157 Variation in canide production between different strains of *Pseudomonas aeruginosa*

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Aims: There is increasing interest in using the cyanogenic properties of *Pseudomonas aeruginosa* (PA) to develop a non-microbiological method for its detection. Prior to this, the variation in cyanide production between different PA strains needs to be investigated.
Methods: Hydrogen cyanide (HCN) released into the gas phase by 96 genotyped PA samples was measured using Selected Ion Flow Tube Mass Spectrometry after 24, 48, 72 and 96 hours of incubation. The HCN produced by a range of non-PA cultures and incubated blank agar plates was also measured.
Results: The 96 samples included 26 different strains; four were previously described clonal strains (Liverpool, Midlands1, Midlands2 and Stoke). All PA strains produced more HCN than the controls samples which generated extremely low levels (<10 parts per billion). Analysis across all time points demonstrated that non-mucoid samples produced more HCN than the mucoid samples ($p = 0.003$) but this relationship varied according to strain. There were clear differences in the headspace HCN concentration for different strains. Multivariate analysis of headspace HCN for the commonest strains (Liverpool, Midlands1 and Stoke) revealed a significant effect of strain ($p < 0.001$) and a borderline interaction of strain and phenotype ($p = 0.051$).
Discussion: Significant levels of HCN were detected in the headspace of all the analysed PA samples. Different strains produced varying amounts of HCN. PA phenotype also affected HCN production. This will create further interest in using the cyanogenic properties of PA to develop a diagnostic test.

158 *In vitro* co-culture of *Pseudomonas aeruginosa* and *Prevotella* spp.: Interaction between bacteria common to cystic fibrosis lung infection

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Introduction and Aims: Anaerobic bacteria are present in CF sputum as part of a diverse and metabolically active polymicrobial community. The aim of this study was to characterize the growth of *Pseudomonas aeruginosa* (PA) and *Prevotella* spp. in mixed culture and to investigate the interaction between these two species *in vitro*.
Methods: Growth curves were performed for *Prevotella* and PA alone and in co-culture. Where interaction between the two species was detected, PA were grown overnight and filter-sterilised to remove all cells and debris. Clinical *Prevotella* isolates ($n = 3$) were then grown under anaerobic conditions in the presence of the PA supernatants. Aliquots were removed at time intervals (0–56 h) and the *Prevotella* total viable count (TVC) determined following serial dilution.
Results: Co-culture of *Prevotella* with PA live cells resulted in a one $\log_{10}$ increase in *Prevotella* TVC at 32 hrs from 1×10^7 (control) to 1×10^8 cfu/ml (PA life cells). To determine if this increase in TVC was due to factors secreted by PA into the surrounding media, *Prevotella* were grown in the presence or absence of PA supernatant; *Prevotella* TVC at 58 hrs increased from 1×10^7 (control, no PA supernatant) to 1×10^8 cfu/ml (PA supernatant) indicating that factors secreted by PA were responsible for this increase.
Conclusions: Cell-cell signalling between PA and *Prevotella* spp. may play an important role in the growth of this anaerobe within the CF lung. Such an interaction has not previously been described between these two species and warrants further investigation.

159* Proteomic analysis of the *Burkholderia cepacia* complex outer membrane proteins involved in adhesion to lung epithelial cells

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Burkholderia cepacia complex (BCC) is a group of opportunistic pathogens in cystic fibrosis (CF) patients. Their inherent resistance to antibiotics suggests that prevention of colonization may be a more effective means of improving the quality of life for CF patients. Identification of the bacterial outer membrane proteins (OMP) that are involved in host cell attachment may help in the design of prophylactic therapies to prevent colonization.
Objectives: (1) to identify the OMPs that are involved in the adhesion of Bcc to lung epithelial cells, CFBE41o- (2) and to identify whether these proteins are immunogenic using CF patient sera.
Methods: The OMPs were isolated individually from two *B. cenocepacia* strains and two *B. multivorans* strains, separated by 2-D electrophoresis and blotted onto PVDF membranes. To identify the proteins involved in lung cell attachment, the blots were probed with CFBE41o- cells, fixed and detected with anti-epithelial antibody. Immunogenic OMPs from CF patients were detected by probing with CF patient sera, from BCC^+ or BCC^- patients. Proteins were detected by chemiluminescence and identified by excising corresponding proteins from a Coomassie blue stained gel, digesting and subsequently analysed by MALDI-TOF/MS.
Conclusions: Fifteen different proteins were identified as being involved in attachment to lung cells from four strains, only two of which were common to all four strains. In contrast, over 60 immunoreactive proteins were identified. Several of the proteins involved in lung cell attachment were also highly immunoreactive to serum from Bcc^+ CF patients, indicating that these are major antigens and may have potential as vaccine candidates.

160* Acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) by patients with cystic fibrosis: risk factors and impact on outcome

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Objectives: Factors associated with MRSA acquisition and its potential impact on CF outcome are studied.
Methods: A retrospective case-control study from 2002 to 2010, comparing variables and outcome of patients having MRSA positive respiratory cultures with age-sex matched controls who never cultured MRSA.
Results: Twenty-seven (14F/13M – mean age 18.5 yrs) of 165 patients (16.3%) identified as MRSA positive at some time, were included in the MRSA group. Chronic infection (3 or more MRSA cultures during follow-up) was found in 21 out of 27 patients. Comparators were 27 age-sex-matched controls with a mean BMI of 19.1 (versus 18.2 in the MRSA group; $p = 0.9$). Following observations in the MRSA group were significant with respect to controls: all were pancreas insufficient (versus 21/27 in the controls; $p = 0.007$), *Pseudomonas* co-infection was present in 74% (versus 33%; $p = 0.006$), bronchiectasis was found in 100% (versus 63%; $p = 0.002$), 70% were ΔF 508 homozygote (versus 33%; $p = 0.014$), and 81% was admitted to the hospital (compared to 33% $p = 0.001$). Differences in CFRD prevalence (37% versus 22% in the control group; $p = 0.4$) and decline in FEV_1 (2 ± 2(SD)%pred versus 1 ± 2(SD)%pred; $p = 0.4$) were not significant, yet, there was a clear trend towards a lower mean FEV_1 at diagnosis in the MRSA group (66 ± 28(SD)%pred versus 79 ± 23(SD)%pred; $p = 0.08$). During the study period 2 patients died and 2 others underwent lung transplantation, all 4 in the MRSA group.
Conclusion: The presence of MRSA is associated with higher incidence of pancreasinsufficiency, of *Pseudomonas* infection and bronchiectasis, and with a trend towards a lower FEV_1 at diagnosis. MRSA infection might reflect worse survival.