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

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ABSTRACT

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

INTRODUCTION

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

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

(≤4.7μm in size) for extended durations [4, 5]. The extent of airborne dissemination of other common CF pathogens is poorly understood.

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

METHODS

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

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

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

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

RESULTS

Population description: Thirty participants (19 males (63.3%)) with mean (SD) age 29.9 (10.4) years, FEV_1 61.9 (25.7) % predicted and BMI 23.6 (4.5) kg/m^2 were studied. Twelve participants had a history of GNB infection, twelve participants had pre-existing *S. aureus* infection, and 6 participants harboured both a GNB and *S. aureus*; thereby 18 participants were assigned to each organism group (Figure 1). One participant (GNB) could not tolerate or complete the 15 and 45-min duration experiments.

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

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

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

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

CFU correlations at 2-m: Bacterial sputum and aerosol concentrations were correlated for 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) for *P. aeruginosa*.

DISCUSSION

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

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

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

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

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

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Contributors: SCB, CEW, TJK, GRJ, LDK, LM, PDS led the funding application and conceived the study design. MEW, SCB and JC contributed to subject recruitment. MEW, RES, GRJ and NJ conducted the studies and collected the participant data and samples. RES, KAR and LJS performed the microbiological analysis. TJK and KAR undertook the genotypic analyses for GNB and Pathology Queensland for *S. aureus*. ELB and PO'R led the statistical analysis. MEW and SCB oversaw the overall study and wrote the manuscript, with input from all authors.

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Ethics approval: The project was granted approval by the Children's Queensland Human Research Ethics Committee HREC/14/RCH/88 and The Prince Charles Hospital Research Governance Office SSA/14/TPCH/202. Participants provided written consent/assent.

Competing interests: During conduct of the study: SCB reports grants from Cystic Fibrosis Foundation Therapeutics USA and The Prince Charles Hospital Foundation and outside of the submitted work, travel support to attend conferences from Novartis and Gilead and meetings for clinical trials sponsored by Vertex, Abbvie, Raptor. LDK reports grants from the NHMRC during the conduct of the study. GRJ reports grants from Cystic Fibrosis Foundation Therapeutics USA and The Prince Charles Hospital Foundation during the conduct of the study. CEW reports outside of the submitted work: research grant from Novo Nordisk Pharmaceuticals; honorarium fees as speaker for Vertex, DKBmed; honorarium for: consulting work (BMJ, Vertex), advisory board (Vertex); to present at conference (Novartis), attendance at meetings (University of Miami), Associate Editor duties (Thorax) and travel support to attend meetings for clinical trials sponsored by Vertex. CEW is Associate Editor Thorax and Associate Editor Respirology. MEW reports outside of submitted work: travel support to attend

clinical trial meetings sponsored by Vertex and Galapagos. Other authors have no competing interests to disclose.

Table 1: Comparison of the sputum and aerosol concentrations between the GNB and *S. aureus* groups.

Sputum parameter; mean* (95% CI)	Stratified by organism/s identified in sputum				p-value
	GNB, n=18†		*S. aureus*, n=16		
Sputum bacterial concentration; CFU/mL x 10^6	7.0 (1.6 – 31)		1.3 (0.2 – 7.5)		0.13
Aerosol parameter; mean* (95% CI)	n#	GNB aerosol CFU	n#	*S. aureus* 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 ‡	9	10 (3 – 32)	6	4 (2 – 7)	0.12
45-minutes ‡	9	12 (3 – 40)	4	4 (1 – 12)	0.10

***** Geometric mean

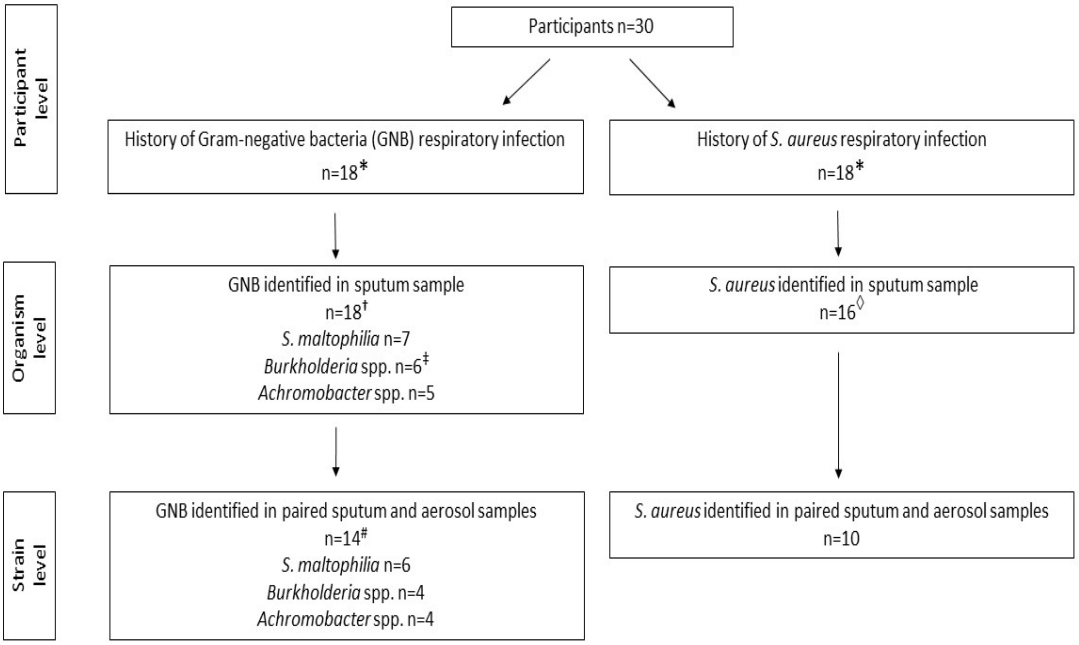
† 18 GNB organisms identified from the sputum of 15 participants (three participants had two GNB species detected)

‡ One GNB group participant did not complete the 15 and 45-min duration experiments

Target organisms identified in sputum that had a positive aerosol detected

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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