Molecular epidemiology of *Pseudomonas aeruginosa* in an unsegregated bronchiectasis cohort sharing hospital facilities with a cystic fibrosis cohort

Corresponding author:

Philip J. Mitchelmore, Department of Respiratory Medicine, Royal Devon and Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW, United Kingdom, pm339@exeter.ac.uk, 07712124799

Authors:

Philip J. Mitchelmore[1,2], Joanna Randall[3], Matthew J. Bull[4], Karen A. Moore[5], Paul A. O'Neill[5], Konrad Paszkiewicz[5], Eshwar Mahenthiralingam[4], Chris J. Scotton[1], Christopher D. Sheldon[2], Nicholas J. Withers[2], Alan R. Brown[5].

[1]Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, Exeter, UK.

[2]Department of Respiratory Medicine, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

[3]Department of Microbiology, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

[4]Organisms and Environment Research Division, Cardiff School of Biosciences, Cardiff University, Cardiff, UK.

[5]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK.

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Abstract

Whilst *Pseudomonas aeruginosa* (PA) cross-infection is well-documented amongst cystic fibrosis (CF) patients, the equivalent risk amongst non-CF bronchiectasis (NCFB) patients is unclear, particularly those managed alongside CF patients. We performed analysis of PA within a single centre that manages an unsegregated NCFB cohort alongside a segregated CF cohort. We found no evidence of cross-infection between the two cohorts, nor within the segregated CF cohort. However, within the unsegregated NCFB cohort, evidence of cross-infection was found between three (of 46) patients. Whilst we do not presently advocate any change in the management of our NCFB cohort, longitudinal surveillance is clearly warranted.

Pseudomonas aeruginosa (PA) is a significant pathogen within cystic fibrosis (CF) and non-cystic fibrosis bronchiectasis (NCFB) cohorts. Transmissibility of PA amongst CF patients has been widely-documented,[1] leading to widespread segregation policies.[2] In contrast, the cross-infection risk amongst NCFB patients is unclear. One UK study concluded that PA cross-infection was rare in NCFB.[3] However, in that study, NCFB patients were managed at a different site from the local CF cohort. Similarly, a recent multi-centre study highlighted the potential for PA cross-infection, although this was again exclusively focused on NCFB cohorts.[4] In many hospitals, including ours, CF and NCFB patients share facilities and healthcare professionals. In this context, we conducted a cross-sectional study of PA within our NCFB and CF cohorts to assess the likelihood of cross-infection. In parallel, analysis of local non-respiratory isolates allowed comparison with PA in the wider population.

Sixty-three NCFB and 32 CF patients were recruited from out-patient clinics based on a documented diagnosis of NCFB/CF and previous PA-positive sputum. PA was subsequently obtained from 46/63 NCFB and 22/32 CF patients. Ten representative colonies were stored from each PA-positive sputum, and were initially genotyped by Random Amplification of Polymorphic DNA (RAPD)[5], ahead of Multi-Locus Sequence Typing (MLST).[6] In brief, RAPD was performed on all 10 isolates per patient, and all unique profiles underwent additional evaluation using microfluidic amplicon separation and cluster analysis as described previously.[7] This same panel of isolates with unique RAPD profiles was subjected to MLST, enabling strain identification in a global context. Patient demographics and methodologies are detailed in online supporting information.

Through this approach, 25/46 NCFB patients (54%) and 13/22 CF patients (59%) were found to harbour their own unique strain by MLST. The remaining patients harboured strains that were shared within or between cohorts (Table 1).

Table 1. Shared strains of *Pseudomonas aeruginosa* identified within the respiratory (CF and NCFB) and non-respiratory cohorts, as defined by MLST. One NCFB patient was co-infected with ST17 and ST564. Isolates in the non-respiratory cohort originated from genitourinary, wound, ENT and faecal samples from community and hospital investigation.

MLST type	**Alias**	**NCFB** (n=46)	**CF** (n=22)	**Non-resp** (n=76)	**Total** (n=144)
ST17	Clone C	8 (17%)		7 (9%)	15 (10%)
ST27		1 (2%)	3 (14%)	5 (7%)	9 (6%)
ST146	LES		2 (9%)		2 (1%)
ST235		1 (2%)	1 (5%)		2 (1%)
ST252		3 (7%)	1 (5%)		4 (3%)
ST253	PA14	2 (4%)		10 (13%)	12 (8%)
ST274		1 (2%)	1 (5%)	2 (3%)	4 (3%)
ST395		3 (7%)	1 (5%)	3 (4%)	7 (5%)
ST564		3 (7%)			3 (2%)

All shared strains within our respiratory cohorts are globally-distributed according to the MLST database, and the majority have been isolated from diverse clinical and environmental sources. Consistent with this, we observed many of the same strains within our non-respiratory cohort (Table 1). Given the ubiquitous nature of these strains, their presence in multiple patients may reflect independent acquisition rather than cross-infection, and neither RAPD nor MLST provide sufficient resolution to address this. Consequently, whole genome sequencing (WGS) was performed on the shared strains from the respiratory cohorts to assess relatedness at a

whole-genome level. For ST17 (the most prevalent strain observed), three isolates per patient were sequenced to enable assessment of inter- and intra-patient diversity. For all other shared strains, one isolate was sequenced per patient.

In WGS-based studies, patient-to-patient transmission cannot be defined based on a simple threshold of the number of single nucleotide polymorphisms (SNPs) between isolates. Hypermutation accelerates genetic divergence, and hypermutable PA are commonly observed within chronic lung infections.[8] Consistent with this, *in silico* prediction of hypermutators revealed putative hypermutable PA within our respiratory cohorts (online supporting information) and predicted hypermutator status correlated strongly with SNP distance (Fig. 1B), highlighting the difficulty in setting a SNP threshold. Consequently, to aid interpretation, we incorporated relevant publicly-available genome sequences into our WGS-based analysis, enabling us to compare PA isolates from our respiratory cohorts with representative PA that belong to the same sequence type but are not epidemiologically-linked to our cohorts (see online supporting information). Furthermore, when considering likelihood of cross-infection, relevant patient-specific and strain-specific information was reviewed, including potential cross-infection events, duration of infection, change in culture status and knowledge of strain distribution and transmissibility.

The WGS analysis revealed that the CF and NCFB isolates belonging to ST17, ST27, ST235, ST252, ST253, ST274 and ST395 are as divergent from each other (Fig. 1A; circles) as they are from unconnected representatives of the same sequence type (Fig. 1A; grey crosses). Furthermore, with the exception of two intra-patient pairwise comparisons (one involving a predicted hypermutator), analysis of the ST17 isolates revealed significantly greater ST17 diversity between patients than within patients. Whilst cross-infection cannot be completely ruled out, particularly for the divergent ST17 group, we conclude (on the basis of inter-patient diversity, the ubiquitous nature of these strains and a review of relevant clinical information)

that the occurrence of these seven sequence types in multiple patients most likely reflects independent acquisition.

In contrast, two shared strains exhibited low inter-patient diversity that we believe to be indicative of cross-infection. Firstly, two CF isolates of ST146 (Liverpool epidemic strain, LES) differed by only 31 SNPs, and were more closely-related to each other than to unconnected representatives of the LES (Fig. 1A). The two patients involved were siblings who had become colonised with PA before coming under the care of our unit. The genetic relatedness of the isolates coupled with the high level of personal contact between patients and the known transmissibility of LES strongly supports cross-infection.

More significantly, the ST564 isolates from three unrelated NCFB patients are near-identical, differing by only 4-12 SNPs. Whilst no publicly-available genomes of ST564 representatives were available for comparison, we believe this extremely high level of genetic relatedness is indicative of cross-infection, a conclusion further supported by clinical records that revealed two of the three patients shared a waiting area and lung function room approximately 17 months prior to recruitment. This potential cross-infection event did not coincide with a clear change in PA culture status as one of the patients intermittently isolated PA before and after this event whilst the other patient had evidence of multiple PA strains (and therefore super-infection may have occurred). Whilst we were unable to identify potential cross-infection event(s) involving the third ST564-infected patient (who also carried multiple strains), a difference of only 4 SNPs strongly supports cross-infection. Interactions may have occurred in or outside the hospital that are not apparent via the review of clinical notes. Furthermore, we believe ST564 acquisition from a common environmental source is highly unlikely due to its absence from other cohorts. In agreement with previous literature,[3] but expanding it to high-resolution WGS analysis, we therefore conclude that PA cross-infection is highly likely to have occurred within our NCFB cohort. Whilst we believe this to be restricted to ST564, additional cross-infection events

involving other sequence types cannot be definitively ruled out, particularly given the confounding role of hypermutators. Similarly, on the balance of evidence, we consider it unlikely that cross-infection has occurred between CF and NCFB cohorts despite them sharing facilities, and notable differences in strain distribution between the cohorts argue against the presence of an environmental reservoir within the unit. Our studies suggest that ST564 has the potential for transmissibility and super-infection. Although not reported in the literature, the MLST database (https://pubmlst.org/paeruginosa; [9]) reports ST564 as having been isolated from sputum (Netherlands) and water (Australia and France).

At present, we believe the negative impacts that would be associated with implementing a segregated NCFB cohort (including reduced patients per clinic and reduced access to pulmonary rehabilitation courses) outweigh the low risk of cross-infection. However, with growing NCFB cohorts nationwide[10] and cross-infection possible, ongoing longitudinal surveillance is clearly warranted.

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Competing Interests

EM declares a grant from AlgiPharma AS held as a service contract on a CF clinical trial (unrelated to this work).

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Contributors

Study Concept & Design, CDS, NJW, PJM & ARB; Patient recruitment, PJM, NJW & CDS; Methodology & Investigation, PJM, JR & KAM; Data Analysis & Interpretation, PJM, ARB, MJB, EM, PAO & KP; Writing – Original Draft, ARB & PJM; Writing – Review & Editing, CDS, NJW, CJS, EM, MJB, ARB & PJM.

Ethics approval

Ethical approval for the study of our NCFB cohort was obtained through the NRES Committee South West- Exeter (14/SW/0080). Our CF samples and data were collected through the RD&E tissue bank (11/SW/0018).

Provenance and peer review

Not commissioned

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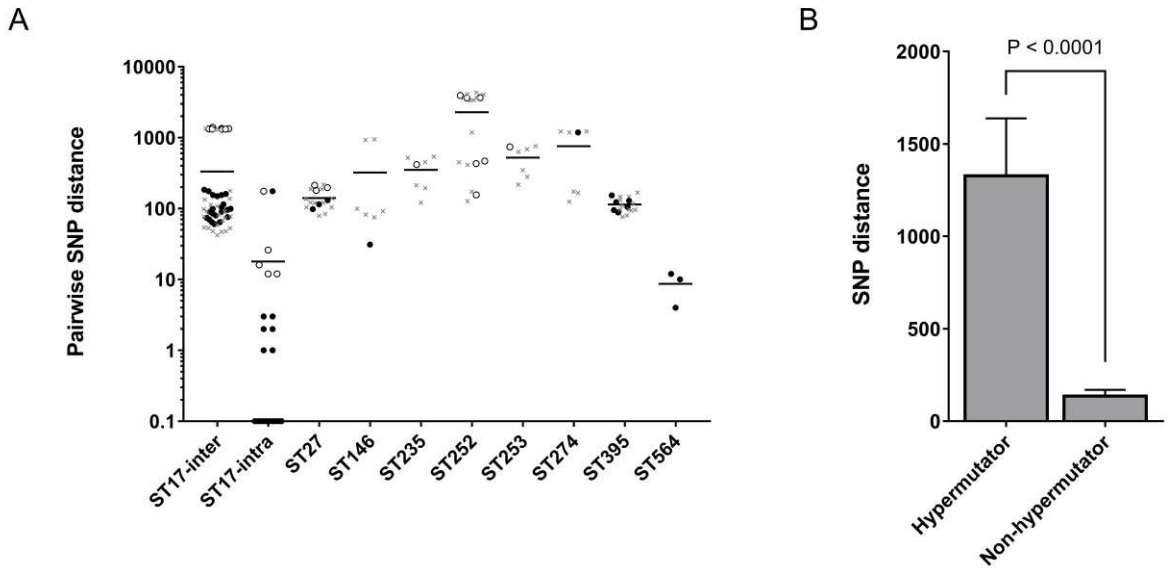
Figure legends

Figure 1. Genetic diversity within *P. aeruginosa* isolates, as defined by whole genome sequencing. (A) The number of single nucleotide polymorphisms (SNPs) was calculated across the core genome of all sequenced isolates. Each data point represents a pairwise comparison

within each ST, with the bar representing the mean. Circles represent pairwise comparisons that are exclusively between PA isolates from our own respiratory cohort (CF or NCFB), with the open circles representing those comparisons in which at least one isolate is a predicted hypermutator. The grey crosses represent pairwise comparisons in which one isolate is from our respiratory cohort and the other is an unconnected representative of the same sequence type (using publicly-available genomes). For ST17, SNP numbers are shown that reflect the diversity observed between patients (ST17-inter) and within individual patients (ST17-intra; based on sequencing of three isolates per patient). (B) Predicted hypermutable PA isolates exhibited significantly elevated levels of genetic divergence (SNP distance) relative to predicted non-hypermutable PA.



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