135 The use of rep-PCR (Diversilab®, bioMérieux) in combination with multiplex PCR (targeting virulence genes) reveals the transmission of *Pseudomonas aeruginosa* isolates among cystic fibrosis patients in a hospital background

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Objectives: To rapidly detect transmission of *Pseudomonas aeruginosa* (*P. aeruginosa*) among cystic fibrosis (CF) patients in a hospital environment, so that the necessary measures can be taken to avoid future transmissions.
Methods: The use of molecular typing techniques such as rep-PCR (Diversilab®, bioMérieux), in combination with multiplex PCR targeting ferripyoverdine receptor genes and S-type pyocin genes to distinguish between *P. aeruginosa* isolates.
Conclusion: In order to quickly detect and respond to bacterial transmissions and outbreaks in hospitals, an appropriate detection method to identify these transmission events and strains is indispensable. Although the use of rep-PCR to determine the clonally relatedness of potential transmissible strains is very useful, we have shown that a combination of the latter technique together with a specific multiplex PCR targeting virulence factors such as ferripyoverdine receptors and pyocins allows for the accurate detection of transmission of *P. aeruginosa* among CF patients in a hospital environment. We showed that intra CF patient *P. aeruginosa* transmission can occur but that there is not really one highly transmissible CF clone spreading.

136 Development of a sensor for detection of *Pseudomonas aeruginosa* lung infection in the breath of CF patients

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Background: *Pseudomonas aeruginosa* (PA) is one of a few bacteria emitting the poisonous gas hydrogen cyanide (HCN) to effectively outmatch competitive flora using HCN synthase to produce HCN from glycine under microaerobic conditions.
Methods: Surface-Enhanced Raman Spectroscopy (SERS) is a method which holds great potential in the detection of small molecules at very low concentrations because our nanostructured SERS surface enhances the Raman signal >1,000,000 times.
Objectives: The objective of the project is to further develop the nanostructured SERS chip originally made for explosives detection to build a point-of-care sensor able to detect hydrogen cyanide emissions in the breath of PA infected CF patients.
Results: In a serial dilution experiment on the SERS chip a direct proportional relationship between the concentration of applied cyanide and the resulting enhanced Raman signal was demonstrated. SERS measurements on physiologically relevant concentrations of HCN gas will be presented as well as incubation results of the SERS chip with PA in various environments.
Conclusion: Using our SERS chip it is possible to measure HCN at physiologically relevant concentrations demonstrating the potential of detecting PA at an earlier stage than allowed by current technology.

137 Hydrogen cyanide concentrations in the breath of adult cystic fibrosis patients with and without *Pseudomonas aeruginosa* infection

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Objectives: Elevated concentrations of hydrogen cyanide (HCN) have been detected in the headspace of *Pseudomonas aeruginosa* (PA) cultures and in the breath of children with cystic fibrosis (CF) and PA infection. The use of mouth-exhaled breath HCN as a marker of PA infection in adults is more difficult to assess as some without PA infection generate HCN in their mouth.
Hypothesis: The analysis of breath exhaled via the nose, thereby avoiding volatile compounds produced in the mouth, will demonstrate elevated concentrations of HCN in adult CF patients chronically infected with PA.
Methods: Using selected ion flow mass spectroscopy (SIFT-MS), the mouth and the nose-exhaled breaths of 20 adult CF patients; 10 with chronic PA infection and 10 free from PA infection were analysed for HCN. Acetone and ethanol were also measured as controls. This analytical technique allows direct sampling of single breath exhalations, obviating the need to collect samples into bags or onto traps, which can compromise samples.
Results: HCN was detected in the mouth-exhaled breath of patients in both groups and in the nose-exhaled breath of patients with chronic PA infection. The median (IQR) nose-exhaled HCN was significant higher in those with chronic PA infection compared to those free from PA (11 (0.8–18) ppbv vs 0 (0–3.2) ppbv, $p = 0.03$). The concentrations of acetone and ethanol in nose-exhaled and mouth-exhaled breath are in keeping with previous studies.
Conclusion: HCN in nose-exhaled breath is a biomarker of chronic airway infection with PA in adults with CF. Its application as a non-invasive diagnostic test for early PA infection warrants further investigation.

138 Sampling and processing for *Pseudomonas aeruginosa* identification across ECFS-CTN sites: Standardisation of clinical practice is important for patient selection for research studies

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Background: The definitions of "chronic" and "new/early" infection are assessed retrospectively. In order for these definitions to be consistently applied in research practice, standardised clinical practice is required.
Objectives: To gain information on clinical practice within ECFS-CTN sites with regards sampling and processing of respiratory samples for identification of *P. aeruginosa*.
Methods: A survey was distributed to investigators in all ECFS-CTN sites.
Results: 43 sites/subsites completed the survey giving a representative sample of the whole group. 72% of sites had both adult and paediatric patients registered, with remaining centres having either adults only (14%) or paediatrics only (14%). 52% of ECFS-CTN sites collected respiratory samples for routine culture at least every 3 months (5% monthly; 43% at every hospital visit). In adult patients this was predominantly sputum. Sites with only paediatric patients and sites with both adults and paediatrics use a combination of sputum and oropharyngeal swabs. For patients unable to expectorate, sites had different strategies for escalating to more invasive methods of obtaining samples (i.e. sputum induction and bronchoalvaolar lavage). The two main reasons for performing invasive sampling were unexplained clinical deterioration and suspicion of non-tuberculous mycobacteria.
There was great variability in laboratory practices reported for processing (e.g. homogenisation of samples, medium used, methods used to confirm identity of *P. aeruginosa*).
Conclusion: This survey highlights the challenges to research in applying definitions for infection retrospectively using clinical data.