Canine detection of Pseudomonas aeruginosa (PA) volatile organic compounds (VOCs)

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Objective: To select and professionally train 2 dogs (2 yo foxhound cross and 1 yo retriever cross) using established technologies for the detection of blood, narcotics and explosives.

Method: Training was given over a 5 month period comprising 54 individual sessions involving both dogs with PA VOCs from wildtype clinical PA isolate grown to stationary phase. Dogs were then challenged with control VOCs obtained from 10 species of yeasts and fungi were also challenged, taken from 5 genera including Aspergillus flaveus/fumigatus/niger, Candida albicans/glabrata/krusei/parapsilosis, Exophiala dermatitidis, Penicillium sp. & Scedosporium apiospermum. On successful completion of this stage (non detection of PA), dogs were further challenged by introducing distractor VOCs ranging from one through 20 bacterial/fungal species in combination with/without the presence of PA VOCs.

Conclusion: Overall, the mean specificity was 96.2%. With 338 challenges, dog #1 successfully detected PA in all distractor combinations, had 3 false positive and 0 false negatives, whereas dog #2 had 9 false positive and 1 false-negative indications. To date, we have not attempted to estimate the sensitivity of detection. Phase III clinical trials are now being planned to detect PA VOCs directly from patient sputum and captured breath.

Molecular comparison of Pseudomonas aeruginosa isolates from patients with CF and non-CF bronchiectasis

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Introduction: Chronic infection with P. aeruginosa (PA) is common in both CF and non-CF bronchiectasis not related to CF (BE). Although well characterized in CF, less is known regarding PA infection in BE. This study aimed to compare PA isolates from both CF and BE.

Methods: PA from CF (n = 7) Liverpool epidemic strains (LES); n = 8 Belfast (BFS) isolates were compared to PA from BE (n = 16 isolates from 10 patients). BE PA isolates obtained pre- (n = 5) and post-antibiotic (n = 5) treatment of a pulmonary exacerbation were included, together with two distinct PA morphotypes (n = 6 isolates), from one patient. Isolates were characterized by Pulsed Field Gel Electrophoresis (PFGE).

Results: PFGE identified 4 main groups, each with >50% homology: Groups A (n = 7 NCFB BE PA; n = 1 BFS PA), B (n = 10 BFS PA; n = 3 LES PA), C (n = 8 NCFB BE PA), and D (n = 4 LES PA). Isolates taken pre- and post-antibiotics in one NCFB BE patient were >70% similar (group A), with the exception of 1 post-treatment isolate which shared <45% homology (group C). Different morphotypes isolated from this patient were found to be genotypically identical (>95% homology).

Conclusions: PA isolates from a BE patient pre- and post-antibiotic treatment were identical, with one exception. This genotypically distinct isolate may have initially been below detection limits or may represent a new colonization. In general, PA from CF and BE formed distinct PFGE groups, suggesting genotypic differences between isolates from CF and BE. Further numbers of isolates from both diseases are currently under investigation to confirm this finding.

Comparison of pulsed-field gel electrophoresis and DiversiLab system for typing Pseudomonas aeruginosa in cystic fibrosis patients

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Molecular typing of Pseudomonas aeruginosa (P. aeruginosa) in cystic fibrosis (CF) patients is important to monitor for evidence of cross-infection in this cohort of patients. Pulsed field gel electrophoresis (PFGE) has long been the gold standard method for typing, although problems with standardisation, reproducibility and the laborious nature of the technique has led to the development of sequenced based methods such as multi-locus sequence typing (MLST). While MLST has been shown to be more reproducible and can be compared between centres, it is still quite expensive and requires specialised equipment and trained staff.

Objective: In this study, we examined the use of the DiversiLab typing system, which is based on a patented rep-PCR method for typing of P. aeruginosa isolates.

Methods: The DiversiLab typing system consists of three parts: isolation of DNA, Amplification of the isolated DNA with rep-PCR and detection of the amplified material using chip electrophoresis. P. aeruginosa isolates isolated from CF patients were typed in parallel using PFGE and Diversilab.

Results: Preliminary analysis of 12 P. aeruginosa isolates by both methods revealed similar typing patterns in 84% of isolates and divergent results in 16% of isolates. Conclusions: The DiversiLab typing system is more rapid and user friendly than PFGE. Preliminary analysis suggests that the discrimination of P. aeruginosa strains by Diversilab is lower than by PFGE. Analysis of additional isolates will determine the true discriminatory power and reproducibility of the DiversiLab system for CF P. aeruginosa isolates.

Prevalence of transmissible strains of Pseudomonas aeruginosa in a cystic fibrosis tertiary referral centre and lung transplant unit

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Background: For cystic fibrosis (CF) patients colonised with transmissible strains of P. aeruginosa, in particular the Liverpool epidemic strain (LES), the requirement for lung transplantation or the 3-year rate of death has been reported as being greater than for those colonised with non transmissible strains. We examined a collection of P. aeruginosa from CF patients either assessed for transplantation or transplanted at our centre and from patients attending our CF unit to determine the prevalence of transmissible strains.

Method: Species-specific PCR was used to detect genetic markers for the LES, Manchester and Midlands epidemic strains from 86 CF patients in the transplant group and a combination of species-specific PCR, variable number tandem repeats and pulse field electrophoresis on single isolates from 169 CF patients attending our CF unit.

Results: Of the 169 isolates from patients attending our CF unit, 5.3% were colonised with the Manchester strain, 4.8% with the LES and 3.5% clone C. From the lung transplant group 6 isolates were identified as the LES and 2 as the Manchester strain. Three of six patients colonised with the LES strain underwent successful lung transplantation, the remaining three patients died pre-transplant. One of two patients colonised with the Manchester strain died post transplant. This was not attributed to Pseudomonas infection, the second patient died pre-transplant.

Summary: A small number of patients attending our CF unit are colonised with transmissible strains of P. aeruginosa. Our limited data demonstrates lung transplantation for CF patients colonised with LES has good outcomes.