First identification of non-lactose fermenting Gram negative respiratory isolates from an Irish cystic fibrosis population using matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS)

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Background: The identification of nonfermenting organisms from the sputum of cystic fibrosis (CF) patients presents many challenges to the microbiology laboratory. Molecular methods such as real time PCR and 16s rRNA sequencing have been shown to be superior to commonly used biochemical identification methods. MALDI-TOF MS based species identification is now being widely considered for routine use as it is rapid, inexpensive and requires a low level of expertise.

Methods: The purpose of this study is to compare MALDI-TOF MS to molecular methods and to assess if it is a reliable tool for routine use in the laboratory for identification of non-lactose fermenting Gram negative organisms, including Stenotrophomonas maltophilia, Pseudomonas aeruginosa and Burkholderia cepacia complex. A single colony of each isolate was initially deposited in duplicate on a MALDI-TOF MTP target plate and overlaid with matrix solution. A score of >2 indicated a high confidence of species level. Where the score yielded was <2 an extraction step was performed. The results were compared to those obtained using molecular methods.

Results: A total of 35 isolates were processed using MALDI-TOF MS. 26 isolates correctly identified to species level (score >2), 3 identified to genus level and one failed to identify. 5 isolates were misidentified. All genovars of B. cepacia complex were correctly identified.

Conclusions: MALDI-TOF MS is a useful, reliable tool for the identification of Gram negative organisms from the sputum of CF patients. However for infrequently isolated organisms molecular methods may still be required to confirm identification until more information is available about the use of MALDI-TOF for this purpose.

Exhaled breath hydrogen cyanide concentrations using selected ion flow tube mass spectroscopy (SIFT-MS): a comparison of on-line and off-line techniques

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Objective: The Space (Sensitivity of Pseudomonas aeruginosa [PA] detection by hydrogen Cyanide [HCN] in Exhaled breath) Study is taking place in 8 UK CF centres, it measures HCN concentrations in breath samples collected from children. The SIFT-MS instrument is not portable, so breath is collected in special sampling bags and transferred to the instrument for analysis. We investigated which bag to use, the maximum storage time before analysis and the benefit of warming samples to 37°C.

Methods: We studied 7 CF children with PA infection and 7 without. Each exhaled directly into the instrument (online) and also into two 25 micron Nalophan (25N), two 70 micron Nalophan (70N) and two Tedlar® (TED) bags. Bags were stored at 20 or 37°C. HCN concentrations were analysed at 1, 6, 24 and 48 hours (off-line).

Results: Mean(SD) on-line HCN concentration was 8.9 (5.4) parts per billion by volume. At 20°C on and off-line HCN concentrations had good correlation at 1 hr (R²=0.88) in the 25N bag and up to 6 hrs in the 70N and TED bags (R²=0.80 and 0.84). At 37°C the correlation was good in the 25N bag up to 6 hrs (R²=0.79) but up to 24 hrs for the 70N and TED bags (R²=0.82 and 0.86). Subsequent correlations were poor. Even when correlation was good, offline concentrations were lower as bag samples contain mixed expired breath, whereas online analysis only uses the alveolar portion.

Conclusions: Samples stored in TED or 70N bags, warmed to 37°C and analysed within 24 hours give HCN concentrations which correlate well with on-line measurements. The SPACE Study uses 70N bags as they are much cheaper and can therefore be discarded after a single use.

A decade of Burkholderia cepacia complex (Bcc) infection in Queensland, Australia: 2001 to 2010

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Background: Bcc has adverse effects on health status of people with cystic fibrosis (CF) and is a relative contra-indication of lung transplantation. Despite strict segregation policies new cases of Bcc infection continue to occur. However, the origins and transmission pathways of new infections are poorly understood.

Objectives: To determine if recent cases of Bcc infection were associated with cross-infection or other contributing environmental factors.

Methods: All cases of Bcc infection (n=63) that occurred in two Queensland CF centres (n=42 adults; n=18 children) between 2001 and 2010 were analysed. Multilocus sequence typing (MLST) was used to confirm identification and to determine relatedness. Prevalence and incidence rates were calculated. Contact tracing of patients and analyses of climatic changes were also assessed.

Results: The prevalence of Bcc infection was relatively stable over the study period (4.3% in 2001 and 3.2% in 2010). However, the incidence rate increased from 0.5% in 2001 to 1.3% in 2010. MLST was performed on 60 isolates resulting in 45 individual genotypes and 11 species. 67% of the incidence cases represented unique genotypes. Of the 15 patients infected with the seven shared genotypes, only 6 were potentially associated with cross-infection. After an initial period of drought (2001–2006) an increase in incidence cases coincided with above average rainfall.

Conclusions: Since 2007, there has been an increase in new cases of Bcc infection. MLST confirmed effective infection control measures, with little evidence of cross-infection during the study period. Changes in environmental conditions may play a role in the acquisition of Bcc in CF.