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**Transmission of bacteria in bronchiectasis and chronic obstructive pulmonary disease:
low burden of cough aerosols.**

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Summary at a glance

Our study shows that people with bronchiectasis and chronic obstructive pulmonary disease (COPD) can release potentially infectious aerosols during coughing; however, no shared strains of *Pseudomonas aeruginosa* were identified in our study. The results suggest that aerosol transmission is an unlikely mode of cross-infection in people with bronchiectasis and COPD.

Abstract

Background and objectives: Aerosol transmission of *Pseudomonas aeruginosa* has been suggested as a possible mode of respiratory infection spread in people with cystic fibrosis (CF); however, whether this occurs in other suppurative lung diseases is unknown. Therefore, we aimed to determine if 1) people with bronchiectasis (unrelated to CF) or chronic obstructive pulmonary disease (COPD) can aerosolise *P. aeruginosa* during coughing and 2) if genetically indistinguishable (shared) *P. aeruginosa* strains are present in these disease cohorts.

Methods: People with bronchiectasis or COPD and *P. aeruginosa* respiratory infection were recruited for two studies. *Aerosol study:* Participants (n=20) underwent cough testing using validated cough rigs to determine the survival of *P. aeruginosa* aerosols in the air over distance and duration. *Genotyping Study:* *P. aeruginosa* sputum isolates (n=95) were genotyped using the iPLEX20SNP platform with a subset subjected to the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) assay to ascertain their genetic relatedness.

Results: *Aerosol study:* Overall, 7/20 (35%) participants released *P. aeruginosa* cough aerosols during at least one of the cough aerosol tests. These cough aerosols remained viable for 4-metres from source and for 15-minutes after coughing. The mean total aerosol count of *P. aeruginosa* at 2-metres was two colony forming units. *Typing study:* No shared *P. aeruginosa* strains were identified.

Conclusions: Low viable count of *P. aeruginosa* cough aerosols and a lack of shared *P. aeruginosa* strains observed suggesting that aerosol transmission of *P. aeruginosa* is an unlikely mode of respiratory infection spread in people with bronchiectasis and COPD.

Key words (five key words in alphabetical order from MeSH list)

Bronchiectasis, chronic obstructive pulmonary disease, *Pseudomonas aeruginosa*, infection control, person-to-person transmission

Short title (fewer than 40 characters including spaces)

- Infection spread in chronic lung disease

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1 List of abbreviations

%	Percentage
°C	Celsius
µg	Microgram
kg	Kilograms
m	Metres
mL	Millilitre
ACI	Andersen cascade impactor
BMI	Body mass index
CF	Cystic fibrosis
CFU	Colony forming unit
CI	Confidence interval
<i>C. koseri</i>	<i>Citrobacter koseri</i>
COPD	Chronic obstructive pulmonary disease
<i>E. coli</i>	<i>Escherichia coli</i>
ERIC	Enterobacterial repetitive intragenic consensus
FEV ₁	Forced expiratory volume in one-second
FVC	Forced vital capacity
GNB	Gram-negative bacteria
<i>H. influenzae</i>	<i>Haemophilus influenzae</i>
HRCT	High resolution computed tomography
HREC	Human Research and Ethics Committee
IQR	Inter quartile range
MALDI-TOF	Matrix-assisted laser desorption/ionisation-time-of-flight

<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PCR	Polymerase chain reaction
<i>S. maltophilia</i>	<i>Stenotrophomonas maltophilia</i>
SD	Standard deviation
SNP	Single nucleotide polymorphism
spp.	Species
TPCH	The Prince Charles Hospital

2

3 **Introduction**

4 *Pseudomonas aeruginosa* is an opportunistic pathogen isolated from the sputum of people
5 with underlying lung conditions. In people with cystic fibrosis (CF) over 12 years of age, *P.*
6 *aeruginosa* is the dominant bacterial pathogen¹ and cross-infection between people with CF
7 attending specialist centres has been well-documented.^{2,3} The transmission route of
8 *P. aeruginosa* cross-infection has been suggested as aerosol transmission with evidence that
9 viable *P. aeruginosa* (and other CF pathogens) cough aerosols could travel for four metres
10 from the source (person with CF) and remain in the air for 45-minutes after cough^{4,5}. Yet,
11 shared strains of *P. aeruginosa* have not been found in environmental sampling,⁶ further
12 supporting aerosol transmission as a possible mode of cross-infection.

13

14 In (non-CF) bronchiectasis and chronic obstructive pulmonary disease (COPD),
15 *P. aeruginosa* predominantly causes infection in those with severe disease^{7,8} and is
16 associated with poorer prognosis⁹, higher mortality¹⁰⁻¹² and increased hospital admissions¹³.
17 Yet unlike CF, cross-infection with *P. aeruginosa* is reported to be uncommon in people
18 with bronchiectasis and COPD.¹⁴⁻²⁰ Although the evidence for cross-infection is infrequent
19 in non-CF suppurative lung diseases,^{15,16} the transmission mechanism of possible person-to-
20 person transmission events has not been studied previously. Therefore, we sought to
21 determine if 1) people with bronchiectasis or COPD can produce cough aerosols containing
22 *P. aeruginosa*, and 2) if respiratory infections with shared *P. aeruginosa* strains occurs in
23 people with bronchiectasis and COPD attending a centre which is co-located with a large
24 adult CF centre.

25

26 **Methods**

27 Clinically stable adult participants (>18 years) who had at least one prior *P. aeruginosa*
28 positive sputum culture were recruited from respiratory clinics at The Prince Charles
29 Hospital (TPCH), Brisbane, Australia. Participants with a confirmed diagnosis of
30 bronchiectasis had evidence of consistent radiological changes affecting ≥ 2 lobes by high
31 resolution computed tomography [HRCT].²¹ In cases where clinical features and
32 investigations were suggestive, *CFTR* mutation analysis and sweat electrolytes were
33 performed to exclude a diagnosis of CF. The diagnosis of COPD was based on standard
34 diagnostic criteria including symptoms and physiology. Clinical stability was defined as: no
35 recent change in symptoms and no change in therapy including acute administration of
36 antibiotics in the prior two weeks. Participants in the Aerosol Study were excluded if:
37 clinically unstable and/or experienced recent haemoptysis or pneumothoraces. Written,
38 informed consent was obtained from all participants and the studies were approved by the
39 relevant Human Research and Ethics Committee (HREC/15/QPCH/29;
40 HREC/11/QPCH/71).

41

42 **Aerosol study**

43 ***Cough aerosol sampling***

44 Twenty participants with bronchiectasis (n=16) or COPD (n=4) underwent cough aerosol
45 testing using two validated cough rigs – “distance” and “duration” rigs.^{4,22} Participants
46 performed up to five cough tests: two tests in the distance rig with aerosol collection points
47 at 2- and 4-metres (order randomised), and three tests in the duration rig with aerosol ageing
48 periods of 5-, 15- and 45-minutes. The distance and duration testing methodology have been
49 described in detail previously with participants monitored by a healthcare professional.^{4,5,22}
50 In brief, participants performed respiratory function testing on the day of testing to measure
51 forced expiratory volume in one-second (FEV₁) and forced vital capacity (FVC) according

52 to ATS guidelines.²³ Weight, height and age were recorded and the percent predicted values
53 calculated from the Global Lung Index.²⁴

54

55 ***Distance testing***

56 For each cough test (2- and 4-metres), participants entered into the “distance” rig, completed
57 2-minutes of tidal breathing to purge the lungs of room air and then proceeded to cough for
58 5-minutes at a comfortable pace determined by each study participant. Cough aerosols were
59 extracted continuously during this time using an Andersen Cascade Impactor (ACI) (Thermo
60 Fisher Scientific, USA). The ACI for both distance and duration testing (see below) was
61 loaded with Chocolate-Bacitracin media (300 µg/mL) to determine the viability of *P.*
62 *aeruginosa* in cough aerosols.⁴

63

64 ***Duration testing***

65 Participants with COPD were excluded from the duration testing due to airflow obstruction
66 severity. For each test, the remaining bronchiectasis participants completed 2-minutes of
67 tidal breathing to purge the lungs of room air followed by coughing for 2-minutes at a
68 comfortable pace determined by each study participant. The cough aerosols were sealed in
69 the rotating drum inside the duration rig, aged (5-, 15- or 45-minutes) and then extracted
70 using an ACI as previously described.²²

71

72 ***Microbiology***

73 Qualitative and quantitative sputum cultures were performed.^{4,5} The aerosol agar plates were
74 incubated aerobically at 37 °C for 72-hours. Presumptive identification of
75 *P. aeruginosa* isolates was based on positive oxidase reaction and growth at 42 °C. All
76 bacterial isolates had confirmatory identification using matrix-assisted laser

77 desorption/ionisation-time-of-flight (MALDI-TOF) mass spectrometry and real-time
78 PCR.²⁵ Sputum and aerosol *P. aeruginosa* colony forming units (CFU) for individual
79 *P. aeruginosa* morphotypes were enumerated. The total viable count in sputum (CFU/mL)
80 and total bacterial species aerosol count across the six-stages of the ACI were determined.
81 Participants were defined as low (<10 total aerosol CFU) or high (\geq 10 total aerosol CFU
82 count) aerosol producers.²⁶ A hole-correction factor was applied to account for possible
83 ‘stacking’ of bacterial colonies on the agar plates inside the ACI.²⁷ All confirmed
84 *P. aeruginosa* isolates underwent genotyping using an iPLEX20SNP assay (Sequenom) for
85 genotyping as previously published.²⁸

86

87 **Genotyping Study**

88 ***Sputum microbiology***

89 Sputa were collected from 30 eligible participants with a recent history of *P. aeruginosa*
90 infection (bronchiectasis, n=29; COPD, n=1) and cultured in an accredited clinical
91 microbiological laboratory in accordance with local protocols (Pathology Queensland).
92 Longitudinal sputum samples were included for analysis where available. Clinical
93 measurements were recorded as detailed above.

94

95 ***Genotyping***

96 Purified presumptive *P. aeruginosa* isolates representing different colonial morphotypes
97 from each specimen (where possible) were selected and stored at -80 °C, with identification
98 subsequently confirmed by real-time PCR.²⁵ All confirmed *P. aeruginosa* isolates
99 underwent iPLEX20SNP genotyping.²⁸ The genotyping results were evaluated using a
100 database of multilocus sequence profiles from local environmental, animal, CF and non-CF
101 associated clinical isolates.^{6,28} Fourteen isolates (from nine participants) had

102 indistinguishable iPLEX20SNP profiles and subsequently underwent ERIC-PCR analysis²⁹
103 (200kb ladder was used for comparison and the gel was run at 80V for 5 hours). ERIC-PCR
104 banding patterns were visually analysed, with isolates showing a variance of ≥ 1 band
105 allocated to a different rep-PCR type. Furthermore, patterns of infection within-patients
106 were determined.¹⁷ Clinical records were reviewed to determine possible opportunities for
107 cross-infection such as overlapping hospital admissions, outpatient clinic appointments
108 (including lung function appointments if available) and emergency admissions. During the
109 study period, there were no specific infection control policies to segregate patients with
110 bronchiectasis or COPD from each other or patients with CF when receiving inpatient care,
111 outpatient care or during lung function testing. While the participants with bronchiectasis or
112 COPD recruited to this study may have had contact with patients with CF, we were unable
113 to access specific data to determine if any overlapping contact occurred (and the
114 nature/extent of the contact.

115

116 **Statistical analysis**

117 Data was analysed using SPSS version 23 (IBM Corp). Categorical variables were
118 summarised as frequency and percentage and continuous variables as mean and standard
119 deviation. The total CFU count present in both sputum and aerosols were log transformed
120 and reported as geometric mean and 95% confidence interval (CI). The Jeffreys 95% CI is
121 given for the proportion of participants with *P. aeruginosa* detected in cough aerosols. A
122 two-tailed Pearson's correlation was used to examine the correlation between the mean
123 concentration of *P. aeruginosa* in the sputum and total mean *P. aeruginosa* aerosol count at
124 2-metre testing. A linear mixed effect model with participant as the random effect and cough
125 test as a fixed effect was used to calculate the overall mean and 95% CI for the total mean
126 count of Gram-negative bacteria other than *P. aeruginosa*. Values presented in Table 1 for

127 the Genotyping Study are from the most recent sputum collection time point with the
128 exception of height and weight. If the height and weight data was missing, the values
129 recorded for the previous collection time point were used in the analysis.

130

131 **Results**

132 **Participants**

133 The clinical characteristics of participants in the Aerosol Study (n=20) and the Genotyping
134 Study (n=30) are summarised in Table 1. Thirteen participants were enrolled in both the
135 aerosol and genotyping study.

136

137 **Aerosol study**

138 Sputum samples were obtained from 15 (75%) participants on the cough aerosol sampling
139 testing day (Table 2) (five participants were unproductive). *P. aeruginosa* was cultured from
140 12 (80%) participants who produced a sputum sample and of these participants, 7 (58%)
141 produced cough aerosols containing *P. aeruginosa*. The mean concentration of
142 *P. aeruginosa* in the sputum was 1.1×10^7 CFU/mL (95% CI 0.2×10^7 to 8.0×10^7) (n=12).

143

144 ***Cough aerosol testing: P. aeruginosa***

145 All 20 participants completed the distance tests of 2- and 4-metres. Sixteen of the
146 participants completed the 5- and 15-minute duration tests, and of these only 10 participants
147 completed the 45-minute duration test. Seven participants (35%, 95% CI 17 - 57) produced
148 cough aerosols containing *P. aeruginosa* during at least one cough tests (Table 2) and also
149 had *P. aeruginosa* detected their sputum sample provided on the day of testing. *P.*
150 *aeruginosa* positive aerosols were detected in 5/20 (25%) participants (bronchiectasis, n=4;
151 COPD, n=1) at 2-metres, 4/20 (20%) bronchiectasis participants only at 4-metres and 2/16

152 (13%) bronchiectasis participants only at 15-minutes (Table 2). All participants were
153 considered as low producers²⁶ of *P. aeruginosa* cough aerosols with a total mean aerosol
154 count of 2 CFU at 2-metres (n=5), 3 CFU at 4-metres (n=4), and 1 CFU at 15-minutes (n=2)
155 (Table 2). No viable *P. aeruginosa* containing aerosols were detected in either the 5-minute
156 test or 45-minute duration tests. The viable burden of potentially infectious aerosols released
157 during coughing was much lower in the bronchiectasis and COPD than we have seen in CF
158 participants.⁴

159

160 ***Sputum sampling and cough aerosol testing: P. aeruginosa***

161 Genotyping of the *P. aeruginosa* cough aerosol isolates revealed genetically
162 indistinguishable *P. aeruginosa* from paired sputum and cough aerosol isolates for the seven
163 participants. One participant had an additional *P. aeruginosa* strain identified in the aerosol
164 cultures that was not detected in the sputum sample. The total viable count of
165 *P. aeruginosa* in sputum did not correlate with the total *P. aeruginosa* aerosol count
166 ($r=0.416$, $n=15$, $p=0.12$) at 2-metres.

167

168 ***Sputum sampling: Other Gram-negative bacteria***

169 Three (15%) participants cultured other Gram-negative bacteria (*Haemophilus influenzae*,
170 *Escherichia coli*, *Stenotrophomonas maltophilia*) from the sputum (Table S1,
171 Supplementary Information). The mean concentration of these Gram-negative bacteria in
172 the sputum was 5.8×10^7 CFU/mL (95% CI $0.15 \times 10^7 - 224 \times 10^7$) (other GNB sputum
173 counts, $n=4$).

174

175 ***Cough aerosol testing: Other Gram-negative bacteria***

176 The three participants that cultured other Gram-negative bacteria from the sputum also had
177 these bacteria recovered from ≥ 3 of their cough aerosols samples (*H. influenzae*, n=2;
178 *E. coli*, n=1; *S. maltophilia*, n=1; Table S1, Supplementary Information); including one
179 participant who also produced cough aerosols with *P. aeruginosa* (Table S1, Supplementary
180 Information). One COPD participant did not provide a sputum sample yet produced
181 *Citrobacter koseri* and *Achromobacter* spp. in the cough aerosol samples (Table S1,
182 Supplementary Information). The total mean aerosol count of other Gram-negative bacteria
183 from all distance and duration cough aerosol tests (total=19) was 22 (95% CI 2 – 181).

184

185 **Genotyping Study**

186 ***Sputum collection***

187 Sixteen (53%) of the 30 participants provided a single sputum sample. Fourteen (47%)
188 participants provided multiple sputum samples (two, n=10 participants or three, n=4
189 participants) and the median duration between the initial and final samples was 8.1 months
190 (IQR 2.8 – 45.2) (Figure S1, Supplementary Information). *P. aeruginosa* sputum isolates
191 were confirmed by PCR.

192

193 ***Prevalence of shared P. aeruginosa strain infection***

194 A total of 95 confirmed *P. aeruginosa* sputum isolates (range: 1 to 8 isolates per participant
195 and 1 to 4 isolates per sample) were genotyped (iPLEX20SNP) (Table S2, Supplementary
196 Information). Of these, 3 isolates were classed as non-typeable. No dominant Australian
197 shared CF *P. aeruginosa* strains (e.g. AUST-01, AUST-02 and AUST-06)^{6,28} were observed.
198 In contrast, our analysis revealed 20 (67%) participants had infection with *P. aeruginosa*
199 strains with genotype profiles that showed close genetic relationships to locally-derived
200 genotypes found in the environment, animals and other non-CF clinical presentations.⁶

201 There were eight possible transmission events: one overlapping hospital admission of two
202 participants, one overlapping emergency department attendance of two participants and six
203 same day outpatient attendance at TPCCH. None of the participants with genetically
204 indistinguishable profiles had likely transmission events (common admissions, emergency
205 department or outpatient attendance). The indistinguishable genotype profiles related to
206 sequence type (ST)-17 (Clone C) (participants 1 and 9), ST-155 (participants 4 and 20), ST-
207 274 (participants 16 and 18) and ST-253 (PA14) (participants 5, 13 and 17). Representative
208 isolates from these participants subsequently underwent ERIC-PCR and no genetically
209 indistinguishable *P. aeruginosa* strains were found between the three sets of pairs or in the
210 group of three participants.

211

212 ***P. aeruginosa* infection patterns**

213 Of the 14 participants who had multiple samples analysed, 12 (80%) harboured a single
214 *P. aeruginosa* strain over time, one cultured different strains in their sputum over three time
215 points (between 2013 and 2016) and one showed evidence of a new strain then subsequently
216 reverting back to the original strain.

217

218 **Discussion**

219 Our study demonstrates that people with bronchiectasis and COPD can release aerosols
220 containing viable *P. aeruginosa* during coughing; however, no shared strains of
221 *P. aeruginosa* respiratory infection were detected in study participants. Our results support
222 the published data that cross-infection of *P. aeruginosa* affects a minority of people with
223 bronchiectasis¹⁴⁻¹⁶ and provides much needed evidence to understanding cross-infection in
224 bronchiectasis, which was highlighted as a research priority in a recent review.³⁰ Whilst we
225 have demonstrated that aerosol transmission is an unlikely transmission route, it is worth

226 noting that the participants selected for the study were all low producers²⁶ of
227 *P. aeruginosa* cough aerosols and also, that the study participants had very few opportunities
228 for transmission events to occur during hospital visits; thus reducing the risk of potentially
229 being exposed to each other's cough aerosols.

230

231 The results of our cough aerosol study were in contrast to the results of previous studies in
232 people with CF (Table 2) despite that the participant numbers were almost the same (CF
233 cough study, n=19⁴ versus this study, n=20). Firstly, only 25% of all participants in this
234 study produced cough aerosols containing viable *P. aeruginosa* at two-metres whereas most
235 participants with CF produced cough aerosols containing *P. aeruginosa* at the same
236 distance.⁴ Secondly, the total mean *P. aeruginosa* aerosol count at 2-metres was much lower
237 in participants with bronchiectasis or COPD compared to people with CF (2 CFU versus 39
238 CFU, respectively) (Table 2).⁴ Thirdly, the distance that viable *P. aeruginosa* cough aerosols
239 could travel in people with bronchiectasis, COPD or CF⁴ were similar (four-metres);
240 however, the duration that *P. aeruginosa* cough aerosols could remain suspended in the air
241 was shorter in people with bronchiectasis at 15-minutes compared to 45-minutes for people
242 with CF.⁴ Lastly, the mean concentration of *P. aeruginosa* in sputum in the bronchiectasis
243 and COPD cohort did not correlate with the total aerosol count observed at two-metres and
244 this was in contrast to our findings in the CF cough aerosol studies.^{4,5,31}

245

246 Our genotyping study is the first Australian study to investigate the possibility of cross-
247 infection in people with bronchiectasis and COPD attending a facility which has shared
248 inpatient and outpatient facilities with CF. Our results found that no major Australian CF
249 shared *P. aeruginosa* strains³³ were detected in our current cohort. In fact, our study found
250 no evidence of shared *P. aeruginosa* strain infections, which is in keeping with the published

251 data that shared *P. aeruginosa* strains are uncommon in people with bronchiectasis or
252 COPD.^{14-17,19,20} The *P. aeruginosa* strains detected in our study are commonly found in other
253 niches such as the natural environment and non-CF infections.^{3,6,14,34} Our longitudinal
254 analysis of *P. aeruginosa* isolates showed that the majority of participants retained the same
255 unique *P. aeruginosa* strain over time which is consistent with other recent
256 studies.^{15,17,19,20,35} These results suggest that person-to-person transmission of *P. aeruginosa*
257 is unlikely to occur in people with bronchiectasis and COPD. Instead, *P. aeruginosa*
258 respiratory infection is likely acquired from the natural environment.

259

260 Interestingly, our study found four of the 20 participants produced cough aerosols containing
261 other Gram-negative bacteria. This was a higher proportion than in our previous CF *P.*
262 *aeruginosa* cough aerosol studies^{4,31,32} which is likely to be related to the difference in
263 infection profile in people with bronchiectasis and COPD compared with CF populations.
264 Incidentally, we found two study participants with bronchiectasis who were high producers
265 of *H. influenzae* cough aerosols,²⁶ a common respiratory pathogen of people with
266 bronchiectasis and COPD.³⁶⁻⁴⁰ Whilst *H. influenzae* cross-infection is not thought to occur
267 in people with bronchiectasis,¹⁸ it has been recently reported in a single study of people with
268 CF;⁴¹ though it is presently unclear if aerosol transmission plays a role in *H. influenzae*
269 acquisition. Our study reported one non-expectorating participant with COPD who produced
270 cough aerosols containing *C. koseri* and *Achromobacter* spp. The finding of potentially
271 infectious cough aerosols in the absence of sputum production was also reported in our
272 earlier cough studies in people with CF^{4,42} yet was in contrast to our two most recent studies
273 in people with CF which found that people with CF who could not expectorate sputum were
274 unable to generate potentially infectious cough aerosols.^{31,32}

275

276 This study had several limitations. Firstly, most people with COPD and *P. aeruginosa*
277 respiratory infection were unsuitable for participation because they had severe airflow
278 obstruction which impacts on the generalisability of our results in these patients. Therefore,
279 a larger study using altered study protocols may better include participants with COPD and
280 may support stronger correlations between clinical and microbiological measures and
281 aerosol CFU counts. Secondly, our sample size was small and the number of participants
282 which produced viable *P. aeruginosa* in their cough aerosols was low. Therefore robust
283 estimates cannot be determined however, the estimates obtained in this study are useful for
284 calculation of sample size for future cough aerosol studies. Similarly, given that the number
285 of participants in the Genotype Study had a median follow-up time of less than 12 months,
286 the diversity of genetic variation of *P. aeruginosa* in patients with bronchiectasis may have
287 also been underestimated. Thirdly, the infectious dose of
288 *P. aeruginosa* and other Gram-negative bacteria is not known and therefore, the risk of
289 infection from exposure to potentially infectious aerosols remains uncertain. Fourthly, the
290 study participants were tested when clinically stable and therefore, may underestimate the
291 *P. aeruginosa* aerosols released during pulmonary exacerbations. Fifthly, the media used to
292 capture the cough aerosols was selective for Gram-negative bacteria and thus, the results of
293 this study cannot be generalised to those people with bronchiectasis and COPD harbouring
294 Gram-positive bacterial respiratory infections. Finally, the longitudinal analyses, at times,
295 included one isolate per sputum which limited the capacity to detect strain diversity.

296

297 Our study has demonstrated that people with bronchiectasis and COPD can release low
298 amounts of viable *P. aeruginosa* aerosols during coughing. The result confirms the finding
299 that *P. aeruginosa* cross-infection is uncommon in bronchiectasis and that aerosol
300 transmission seems unlikely to be a major contributor to *P. aeruginosa* cross-infection.

Table 1: Demographics and clinical characteristics of study participants

Patient Characteristics	Aerosol study (n=20)	Typing study* (n=30)
Age (years), mean (SD)	62.5 (11.0)	64.0 (8.8)
Sex, male, n (%)	6 (30%)	10 (33%)
FEV ₁ % predicted, mean (SD)	56.7 (20.7)	58.7 (18.1)
FVC % predicted, mean (SD)	76.5 (16.6)	75.0 (17.0)
BMI (kg/m ²), mean (SD)	25.3 (4.3)	26.7 (5.6) [^]
Ethnicity		
Caucasian	19 (95%)	29 (97%)
Asian	1 (5%)	1 (3%)
Clinical disease		
Bronchiectasis, n (%)	16 (80%)	29 (97%)
<i>Idiopathic</i> , n (%)	1 (6%)	10 (34%)
<i>Childhood infection</i> , n (%)	14 (88%)	15 (52%)
<i>Pink's Disease</i> , n (%)	1 (6%)	2 (7%)
<i>Kartageners Syndrome</i> , n (%)	0 (0%)	1 (3%)
<i>Aspiration</i> , n (%)	0 (0%)	1 (3%)
COPD, n (%)	4 (20%)	1 (3%)
Subjects that contributed multiple sputum samples, n (%)	n/a	14 (47%)
Time under observation (months), median (IQR)	n/a	8.1 (2.8 – 45.2)
Chronic <i>P. aeruginosa</i> infection, n (%)	17 (85%)	25 (83%)
Smoking history		
Bronchiectasis cohort:		
Never, n (%)	14/16 (88%)	22/29 (76%)
Former, n (%)	2/16 (13%)	7/29 (24%)
- Pack years, median (IQR)	(1, 2) [~]	8 (2 – 20)
COPD cohort:		
Never, n (%)	2 [#] /4 (50%)	1/1 (100%)
Former, n (%)	2/4 (50)	n/a
- Pack years, median (IQR)	(45, 85) [~]	n/a

*n=13 also participated in the Aerosol Study; [^]n=28; [~]individual pack years; [#]One COPD participant had alpha-1 antitrypsin deficiency and the other COPD participant had longstanding asthma); n/a, not applicable.

Table 2: Comparison of *P. aeruginosa* in sputum and in cough aerosols

	Participants n = 20		Previously published CF cough study ^{4*} n = 19 [~]	
Sputum	n (%)	CFU/mL, geometric mean (95% CI)	n (%)	CFU/mL, geometric mean (SD)
Sputum provided				
- All participants	15 (75)		18 (95)	
○ Bronchiectasis	14/16 (88)			
○ COPD	1/4 (25)			
<i>P. aeruginosa</i> detected in sputum				
- All participants	12 [^] /15 (80)	1.1 x 10 ⁷ (0.2 x 10 ⁷ – 8.0 x 10 ⁷)	18/18 (100)	13.7 x 10 ⁷ (2.2 x 10 ⁷ – 106.0 x 10 ⁷)
○ Bronchiectasis	11/14 (79)			
○ COPD	1/1 (100)			
Cough aerosol		Count (CFU), geometric mean (95% CI)		Count (CFU), geometric mean (95% CI)
<i>P. aeruginosa</i> detected in cough aerosol				
○ All participants	7/20 (35)	-	18/18 (100)	-
○ Bronchiectasis	6/16 (38)	-	-	-
○ - COPD	1/4 (25)	-	-	-
Distance				
- 2-metres	5 [^] /20 (25)	2 (1 - 7)	17/18 (94)	39 (30 – 51)
- 4-metres	4 [^] /20 (20)	3 (1 - 9)	17/18 (94)	26 (20 – 34)
Duration				
- 5-minutes	0/16 (0)	0	15/18 (83)	15 (11 – 20)
- 15-minutes	2 [^] /16 (13)	1 (-1 – 31)	14/18 (78)	12 (9 – 16)
- 45-minutes	0/10 (0)	0	14/18 (78)	8 (6 – 11)

CF, cystic fibrosis; CFU, colony forming unit; mL, millilitre; CI, confidence interval; COPD, chronic obstructive pulmonary disease; [~]includes cough swab from one participant; [^]numerator represents the number of participants included in the geometric mean calculations; * data taken from Knibbs *et al*⁴ online supplement, table S3.

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