Molecular Epidemiological Analysis Suggests Cross Infection with *Pseudomonas aeruginosa* is Rare in Non-Cystic Fibrosis Bronchiectasis.

A De Soyza\textsuperscript{1,2,*}, A Perry\textsuperscript{3}, A J Hall\textsuperscript{4}, S Sunny\textsuperscript{2}, KE Walton\textsuperscript{3}, N Mustafa\textsuperscript{5}, J Turton\textsuperscript{5}, DT Kenna\textsuperscript{5}, C Winstanley\textsuperscript{4}

1) Newcastle University, Institute of Cellular Medicine and 2) Sir William Leech Centre for Respiratory Research and Freeman Hospital Adult Bronchiectasis Unit, Newcastle upon Tyne Hospitals Trust, 3) Department of Medical Microbiology, The Newcastle upon Tyne Hospitals NHS Foundation Trust, 4) Institute of Infection and Global Health, University of Liverpool 5) Antibiotic Resistance and Healthcare Associated Infections Reference Unit, Public Health England, Colindale, London

Key words

Epidemic strain, Bronchiectasis, *Pseudomonas aeruginosa*, Genotyping, Variable Number Tandem Repeat, Array Tube

*Corresponding author*\textsuperscript{*} Anthony De Soyza, anthony.de-soyza@ncl.ac.uk, Institute of Cellular Medicine, Sir William Leech Building, The Medical School, Newcastle University, NE2 4HH
In both Cystic fibrosis (CF) and non CF bronchiectasis (NCFBr) chronic *Pseudomonas aeruginosa* infection is adversely prognostic.\textsuperscript{1,2} In CF, epidemic infections with specific clones of *P. aeruginosa* are associated with further adverse outcomes.\textsuperscript{3,4} This cross infection risk has led to segregation of patients.\textsuperscript{5} There are few data on *P. aeruginosa* cross infection in NCFBr. As a result segregation in NCFBr has not been addressed in guidelines.\textsuperscript{6}

Our aim was to undertake a cross infection study in NCFBr. This was undertaken in an adult Bronchiectasis service, North East of England, U.K that is separated from the regional CF unit (sited 2 miles away). The service was initiated in 2007 with a weekly specialist clinic without a *Pseudomonas*-specific clinic. When NCFBR patients are hospitalised there is a preference for cubicle-based (single patient room) management, but when cubicles are unavailable patients are managed in 6-bedded bays. All patients had Computed Tomographic (CT) confirmation and were predominantly idiopathic or post-infectious bronchiectasis with CF excluded following current guidelines.\textsuperscript{6} The study had ethical permission and Caldicott approval.

Fifty-six isolates were selected for analysis. Six were chosen from CF patients as laboratory controls. Fifty were NCFBr isolates collected between 2008 and 2011 from 40 NCFBr patients. Thirty-six patients (patients 1-36) attending the Adult Bronchiectasis service were randomly selected. Longitudinal isolates were included from ten patients with on average 16 months (range 2-35 months) between isolates. Additionally single isolates were chosen from four NCFBr patients (patients 37-40) as potential patient controls who had not attended the specialist clinic nor had been hospitalised. Isolates were cultured from spontaneous sputum and identified as *P. aeruginosa* by routine biochemical methods and MALDI-TOF mass spectrometry.

We used two genotyping methods; in each case testing was blinded. We used the ArrayTube (AT genotyping, Alere, Germany) as per manufacturer’s protocol. This system detects 13 single-nucleotide polymorphisms within conserved *P. aeruginosa* genes and the presence / absence of 38 variable genetic markers in the accessory genome. The data was converted into a four digit hexadecimal code and then compared to a database.\textsuperscript{7,8} Secondly we used Variable Number Tandem Repeat (VNTR) analysis based on variation in the number of DNA repeats at specified sites across the genome at nine variable loci\textsuperscript{9} with comparison to a VNTR database.\textsuperscript{10}
The vast majority of the 36 NCFBr patients attending the service (patients 1-36) harboured their own strains (Figure 1). Patients 11 and 27 had distinct variants of clone C, which is a lineage that is widely found globally\textsuperscript{10}; two patients in the non-hospitalised control group also had further variants of this clone (patients 39 and 40). Among the 34 remaining NCFBr patients attending the service, there were three pairs (patients 2 and 34, 12 and 19 and 16 and 17) whose isolates of \textit{P. aeruginosa} shared very similar profiles by at least one method (Fig 1). For patients 12 and 19, this was confirmed by pulsed-field gel electrophoresis of \textit{Spe}I-digested genomic DNA; this is an unusual profile and that these patients shared the same strain almost certainly does reflect cross-infection. Patients 2 and 34 both had a widely found type belonging to ST 27, while patients 16 and 17 had clearly distinct strains by VNTR, suggesting only one probable case of cross-infection.

The location of the NCFBr isolates amongst the wider population structure of \textit{P. aeruginosa}, based on AT genotype, was mapped (not shown) and demonstrated that NCFBr isolates were widely distributed. AT analysis found several pattern matches with the AT database: Matches were with AT clones A2, K, E, I, A7, A, L, J, A5 and U. Both methods correctly identified the known control strains from CF patients.

Longitudinal paired isolates from ten patients were examined (patients 3, 6, 7, 8, 9, 10, 11, 14, 16 and 18). For nine patients, the first and second were indistinguishable by both AT and VNTR genotyping, confirming persistence of the initial strain. In one patient (patient 11) VNTR analysis indicated that both isolates were identical but AT genotyping did not.

Our key finding was the absence of dominant clones of \textit{P. aeruginosa} with little evidence of cross infection and many strains “unique”. Whilst there is distinct clustering of AT clone types in ocular keratitis, the isolates from NCFBr were widely distributed in the \textit{Pseudomonas} population structure. Collectively this suggests that sporadic infection is the most common mode of infection in NCFBr. Clone C was only found in 6 % of the NCFBr patients and is known to be widely distributed within the environment.\textsuperscript{8} Cross infection with clone C seems unlikely as the VNTR profiles varied at two or three loci, suggesting that they were independently acquired. Other isolates matched those previously isolated from various clinical and environmental sources.\textsuperscript{8} These data may suggest a risk of environmental acquisition in NCFBr.\textsuperscript{5,8}

The absence of a dominant \textit{P. aeruginosa} clone in NCFBr may reflect many factors, including the lower incidence of \textit{P. aeruginosa} infection in NCFBr (less than 50%) as
compared to CF (up to 80%). The probable lower rate of hospitalisation in NCFBr as compared to CF, may also limit cross-infection exposures. The standard infection prevention and control measures used in our NCFBr centre are less stringent than those implemented in designated CF centres. The absence of significant cross infection herein is therefore unlikely to be due to a higher standard of infection control practices.

Notably our study is solely focussed on *P. aeruginosa* cross infection and we cannot exclude cross infection in the NCFBr population with *S. aureus*, *H. influenzae* or non-tuberculous mycobacteria. Furthermore limitations of our study include the single centre design. Our observations may have been different if our NCFBr centre facilities were shared with our regional CF clinic. We therefore advocate further multicentre studies to delineate the cross infection risks.
References


Acknowledgements

ADS acknowledges a HEFCE Senior lectureship and funding from the National Institute for Health Research (NIHR) Biomedical Resource Centre for Ageing (Newcastle).
Figure 1