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

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22

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26 **Abstract**

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

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

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

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

59

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

64

<b>MLST type</b>	<b>Alias</b>	<b>NCFB</b> (n=46)	<b>CF</b> (n=22)	<b>Non-resp</b> (n=76)	<b>Total</b> (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%)

65

66

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

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

77 In WGS-based studies, patient-to-patient transmission cannot be defined based on a simple  
78 threshold of the number of single nucleotide polymorphisms (SNPs) between isolates.  
79 Hypermutation accelerates genetic divergence, and hypermutable PA are commonly observed  
80 within chronic lung infections.[8] Consistent with this, *in silico* prediction of hypermutators  
81 revealed putative hypermutable PA within our respiratory cohorts (online supporting  
82 information) and predicted hypermutator status correlated strongly with SNP distance (Fig.  
83 1B), highlighting the difficulty in setting a SNP threshold. Consequently, to aid interpretation,  
84 we incorporated relevant publicly-available genome sequences into our WGS-based analysis,  
85 enabling us to compare PA isolates from our respiratory cohorts with representative PA that  
86 belong to the same sequence type but are not epidemiologically-linked to our cohorts (see  
87 online supporting information). Furthermore, when considering likelihood of cross-infection,  
88 relevant patient-specific and strain-specific information was reviewed, including potential  
89 cross-infection events, duration of infection, change in culture status and knowledge of strain  
90 distribution and transmissibility.

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

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

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

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

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

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

137

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### 147 **Competing Interests**



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

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## 154 **Contributors**

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

## 159 **Ethics approval**

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

## 163 **Provenance and peer review**

164 Not commissioned

165

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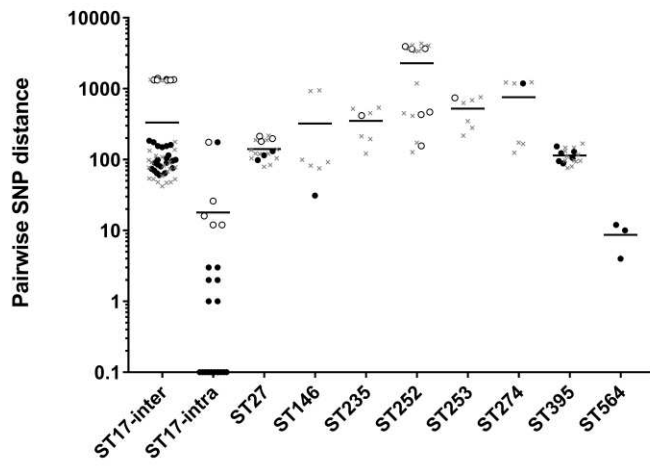
## 196 **Figure legends**

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

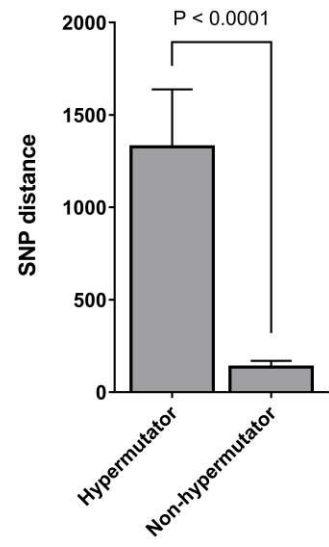
200 within each ST, with the bar representing the mean. Circles represent pairwise comparisons  
201 that are exclusively between PA isolates from our own respiratory cohort (CF or NCFB), with  
202 the open circles representing those comparisons in which at least one isolate is a predicted  
203 hypermutator.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

210

A



B



211

212 **Figure 1**