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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 among 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 m and remain viable within droplet nuclei for up to 45 min. These results suggest that airborne person-to-person transmission is plausible for the CF pathogens we measured.

INTRODUCTION

Recurrent pulmonary infection characterises cystic fibrosis (CF). While *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 (≤4.7 µm 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 m and remain viable for up to at 45 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 2 years. On the testing day, spirometry was performed and sputum was collected.

The experimental equipment 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, while the duration studies involved the ageing 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 Supplementary file 1).

Data were analysed using SPSS V.23. 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 the 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 body mass index 23.6 (4.5) kg/m^2 were studied. Twelve participants had a history of GNB infection, 12 participants had pre-existing *S. aureus* infection and six 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;

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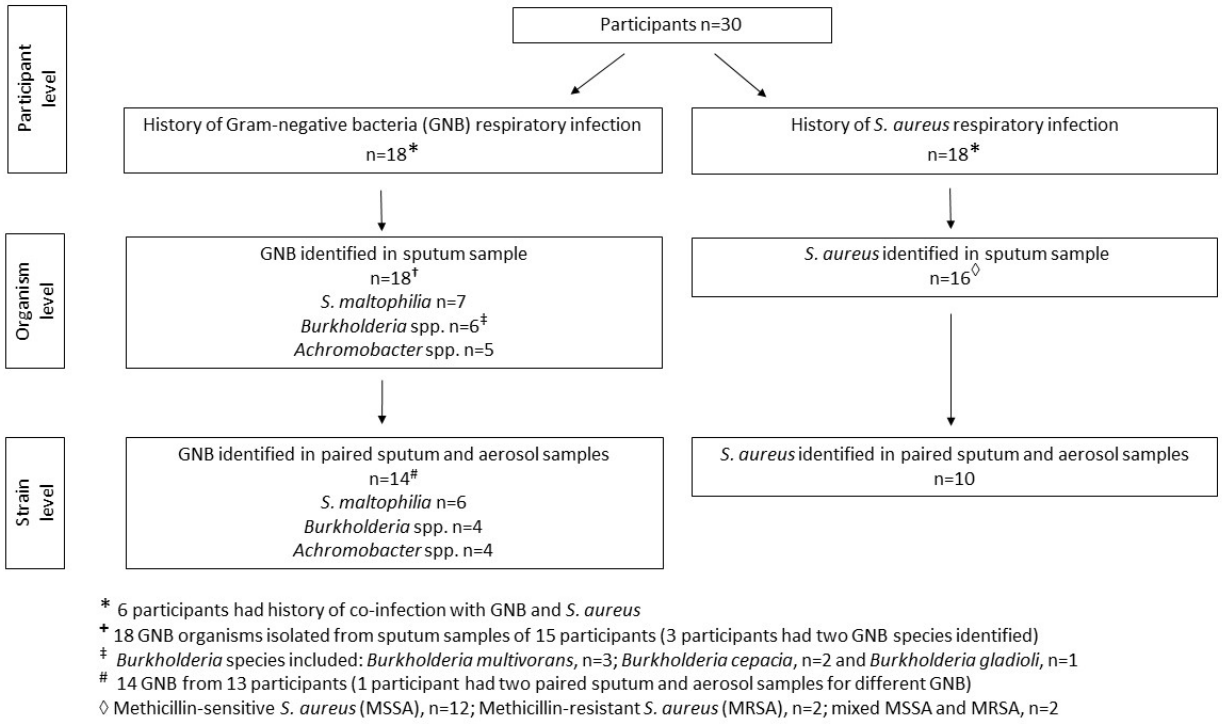

Figure 1 Flow chart of participant stratification and matched sputum/aerosol findings.

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×10^6) for the GNB group was 7.0 (95% CI 1.6 to 31) and for the *S. aureus* group, 1.3 (95% CI 0.2 to 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). Eleven out of 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 (≤4.7 µm) 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 multilocus sequence typing-derived from whole genome sequences) including: *S. maltophilia* (n=6); *Achromobacter* spp. (n=4) and *Burkholderia* spp. (n=4). Aerosolised bacteria were not

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

	Stratified by organism/s identified in sputum				
Sputum parameter; mean* (95% CI)	**GNB, n=18†**		***Staphylococcus aureus*, n=16**		**P values**
Sputum bacterial concentration (CFU/mL×10^6)	7.0 (1.6 to 31)		1.3 (0.2 to 7.5)		0.13
Aerosol parameter; mean* (95% CI)	**n‡**	**GNB aerosol (CFU)**	**n‡**	***S. aureus* aerosol (CFU)**	**P values**
Distance (m)					
2	14	11 (4 to 28)	9	5 (2 to 10)	0.22
4	11	20 (7 to 50)	8	7 (2 to 23)	0.14
Duration (min)					
5	10	13 (4 to 38)	8	3 (1 to 8)	0.062
15§	9	10 (3 to 32)	6	4 (2 to 7)	0.12
45§	9	12 (3 to 40)	4	4 (1 to 12)	0.10

*Geometric mean.
†18 GNB organisms identified from the sputum of 15 participants (three participants had two GNB species detected).
‡Target organisms identified in sputum that had a positive aerosol detected.
§One GNB group participant did not complete the 15 and 45 min duration experiments.
CFU/mL, colony forming units per millilitre of sputum; GNB, Gram-negative bacteria.

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 with 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 *B. 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 GRJ, TJK, CEW, LDK, PDS, LM and SCB led the funding application and conceived the study design. MEW, JC and SCB 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 *Staphylococcus aureus*. ELB and POR led the statistical analysis. MEW and SCB oversaw the overall study and wrote the manuscript, with input from all authors.

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Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

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.

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REFERENCES

1 Kidd TJ, Soares Magalhães RJ, Paynter S, *et al*. The social network of cystic fibrosis centre care and shared *Pseudomonas* aeruginosa strain infection: a cross-sectional analysis. *Lancet Respir Med* 2015;3:640–50.
2 Scott FW, Pitt TL. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. *J Med Microbiol* 2004;53(Pt 7):609–15.
3 Govan JR, Brown PH, Maddison J, *et al*. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *Lancet* 1993;342:15–19.
4 Bryant JM, Grogono DM, Rodriguez-Rincon D, *et al*. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 2016;354:751–7.
5 Knibbs LD, Johnson GR, Kidd TJ, *et al*. Viability of *Pseudomonas aeruginosa* in cough aerosols generated by persons with cystic fibrosis. *Thorax* 2014;69:740–5.
6 Saiman L, Siegel JD, LiPuma JJ, *et al*. Infection prevention and control guideline for cystic fibrosis: 2013 update. *Infect Control Hosp Epidemiol* 2014;35(Suppl 1):S1–S67.
7 Wood ME, Stockwell RE, Johnson GR, *et al*. Face masks and cough etiquette reduce the cough aerosol concentration of *Pseudomonas aeruginosa* in people with cystic fibrosis. *Am J Respir Crit Care Med* 2018;197:348–55.
8 Jones AM, Govan JR, Doherty CJ, *et al*. Identification of airborne dissemination of epidemic multiresistant strains of *Pseudomonas* aeruginosa at a CF centre during a cross infection outbreak. *Thorax* 2003;58:525–7.

9 Ridderberg W, Bendstrup KE, Olesen HV, *et al*. Marked increase in incidence of *Achromobacter xylosoxidans* infections caused by sporadic acquisition from the environment. *J Cyst Fibros* 2011;10:466–9.

10 Muhlebach MS, Heltshe SL, Popowitch EB, *et al*. Multicenter observational study on factors and outcomes associated with various methicillin-resistant *Staphylococcus aureus* types in children with cystic fibrosis. *Ann Am Thorac Soc* 2015;12:864–71.