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1 **Cystic fibrosis pathogens survive for extended periods within cough generated droplet**  
2 **nuclei**

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26

## 27 **ABSTRACT**

28 The airborne route is a potential pathway in the person-to-person transmission of bacterial  
29 strains amongst cystic fibrosis (CF) populations. In this cross-sectional study we investigate  
30 the physical properties and survival of common non-*Pseudomonas aeruginosa* CF pathogens  
31 generated during coughing. We conclude that Gram-negative bacteria and *Staphylococcus*  
32 *aureus* are aerosolised during coughing, can travel up to 4-metres and remain viable within  
33 droplet nuclei for up to 45-minutes. These results suggest airborne person-to-person  
34 transmission is plausible for the CF pathogens we measured.

35

## 36 **INTRODUCTION**

37 Recurrent pulmonary infection characterises cystic fibrosis (CF). Whilst *Pseudomonas*  
38 *aeruginosa* is generally the most prevalent respiratory pathogen, *Staphylococcus aureus*,  
39 *Stenotrophomonas maltophilia*, *Achromobacter* and *Burkholderia* species are common.

40 Studies have demonstrated genetically indistinguishable strains of *P. aeruginosa* [1, 2],  
41 *Burkholderia cepacia* complex species [3] and *Mycobacterium abscessus* [4] both within and  
42 between CF centre populations. Environmental reservoirs are infrequently identified for these  
43 shared bacterial strains, suggesting possible cross-infection. The airborne route is a possible  
44 mode of person-to-person transmission of *P. aeruginosa* and *M. abscessus*, which can be  
45 aerosolised during coughing by people with CF and remain viable within droplet nuclei

46 ( $\leq 4.7\mu\text{m}$  in size) for extended durations [4, 5]. The extent of airborne dissemination of other  
47 common CF pathogens is poorly understood.

48 We studied survival of CF pathogens (other than *P. aeruginosa* and *M. abscessus*) in the air  
49 over distance and duration, and compared the results with the survival of *P. aeruginosa* during  
50 voluntary coughing. It was hypothesised that individuals with CF produce similar levels of  
51 droplet nuclei containing Gram-negative bacteria (GNB) and *S. aureus* during coughing, which  
52 can travel up to 4-metres (m) and remain viable for up to at 45-minutes (min).

### 53 **METHODS**

54 Participants  $\geq 14$  years, with CF were assigned to either GNB or *S. aureus* groups based on  
55 positive sputum microbiological results in the prior two years. On the testing day, spirometry  
56 was performed and sputum was collected.

57 The experimental equipment was comprised of two validated, independent systems to study the  
58 distance travelled and survival duration of bacteria contained in aerosols generated during  
59 coughing [5]. Participants completed five cough experiments; distance studies involved aerosol  
60 sampling at 2 and 4-m, whilst the duration studies involved the aging of cough aerosol samples  
61 for 5, 15 and 45-min prior to extraction [5]. Aerosol sampling was undertaken through an  
62 Andersen Cascade Impactor and cough aerosol cultures were performed. (See online  
63 supplement).

64 Data were analysed using SPSS version 23 (IBM Corp., N.Y., USA). The experimental unit  
65 was organism. The total colony-forming unit (CFU) counts for sputum and aerosol plates were  
66 compared between GNB and *S. aureus* after  $\log_{10}$  transformation for analysis and back-  
67 transformation to the geometric mean for reporting. Where the organism was detected in  
68 sputum samples, a Pearson's correlation examined correlations between sputum and total  
69 viable aerosol at 2-m for each of the GNB and *S. aureus* organisms detected. The 2-m distance

70 was selected in accordance with current infection control recommendations for separation  
71 between people with CF [6] and correlation data for *P. aeruginosa* from our recent study was  
72 also reported as a comparison [7].

## 73 RESULTS

74 **Population description:** Thirty participants (19 males (63.3%)) with mean (SD) age 29.9 (10.4)  
75 years, FEV<sub>1</sub> 61.9 (25.7) % predicted and BMI 23.6 (4.5) kg/m<sup>2</sup> were studied. Twelve participants  
76 had a history of GNB infection, twelve participants had pre-existing *S. aureus* infection, and 6  
77 participants harboured both a GNB and *S. aureus*; thereby 18 participants were assigned to each  
78 organism group (Figure 1). One participant (GNB) could not tolerate or complete the 15 and 45-  
79 min duration experiments.

80 **Sputum bacteriology:** Expecterated sputum samples were provided by 29/30 participants.  
81 Participants with negative or missing sputum cultures were excluded from the analysis. Of the 18  
82 participants with previous GNB infection, 18 GNB organisms were identified in sputum from 15  
83 participants (three participants harboured two different GNB species): *S. maltophilia*, n=7;  
84 *Achromobacter spp.*, n=5; *Burkholderia spp.*, n=6; Figure 1. *S. aureus* was recovered from  
85 16/18 participants with history of infection (Figure 1). The mean (95% CI) sputum bacterial  
86 concentration (CFU/mL x 10<sup>6</sup>) for the GNB group was 7.0 (1.6 – 31) and for the *S. aureus*  
87 group, 1.3 (0.2 – 7.5) (p=0.13; Table 1).

88 **Aerosol sampling:** During the cough experiments, at least one positive aerosol was detected for  
89 15/18 (83%) organisms in the GNB group and 10/16 (63%) in the *S. aureus* group (p=0.25). 11/18  
90 (61%) GNB organisms were cultured at 4-m, and 9/17 (53%) at 45-min; whereas for the *S. aureus*  
91 group, 8/16 (50.0%) had viable aerosol at 4-m and 4/16 (25%) at 45-min, with no significant  
92 difference in the number of bacterial CFUs between the groups at any distance or duration (Table  
93 1). The mean percentage of viable particles cultured in the droplet nuclei size range ( $\leq 4.7\mu\text{m}$ )

94 was 66.5 (SD 26.1) for the GNB organism group and 58.2 (SD 26.0) for the *S. aureus* group  
95 (p=0.46).

96 **Sputum and aerosol bacterial typing:** Fourteen viable GNB cultures were detected in cough  
97 aerosols from 13 participants (Figure 1) and each organism had an identical genotype identified  
98 in paired sputum (confirmed by MLST-derived from whole genome sequences) including: *S.*  
99 *maltophilia* (n=6); *Achromobacter spp.* (n=4); and *Burkholderia spp.* (n=4). Aerosolised  
100 bacteria were not detected for five participants in the GNB group, including the participant who  
101 did not provide a sputum sample. Each participant had distinct strains of GNB species. Ten of  
102 16 participants had *S. aureus* cultured from their paired sputum and aerosol samples (Figure 1)  
103 and 8/10 had concordant genotypes. Isogenic strains were identified in the aerosol and sputum  
104 samples of the remaining two participants (as determined by single nucleotide polymorphism-  
105 based genotyping).

106 **CFU correlations at 2-m:** Bacterial sputum and aerosol concentrations were correlated for  
107 GNB species (r=0.50, p=0.035) and *S. aureus* (r=0.66, p=0.005) compared to r=0.55 (p=0.005)  
108 for *P. aeruginosa*.

## 109 **DISCUSSION**

110 Cross-infection of CF pathogens remains a concern, with the airborne route considered a  
111 potential transmission pathway [4, 8]. This study demonstrates that GNB species and *S. aureus*  
112 commonly recovered from people with CF can be aerosolised during coughing, travel up to 4-  
113 m from source and survive within droplet nuclei for up to 45-min, which is similar to airborne  
114 characteristics of *P. aeruginosa* and *M. abscessus* [4, 5]. The majority of viable particles were  
115 within the size range potentially capable of airborne dispersal and inhaled airway deposition.

116 Evidence demonstrating cross-infection of *Burkholderia cepacia* complex species and  
117 Methicillin-resistant *S. aureus* is clearly established and possible for some *Achromobacter spp.*

118 strains [3, 6, 9]. With each of the organisms of interest investigated in the current study, routes  
119 of acquisition could also be related to healthcare contact [6, 10]. This study highlights the  
120 potential for person-to-person transmission of common CF bacterial pathogens via the airborne  
121 route. As found in our earlier cough aerosol studies with *P. aeruginosa* [5, 7] an association  
122 between aerosol CFUs and sputum CFU concentrations for GNB and *S. aureus* has been  
123 demonstrated, suggesting those with a higher burden of microbial load in the sputum may pose  
124 a greater risk of airborne transmission. Taken together, these data provide further support for  
125 surgical mask wear to minimise potential cross-infection within CF healthcare facilities [7].

126 Study limitations include that the infectious dose to cause bacterial infection in CF is unknown  
127 and it is not possible to quantify individual risk of transmission via the airborne route.  
128 Similarly, the implications for younger children remains undetermined and our findings may  
129 not be representative for all people with CF.

130 This study has demonstrated that common CF pathogens can be aerosolised during coughing  
131 and survive within droplet nuclei for extended durations, highlighting the importance of  
132 universal infection control practices for all people with CF.

133

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139 (<https://pubmlst.org/bcc/>) developed by Keith Jolley and sited at the University of Oxford  
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143 conceived the study design. MEW, SCB and JC contributed to subject recruitment. MEW,  
144 RES, GRJ and NJ conducted the studies and collected the participant data and samples. RES,  
145 KAR and LJS performed the microbiological analysis. TJK and KAR undertook the genotypic  
146 analyses for GNB and Pathology Queensland for *S. aureus*. ELB and PO'R led the statistical  
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168 **Table 1:** Comparison of the sputum and aerosol concentrations between the GNB and *S. aureus* groups.  
 169

Sputum parameter; mean* (95% CI)	Stratified by organism/s identified in sputum				p-value
	GNB, n=18 <sup>†</sup>		<i>S. aureus</i> , n=16		
Sputum bacterial concentration; CFU/mL x 10 <sup>6</sup>	7.0 (1.6 – 31)		1.3 (0.2 – 7.5)		0.13
Aerosol parameter; mean* (95% CI)	n <sup>#</sup>	GNB aerosol CFU	n <sup>#</sup>	<i>S. aureus</i> aerosol CFU	p-value
Distance					
2-metres	14	11 (4 – 28)	9	5 (2 – 10)	0.22
4-metres	11	20 (7 – 50)	8	7 (2 – 23)	0.14
Duration					
5-minutes	10	13 (4 – 38)	8	3 (1 – 8)	0.062
15-minutes <sup>‡</sup>	9	10 (3 – 32)	6	4 (2 – 7)	0.12
45-minutes <sup>‡</sup>	9	12 (3 – 40)	4	4 (1 – 12)	0.10

170 \* Geometric mean

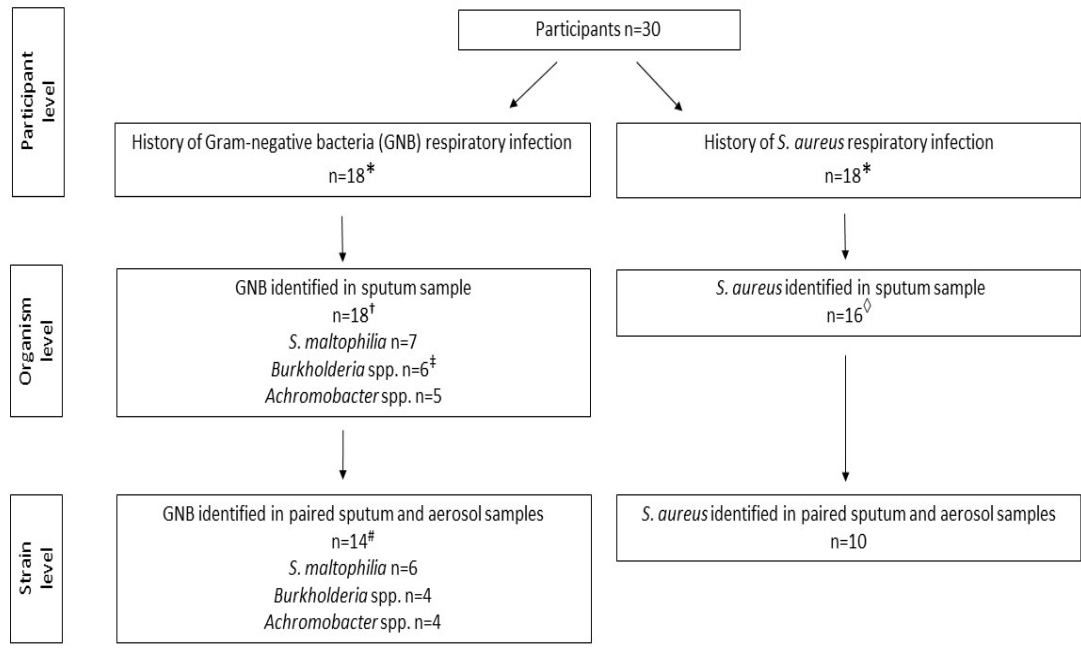
171 <sup>†</sup> 18 GNB organisms identified from the sputum of 15 participants (three participants had two GNB species detected)

172 <sup>‡</sup> One GNB group participant did not complete the 15 and 45-min duration experiments

173 <sup>#</sup> Target organisms identified in sputum that had a positive aerosol detected

174 *Definitions:* CFU, colony forming unit; CFU/mL, CFU per millilitre of sputum; CI, confidence interval; GNB, Gram-negative bacteria

**Figure 1:** Flow chart of participant stratification and matched sputum/ aerosol findings



\* 6 participants had history of co-infection with GNB and *S. aureus*  
 † 18 GNB organisms isolated from sputum samples of 15 participants (3 participants had two GNB species identified)  
 ‡ *Burkholderia* species included: *Burkholderia multivorans*, n=3; *Burkholderia cepacia*, n=2 and *Burkholderia gladioli*, n=1  
 # 14 GNB from 13 participants (1 participant had two paired sputum and aerosol samples for different GNB)  
 ◇ Methicillin-sensitive *S. aureus* (MSSA), n=12; Methicillin-resistant *S. aureus* (MRSA), n=2; mixed MSSA and MRSA, n=2

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