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

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Online Data Supplement

METHODS

Between May 2015 and July 2016, thirty participants aged ≥ 14 years (adult n=26; adolescent n=4) with a confirmed diagnosis of CF were enrolled from CF Centres at The Prince Charles Hospital and the Lady Cilento Childrens' Hospital, Brisbane, Australia. Exclusion criteria included recent haemoptysis (>50mL), recent pneumothorax, pregnancy and history of cough syncope.

Clinical measurements: Clinical demographics and measurements of age, gender, weight, and height were recorded. Spirometry was performed on the testing day [1] and the Global Lung Index predicted scale applied [2]. An expectorated sputum sample was collected and processed immediately by bacterial culture.

Aerosol collection: The Distance Rig comprised a closed-system, 4.5-m perspex tunnel with HEPA-filtered airflow [3, 4]. Participants were seated and elevated into the rig to their shoulder level. They undertook 2-min of tidal breathing and were then requested to cough at a comfortable strength and frequency for 5-mins. Two tests were undertaken in the Distance Rig with the collection point at 2-m and then 4-m. The Duration Rig consisted of a rotating drum to prevent aerosol settling, with the participants connected to the system via external

tubing and mouthpiece [3, 4]. Three tests were undertaken with the Duration Rig; participants performed 2-mins of tidal breathing of HEPA-filtered air and then coughed for 2-mins, expelling the aerosol directly into the drum. The drum was then sealed and the sample isolated and aged for the designated time period (5, 15 and 45-min) before aerosol extraction. A pump drew the aerosol sample through six-stage Andersen Cascade Impactors (ACI) (Thermo Scientific, Franklin, MA, USA), stacked with selective culture media for the target organism/s (Gram-negative bacteria [GNB]: chocolate bacitracin [300 mg/mL]) agar; *Staphylococcus aureus*: colistin nalidixic acid agar [Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia]). Continuous aerosol sampling for 5-min was undertaken during the coughing period for the distance studies and at the designated time following cough manoeuvres for the duration tests [3, 4]. The ACI allowed particle size distribution (65µm to >7µm) of the aerosol samples, with droplet nuclei sized particles (≤4.7µm) collected on stages 3–6 [5].

Bacterial culture: The aerosol plates were incubated aerobically at 35°C and the total number of bacterial colony forming units across all stages of the ACI was assessed daily for 72 hours and adjustments made for hole correction [6]. Standard quantitative and qualitative microbiological processing was performed on sputum samples [4].

Bacterial typing: Identification of the *S. aureus* isolates was performed by latex agglutination using the Staphytech Plus kit (Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) and *S. aureus* genotyping was undertaken using a single nucleotide polymorphism-plus-binary-marker-based typing system as previously described [7]. Presumptive GNB identification was carried out using the VITEK® MS Mass spectrometry identification system (bioMérieux Australia Pty Ltd, Queensland, Australia), with identity confirmation and multilocus sequence typing (MLST) achieved by whole genome sequence (Australian Genome Research Facility, Victoria, Australia) analysis using the Centre for

Genomic Epidemiology Pathogen Finder [8] and MLST [9] platforms, in conjunction with the *Burkholderia cepacia* complex, *Achromobacter* and *Stenotrophomonas maltophilia* PubMLST databases [10]. Molecular typing data were used to compare the relatedness between positive aerosol and sputum isolates cultured from individual participants and across the study cohort.

Additional Statistical Methods: Categorical variables were examined using Pearson Chi-Squared or the Fisher's Exact test when more than 20% of the expected counts were less than 5. Continuous variables were examined using the Student t-test and the Levene's test used to check for equality of variances.

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