124 Saliva IgA antibodies is a diagnostic tool for sinusitis caused by *Pseudomonas aeruginosa* in patients with cystic fibrosis

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Background: When cystic fibrosis (CF) patients become chronically infected with *P. aeruginosa*, constantly elevated specific immunoglobulin G (IgG) and immunoglobulin A (IgA) against *P. aeruginosa* are seen. We believe that *P. aeruginosa* often colonizes and adapts in the paranasal sinuses, which later results in an IgG elevation, years before establishing the chronic lung infection. We therefore hypothesize that an early *P. aeruginosa*-sinusitis simultaneously causes a local rise in the specific IgA in mucous secretions, preventing the adherence of *P. aeruginosa*, thus inhibiting a local and a systemic immune response.
Material and Method: 108 CF patients were enrolled in this cross-sectional study. Antibodies against *P. aeruginosa* standard antigen (St-Ag) and *P. aeruginosa* alginate were measured in serum, saliva and nasal secretion by means of ELISA.
Results: Patients with chronic *P. aeruginosa* infection had high IgA and IgG antibody responses against alginate and St-Ag. Patients intermittently colonised with *P. aeruginosa* had a higher mean IgA St-Ag than CF patients with other pathogens being 70–75% higher ($P = 0.02$). Patients intermittently colonised with *P. aeruginosa* had a higher St-Ag "IgA saliva: IgG saliva ratio" compared with chronically infected patients ($P = 0.04$) and compared with CF patients with other pathogens ($P = 0.02$).
Conclusion: We present a cheap method with high sensitivity that early can diagnose *P. aeruginosa* sinusitis and differentiate between patients chronically infected, intermittently colonised with *P. aeruginosa* and other CF patients.

125* Comparison of real time diagnostic chemistries to detect early and chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis patients

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Infection by *P. aeruginosa* usually occurs at some point during childhood, often acquired from the environment, and once established can be difficult to eradicate. Early, aggressive treatment is key to keeping airways free of *P. aeruginosa*, however this requires rapid identification of this opportunistic pathogen by diagnostic laboratories.
Although the use of real time PCR (RT-PCR) in diagnostic microbiology is increasing, in CF microbiology diagnostics, culture-based methods still dominate. Here, we describe a rapid DNA extraction method and assays for the quantitative detection in patient samples of (1) all bacteria, (2) *P. aeruginosa* and (3) the Liverpool epidemic strain (LES), using two different RT-PCR chemistries: SYBR green and multiplex probe-based assays. Initial comparisons revealed that RT-PCR can be up to 6 log units more sensitive when comparing copy number to colony forming units identified on culture medium. Using >180 sputum and cough swabs from both adult and children CF patients we have compared both these methods to the existing culture-based methods in terms of sensitivity and time to result. There was 100% concordance between the 3 methods in terms of bacterial status of patients; however the time to result was dramatically reduced. 85% of adult patients were found to be infected with *P. aeruginosa* and 90% of these had the LES. Furthermore, fluctuations in *P. aeruginosa* number could also be detected using RT-PCR.
Rapid identification and subsequent antibiotic treatment of *P. aeruginosa* could be pivotal in prolonging infection-free airways in CF patients.
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126 Characterisation of a cluster of *Pseudomonas aeruginosa* isolates from cystic fibrosis (CF) and non-CF patients

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The prevalence of the Liverpool, Manchester and Midlands 1 strains of *Pseudomonas aeruginosa* in UK CF centres was determined in a study conducted in our national reference laboratory in 2004 [1]. The segregation of CF patients based on infection "status" is widely practised, and though there is limited published evidence for its success, it is believed to have reduced the transmission of shared strains. Our laboratory routinely performs national surveillance of *P. aeruginosa* from UK hospitals using "Variable Number Tandem Repeat" (VNTR) analysis [2]. In 2010 we discovered a cluster of isolates with the VNTR profile 8, 3, 4, 5, 2, 3, 5, 2 with a variable ninth locus. In all, we received 38 isolates from 35 patients across 23 hospitals with this profile. Twenty-five were from CF patients, 10 from non-CF patients and 3 from hospital taps. Both pulsed-field gel electrophoresis analysis, and PCR screening of the accessory genome supplied further evidence that these isolates represent a single strain. The clinical relevance of this cluster remains unclear. This VNTR profile was first noted in non-CF patients in 2006, while the earliest detection in CF patients was in 2009, perhaps suggesting this strain is currently adapting to the CF lung.

Reference(s)

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127* Array tube genotyping of *Pseudomonas aeruginosa* isolates as a strategy for identifying potential emerging transmissible strains

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Background: *Pseudomonas aeruginosa* is a major pathogen in the CF lung and once established is impossible to eradicate. The highly transmissible Liverpool Epidemic Strain (LES) was already widespread in the community prior to its discovery. A previous study of non-LES isolates (2005–06) from a paediatric CF unit, using Clondiag Array Tube genotyping, revealed a high prevalence of a single clone (D). The aim of this study was to genotype more recent isolates (2009–11) from non-LES patients within the paediatric and adult CF Units in Liverpool.
Methods: 37 isolates were analysed (22 from the paediatric unit and 15 from the adult unit) using Array Tube genotyping. The formerly identified Clone D isolates (6) were additionally genotyped by PFGE (using *Spe1*) and by RAPD-PCR.
Results: Of the 22 paediatric isolates 18 Clone types were identified with 3 (C, P and A3) being found more than once. 13 clone types were identified from the 15 adult isolates with 2 types found in more than one patient. None of the more recent isolates were clone D. Isolates identified previously as clone D using the Array Tube system varied according to the other genotyping methods used (PFGE and RAPD). There was some concordance between the groupings obtained using PFGE and RAPD typing.
Conclusion: This study demonstrates that clone D was most likely transient in the CF population. Regular surveillance, involving genotyping of *P. aeruginosa* isolated from patients and the hospital environment, would be beneficial in the identification of newly emerging problem strains and the prevention of transmission.
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