equimolar ratio and displays a marked different architecture to other bacterial secretion systems.
The ESX-5 structure reveals highly flexible extensions at its cytoplasmic face that have not been observed in other secretion systems. We identified these subunits as the ATPase component of the ESX-5 system that is responsible for mediating interactions with secreted substrates. The unusual flexibility of these domains suggests a novel mode of substrate recognition and/or accommodation. We were able to locate two additional components in the membrane complex by modelling, gold-labeling and producing complexes of a mutant strain. The T7S channel is not large enough to span both membranes, indicating that this core complex is located solely in the inner membrane.
Conclusions
Together, these experiments yielded the first structural image of the T7S system membrane complex and provides a first insight into a possible mechanism of protein transport through these systems.

P048
Third generation cephalosporine and carbapenem resistance in Streptococcus mitis/oralis. Results from a nationwide registry in the Netherlands.
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Background: Beta-lactam resistance in *Streptococcus mitis* group bacteria is especially important in hematological patients. Mucositis, neutropenic fever and sepsis are common complications in these patients and adequate empirical antibiotic treatment is paramount. A neutropenic patient with *S. mitis/oralis* bacteraemia with borderline meropenem susceptibility (MIC=2.0 mg/L) prompted us to investigate the prevalence of third generation cephalosporine and carbapenem resistance in the Netherlands.
Materials/methods: The Dutch National antimicrobial susceptibility databank (ISIS-AR) was searched for susceptibility results of *S. mitis/oralis* isolates from 2012 through 2016. ISIS-AR is managed by the Dutch National Institute for Public Health and the Environment and collects susceptibility test results of all bacterial isolates of 40 clinical microbiology laboratories, thereby covering 75% of hospitals in the Netherlands. The Dutch Society of Medical Microbiology advises laboratories to use the EUCAST guidelines for susceptibility testing and reporting. Only the first isolate of each patient was included for analysis.
Results: 3665 *S. mitis/oralis* isolates were included. Penicillin resistance and intermediate resistance was reported in 223 (6.1%) and 242 (6.6%) isolates. Ceftriaxon/cefotaxim resistance was reported in 152 isolates of 1907 tested for ceftriaxon/cefotaxim (8,0%). Meropenem resistance was reported in 2 isolates of 226 tested for meropenem (0.9%). Median penicillin MIC of isolates susceptible to third generation cephalosporines was 0.064 mg/L (IQR 0.032-0.19, n=467); 2.0 (IQR 0.875-6.0, n=59) in non-susceptible isolates; and 0.064 (IQR 0.032-0.25, n=341) in isolates with no third generation cephalosporine susceptibility reported. In the two isolates with meropenem resistance, penicillin MIC was >4 and 32 mg/L, and ceftriaxon/cefotaxim MIC was 4 and 12 mg/L.
Conclusions: In the Netherlands, from 2012 through 2016, third generation cephalosporin resistance was detected in 6% of *S. mitis/oralis* isolates tested. Carbapenem resistance was very rare. Elevated penicillin MIC in *S. mitis/oralis* isolates should prompt additional cephalosporine and carbapenem susceptibility testing. This is particularly relevant in isolates of hematological patients.

P049
Optimizing high-molecular weight DNA extraction protocols from complex samples.
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The intestinal microbiota is an important reservoir of resistance genes. The probability of transfer of these resistance genes to potential pathogens depends on their association with mobile genetic elements (MGE's). Current optimized metagenomic sequencing techniques result in short DNA reads (between 100bp to 300bp), and assembly of these short reads often only covers the resistance gene but does not provide information on its genetic context, such as association with MGE's. Oxford Nanopore sequencing does allow sequencing of long reads and will thus cover long genomic regions in the microbiota thereby revealing the genomic context of these resistance genes. However, this does require large amounts of high-molecular weight (HMW) input DNA between 5-12 Kb in size. In this study, we aim to redesign protocols to a fast and efficient method to extract HMW DNA from low amounts of human feces in order to study the dynamics of the gut microbiota and resistome using long-reads metagenomics. Both DNA yield and size were compared between different methods, including commercially available kits. As part of the optimization of protocols to further reduce DNA shearing we determined the effect of replacing bead-beating, a frequently used method to lyse bacteria, by enzymatic lyses.
We compared 11 different methods and observed that the protocols yielding the highest molecular DNA were 'Bioline isolate fecal DNA kit' and 'PSP spin stool DNA plus kit' both using a bead-beating step. Compared to the enzymatic lysis, the incorporation of bead-beating does results in fierce fragmentation of DNA when applied on the Bioline kit (majority of DNA between 2-12 Kb), but in a significant lesser extend in case of the PSP kit (majority of DNA around 12 Kb). In conclusion, these two methods are the most promising in terms of total yield (around 2.5 µg of DNA, while using 200mg

of feces) and DNA molecular size. The efficiency of the tested methods in lysing most of the bacteria present in the intestinal microbiota will be confirmed first through analysis of the microbiota composition using 16S rRNA profiling.

P050

The urogenital microbiome as a predictor for in vitro fertilization with or without intracytoplasmic sperm injection outcome

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Introduction Success rates for in vitro fertilization (IVF) vary between 25 and 35%. It is hard to predict who does or doesn't get pregnant after an embryo transfer (ET). Recently, by serendipity, it was discovered that prior to treatment, the urogenital microbiome can predict pregnancy outcome after IVF with or without intracytoplasmic sperm injection (ICSI).
Methods In this prospective study a total of 301 IVF/IVF-ICSI patients were included between June 2015 and March 2016. Women obtained a vaginal sample before the start of the actual IVF/IVF-ICSI procedure. Microbiome composition and evaluation of its predictive accuracy for IVF/IVF-ICSI outcome was determined with the IS-pro technique.
Results In total 192 of the 301 patients had an ET and an evaluable vaginal microbiome. Profiles could be assigned a community state type based on dominant microbial species. A low percentage of Lactobacillus had a strong association with embryo implantation failure. With a combined prediction model based on a small number of microbial parameters, a subgroup of patients (26%) could be identified with a low chance of pregnancy following ET (specificity 97%). This model predicted the non-pregnant women after the first ET. This failure to implant was correctly predicted in 32 out of 34 patients based on the vaginal microbiome, resulting in a predictive accuracy of 94%.
Conclusion Microbiome profiling with IS-pro enables correct prediction of embryo implantation failure prior to the start of an IVF/IVF-ICSI treatment with a high predictive accuracy in a subgroup of patients. Knowledge of their microbiome profile enables couples to make a more balanced decision whether they want to continue with the treatment or not. Hence, the physical and emotional burden of an IVF treatment can be avoided.

P051

Nitrogen removal by a co-culture of comammox and anammox bacteria

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Nitrification is a key step in the biogeochemical nitrogen cycle and is greatly impacted by anthropogenic activities. It is a two-step process, carried out by different microorganisms. First, ammonia is oxidized to nitrite by aerobic ammonia-oxidizing bacteria or archaea, and subsequently to nitrate by nitrite-oxidizing bacteria. Although it would be energetically advantageous to perform complete nitrification, no microorganism was known to catalyze this until recently, when complete ammonia oxidation (comammox) carried out by bacteria of the genus *Nitrospira* was reported. The comammox enrichment described in 2015 had been derived from biomass of a recirculating aquaculture system in a hypoxic bioreactor. The biomass consisted of a co-culture of *Nitrospira* and anammox bacteria, a puzzling combination given the fact that *Nitrospira* spp. are aerobic and anammox bacteria need anoxic conditions to be active. In addition, the oxidation of ammonia by the comammox bacteria would require oxygen for ammonia activation. Two different hypotheses were contemplated to explain the activity of this co-culture. In the first one, *Nitrospira* was considered to act as an ammonia oxidizer or a canonical nitrite-oxidizing bacterium, oxidizing either ammonia or nitrite to nitrate and thus competing with anammox for its substrates. In the second and more plausible conjecture *Nitrospira* would act as nitrite reducer, using the nitrite oxidoreductase in the reverse direction, coupled to ammonia oxidation. The produced nitrite would be used together with ammonium by anammox to generate dinitrogen gas.
The aim of this project is to characterize the microbial community in a novel anammox/comammox enrichment culture sustained on synthetic medium, and to elucidate the metabolic link between the two nitrogen cycle microorganisms. In order to answer these questions, activity assays were performed using stable isotopes (^{15}N-labelled ammonium and nitrate) to test for anammox and denitrification activity. The isotopic composition of the produced nitrogen gas was analyzed, showing a stable increase of $^{29}N_2$ from ^{15}N-ammonium, characteristic for the anammox reaction. In addition, an increase in $^{30}N_2$ was detected, indicating the production of ^{15}N-labelled nitrite from ammonia through aerobic ammonium oxidation. Furthermore, by employing fluorescence *in situ* hybridization (FISH), we observed that the culture was dominated by anammox (>40% of the total bacterial biomass), and *Nitrospira* contributed the majority of the remaining population. The unraveling and better understanding of the comammox/anammox interaction will help to better comprehend nitrogen cycling in engineered systems and change established perceptions about the roles of ammonia-oxidizing microorganisms.

P052

Subcellular localization of the nisin biosynthesis machinery and its interactions

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The nisin synthetase complex consists of dehydratase NisB, cyclase NisC and ABC transporter NisT. However, direct isolation of such a complex was reported to be unsuccessful. The aim of this study is to characterize the subcellular localization of the NisBTC synthetase complex and understand the interactions of its components with the ultimate goal to develop more efficient production systems for engineered lantibiotics. For this, we use the fluorescent proteins GFP and mKate2 to label NisB, NisC and NisT and determine the spatial distribution of these fusion proteins in *Lactococcus lactis* using single molecule fluorescence microscopy. In total, 12 fusion proteins were constructed and the fluorescent signal was determined for all of them. The fluorescent signal of the NisT fusion protein is distributed uniformly and circumferentially, in a pattern consistent with membrane localization. The majority of NisB fusion protein accumulates at only one of the two poles. The NisC fusion protein is shown to be localized to both membrane and cytoplasm. In addition, all of fusion proteins are able to produce active nisin, which indicates that results obtained from microscopy are reliable and not artifacts. The localization of NisB, NisT and NisC from fluorescence microscopy are consistent with previous reports. However the polar localization of NisB and the circumferential distribution of NisT suggest that the modification and export of nisin may occur at different places.

P053

Novel small lantibiotics against *Streptococcus pneumonia*

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Introduction:

S. pneumoniae is an important pathogenic streptococcus which causes invasive diseases such as sepsis, meningitis, and pneumonia. Lantibiotics are lanthionine-containing antibiotic peptides. Lantibiotics are a subclass of lanthipeptides that exhibit activity against pathogenic bacteria comparable to that of medically used antibiotics. In a recent annual report on global risks, the World Economic Forum concluded that “arguably the greatest risk to human health comes in the form of antibiotic-resistant bacteria”. This urgently necessitates further search for new antibiotics. Nisin produced by *Lactococcus lactis*, is the best studied lantibiotic to date. A precursor peptide containing a leader sequence is involved in recognition by the modification enzymes, the serine/threonine dehydratase NisB and the cyclase NisC, which couples the dehydrated residues to cysteines thus forming lanthionines, and the transporter NisT. The mature active nisin molecule is released after translocation and cleavage of the modified precursor by the protease NisP and contains five (methyl)lanthionine rings and three dehydrated residues. Nisin forms pores in the cell membrane and also inhibits the cell wall synthesis by binding lipidII, a cell wall precursor.

Truncated nisin-(1-22) variants do not form pores but still have antimicrobial activity as do other small lantibiotics like epidermin and gallidermin. Previous studies demonstrated that libraries of lantibiotics can be obtained via randomization of amino acid ring positions [Rink 2007; Plat 2011]. It was furthermore demonstrated that from these libraries lantibiotics with enhanced target-cell specific activities could successfully be obtained. These and new libraries will be tested against *S. pneumoniae*. In a second approach different lipidII binding domains will be combined in one lantibiotic, for instance the lipid II binding domains of nisin and of lacticin 3147.

Methods:

For novel libraries and randomization of lanthibiotic rings methods described in Rink 2007 has been followed.

Results:

The work is ongoing and possibly by the time of the KNVM & NVMM 2018 meeting some results can be presented.

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P054

Welcome to the Neighborhood - The Capability of Planctomycetes to shape Biofilms

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In the environment biofilms can be found on almost all surfaces, allowing organisms to colonize even extreme ecological niches. This comes with negative impacts on mankind. Biofilms on wounds or the urinary tract can cause major health issues as antibiotic resistance is strengthened. Additionally, biofilms are major drivers of biofouling causing economic

losses. In many biofilms, like on the surfaces of phototrophs, Planctomycetes are abundant. Since these bacteria are rather slow growing, it is still unknown how they maintain their abundance in biofilms. Planctomycetes are amongst the 'talented producers' of novel secondary metabolites and we recently elucidated the first planctomycetal bioactive structure. Thus, Planctomycetes have the potential to maintain their position in the biofilm by 'chemical warfare'. Especially biofilms on algae have been a rich source to isolate novel Planctomycetes. We tested the ability of Planctomycetes to colonize algal structures in an artificial system, where algal powder was added to a planctomycetal culture. The used planctomycetal strain was previously isolated from an algal biofilm and immediately attached to the algal surfaces. Subsequently, we investigated biofilms on seagrass leaves by scanning electron microscopy (SEM), amplicon sequencing and metagenomics. We found complex communities in which each bacterium must stand its ground to thrive. Based on amplicon analyses, Planctomycetes can constitute up to 80% of the analyzed sequences obtained from such biofilms. But how is such a dominance even possible? By evading areas of the biofilm where too many competitors are present e.g. by a nomadic lifestyle? Or by making use of bioactive molecules, to fight against their competitors or invite others to join their neighborhood.

P055

Prevalence of *vanC* and *vanD* within a tertiary hospital in the Netherlands How isolation of a non-vanA/non-vanB vancomycin-resistant Enterococ provoked development of vanC and vanD PCR's and hospital-wide screening

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Background: Vancomycin-resistant *Enterococci* (VRE) can rapidly spread through hospitals and possess health risks, particularly for the immunocompromised. Therefore, our hospital, a tertiary hospital in the south of the Netherlands, employs a screening program, whereby. rectal swabs are collected from patients and inoculated overnight in broths prior to screening by PCR for two vancomycin resistance genes: *vanA* and *vanB*. PCR-positive broths are streaked on VRE selection agar to check for growth of VRE.
Early November 2016, a clinical *vanA*- and *vanB*-negative isolate of VRE was detected in a patient, which was missed in our screening.. This finding raised the possibility that an undetected VRE was spreading within our hospital. Here, we describe our actions to unravel the resistance mechanism of this isolate and how we subsequently determined the prevalence of vancomycin resistance genes in our hospital.
Methods: The non-*vanA*, non-*vanB* VRE was analyzed by whole-genome-sequencing (WGS) to unravel the resistance mechanism, and was shown to be *vanD*-mediated. To determine whether our surveillance policy had to be changed, we designed and validated a real-time PCR for *vanC1/2/3*- and *vanD*-positive VRE. Rectal swabs were collected from 360 patients of 12 different wards and screened for *vanA*, *vanB*, *vanC*, and *vanD*. PCR-positive broths were streaked on CNA agar and VRE selection agar to determine the prevalence of VRE with either of the genes.
Results: WGS showed that the clinical isolate was *vanD*-positive and belonged to MLST type ST17/CT154, a VRE type previously described in Germany. Yet, the German isolates were *vanA*-positive, besides other additional resistance genes, suggesting that independent evolution.
Based on our in-house *vanA/vanB/vanC/vanD* PCRs, we determined the prevalence of these resistance genes in our hospital. Out of 360 patients, 139 (38.6%) proved positive for any vancomycin-resistance genes; 2 *vanA*, 38 *vanB*, 28 *vanC1*, 26 *vanC2/3*, 100 *vanD*. However, culture of PCR-positive broths on VRE selection agar only detected VRE in the two *vanA*-positive broths. To exclude a selection bias, broths were also streaked on CNA agar with a disc of vancomycin, visualizing VRE as colonies within a zone of inhibition. This resulted in 5 *E. gallinarum* (*vanC1*-positive), 1 *E. casseliflavus* (*vanC2/3*-positive), and two vancomycin-susceptible microorganisms: *E. faecalis* (negative for *vanA/vanB/vanC/vanD*), and *S. haemolyticus*. No *vanD*-positive VRE were found during our screening, limiting the possibility that *vanD*-positive VRE had spread within our hospital.
Conclusion: The prevalence of the four vancomycin-resistance genes was 0.6%(*vanA*), 10.6% (*vanB*), 7.8% (*vanC1*), 7.2% (*vanC2/3*), and 27.8% (*vanD*), respectively. Moreover, we retrospectively calculated the positive predictive value (PPV) of the *vanA* and *vanB* PCRs: *vanA* 82% PPV(N=65), *vanB* 8% PPV(N=426). No *vanD* was found (N=100), while *vanC1/2/3*-positive microorganisms do not grow on VRE-selective agars and are considered of low clinical relevance. We conclude that: 1. the *vanD*-positive VRE was an isolated finding. 2. The high prevalence of *vanD* in anal swabs and its low PPV makes it infeasible to include *vanD* in our VRE screening. 3. Yet, having validated the *vanC1/2/3* and *vanD* PCRs allows to rapid check for the presence of *vanC* and *vanD* in future *vanA/B*-negative VREs.

P056

Antimicrobial stewardship in companion animal clinics in the Netherlands (preliminary data)

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Introduction:
Due to the growing threat of antimicrobial resistance, more attention is paid to prudent antimicrobial use (AMU) both in human and veterinary medicine. In veterinary medicine, first efforts went into the reduction of AMU in farm animals. Since recent years, focus has also shifted towards AMU in companion animals. To encourage prudent AMU in companion animals, an antimicrobial stewardship programme (ASP) was developed. Aim of present study is to implement and to evaluate this ASP in 22 Dutch companion animal clinics.

Methods:
In total, 22 companion animal clinics dispersed throughout the Netherlands were included in the study, using a Stepped Wedge design. In the study period, finally all clinics were exposed to the ASP interventions separately. Antimicrobial prescription data from the participating clinics were gathered via the Practice Management Systems. DDDA's (Defined Daily Dose Animal) for 1st, 2nd and 3rd choice antimicrobials according to Dutch guidelines were calculated prior to, during and after the ASP, using these prescription data. The trend line data from the 3 years prior to the implementation of the ASP were used as baseline information. The ASP comprised three pillars: 1) theoretical training on rational AMU 2) communication training 3) individual feedback per clinic on prescription data from a so-called Support team (containing a veterinary pharmacologist, microbiologist, clinician and a hospital pharmacist).
Results:
Prior to start of the ASP, a decreasing trend in the total AMU of the participating clinics was visible. The use of 3rd choice antimicrobials (i.e. fluoroquinolones and 3rd generation cephalosporines) already showed a significant decrease. Preliminary data, during and after the ASP, from the first clinics indicate a continuing decrease in DDDA and a shift towards increased prescription of 1st choice antimicrobials at the expense of 2nd (e.g. penicillins with β-lactamase inhibitors and first generation cephalosporins) and 3rd choice antimicrobials.
Conclusions:
Prior to start of the ASP changes in AMU could already be recognized. Whether the ASP affects the AMU in all 22 clinics is currently being analyzed. These results will be used to evaluate the effect of the ASP.
Keywords:
veterinary medicine, companion animals, antimicrobial stewardship, antimicrobial prescription

P057
Clinical impact of genotypic characterization of an *in vivo* multi-resistant Herpes Simplex Virus Type 1
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Introduction:
Mutations in the thymidine kinase (TK) and the polymerase (*pol*) gene in herpes simplex virus (HSV) may lead to resistance of antiviral agents, particularly in the setting of immunocompromised patients with HSV reactivation. We present a 62-year old male with HSV-1 gingivostomatitis after cord blood allogenic stem cell transplant (alloSCT) for chronic lymphatic leukemia. During treatment with first acyclovir and subsequently foscarnet the painful oral lesions did not improve. Based on resistance analysis the therapy was effectively switched to cidofovir. We present the *in vivo* finding of an A605V mutation of the HSV-1 *pol* gene in an immunosuppressed patient. The genotypic characterization of the TK and *pol* gene of 3 sequential HSV-1 isolates of our patient are presented in this report.
Methods:
Three clinical HSV-1 strains were isolated from swabs of oral ulcerations collected on day 34, 49 and 104 after alloSCT. The antiviral treatment on these days consisted of acyclovir, foscarnet and cidofovir with famciclovir respectively. In addition, the 3 strains were cultured on Vero cells. Genotypic resistance analysis was performed by sequencing the TK (UL23) and *pol* (UL30) genes on the isolates of the original swab as well as on the 3 strains from viral culture.
Results:
Sequencing of the *pol* gene in the swab collected 49 days after alloSCT showed an A605V mutation, which is associated with *in vitro* resistance to both acyclovir and foscarnet. The 2 other isolates did not show relevant mutations in the *pol* gene. Analysis of the TK gene showed a premature stopcodon on position 281 only in the isolates collected 34 and 104 days after alloSCT, which is associated with reduced susceptibility to acyclovir and *in vitro* to brivudin and famciclovir. *In vivo* the A605V mutation was undetectable on day 104 post-alloSCT after 13 days without antiviral pressure with foscarnet. However, resistance associated mutations persisted *in vitro* when the strains were cultured in absence of antiviral pressure.
Conclusion:
1) We report an immunosuppressed patient with multi-resistant HSV-1 reactivation. Genotypic characterization revealed a rare mutation in the *pol* gene (A605V) which is associated with *in vitro* resistance to acyclovir and foscarnet.
2) The finding of resistance associated mutations corresponds with the clinical failure on acyclovir and foscarnet. Based on this finding treatment was successfully switched to cidofovir.
3) Although the A605V mutation was not detectable on day 104 after alloSCT, it is unclear whether the patient can be treated with foscarnet in the future. The premature stopcodon on position 281 reappeared under antiviral pressure and resistance associated mutations persisted *in vitro* without reversion to wild-type virus in absence of antiviral pressure. Therefore it could be possible that resistance associated mutations also persists in latent HSV.

Phenotypic susceptibility testing for ACV and FOS is still in progress and will follow.

P058

How to count our microbes: the effect of different quantitative microbiome profiling approaches

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Introduction

Recently, Quantitative Microbiome Profiling (QMP) has been proposed as a way to deal with many of the statistical and interpretative challenges that arise from the compositional structure of microbiome sequencing data. In this approach, microbiome sequencing is combined with flow cytometry to generate a quantitative microbiome profile (as bacterial cell numbers per gram of feces), rather than a relative profile. Since microbiome sequencing data are generated from metagenomic DNA whereas flow cytometry only counts bacterial cells, a large amount of free extracellular DNA might potentially introduce bias in this QMP approach.

We aimed to investigate the effect of two variations on the QMP approach:

1. removal of free extracellular DNA (by propidiummonoazide treatment) prior to microbiome sequencing in combination with flow cytometry (QMP-PMA), and;
2. combining microbiome sequencing with qPCR to quantify bacterial load (QMP-qPCR). Both methods were compared to the standard QMP and relative microbiome profiling (RMP) methods.

Methods

Fecal samples of 17 healthy volunteers were analysed by RMP, QMP, QMP-PMA and QMP-qPCR methods in duplo. Microbiome composition was determined by amplification of the V4 hypervariable 16S rRNA gene region and sequencing on a MiSeq platform.

Results

A moderate significant correlation was found between the quantification of bacterial load by flow cytometry and Ct-values from qPCR was moderate (Pearsson correlation:-0.501, p=0.047). Removal of free extracellular DNA by PMA resulted in a median decrease in the total number of 16S copies/gram faeces of 2.12^{10} [IQR: 7.31^{9}-3.86^{10}] as determined by qPCR, but failed to improve the correlation with bacterial cell counts as assessed by flow cytometry (Pearsson correlation:-0.413, p=0.112).

To assess the influence of the different microbiome profiling methods on the outcome of microbiome analyses, we examined the effect on genus abundance profiles. For this purpose, we assessed the concordance of the sample ranking for each genus (the sample with the highest abundance of a specific genus gets rank #1) between the different techniques measured as Kendall correlation coefficient (Tau).

QMP-PMA and standard QMP showed the highest concordance and the lowest spread, indicating that the rank order was not significantly affected by PMA-treatment.On the other hand,lowest concordance was observed for QMP-qPCR against all other methods. We next performed Bray-Curtis based PCoA for all the samples. We found that on average the dissimilarity between replicates(technical error) much lower compared to the distance between techniques.

Moreover, we confirmed that the microbial community structure of samples upon PMA-treatment is highly similar to standard QMP without PMA-treatment, whereas the community structure of samples upon QMP-qPCR is highly divergent

Finally, we performed enterotype analysis confirming that the observed enterotypes are no longer driven by a trade-off between Prevotella and Bacteroideswhen moving from relative abundance profiling (RMP) to quantitative profiling (both for QMP and QMP-PMA methods).

Conclusion

PMA treatment did not significantly improve quantitative microbiome profiling when compared to standard QMP, moreover qPCR-based determination of bacterial load appears inappropriate for quantitative microbiome profiling.

P059

Identification and characterization of beneficial Bacilli for tomato disease control

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Abstract:

Introduction: Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in the world and it has the highest acreage among all edible crops. However, tomato growing is frequently limited due to the high susceptibility to pests and diseases. The traditional chemical control causes a serious impact on both environment and human health. Therefore, seeking for environment-friendly and cost-effective green methods in agricultural production becomes crucial nowadays. Plant Growth Promoting Rhizobacteria (PGPR) can promote plant growth through direct solubilization of phosphate, production of phytohormones and fixation of nitrogen and/or inhibition of phytopathogens, and by triggering induced system resistance (ISR) of plants. It is considered to be a promising sustainable approach for crop disease control.

Methods: In our research, we aim at isolating and characterizing novel PGPRs and deciphering the molecular mechanisms during plant-microbe interactions, as well as mining and characterizing novel anti-bacteria/fungi compounds produced by PGPRs. To this end, we isolated 343 bacteria strains (of which 191 are Bacillus *sp*., and 28 endophytes) from healthy tomato, pepper and green onion rhizosphere soil and plant tissues of the Netherlands and Spain.

Results: *In vitro*antagonistic assays revealed that 20 strains have antimicrobial activity against *Rhizoctonia solani*; 24

are against *Botrytis cinerea*; 30 are against of *Verticillium dahliae* and 36 are against *Phytophthora infestans*. Meanwhile, 16s RNA analyses of isolated bacteria showed that most of them are gram positive bacteria, *Bacillus*, except 2 sequences from *Pseudomonas* and 1 sequence from *Paenibacillus*. Currently, *in vitro* and *in vivo* plant assays, together with genome sequencing and chemical characterization are ongoing to discover bioactive compounds.as well as the molecular mechanisms of the plant growth promoting effects.

P060

Cost analysis of outbreaks with Methicillin-resistant *Staphylococcus aureus* (MRSA) in Dutch nursing homes

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Introduction

Highly resistant microorganisms (HRMOs) are spreading over the world and are becoming increasingly less susceptible for antibiotics. To study the cost effectiveness of infection prevention measures in long-term care, it is essential to first fully understand the impact of HRMOs. The objective of this study is to identify the costs associated with multiple outbreaks caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) in Dutch long-term care institutions.

Methods

After an outbreak with MRSA, Dutch long-term care institutions can submit a reimbursement form at the Dutch Healthcare Authority (“Nederlandse Zorgautoriteit”; NZa) and get a part of the total costs reimbursed. These forms have been requested from the NZa. Details regarding the costs of the outbreak have been extracted from these forms and certain long-term care institutions have been visited in person to validate the data.

Results

34 complete reimbursement forms from the period between 2011 and 2016 were received from the NZa and have been included. The median of the cost per patient per day, was estimated at €83.80, varying between €16.89 and €1,820.09. We validated five reimbursement forms by visiting the institution and recalculating the costs. We found a non-significant positive difference of €26.07 with the original data (p=0.068).

Conclusions

This research is the first to give a national overview of total costs associated with an MRSA outbreak in long-term care institutions in the Netherlands. Overall, costs per patient per day seem lower than in a hospital setting, although total costs are much higher.

P061

Intravascular catheters colonized with *Candida* species without prior candidemia: An indication for pre-emptive antifungal therapy?

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Introduction:

Intravascular catheters are identified as a risk factor for candidemia. However, it is unclear what the strategy should be when a catheter tip is colonized with *Candida* species without prior positive blood cultures. In this situation, it is policy in the University Medical Center Utrecht to take control blood cultures and start pre-emptive antifungal therapy awaiting the results of the blood cultures. After 5 days, pre-emptive treatment is stopped on condition that the blood cultures remain negative and the patient is stable. The primary aim of this study was to evaluate the utility of this policy. A secondary aim was to make an inventory of the policy in other Dutch hospitals.

Methods:

A retrospective study was performed in the UMCU. Catheter tips colonized with *Candida* spp. without preceding candidemia were identified over a 7-year period (2011-2017). Primary outcome was candidemia or other forms of invasive candidiasis within 90 days. Patient and catheter characteristics were collected, as well as details on executed policy. Protocols in other Dutch hospitals were collected from the internet and by personal communication.

Results:

81 catheter tips comprising 77 episodes from 77 unique patients were included in the analysis. Candidemia developed in 3 patients (4%), all within one day after *Candida* spp. were isolated from a catheter. Two of these 3 patients also had other forms of invasive candidiasis, not yet diagnosed at the time of catheter removal. 91% of patients received systemic antifungal therapy with a mean of 10 days (14 days in the period 2011-2013, and 7 days in the period 2014-2017). No candidemia or other forms of invasive candidiasis were found in the rest of the patients during the 90 day follow-up. The policy was documented for 12 Dutch hospitals, including all 8 academic centers. The protocols differ substantially, with respect to starting antifungal therapy and, if started, the duration of pre-emptive antifungal therapy.

Conclusions:

- Colonization of intravascular catheter tips with *Candida* spp. is associated with candidemia in 4% of patients in this single center study.
- All episodes of candidemia were seen within the first days after catheter removal.
- The protocols in Dutch hospitals differ considerably.
- Since 4% of patients are candidemic, taking control blood cultures and starting pre-emptive treatment is reasonable.
- More study is needed to determine whether starting pre-emptive treatment can be administered only to a subset of patients based on clinical criteria.

P062

Methodological validation of a Zika, Dengue and Chikungunya virus PCR diagnostics using dried blood spots

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The causes of fever amongst febrile children are mostly unknown in developing countries. Given the complexity of collecting and transporting whole blood in a tropical climate with poor infrastructure, Dried Blood Spots (DBS) as an alternative sampling methodology was developed to investigate the causes of fever amongst children attending outpatient clinics in Myanmar.
DBS were validated for the detection of Zika, Dengue and Chikungunya virus by in-house PCR[1]. PCR efficiency and limit of detection were determined for each target in combination with three extraction methods; chloroform based TriPure Isolation Reagent (Roche), automated extraction using the MagNA Pure 24 (Roche), and isopropanol precipitation. EDTA-Whole blood was spiked with the three viruses and spotted onto filter paper. Four dilutions were extracted in four-fold with each extraction protocol.
The limit of detection (LOD_{95}) of the PCR was 7 copies/Âµl for Zika and Dengue, and 84 copies/ Âµl for Chikungunya. Compared to whole blood, DBS samples led to a 10x RNA yield reduction for all viruses (~3Ct). Extraction by means of chloroform (Tripure) was the most efficient manual method and was 3-4Ct more efficient compared to the automated MagNA Pure 24 robot. Using Tripure as extraction method, the limit of detection for Zika virus using DBS samples was estimated to be 100 copies/Âµl.
The in-house multiplex PCR proofed to be sensitive for all three viruses, albeit the LOD for chikungunya could be improved. Chloroform RNA manual extraction provided the best extraction method. In a follow-up study the diagnostic performance using clinical DBS and plasma samples collected in the field from febrile children from Myanmar will be assessed. Even though the detection limit in DBS is higher than in venous blood, the higher viral loads found in capillary blood samples for these viruses may further increase the sensitivity of DBS samples.

P063

Respiratory bursts in the White button mushroom

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Introduction
The White button mushroom or *Agaricus bisporus* produces edible mushrooms. It is of high industrial interest, since the Netherlands facilitates 25% of the world production, totalling to 400 million euros annually. Previously we have observed temperature peaks in compost during cultivation with *A. bisporus*. These peaks correlated with O_2 consumption and CO_2 production, indicating respiratory bursts.
Methods
Using temperature probes, the temperature of the compost is measured in real-time in able to study the behaviour during respiratory bursts. Compost samples will be taken during and between respiratory bursts to assess whether they correlate with extracellular enzyme secretion.
We will try to find factors that modulate these respiratory bursts in time and in amplitude. For instance, the inoculum composition, size and quantity will be varied.
Results
During the growth process of *A. bisporus* sudden respiratory bursts are observed in the form of temperature peaks in a ~20 hour rhythm. There was a clear difference between the patterns of the peaks when inoculating with one inoculum or using multiple inocula like in industry, though all were increasing in amplitude over time, then decreasing and finally disappearing. The peaks are all of slowly increasing height, when inoculated with multiple inocula. In contrast, when inoculating with one inoculum a different pattern occurs, now there is a low and a high peak in an interchanging pattern that increases slowly over time. When 2 inocula are spatially separated, it begins with the pattern of a high peak followed by a low peak, however when the hyphae of the two inocula meet, it changes to the situation as is observed with the multiple inocula.
Conclusion
Investigating the nature of these respiratory bursts could provide a greater understanding of the growth process and potentially aid in increasing the overall production of *A. bisporus* mushrooms. Future efforts will be made to directly correlate it to biomass, enzymatic activity and how the compost is inoculated.

P064

Development of personalized anti-infective medical devices in EU training consortium PRINT-AID

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PRINTing Anti-Infective Devices (PRINT-AID) is a multidisciplinary European training partnership for the development of personalized anti-infective medical devices combining printing technologies and antimicrobial functionality. Six academic groups and four industrial partners in six European countries have joined forces in collaboration to address the challenge of alternative antimicrobial strategies.
Antimicrobial resistance (AMR) and Healthcare-Associated Infections (HAI) are the most serious public health concerns globally. The European Center for Disease Prevention and Control determined that almost 4 million patients acquire

HAIs each year in the EU resulting in close to 37,000 deaths/year. Biofilm infections in medical devices are responsible for a considerable number of HAIs. It is essential to study novel antimicrobial methods to confront the problem at hand. One strategy to prevent or decrease medical device-associated infections is altering the surface properties of these devices by incorporating antimicrobial compounds.
PRINT-AID aim consists in an educational platform for the development of novel generation of 3D-printed medical devices with antimicrobial functionalities. Experimental drugs which inhibit bacterial colonization or kill bacteria will be added in the printed implant. These compounds will be added in the medical device structure itself during the 3D-printing process. The use of 3D-printing technology makes possible the customization of personalized devices that fit the needs of the patients.
PRINT-AID is structured in four consecutive work packages with the contribution of each institution. The first package consists of the identification and characterization of anti-biofilm compounds. The second part is based on the development and printing devices. The third part involves the implant evaluation with *in vivo* studies. The forth package entails the data integration and standardization.
As part of PRINT-AID, my study will focus on the development, characterization and *in vivo* evaluation of novel 3D-printed femur implants by incorporating newly developed highly potent synthetic antimicrobial and anti-biofilm peptides to prevent *Staphylococcus aureus* and *Staphylococcus epidermidis* infection in a murine model. Furthermore, anti-biofilm activity and possible influence on the host immune response and tissue repair using invasive and non-invasive techniques will also be evaluated.
Overall, the consortium aims to educate the young scientists in the study and development of novel approaches to prevent implant infections. The technologies for antimicrobial personalized implants will avoid the difficulties related to treatment of persistent infections, which are promoted by the insertion of medical devices.

P065

Efficacy of Meningococcal B Vaccine in patients with alternative pathway and terminal pathway complement deficiencies

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Introduction

Meningococcal serogroup B vaccine is protective in the general population as assessed following introduction of MenB-4C vaccine (Bexsero) in routine childhood vaccination in the United Kingdom. Children and adults with complement deficiencies have increased risk of serogroup B meningococcal disease and several guidelines recommend Meningococcal serogroup B vaccine for inherited complement deficiencies including C3, C5-C9, properdin, factor D, or factor H deficiencies. However, the efficacy of vaccines may me decreased in patients with complement deficiencies because important mechanism for vaccine-induced meningococcal killing may be deficient, including complement opsonization, complement anaphylatoxin release and serum bactericidal activity.
Previous studies in patients with terminal complement deficiencies have demonstrated that vaccine-induced anticapsular antibodies against serogroups A, C, Y and W confer protection against meningococcal disease by increasing the opsonophagocytic activity despite inability to kill bacteria by direct serum bactericidal activity (SBA).
Here, we assessed the efficacy of the MenB-4C vaccine on SBA and opsonophagocytic killing of meningococci in patients with alternative pathway or terminal complement pathway deficiencies.

Methods

We collected pre- and post-vaccination serum from two patients with alternative pathway deficiency and from three patients with terminal complement pathway deficiency. As control we collected pre- and post-vaccination serum from three healthy volunteers.
For the SBA with autologous serum, we made two-fold serum dilutions of the participants' autologous serum and incubated the serum with ~1,5*10^4 colony forming unit (CFU)/ml of either *Neisseria meningitidis* serotype B strain H44/76, 5/99 or NZ98/254 for 45 min at 37 °C. After incubation, serum samples were plated to determine bacterial survival. The titer was the serum dilution which killed 90% of the bacteria.
The SBA with external complement was performed as the SBA described above, except that the sera were heat inactivated before use and human serum absent for Meningococcal serogroup B antibodies was used as external complement source.
In the whole blood killing, we used blood from healthy volunteers from which serum was replaced with serum from participants. Reconstituted whole blood was incubated with ~1.0*10^5 CFU/ml of either *Neisseria meningitidis* serotype B strain H44/76, 5/99 or NZ98/254 for 1 hour at 37 °C. The number of bacteria were determined before and after incubation by plating two-fold serial dilutions and the percentage of bacteria that survived was calculated.
Antibody titers were determined with a whole cell ELISA using either *Neisseria meningitidis* serotype B strain H44/76, 5/99 or NZ98/254.

Results

MenB-4C vaccination induced effective anti-meningococcal antibodies in complement deficient patients and healthy controls. As predicted patients with a terminal complement deficiency failed to show significant vaccination induced killing in the SBA, whereas patients with alternative pathway deficiency and healthy controls showed significant vaccination induced increases in the SBA. Vaccination did induce significant opsonophagocytic killing of serogroup B meningococcus as measured by the whole blood killing assay in both terminal and alternative pathway deficient patients and healthy controls.

Conclusion
Patients with alternative pathway and terminal pathway complement deficiencies show effective opsonophagocytotic killing after MenB-4C vaccination. Therefore these results underline the recommendation to vaccinate complement deficient patients with MenB-4C.

P066
Harnessing plant-microbe interactions for the discovery of new antimicrobials
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The emergence and rapid spread of antimicrobial resistance (AMR) is a major threat to human health. Particularly alarming is the spread of the 'ESKAPE' pathogens, some of which can hardly be treated with conventional antibiotics. Actinomycetes, a diverse family of filamentous bacteria, are highly versatile natural product (NP) producers relevant for agriculture, biotechnology and medicine, including the majority of the antibiotics we use in the clinic. Genome sequencing has unveiled that many antibiotic biosynthetic gene clusters (BGCs) are poorly expressed under routine laboratory conditions. This forms a potentially vast reservoir of untapped antibiotics; to identify them and elucidate their bioactivity, we need to uncover the triggers and cues that elicit their production. Importantly, many actinomycetes live in symbiosis with eukaryotes, and in particular with plants. These interactions have likely played a key role in the evolution of the high chemical diversity of actinomycete-derived NPs. The signals that control the production of antimicrobials are likely tied to the biotic interactions in the rhizosphere, the zone of the soil that is directly influenced by plant roots [1]. The STW Perspective program BacktoRoots aims to enhance plant growth and productivity by exploring and ultimately exploiting microbial communities [2]. The 'cry for help' hypothesis entails that plants suffering from biotic stress produce signals that activate the production of protective molecules by plant-associated microbes, and notably actinomycetes. We thereby aim to uncover these signals and harness them for the screening of new antibiotics . We have obtained proof of principle that the plant-defense hormone jasmonic acid (JA) elicits the production of antibiotics by actinomycetes [3]. In this poster recent results on how plant hormones influence secondary metabolite production in actinomycetes and the implication for drug discovery will be discussed.
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P067
Antimicrobial supramolecular ureido-pyrimidinone-based Biomaterials
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The use of biomaterials inside the body always entails the risk of infection. The risk of infection might even be higher in so-called *in situ* tissue engineering applications, where population/infiltration of the scaffold material by endogenous cells and thereby the formation of new/healed tissue occurs as a spatiotemporal process. Since the porous scaffold materials can form a niche for invading bacteria, the intended *in situ* production of novel tissue may be severely compromised by infection. Therefore, we aim to develop a new polymeric supramolecular scaffold material, exerting two important functions: preventing microbial adhesion and thereby preventing biofilm formation, and inducing endogenous (eukaryotic) cells to repair the body. For the antimicrobial function the antimicrobial polymers will be prepared by mixing-and-matching of Ureido-Pyrimidinone (UPy) based supramolecular polymers (Dankers *et al.* 2005) with antimicrobial peptides (AMPs) modified with a supramolecular UPy moiety. Initial experiments showed that UPy-functionalization of AMPs did not affect their secondary structure and antimicrobial activity in solution, and incorporation of the UPy-AMPs into a UPy-polymer (PCL_{2k}-UPy) protected the resulting material against colonization by *Escherichia coli* and (methicillin-resistant) *Staphylococcus aureus* (Zaccaria *et al.*, in preparation). Next, we will incorporate the Synthetic Antimicrobial and Anti-biofilm Peptide SAAP-148 into the scaffold. SAAP-148 kills multidrug-resistant pathogens without inducing resistance, prevents biofilm formation and eliminates established biofilms and persister cells, and is effective against acute skin infections (de Breij & Riool *et al.* 2018). Moreover, we will functionalize UPy-units with the cell-adhesive peptide RGD sequence to control cell adhesion. Ultimately we aim to use such materials for *in situ* infection-free tissue engineering of vascular tissues, such as vascular grafts and heart valves.
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number 310389 (BIP-UPy). Furthermore, this work was supported by COST (European Cooperation in Science and Technology) Action iPROMEDAI (Project No. TD1305) and NWO NEWPOL grant SuperActive (Project No. 731.015.505) in collaboration with the Dutch Polymer Institute (DPI).

P068

A case of disseminated histoplasmosis after lung transplantation in a non-endemic area.

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A 56 year old dutch woman was admitted in our hospital because 3 days of fever. She had a history of bilateral lung transplantation 9 months before presentation. Her treatment included prednisone, tacrolimus and mycophenolic acid. Besides fever of 40^0C, she had no other complaints and physical examination was not remarkable. Despite broad spectrum antibiotics, fever persisted and renal and hepatic function worsened. A PET CT showed diffuse increased FDG uptake in colon transversum, four liver lesions, intra-abdominal lymph nodes, bone marrow and spleen.
A liver biopsy was very suggestive of abscess, and microscopically we saw yeast structures. Micafungin was started but 3 days later there was no clinical response. Then, very inhomogeneus growth on the blood agar plate was seen. A Gram stain showed yeasts and hyphae. The combination of a disseminated yeast infection in vivo with hyphae growth in vitro, in a T-cell immunocompromised patient with prolonged fever and no response to antibiotics or echinocandines, made us thinking about the possibility of a dimorphic fungi infection: Amphotericine B liposomal was started.
Later, a mold colony grew on de Saboureaud agar at 25oC. It was morphological identified as Histoplasma capsulatum. 18S PCR performed on the sample yielded the same result. The patient condition improved rapidly and Amphotericine B could be switched into Itraconazol, which she continued for 1 year.
Histoplasmosis is intensely endemic in the Ohio and Mississippi River Valleys in the United States and much of Latin America, but our patient had never travelled outside Europe.
Histoplasma is more ubiquitous than first thought, but it likes that only in those places with a very high load of Histoplasma in soil the inoculum is high enough to cause acute histoplasmosis in healthy individuals. A low inoculum by an immunocompetent patient gives no symptoms, but the fungus persists in the macrophages and reactivation can occur anytime in life.
In endemic regions, transplant patients are a known particular group at risk for histoplasmosis, where three different pathogenic mechanisms of transmission have been described: new Infection, reactivation and donor transmitted.
We estimated our case as having a very low probability of donor-derived infection, because of absence of disease in the transplanted organ, but considered it as a reactivation of a latent histoplasmosis acquired pre-transplantation in a non-endemic area. Serology (ID and CBR) pre-transplantation was negative, but sensitivity is very low in asymptomatic patients (CBR is positive in 5-15% of the patients after 3 weeks exposure, and in 75-95% of symptomatic patients).
This is not an isolated case. Multiple case reports of histoplasmosis in immunocompromised patients who had never travelled to an endemic zone can be found in the literature.
Histoplasmosis in immunocompromised patients as reactivation of an asymptomatic latent infection as consequence of a low inoculum in the past can occur everywhere in the world. We should take it into account in our differential diagnosis of unexplained fever in these patients.

P069

FtsZ-less binary fission and budding Planctomycete bacteria

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Cell division is a complex and well-orchestrated process essential for living things to thrive. The study of cell division in bacteria has mostly focussed on a small number of model organisms, ignoring a wealth of cell division systems that exist outside of the select few. Here we focussed on the non-model cell division of the Planctomycetes, which are an enigmatic phylum of bacteria that lack many of the otherwise essential cell division genes such as *ftsZ*. The question therefore remains: How do these Planctomycetes perform cell division? Here we investigated the cell division of both budding and binary fission division type Planctomycetes using mutagenesis, inhibitor studies in a continuous bioreactor system, time-lapse microscopy, and advanced (cryo) transmission electron microscopy techniques. The analysis revealed that a gene normally not essential for cell division is essential for the cell division in both the budding Plancomycete *Planctopira limnophila* and in the binary fission Planctomycete *Kuenenia stuttgartiensis.*

P070

The SKML quality assessment scheme for DNA-based detection of gastrointestinal protozoa; evaluation of participating-laboratories' results

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Introduction:
In 2012 the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) started an external quality assessment scheme (EQAS) for the detection of gastrointestinal protozoa in faecal specimens by DNA-detecting methods. This SKML scheme distributes unpreserved, stable clinical stool samples and therefore allows monitoring of the entire analytical procedure starting from faeces pre-treatment to DNA extraction and amplification.
Methods:
Sets of stool samples were distributed 3 to 4 times per year to 25-40 participating laboratories within the European Union. Depending on the PCR tests available in each laboratory, results for one or more of the protozoa *Giardia lamblia, Cryptosporidium spp, Entamoeba histolytica, Entamoeba dispar* and *Dientamoeba fragilis* were reported. Participants were asked to provide data on methods and platforms used, regarding sample pre-treatment, DNA extraction and PCR.
Results:
Evaluation of results of participating laboratories over the last 4 years shows: (1) Both false-positives as well as false-negatives have been reported. Overall-sensitivity is highest for *G.lamblia* and varies between 96-99%, and lowest for *E.histolytica*: 77-97%. (2) Reported semi-quantitative protozoan DNA loads (expressed as Cq-values) differ enormously between laboratories (>10 Cq-values). (3) Laboratories presenting relatively low Cq-values never reported false-negative results for that particular pathogen. Results indicate that the majority of laboratories reporting *G.lamblia* with relative high Cq-values, have used less efficient pre-treatment and/or DNA extraction protocols. Moreover, highest sensitivity of detecting *G.lamblia*, *Cryptosporidium spp*, and *D.fragilis* was found to be associated with an optimum amount of extracted faeces as input for the PCR. This relation was not seen for *E.histolytica*.
Conclusions:
Quality assessment schemes for DNA-based detection of gastrointestinal protozoa are essential to establish high-standard routine laboratory diagnostics. In particular, intra-laboratory comparison is only possible when results are derived from well validated clinical materials and not by spiking solutions with purified DNA. Our results indicate that about half of the participating laboratories could substantially increase the sensitivity of *G.lamblia*, *Cryptosporidium spp* and *D.fragilis* detection by optimizing the amount of stool to be extracted for DNA amplification.

P071

Understanding the interaction between vaginal microbiota and mucosal immunity using flow cytometry; the role of Immunoglobulin A

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Introduction: At mucosal surfaces a healthy balance between microbiota and mucosal immunity is crucial. Imbalance of the vaginal microbiota is associated with bacterial vaginosis and several adverse clinical outcomes, like the acquisition of sexually transmitted infections and an increased risk of preterm birth. Immunoglobulin A (IgA) is the most prevalent antibody at mucosal surfaces and an important mediator of immunity. In the gut, alterations in IgA coating of resident bacteria have been associated with multiple diseases. However, less is known about the relation between mucosal IgA and commensal bacteria present in the female genital tract. We aim to provide insight in the IgA coating of vaginal bacteria under normal circumstances using flow cytometry based techniques.
Methods: Strains of common vaginal bacteria, *Lactobacillus crispatus, L. iners, L. jensenii, and Gardnerella vaginalis,* were coated with pooled human serum IgA. Binding of IgA was detected using flow cytometry. Vaginal swabs were collected from healthy donors in which microbial IgA coating was detected using the same technique.
Results: *L. crispatus, L. iners, L. jensenii, and Gardnerella vaginalis* were highly coated with serum IgA . Similar, large numbers of IgA coated bacteria were detected in vaginal swabs
Conclusion: Vaginal bacteria show high levels of IgA coating, indicating the importance of IgA in maintaining a healthy vaginal microbiome. Disruption in microbial composition can possibly identify women at risk for diseases of the reproductive tract and adverse pregnancy outcomes. We will use bacterial cell sorting and molecular microbiota analysis to further study taxa-specific IgA-coating.

P072

Sensitivity and specificity of Vitek-2 antimicrobial susceptibility testing for first-line phenotypic screening of carbapenemase production in *Pseudomonas aeruginosa*

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Introduction: There are currently no guidelines on first-line phenotypic screening for the detection of carbapenemase-producing *Pseudomonas aeruginosa*. Our objective was to define sensitivity and specificity of Vitek-2® minimum inhibiting concentrations (MICs) of six antimicrobials for detection of carbapenemase-producing *P. aeruginosa*.
Methods: We retrospectively determined Vitek-2® MICs of ceftazidime, ciprofloxacin, imipenem, meropenem, piperacillin/tazobactam and tobramycin in 259 *P. aeruginosa* isolates. Isolates were collected from the national surveillance of carbapenemase-producing *Enterobacteriaceae* between 2012 and 2016. This national program invited Dutch laboratories to send in *Enterobacteriaceae* with a meropenem MIC>0.25 or imipenem MIC>1. Although the main focus of the national surveillance has been on *Enterobacteriaceae*, a considerable amount of non-fermenting gram-negative bacilli, such as *P. aeruginosa* have also been submitted. From the surveillance database, we randomly selected 130 unrelated, carbapenemase-producing *P. aeruginosa* isolates and 129 carbapenemase negative *P. aeruginosa* isolates, matched on year of isolation. Carbapenemase activity was defined by the carbapenemase inactivation method (CIM) test (reference standard). MIC testing was done with Vitek-2® (card N344, index test). We defined test characteristics of single and combination antimicrobial MICs to detect the presence of carbapenemase activity, using MIC above EUCAST epidemiological cut-off (MIC>ECOFF) values as screening cut-off.
Results: PCR showed that the carbapenemase-producing *P. aeruginosa* isolates harboured the metallo-β-lactamases VIM (*n*=110), IMP (*n*=13), NDM (*n*=1) or an unknown carbapenemase gene (n=6). Using MIC>ECOFF, Vitek-2® sensitivity for detecting carbapenemase-producing *P. aeruginosa* was higher than 92% for all antimicrobials and highest for imipenem (98% [95% confidence interval (CI): 94-100%]), tobramycin (98% [95%CI: 93-100%]) and ciprofloxacin (98% [95%CI: 95-100%]). Vitek-2® specificity was low in meropenem (20% [95%CI: 14 – 28%]) and imipenem (26% [95%CI: 19 – 35%]) and highest in tobramycin (90% [95%CI: 82 – 94%]). Combining Vitek-2® imipenem and tobramycin MIC>ECOFF resulted in a sensitivity of 96% (95%CI: 91-99%) and specificity of 90% (95%CI: 83-94%) for detecting carbapenemase-producing *P. aeruginosa.*
Conclusion: Vitek-2® MIC above *P. aeruginosa* ECOFF value is a sensitive first-line phenotypic screening strategy for carbapenemase-producing *P. aeruginosa*. Imipenem MIC>ECOFF performed better than meropenem MIC>ECOFF, but tobramycin MIC>ECOFF and the combination of imipenem and tobramycin MIC>ECOFF performed best within our selection of isolates. We recommend first-line phenotypic screening of carbapenemase-producing *P. aeruginosa* with Vitek-2® imipenem and/or tobramycin MIC above ECOFF. Future studies should confirm these findings in various prevalence settings of carbapenemase-producing *P. aeruginosa*.

P073

Mimicking the *in vivo* growth conditions of *Streptococcus pneumoniae*

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Introduction
Streptococcus pneumoniae is a bacterial pathogen and a major cause of morbidity worldwide, ranging from otitis media and sinusitis to more severe and invasive diseases such as pneumonia, meningitis and sepsis, associated with high mortality rates. Pneumococcal conjugate polysaccharide vaccines (PCVs) have been very successful in preventing pneumococcal infections. However, only 13 of the more than 90 different serotypes are covered, leading to serotype replacement. In addition, several low-income countries are unable to afford PCVs. Novel vaccines consisting of broadly protective protein-based antigens represent a highly promising alternative or complementary approach to reduce pneumococcal disease.
Our main aim is to identify candidate protein antigens through a better understanding of *in vivo* expression. The most important nice for *S. pneumoniae* is the nasopharynx, a nutrient poor environment. Of all nutrients, especially the divalent cations are essential for survival of *S. pneumoniae*. Since the concentrations of divalent cations in the nasopharyngeal cavity is unknown, we determined them to design a medium mimicking the *in vivo* conditions, which was used to study the growth behavior of pneumococci.
Methods
We collected nasal fluid of ten healthy adults using a Schirmer strip and measured the concentration of divalent cations by induction-coupled plasma mass spectrometry. A chemically defined medium (CDM) was designed containing equivalent divalent cation concentrations as in human nasal fluid, using highly pure chemicals. Growth of *S. pneumoniae* in *in vivo*-mimicking CDM (IVM-CDM) was analysed and compared to standard growth conditions.
Results
We identified trace amounts of manganese and cobalt (<40 µg/L) in human nasal fluid, whereas copper and zinc levels were at least 10-fold higher. *In vivo* divalent cation levels were highly distinct from *in vitro* concentrations and pneumococci grown in IVM-CDM show a delay in growth compared to standard growth conditions. The study of more in depth metabolic adaptation of pneumococci to the *in vivo* mimicking conditions and the characterization of the proteome

is ongoing.

Conclusion

Large differences in divalent cation levels were detected between standard culture conditions of *S. pneumoniae* and nasal fluid. Growth in IVM-CDM required adaptation of pneumococci resulting in a delay in growth compared to growth under standard conditions. Cell wall fractions from pneumococci grown in IVM-CDM will be compared to standard *in vitro* conditions, in order to characterize the potentially *in vivo* expressed antigens.

P074

Comparative genome analyses of two distinct comammox *Nitrospira* from the terrestrial subsurface

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Complete nitrification by a single microorganism (comammox) was shown to be catalysed by members of the genus *Nitrospira*. To catalyse ammonia oxidation, these *Nitrospira* contain an ammonia monooxygenase (AMO) that is phylogenetically divergent from the enzyme in canonical ammonia-oxidizing bacteria. Furthermore, a metagenomics approach revealed that two distinct sister clades of AMOs exist in comammox *Nitrospira.* These are referred to as clade A and clade B.

To date, comammox *Nitrospira* have mainly been found in engineered systems. In this study, we report the high-quality draft genomes of two novel comammox *Nitrospira* from the Rifle Integrated Field Research Challenge site adjacent to the Colorado River, representing the first comammox genomes reported from a natural environment. Intriguingly, one of these contains a clade B AMO, allowing for the first time in-depth phylogenetic and genomic analyses of this understudied group. These genomes were compared to *N*. *moscoviensis*, *Candidatus* N. nitrosa, and *Ca.* N. inopinata, representing both canonical and comammox *Nitrospira*.

For all genomes analysed, pairwise average nucleotide identity values ranged between 74 and 80% which classifies them as separate species. All comammox *Nitrospira* encode the full enzyme set necessary for ammonia oxidation to nitrate. Unlike the two different forms of AMO between clade A and B, the genes for nitrite oxidoreductase (NXR) are highly conserved in both comammox types, as well as in *N. moscoviensis*. This indicates affiliation of both comammox clades with sublineage II, which further was confirmed by phylogenetic analyses based on concatenated ribosomal proteins.

Besides general housekeeping functions including transcription and translation, carbon fixation via the reverse citric acid cycle, and gluconeogenesis, all genes for respiratory complexes I to V are highly conserved in the core genome of the analyzed species. The variable genome involves genes for hydrogen and formate oxidation as an alternative energy source outside the nitrogen cycle. Interestingly, in contrast to *N. moscoviensis* which has a group 2a [Ni-Fe] hydrogenase, the Rifle clade B comammox, *Ca.* N. nitrosa and *Ca.* N. inopinata contain a group 3b bifunctional hydrogenase (sulfhydrogenase). The NADH-dependent formate dehydrogenase is present in *N. moscoviensis* and the Rifle clade A comammox, representing a functional trait which was not reported for other comammox *Nitrospira* genomes previously. Furthermore, all the analyzed genomes also contained genes for oxygen stress and heavy metal resistance, including superoxide dismutase (SOD), catalase peroxiredoxins and arsenic detoxification mechanisms. Like *N. moscoviensis*, the two Rifle comammox contain genes encoding for superoxide dismutase and catalase to cope with reactive oxygen species (ROS), which are absent from *Ca.* N. nitrosa and only partly conserved in *Ca.* N. inopinata. While all analyzed genomes encoded arsenate reductase, only clade B had a mechanism of arsenite [As(III)] resistance through methylation

In conclusion, this is the first study reporting comammox *Nitrospira* derived from natural systems. This is valuable step towards understanding how comammox contribute to nitrification in terrestrial ecosystems.

P075

Can the Homoacetogen *Moorella thermoacetica* turn into a Syntroph?

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Microbial interactions are essential for the conversion of carbon compounds. In natural environments, even the conversion of simple one-carbon molecules, such as CO_2, CO, formate and methanol, may require the metabolic activity of several microorganisms. An example is the utilization of formate and methanol (cobalt-limited) by *Moorella thermoacetica* strain AMP, which can only be done in syntrophy with the methanogen *Methanobacter thermoautotrophicus* [1]. *M. thermoacetia* strain AMP has a hydrogenogenic metabolism on CO, formate and methanol in contrast to the type strain *M. thermoacetia* VPI 12954^T, which can grow axenically on these substrates and produces mainly acetate. In this study a genomic comparison between *M. thermoacetia* strains AMP and VPI 12954^T was performed. Results point to the importance of the hydrogenogenic metabolism of strain AMP in its syntrophic behavior. In addition, strain AMP was unable to grow with H_2 + CO_2, but VPI 12954^T was, despite both *Moorella* having similar hydrogenases. Both *M. thermoacetica* strains could grow axenically on CO. It appears that strain AMP has a carbon monoxide dehydrogenase catalytic subunit (*cooS* gene) in an operon structure coding for a maturation and nickel incorporation proteins, unlike VPI 12954^T, which has a formate dehydrogenase catalytic subunit (FDH) in a similar operon. This could be one of the reasons for the observed physiological differences in hydrogen and CO utilization. Our overall approach is to use omics analysis with pure cultures and co-cultures of each *Moorella* strain with the methanogen on formate, CO and methanol to validate our initial findings and to look at the expression of genes in the three

microorganism to determine if there are other key factors that play a role in the development of syntrophic interactions on one carbon compounds.

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P076

High level resistance to ceftriaxone and azithromycin is well detected by laboratories across the Netherlands, in contrast to low-level resistance to these antibiotics

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Introduction: Standard treatment for gonorrhea in the Netherlands is ceftriaxone monotherapy; some guidelines recommend dual therapy with azithromycin. Resistance to ceftriaxone has sporadically been reported worldwide, but so far not in the Netherlands. Resistance to azithromycin is found in approximately 10% of strains reported in the GRAS (Gonococcal Resistance to Antibotics Surveillance).

Methods: Ten well-characterized WHO strains, of which two in duplicate, were distributed to 19 laboratories across the Netherlands.17 of these labs participate also in GRAS. Of these ten strains, six were susceptible to ceftriaxone, one was low-level resistant (first dilution above EUCAST-breakpoint) and three were high-level resistant. An intermediate category for ceftriaxone does not exist within EUCAST. For azithromycin, three strains were susceptible, three were intermediate, two were low-level resistant and two were high-level resistant. Susceptibility tests were performed with media and E tests routinely used in the 19 laboratories.

Results: Ceftriaxone: High-level resistance in three strains was detected in 19 (100%), 19 and 17 (89%) of the laboratories. The one strain reported to have low-level resistance was reported resistant in only 3 (15%) of the laboratories. All six susceptible strains were correctly reported as susceptible in all labs except one strain which was reported as resistant in one laboratory.

Azithromycin: High-level resistance in two strains was detected in all laboratories. Low-level resistance in two strains was confirmed in 12 (63%) and 7 (37%) of the laboratories, whereas 1 (5%) and 3 (16%) reported these strains as susceptible. The other lab reports on these strains showed intermediate results. Regarding the three strains with an intermediate MIC, 4 (21%), 5 (26%) and 11 (53%) of the labs reported them as resistant whereas 6 (31%), 4 (21%) and 2 (11%) of the labs gave a susceptible result.Resistance was also reported in two of the three strains regarded as susceptible in 4 (21%) and 4 (21%) of the labs.

Strains submitted in duplicate: High-level resistance to azithromycin in one strain and to ceftriaxone in the other strain was recognized by all labs in both samples. Minor discrepancies in azithromycin susceptibility in the duplicate samples of one strain were reported in three (16%) of the labs: one lab reported susceptible and intermediate for these samples, two labs reported intermediate and resistant.

E-tests and media: Sixteen different media (differing either in composition or in supplier) were used by the 19 laboratories. Laboratories reporting results closest to reference values used GC agars with or without supplements. 18/19 labs used the same e-tests (Biomerieux).

Conclusion: High-level resistance to ceftriaxone or azithromycin will easily be detected in routinely cultured NG strains. Low-level resistance may well go undetected, and for azithromycin, intermediate or susceptible strains may well be reported as resistant. It should be noticed that there are no clear molecular mechanisms explaining low-level resistance for ceftriaxone or azithromycin. For azithromycin, the EUCAST breakpoints are chosen within the wild-type distribution of MICs, making errors more likely.

P077

Antimicrobial resistance differs significantly between hospitals: The advantage of a regional analysis of blood culture isolates

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Background: Sepsis is a life-threatening syndrome caused by a dysregulated host response to infection. In case of a suspected case of sepsis empirical antimicrobial treatment is needed. The choice of empiric treatment is primarily an “informed guess” based on national guidelines, but also on knowledge of local and regional resistance of typically isolated microorganisms.

Materials/methods: In this study, bug/drug combinations were analysed of all bacterial blood culture isolates from patients of all 14 secondary and tertiary care hospitals in the Northern Netherlands detected in the last 15 years (between 2002 to 2016). Only first isolates were selected and included by using a novel selection algorithm, based on the M39-A4 guideline of CLSI, taking into account isolate-specific resistance of key antibiotics which were chosen based on the genus and Gram stain. Subsequently, we compared the resistance of blood culture isolates between hospitals using a full-region approach.

Results: Using the novel method, nearly 10% more bacterial strains were included compared to the CLSI guideline, resulting in a total of about 123,000 instead of 105,000 isolates. We show significant differences in antimicrobial resistance between hospitals, even when comparing the resistance of the same microbial species. Importantly,

prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) has increased from 0.3% in 2002 to 1.4% in 2016 and Quinolone and Aminoglycoside Resistant Enterobacteriaceae (QARE) from 0.2% in 2002 to 2.4% in 2016. Nevertheless, their prevalence remains lower compared to neighbouring countries.
Conclusions: Empiric therapy for patients with septicaemia might be improved in single hospitals, by analysing the regional inter-institutional epidemiology of bacteriaemic isolates comprising the correspondent resistance. It has been shown that there are major local epidemiological differences in microbial resistance, but a regional homogeneity in microbial prevalence. This allows for a more specific therapy of first choice using regional or even local hospital protocols, rather than using general national guidelines. We recommend to analyse bacterial surveillance and prevalence of resistance in a full-area approach instead of single hospitals or the national level.

P078

Co-colonization between *Streptococcus pneumoniae* and *Staphylococcus aureus* affects antibiotic resistance

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Background:
Streptococcus pneumoniae and *Staphylococcus aureus* are frequent colonizers of the human upper respiratory tract. Different mechanisms of interaction between these co-colonizing species are described. Here we described the effect of *S. pneumoniae* and *S. aureus* co-colonization on their antibiotic susceptibility in nasal colonizing strains.
Materials/methods:
Bacterial carriage strains were collected as part of 'The Appropriateness of prescribing antimicrobial agents in primary health care in Europe with respect to antimicrobial resistance' study. Shortly, General practitioners from nine European countries (20 per country), were asked to recruit each 200 individuals, visiting their practice for a non-infectious condition, to provide nasal swabs. Patients who had used antimicrobial drugs or were hospitalized in the previous 3 months, immunocompromised patients and nursing home were excluded residents.
Identification and antimicrobial susceptibility of *S. aureus* and *S. pneumoniae* were performed in a central microbiological laboratory (Maastricht University Medical Centre, The Netherlands). The antibiotic resistance of the isolates was performed with microdilution for cefuroxime, cefaclor, ceftazidime, clarithromycin, clindamy-cin, ciprofloxacin, moxifloxacin, benzyl-penicillin, tetracycline and co-trimoxazole for *S. pneumoniae*, and azithromycin, ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, linezolid, oxacillin, benzyl-penicillin, tetracycline, co-trimoxazole, and vancomycin for *S. aureus*. The method used was in accordance with EUCAST guidelines and EUCAST epidemiological cutoffs were used as resistance breakpoints.
Results:
In total, 7622 nasal swabs were analyzed. Of these 6685 were only colonized by *S. aureus,* 721 only colonized *S. pneumoniae* and 216 were positive for both *S. aureus* and *S. pneumoniae*. The prevalence of benzyl-penicillin non-susceptible *S. aureus* was significantly higher among co-colonizing strains compared to single colonizing strains (OR=1.7, 95% CI 1.2-2.3) as was the MIC ≥ 2 mg/l (OR=1.5, 95% CI 1.0-1.7). In *S. pneumoniae*, single colonizing strains were significantly more susceptible to cefaclor compared to co-colonizing strains (OR=0.7, 95% CI 0.5-0.9).
Conclusions:
Our findings might give more insight in the interactions between co-colonizing carriage isolates of S*. pneumoniae* and *S. aureus* and the effect of these interactions on benzyl-penicillin (non)susceptibility. However, more studies are needed with clinical isolates and to clarify the basis of these interactions and the clinical relevance.

P079

An outbreak of *Clostridium difficile* ribotype O17 in an academic hospital in the Netherlands: molecular typing and lessons learned

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Introduction:
Clostridium difficile infection (CDI) continues to be a leading cause of nosocomial diarrhoea. Especially ribotype 027 is well known to spread rapidly in health care facilities. In our hospital all *Clostridium difficile* isolates are routinely typed as part of ongoing surveillance. We describe an outbreak of CDI with a newly introduced strain in an academic hospital.
Methods:
We retrospectively analyzed an outbreak of *C. difficile* PCR ribotype 017 in our university hospital. CDI was defined as diarrhoea and a positive *C. difficile* toxin A/B gene PCR result. All PCR positive samples were cultured and characterized with Amplified Fragment Length Polymorphism (AFLP). PCR ribotyping was performed on 3 representative isolates. To contain the outbreak, two bundles of interventions were implemented.
Results:
The outbreak was first noticed when 10 patients developed CDI with the previously undetected ribotype 017. Eight of these patients had an epidemiologic link with the intensive care unit (ICU). Subsequently, a bundle of interventions was implemented, which involved enhanced awareness of contact isolation in case of diarrhoea, complete disinfection of the wards, and intensification of the cleaning protocols. Despite implementation of the bundle another 5 patients developed CDI with ribotype 017. Evaluation of the bundle revealed that disinfection of the medical equipment on the ICU was omitted. After addressing this shortcoming, no new cases were reported.
Conclusions:

- Routine in-house surveillance and strain typing enabled early detection and containment of an outbreak with a newly introduced *Clostridium difficile* strain. Without routine typing, this outbreak might not have been noticed at this early stage, since the total number of patients with CDI was not increased. PCR ribotyping directly on stool samples could further accelerate typing results and is currently in development.
- We experienced rapid spread of *Clostridium difficile* PCR ribotype 017 in our hospital despite infection control measures. Incomplete disinfection presumable caused the ongoing outbreak, therefore responsibilities for each part of the disinfection protocol need to be clearly defined.

P080

Acidothermophilic bioreduction of sulfur for the recovery of valuable metals

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Base metals and rare earth elements (REE) are important raw materials for a wide range of technological applications. Most mining operations focus on the recovery of one or a limited number of metals from polymetal sulfide ores. The remaining metals are discarded in the liquid waste streams generated during metal extraction and metallurgy procedures, or in crushed metal sulfide rock on solid waste piles. There, the exposure of the metal sulfides results in their oxidation to ferric (Fe^{3+}) iron, sulfuric acid and metal ions, which leach from the waste piles as acid mine drainage (AMD). AMD and metalliferous waste streams from mining and metallurgy operations have great potential as (secondary) sources of valuable metals, provided they can be recovered efficiently.
A preferred approach for metal recovery is selective precipitation of dissolved metals with hydrogen sulfide (H_2S) as metal sulfides (MeS). For the supply of H_2S, microbial sulfidogenesis is preferred over conventional physicochemical methods, as it can be performed on site and adjusted to the metal load of the water stream. Currently industrial microbial sulfidogenic processes are operated under mesophilic and neutrophilic conditions, making the associated microbial communities unfit for *in situ* sulfidogenesis in the hot, acidic metalliferous waste streams typical for metallurgy processes. Furthermore, sulfate (SO_4^{2-}) is used as electron acceptor, which requires 8 electrons for the reduction of 1 mol SO_4^{2-} to H_2S. Use of elemental sulfur (S^0) as electron acceptor requires only 2 electrons for reduction to H_2S, resulting in a reduction of four times the electrons needed.
This research explores the reduction of S^0 to H_2S by thermoacidophilic mixed microbial communities. Active S^0 reducing communities were enriched using as inoculum samples from Yellowstone National Park, a natural hot, acidic environment. Enrichments were carried out at different pH (4, 3, 2) and temperature (60, 70, 80 °C), using acetic acid, ethanol, glycerol or H_2/CO_2 as substrates for sulfur reduction. The activity of the enrichments was monitored through measurement of sulfide production, electron donor consumption and pH.
H_2S production was mainly detected (0.5 – 2.5 mM) in the enrichments performed with H_2/CO_2 at 60 °C and pH 4. In view of these results we conclude that H_2 is preferred to acetic acid, ethanol and glycerol as electron donor for the reduction of elemental sulfur at these conditions.

P081

Combining the best of culture and PCR: V-PCR to determine *Chlamydia trachomatis* viable load

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Introduction:
For different microorganisms (e.g. HIV), total organism load has been associated with disease severity, transmission and acquisition risk. For *Chlamydia trachomatis* (CT) however, the role of organism load in the clinical presentation and transmission is still unclear. In previous research, culture-based studies generally reported a correlation of load and clinical presentation, while most PCR-based studies did not, suggesting that the quantification of viable CT may be more relevant. The current gold-standard for the detection of viable CT is solely based on culture, which is labor-intensive, technically demanding, and lacks sensitivity. Recently, we validated a fast and easy culture-independent approach, called viability-PCR (V-PCR), to assess CT viability in clinical samples. In V-PCR, specimens are treated with a membrane impermeable DNA binding dye (e.g. Propidium mono-azide; PMA) to block the amplification of free bacterial DNA and DNA in membrane comprised bacteria. As a result, only DNA from bacteria with an intact membrane is amplified and detected. In the current study, we utilized V-PCR to quantify the viable load of CT in anorectal samples.
Methods:
COBAS 4800 CT/NG routine testing was used for CT diagnosis. Anorectal CT positive women (n=51), were asked to self-take anal swabs at our outpatient STI clinic (South Limburg Public Health Service) prior to anti-chlamydial treatment. V-PCR was used to quantify the viable organism load. Furthermore, traditional culture was used to confirm V-PCR results.
Results:
Quantification of CT load was achieved in 88% (n=45/51) of anorectal NAAT positive samples, of which 23 specimens gave quantitative results after treatment with PMAxx. In total, 52% (n=12/23) of specimens with a viable load above the detection limit of our qPCR were positive for CT culture. Specimens with a viable load >3.5 $\log_{10}$ gene copies/ml resulted in 89% in a positive CT culture, while specimens with a viable load <2.5 $\log_{10}$ gene copies/ml remained negative.
Conclusion:

This study demonstrated that the viable CT load can be determined in anorectal samples and correlated with culture results. Future research will focus on the correlation between viable CT load (measured by V-PCR) and clinical presentation and transmission.

P082

The ELITe InGenius in a routine laboratory setting

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Currently, the majority of routine molecular diagnostic testing in our lab is performed on the Roche FLOW system. The remaining diagnostic tests are rather diverse and primarily low-volume laboratory-developed-tests (LDT) and some CE-IVD tests. In the latter setting, NucliSENS easyMag (Biomerieux) or MagnaPure 96 (Roche) extraction is performed, followed by PCR analyses with LC480 machines (Roche). To improve traceability and flexibility, further automation and robotisation is necessary, preferably by a “sample-in result-out system”. The ELITe InGenius (ELITechGroup) platform was examined because of its open and flexible characteristics.
Performance of the ELITe InGenius platform was tested using a diverse selection of PCRs (CMV ELITe MGB kit (ELITechGroup; CE-IVD), respiratory viruses (R-DiaXX, Diagenode diagnostics; CE-IVD), *Mycoplasma pneumoniae* (LDT) and Herpes Simplex viruses 1 and 2 (LDT)), together with a diverse range of clinical specimens (EDTA-plasma, sputum, nasopharynx swabs (BD Eswab), cerebrospinal fluid). For these targets and materials sensitivity, specificity and nucleic-acid extraction efficiency were assessed by comparing the ELITe InGenius with the routine diagnostic setting. In addition, prospective testing of clinical samples was performed
The ELITe InGenius gave comparable outcomes regarding sensitivity and specificity when compared to the routine setting for all above mentioned PCRs. Extraction efficiency of the ELITE InGenius was comparable to the NucliSENS easyMag and MagnaPure 96. Results on clinical samples corroborated these findings. No cross-contamination was observed.
In conclusion, our results indicate that the ELITe InGenius, as an open automated platform, is able to carry-out high quality molecular diagnostic testing. With this system it’s possible to perform sample-in result-out molecular diagnostics and allows you to generate a flexible workflow with little hands-on time. Further testing of long-term robustness of the platform is in progress.

P083

Post-translational modifications of G-protein coupled receptors determine the sensitivity of target cells towards the S-component of *Staphylococcus aureus* bi-component toxins

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The staphylococcal bi-component leukocidins Panton-Valentine Leukocidin (PVL) and γ-Hemolysin CB (HlgCB) target human phagocytes. Binding of the toxins’ S-components to human C5a receptor 1 (C5aR1) contributes to cellular tropism and human specificity of PVL and HlgCB. To discover additional factors involved in PVL and HlgCB mediated lysis, a genome-wide CRISPR/Cas9 screen was performed, identifying several sialylation and sulfation genes possibly involved in PVL and HlgCB mediated lysis. We hypothesized that sialylation and sulfation are linked to the S-component target, G-protein coupled receptors (GPCR) on target cells, and that other *S. aureus* bi-component toxins also benefit from these essential enzymatic modifications. In this study we demonstrate that *S. aureus* bi-component toxins profit from host essential intercellular mechanisms, specifically post-translational modifications of GPCRs.
In order to identify additional receptors or cell processes involved in PVL mediated lysis, we incubated a U937 human C5aR1^{+}CRISPR-Cas9 sgRNA library with a half maximal effective concentration of either PVL or HlgCB. Results suggest that the sialylation genes; *slc35a1, cmas* and sulfation genes; *papps1, tpst2, slc35b2* are involved in both PVL and HlgCB mediated susceptibility. A follow up validation by single knock-out U937 cells confirmed that these cells are resistant to PVL (7.5nM) mediated lysis.
Better understanding and knowledge of the mechanisms of action of human specific *S. aureus* toxins could not only identify new targets that could be exploited for anti-virulence strategies to limit *S. aureus* infections, but also improve current in vivo infection models.

P084

The sense or 'nonsense' of a fresh isolate for automated susceptibility testing

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Introduction: The standard procedure on automated susceptibility testing in the VITEK2 (bioMérieux) requires isolates that have been incubated for 16 to 24 hours prior to handling. If samples are not handled during the weekend, isolates will be older than the required 16 to 24 hours after the weekend. To prevent further delay of the susceptibility testing we compared the influence of 3 different scenario’s on the results of the susceptibility testing:
1. Isolates are incubated according to bioMerieux for 16 to 24 hours.
2. Isolates are incubated for 16 to 24 hours and than stored in the fridge for 48 hours.
3. Isolates are stored for 48 hours in the fridge and then incubated for 16 to 24 hours.

Method: The isolates that were included were all clinical isolates. The species were selected so that we covered most of the spectrum we normally test in the VITEK2. Isolates were plated on different blood agar plates, incubated according to the three scenario's described above and susceptibility was tested in the VITEK2. The results of the isolates that were incubated immediately for 16 to 24 hours are considered the "gold standard".
Results: In total, 126 isolates were tested: 70 enterobacteriaceae, 30 non-fermenters, 20 *Staphylococcus spp*. and 6 *Enterococcus spp.* The categorical agreement between scenario 1 (isolates are incubated according to bioMerieux for 16 to 24 hours) and scenario 2 (isolates are incubated for 16 to 24 hours and then stored in the fridge for 48 hours) was 98.4%, with 0.8% minor error, 0.3% major error and 0.5% very major error. The categorical agreement between scenario 1 and scenario 3 (isolates are stored for 48 hours in the fridge and then incubated for 16 to 24 hours) was 98.7%, with 0.6% minor error, 0.2% major error and 0.5% very major error. The categorical agreement between scenario 2 and scenario 3 was 98.9%, with 0.7% minor error, 0.3% major error and 0.1% very major error. The very major errors were mainly found in isolates that were non-fermenters and piperacillin/tazobactam was the main error.
Conclusion: The susceptibility results gave an overall categorical agreement of 98.5% between isolates that have been refrigerated for 48 hours before testing compared to immediate incubation for 16 to 24 hours. The errors we found are considered not significant, as we have more issues with testing piperacillin/tazobactam in non-fermenters with the VITEK2.
It is possible to store isolates of the species we tested in the fridge when they are not handled in the weekend. Susceptibility testing can be performed immediately after the weekend without delay.

P085

MycoSynVac: a European project to engineer *Mycoplasma pneumoniae* as a broad-spectrum animal vaccine

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Mycoplasmas are the smallest cell wall less, free-living microorganisms. Every year, infections caused by Mycoplasmas in poultry, cows, and pigs, result in multimillion euros losses in the USA and Europe. Currently, there are vaccines against *M. hyopneumoniae* in pigs and *M. gallisepticum* and *M. synoviae* in poultry. However, vaccines against many other Mycoplasma species infecting pets, humans and other farm animals do not exist.
The main objective of MycoSynVac is to rationally design a non-virulent *M. pneumoniae* to obtain a universal chassis using synthetic biology. Simultaneously, several methods will be developed to identify and select putative antigens from veterinary relevant Mycoplasma species.
Virulence genes of *M. pneumoniae* will be identified taking into account the sequence variability of 22 clinical isolates and existing literature. These will subsequently be removed from the genome using modified methods for genome editing and the organism wide impact of their removal will be determined using transcriptome and proteome analysis.
To identify putative heterologous mycoplasma antigens, methods are developed in the project to determine which proteins reside on the surface of various Mycoplasma species, which genes are expressed during infection using RNA seq and which putative B-cell epitopes are recognized. Using genome engineering tools, we will clone and surface-express the selected chimeric proteins in the chassis. We will then check by western and immunofluorescence if these chimeric proteins are recognized by the serum of infected animals **.**