Clinical profile of *Pseudomonas aeruginosa* clone C infection in CF children

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Objectives: Several highly transmissible and virulent genotypes of *Pseudomonas aeruginosa* have been recognised in the UK including the Liverpool, Midlands and Manchester genotypes and Clone C. It has been suggested that patients colonised with a transmissible genotype may have a poorer prognosis and increased treatment needs. We describe the clinical features of patients who isolated *P. aeruginosa* clone C in our paediatric CF centre.

Methods: The hospital microbiology database was used to identify the patients. There were five isolations from five patients (four boys, aged 1−12 years, mean 4.4 years). All isolations were new, obtained from cough swabs performed at routine clinics. Four patients had new wet cough, one of whom had reduced lung function. One patient had no new clinical features. Four patients received intravenous antibiotics and one patient oral antibiotics. The organisms were sensitive to standard antipseudomonal antibiotics. The patients were removed from clinic and seen separately until confirmed negative for infection. Four patients remained free of infection in the one year following isolation. Three patients underwent bronchoscopy and BAL was culture negative. One patient had subsequent second isolation in the same year. The frequency of pre and post pseudomonas isolation respiratory exacerbations remains similar. All five are on conventional physiotherapy with no standard antipseudomonal antibiotics. The patients were removed from clinic and seen separately until confirmed negative for infection. Four patients remained free of infection in the one year following isolation. Three patients underwent bronchoscopy and BAL was culture negative. One patient had subsequent second isolation in the same year. All five are on conventional physiotherapy with no additional requirement of pharmacological treatment of chest disease.

Conclusion: *P. aeruginosa* clone C isolates in our centre are currently sensitive to standard antibiotics. We have found no evidence of increased virulence or that the organism is overtly transmissible.

Adaptation of *Pseudomonas aeruginosa* in the CF host: Link between laboratory findings and the clinic

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Objectives: *Pseudomonas aeruginosa* (PA) can adapt to the host environment, adopting a biofilm-like lifestyle associated with increased antibiotic resistance, leading to infections difficult to treat with poor prognosis. We aim to investigate the clinical impact of laboratory found adaptations of PA strains in 3 patients.

Methods: Sputum collection was performed in 2 consecutive years to study the PA biofilm. We reviewed the medical files of 3 patients in whom the lab found evidence of transmission of PA (2002→2012).

Results: The patients, born in 1973 (A), 1982 (B) and 1975 (C), were colonized with PA before 2000. Standard treatment was similar. Subject A suffered from CF-related diabetes (CFRD) since 1990. A and C remained rather stable, with only mildly declining lung functions (FEV1 declining from 83 resp. 57% in 2002 to 72 resp. 43%). B had a greater decline in lung function (58% in 2002 to 26% in 2011), needed more IV treatment, and was eventually transplanted in 2011. The same mucA gene mutation was found in the PA isolates of the 3 patients, however the PA isolate of B also showed a deletion in the lasR gene, which was not present in the PA isolates of A and C.

Conclusion: We conclude that transmission occurred and that PA responds to a rapidly evolving lung environment (a decline in pulmonary status) by accumulating mutations in regulatory genes, such as the lasR gene.

Pseudomonas aeruginosa quorum-sensing molecule inhibits junctional integrity and cell-to-cell communication in human airway epithelial cells

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Cell-to-cell communication via gap junction (GJ) channels coordinates a signaling network to regulate airway epithelial cell homeostasis and to maintain efficient epithelial host defense. In *P. aeruginosa* (Pa), cell-to-cell communication based on quorum-sensing (QS) molecules is known to coordinate the production of virulence factors and biofilms by bacteria. QS signals may also incidentally modulate mammalian cell response to the pathogen. We investigated here whether Pa N-3-oxo-dodecanoyl-L-homoserine lactone (C12) may interfere with the host GJ cell-to-cell communication in human airway epithelial cells (HAEcCs).

We demonstrate that C12, applied extra- or intracellularly, rapidly decreased GJ conductivity in a reversible manner. Other QS molecules, including PQS, C4 or the C12 analog dDHL, had no effect on GJ communication. We also show that C12 promoted calcium influx in HAEcCs by activating store-operated calcium channels, leading to loss of GJ function. In non-polarized HAEcCs, C12 evoked shrinkage and blebbing that could be prevented by inhibitors of Src and ROCK signaling pathways. The latter inhibitors also prevented GJ uncoupling in the presence of C12. Interestingly, the effects of C12 on epithelium integrity were not observed on polarized HAEcCs grown on Transwell filters. We further show that C12 degradation, a reaction catalyzed by intracellular peroxynase 2 (PON2), was more potent in polarized cells. Interestingly, PON2 expression was decreased in non-polarized cells. These results suggest that loss of HAEcC integrity impairs C12 degradation, and thus defense of the epithelium to the QS molecule, providing an additional mechanism for Pa invasion.

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Conclusion: We conclude that transmission occurred and that PA responds to a rapidly evolving lung environment (a decline in pulmonary status) by accumulating mutations in regulatory genes, such as the lasR gene.

Bronchial infection with *Burkholderia cepacia* complex in cystic fibrosis. Experience at a Spanish regional cystic fibrosis centre

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Objectives: Chronic bronchial infection (c.b.i.) with *Burkholderia cepacia* complex (Bcc) in CF is a serious complication. Prevalence In the ECFS Registry 2008−2009 report was 0 to 11% (Spain 3.5%). Differences may be largely due to variable Microbiology testing. Biochemical testing is not reliable and DNA testing is needed. Bcc comprises 17 species. Most patients have infection with *B. cenocepacia* or *B. multivorans* (Bm). Information on the outcome in patients infected with other species is scarce.

Methods and Results: Bcc was grown in 28 CF patients since 1982 to December 2012. Strains were studied at a National Reference Laboratory except in two who died with cepacia syndrome in 1991 and 2002. Eight had Bm, 7 B. contaminans (Bc), 4 B. cepacia (Bcp), 3 B. vietnamiensis (Bv), 3 B. stabiles (Bs) and 1 B. arboris. Their prevalence rose from 2/104 (2%) in 1997 to 22/174 (13%) in 2011 and 17/180 (9.4%) in 2012. Twenty were colonized in 2009−2012. Age at colonization was 19 years (6−36) and mean FEV1 73% Pred (106−34). Mean follow-up is 3.4 years (0.2−14). The outcome was highly variable. Some had steep decline (sd) and others little change. Follow-up was > 2 years in 15. The mean annual % FEV1 change (mac) ranged from −13 to + 15. Five (33%) had sd. (mac < −3): 4/7 with Bm and 1/2 with Bcp, vs none with Bs, Bv or Bc. All with either Bm or Bcp developed c.b.i. vs 0/3 with Bs, 1/2 with Bv and 1/1 with Bc.

Conclusion: Our increasing prevalence of Bcc probably mirrors changing labora-
ty methods with all suspicious strains routinely studied at a Reference Laboratory since 2009. The frequency of sd was higher in patients with Bm or Bcp but numbers are very small. None with Bs has developed c.b.i.