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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 stains 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
C. koseri	*Citrobacter koseri*
COPD	Chronic obstructive pulmonary disease
E. coli	*Escherichia coli*
ERIC	Enterobacterial repetitive intragenic consensus
FEV_1	Forced expiratory volume in one-second
FVC	Forced vital capacity
GNB	Gram-negative bacteria
H. influenzae	*Haemophilus influenzae*
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

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

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Introduction

Pseudomonas aeruginosa is an opportunistic pathogen isolated from the sputum of people with underlying lung conditions. In people with cystic fibrosis (CF) over 12 years of age, *P. aeruginosa* is the dominant bacterial pathogen[1] and cross-infection between people with CF attending specialist centres has been well-documented.[2,3] The transmission route of *P. aeruginosa* cross-infection has been suggested as aerosol transmission with evidence that viable *P. aeruginosa* (and other CF pathogens) cough aerosols could travel for four metres from the source (person with CF) and remain in the air for 45-minutes after cough[4,5]. Yet, shared strains of *P. aeruginosa* have not been found in environmental sampling,[6] further supporting aerosol transmission as a possible mode of cross-infection.

In (non-CF) bronchiectasis and chronic obstructive pulmonary disease (COPD), *P. aeruginosa* predominantly causes infection in those with severe disease[7,8] and is associated with poorer prognosis[9], higher mortality[10-12] and increased hospital admissions[13]. Yet unlike CF, cross-infection with *P. aeruginosa* is reported to be uncommon in people with bronchiectasis and COPD.[14-20] Although the evidence for cross-infection is infrequent in non-CF suppurative lung diseases,[15,16] the transmission mechanism of possible person-to-person transmission events has not been studied previously. Therefore, we sought to determine if 1) people with bronchiectasis or COPD can produce cough aerosols containing *P. aeruginosa*, and 2) if respiratory infections with shared *P. aeruginosa* strains occurs in people with bronchiectasis and COPD attending a centre which is co-located with a large adult CF centre.

Methods

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

Aerosol study

Cough aerosol sampling

Twenty participants with bronchiectasis (n=16) or COPD (n=4) underwent cough aerosol testing using two validated cough rigs – "distance" and "duration" rigs.[4,22] Participants performed up to five cough tests: two tests in the distance rig with aerosol collection points at 2- and 4-metres (order randomised), and three tests in the duration rig with aerosol ageing periods of 5-, 15- and 45-minutes. The distance and duration testing methodology have been described in detail previously with participants monitored by a healthcare professional.[4,5,22] In brief, participants performed respiratory function testing on the day of testing to measure forced expiratory volume in one-second (FEV_1) and forced vital capacity (FVC) according

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

Distance testing

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

Duration testing

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

Microbiology

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

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

Genotyping Study

Sputum microbiology

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

Genotyping

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

indistinguishable iPLEX20SNP profiles and subsequently underwent ERIC-PCR analysis[29] (200kb ladder was used for comparison and the gel was run at 80V for 5 hours). ERIC-PCR banding patterns were visually analysed, with isolates showing a variance of ≥1 band allocated to a different rep-PCR type. Furthermore, patterns of infection within-patients were determined.[17] Clinical records were reviewed to determine possible opportunities for cross-infection such as overlapping hospital admissions, outpatient clinic appointments (including lung function appointments if available) and emergency admissions. During the study period, there were no specific infection control policies to segregate patients with bronchiectasis or COPD from each other or patients with CF when receiving inpatient care, outpatient care or during lung function testing. While the participants with bronchiectasis or COPD recruited to this study may have had contact with patients with CF, we were unable to access specific data to determine if any overlapping contact occurred (and the nature/extent of the contact.

Statistical analysis

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

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

Results

Participants

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

Aerosol study

Sputum samples were obtained from 15 (75%) participants on the cough aerosol sampling testing day (Table 2) (five participants were unproductive). *P. aeruginosa* was cultured from 12 (80%) participants who produced a sputum sample and of these participants, 7 (58%) produced cough aerosols containing *P. aeruginosa*. The mean concentration of *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).

Cough aerosol testing: P. aeruginosa

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

(13%) bronchiectasis participants only at 15-minutes (Table 2). All participants were considered as low producers[26] of *P. aeruginosa* cough aerosols with a total mean aerosol count of 2 CFU at 2-metres (n=5), 3 CFU at 4-metres (n=4), and 1 CFU at 15-minutes (n=2) (Table 2). No viable *P. aeruginosa* containing aerosols were detected in either the 5-minute test or 45-minute duration tests. The viable burden of potentially infectious aerosols released during coughing was much lower in the bronchiectasis and COPD than we have seen in CF participants.[4]

Sputum sampling and cough aerosol testing: P. aeruginosa

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

Sputum sampling: Other Gram-negative bacteria

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

Cough aerosol testing: Other Gram-negative bacteria

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

Genotyping Study

Sputum collection

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

Prevalence of shared P. aeruginosa strain infection

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

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

P. aeruginosa **infection patterns**

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

Discussion

Our study demonstrates that people with bronchiectasis and COPD can release aerosols containing viable *P. aeruginosa* during coughing; however, no shared strains of *P. aeruginosa* respiratory infection were detected in study participants. Our results support the published data that cross-infection of *P. aeruginosa* affects a minority of people with bronchiectasis[14-16] and provides much needed evidence to understanding cross-infection in bronchiectasis, which was highlighted as a research priority in a recent review.[30] Whilst we have demonstrated that aerosol transmission is an unlikely transmission route, it is worth

noting that the participants selected for the study were all low producers[26] of *P. aeruginosa* cough aerosols and also, that the study participants had very few opportunities for transmission events to occur during hospital visits; thus reducing the risk of potentially being exposed to each other's cough aerosols.

The results of our cough aerosol study were in contrast to the results of previous studies in people with CF (Table 2) despite that the participant numbers were almost the same (CF cough study, n=19[4] *versus* this study, n=20). Firstly, only 25% of all participants in this study produced cough aerosols containing viable *P. aeruginosa* at two-metres whereas most participants with CF produced cough aerosols containing *P. aeruginosa* at the same distance.[4] Secondly, the total mean *P. aeruginosa* aerosol count at 2-metres was much lower in participants with bronchiectasis or COPD compared to people with CF (2 CFU *versus* 39 CFU, respectively) (Table 2).[4] Thirdly, the distance that viable *P. aeruginosa* cough aerosols could travel in people with bronchiectasis, COPD or CF[4] were similar (four-metres); however, the duration that *P. aeruginosa* cough aerosols could remain suspended in the air was shorter in people with bronchiectasis at 15-minutes compared to 45-minutes for people with CF.[4] Lastly, the mean concentration of *P. aeruginosa* in sputum in the bronchiectasis and COPD cohort did not correlate with the total aerosol count observed at two-metres and this was in contrast to our findings in the CF cough aerosol studies.[4,5,31]

Our genotyping study is the first Australian study to investigate the possibility of cross-infection in people with bronchiectasis and COPD attending a facility which has shared inpatient and outpatient facilities with CF. Our results found that no major Australian CF shared *P. aeruginosa* strains[33] were detected in our current cohort. In fact, our study found no evidence of shared *P. aeruginosa* strain infections, which is in keeping with the published

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

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

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

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

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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%)
Idiopathic, n (%)	*1* (6%)	*10* (34%)
Childhood infection, n (%)	*14* (88%)	*15* (52%)
Pink's Disease, n (%)	*1* (6%)	*2* (7%)
Kartageners Syndrome, n (%)	*0* (0%)	*1* (3%)
Aspiration, 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 *P. aeruginosa* 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 o Bronchiectasis o COPD	15 (75) 14/16 (88) 1/4 (25)		18 (95)	
P. aeruginosa detected in sputum - All participants o Bronchiectasis o COPD	12^/15 (80) 11/14 (79) 1/1 (100)	1.1×10^7 (0.2×10^7 – 8.0×10^7)	18/18 (100)	13.7×10^7 (2.2×10^7– 106.0×10^7)
Cough aerosol		Count (CFU), geometric mean (95% CI)		Count (CFU), geometric mean (95% CI)
P. aeruginosa detected in cough aerosol o All participants o Bronchiectasis o - COPD	7/20 (35) 6/16 (38) 1/4 (25)	- - -	18/18 (100) - -	- - -
Distance - 2-metres - 4-metres	5^/20 (25) 4^/20 (20)	2 (1 - 7) 3 (1 - 9)	17/18 (94) 17/18 (94)	39 (30 – 51) 26 (20 – 34)
Duration - 5-minutes - 15-minutes – 45-minutes	0/16 (0) 2^/16 (13) 0/10 (0)	0 1 (-1 – 31) 0	15/18 (83) 14/18 (78) 14/18 (78)	15 (11 – 20) 12 (9 – 16) 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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